

## Antioxidant Activity, Phytochemical Screening, and LC/MS-MS Characterization of Polyphenol Content of Jordanian Habitat of *Pennisetum Setaceum* Aqueous Leaf Extract

Lidia K. Al-Halaseh<sup>1\*</sup>, Reem Issa<sup>2</sup>, Rana Said<sup>3</sup>, Rawan Al-suhaimat<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mutah University, Al-Karak, Jordan

<sup>2</sup>Department of basic pharmaceutical sciences, Faculty of Pharmacy, Middle East University, Amman, Jordan

<sup>3</sup>Pharmacological and Diagnostic Research Centre, Faculty of Pharmacy, Al-Ahliyya Amman University, Amman, Jordan

### ABSTRACT

**Background:** *Pennisetum setaceum* is an easy-grow and highly adaptable plant characterized by ravishing stalks and colorful leaves. Therefore, this species has been utilized as a green solution in preserving and restoring the ecological balance and developing biodiversity. In addition, different medicinal uses of the plant have been investigated. Yet, modest research was performed to explore the antioxidant activity and the phytochemical composition of the plant.

**Objectives:** The current research aims to evaluate the phytochemical composition and the antioxidant activity for the Jordanian habitat of *P. setaceum*.

**Methods:** Aqueous extract of leaves was prepared by maceration. Screening tests for the identification of secondary metabolite content were conducted using standard procedures. The free radical scavenging activity for the extract was determined using DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay and compared with ascorbic acid. The LC-MS/MS analysis was performed focusing on the phenolic content of the extract.

**Results:** The screening tests revealed the presence of steroids, triterpenoids, alkaloids, tannins, flavonoids, and polyphenols, while saponins were not observed. At a concentration of 4 mg/ml, the free radical scavenging activity for the extract was only 41.32%, compared to 85.54% for ascorbic acid. The LC-MS/MS analysis revealed the presence of eight different phenolic compounds: Succinic acid, protocatechuic aldehyde, 2,5-dihydroxybenzoic acid, 2,3-trans-3,4-trans-leucocyanidin, apiin, iso-orientin, and apigenin, and 5,6,4'-trihydroxy-7,3'-dimethoxyflavone.

**Conclusion:** The presence of a limited number of phenolic compounds in the *P. setaceum* extract may explain its weak antioxidant activity. Further research is required to identify other (non-phenolic) secondary metabolites content, which would enhance our understanding of the roles this plant species play in agricultural, ecological, or medical applications.

**Keywords:** *P. setaceum*; Chromatography; Mass Spectroscopy; Secondary metabolites; Antioxidants; Phytochemical screening.

### INTRODUCTION

The genus *Pennisetum* (Poaceae) has verified diverse genetic inheritance characteristics according to plant

sources and habitats such as China and the United States.<sup>1</sup> Genetic analysis, for identification, was conducted according to simple sequence repeats (SSRs) as DNA markers, and by identifying polymorphic markers.<sup>2,3</sup> A recent study revealed a wide genetic diversity among *Pennisetum* species in ornamental sources.<sup>4</sup>

The species *Pennisetum setaceum* is highly reputed as

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\*Corresponding author: Lidia Kamal Al-Halaseh

[drhalaseh@mutah.edu.jo](mailto:drhalaseh@mutah.edu.jo)

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an ornamental garden plant,<sup>3</sup> due to its ravishing stalks and colorful leaves.<sup>4</sup> In addition to its aesthetic function, *P. setaceum* has many eco-environmental potential roles; the crop residue has a promising role in adsorbing heavy metal ions in particular, chromium ion from water resources by a non-expensive, efficient absorbent composite based on *P. setaceum* and chitosan. Thus, the plant could be incorporated into a feasible waste management strategy.<sup>5</sup> Besides, a recent study confirmed the efficiency of *P. setaceum* in treating sanitary sewage effluent from pharmaceutical contaminants including caffeine, endocrine disruptors, and other pharmaceuticals.<sup>6</sup> Recent research studied the behavior of some ornamental plants, including *P. setaceum*, and concluded their importance as a green solution in preserving and restoring the ecological balance and developing biodiversity.<sup>7,8</sup>

In regard to the use of *P. setaceum* as a medicinal plant and exploring its biological activities, modest data have been collected from research projects performed to justify the use of the plant for medicinal purposes. Ethnopharmacological and scientific studies revealed that the plant was used in managing urogenital pain in Sudan, lowering blood sugar level in Jordan, and as a slimming agent.<sup>9,10,11</sup>

A review article published by Ojo et al.,<sup>12</sup> mentioned that only a few species' chemical compositions and metabolites have been investigated in the genus *Pennisetum*, with varied health related activities. These secondary metabolites include varied fatty acids, anthocyanin, steroids, triterpenoids, alkaloids, tannins, flavonoids, polyphenols, and saponins. The secondary metabolites content of the plant of our interest are not fully-investigated yet, except for anthocyanin content and biosynthesis.<sup>13,14</sup>

It is well known that oxidative stress has been linked to a variety of degenerative diseases. Therefore, there is a greater demand for the use of antioxidants agents such as phenolic compounds in addition to other micronutrients.<sup>12, 15, 16</sup> The, current study aims to screen the antioxidant

activity (via DPPH assay), and the secondary metabolites content (via general phytochemical screening and LC/MS-MS characterization of polyphenols) of the Jordanian habitat of *P. setaceum* aqueous leaf extract. Findings would shed light on the potential biological activities, as well as the phytochemical composition of the plant, with special focus on the antioxidant and polyphenols components. To our knowledge, this is the first study investigating the antioxidant activity, phytochemical composition, and phenolic content of this plant species.

## MATERIALS AND METHODS

### *Plant material*

Fresh leaves were collected from widely grown plants in South Jordan. The plant material was identified by the Royal Society for the Conservation of Nature (RSCN), Amman, Jordan. A voucher sample was deposited in the RSCN herbarium with the reference number RS/10/1/518.

According to Al-Halaseh et al.<sup>11</sup>, the leaves were dried under shade before reducing in size. For extraction, each 250 g of dried leaves was macerated with 100 ml distilled water with continuous stirring using mmemert shaker waterbath® at 25°C for 16 h. This process was repeated twice, then the suspension was filtered and concentrated until a fine powder was obtained using Benchtop Manifold Freeze Dryer from Millrock Technology®.

### *Preliminary phytochemical analysis*

Phytochemical screening tests were performed to identify the major secondary metabolite content. Polysteroids and triterpenoids content were checked according to Leiberman–Buchard and Salkowski assays, respectively.<sup>16</sup> Saponin content was checked by the frothing test, and ferric chloride solution (1%) was used to identify the soluble polyphenol and tannin content. The Kumar method was used to identify the presence of flavonoids.<sup>17</sup>

### *Antioxidant activity for P. setaceum aqueous leaf extract*

A colorimetric assay based on using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) reagent was used to evaluate the

radical scavenger activity of *P. setaceum* aqueous leaf extract following published data.<sup>18</sup> For the reaction reagent, 0.4 g of DPPH was dissolved in 100 ml methanol. The reaction was performed by mixing 0.1 g of plant extract with 10 ml methanol. 1 ml of the plant extract solution was mixed with 3 ml of DPPH and completed to a final volume of 10 ml using methanol, then allowed to stand in darkness for 30 minutes. The calibration curve was done using ascorbic acid at serial concentrations as a standard antioxidant (Sigma Aldrich, Germany).

The effective concentration required for scavenging of DPPH free radicals (inhibition%) was calculated using the plotted graph of scavenging activity verses the extract concentrations and compared to ascorbic acid. The percentage inhibition (I%) was computed for each sample after measuring the absorbance at  $\lambda_{\text{max}}=517$  nm using Equation 1.

$$\% \text{ inhibition} = \left[ \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right] \times 100$$

Where:  $A_{\text{control}}$  = absorbance of the control sample, and  $A_{\text{sample}}$  = absorbance of the sample

#### Equation 1. DPPH inhibition percentage equation

### LC/MS-MS Methodology

#### Instrumentation and MS parameters

A Bruker Daltonik (Bremen, Germany) Impact II ESI-Q-TOF System equipped with Bruker Daltonik Elute UPLC system (Bremen, Germany) was used for screening the compounds of interest. This instrument was operated using the Ion Source Apollo II ion funnel electrospray source. The capillary voltage was 2500 V, the nebulizer gas was 2 bar, the dry gas (nitrogen) flow rate was 8 L/min and the dry temperature was 200 °C. The mass accuracy was < 1 ppm; the mass resolution was SR (Full Sensitivity Resolution) and the TOF repetition rate was up to 20 kHz. UHPLC was coupled to a Bruker impact II QTOFMS.

Chromatographic separation was performed using Bruker Solo 2.0\_C-18 UHPLC column (100 mm x 2.1 mm x 2.0  $\mu\text{m}$ ) at a flow rate of 0.51 ml/min and a column

temperature of 40 °C. The mobile phase consists of (A) water with 0.05% formic acid and (B) acetonitrile. Gradient mode was applied according to the following: 0 – 27 min linear gradient from 5% - 80% B; 27-29 min 95% B; 29.1 min 5% B. The total analysis time was 35 min with an injection volume of 3  $\mu\text{l}$ .

A previously developed integrated library of natural compounds was used for identification of the phenolic compounds based on the RT and m/z with high resolution. Samples stock solutions were prepared by dissolving an appropriate amount of the plant extract in 2 ml dimethyl sulfoxide-DMSO (analytical grade), followed by dilution with acetonitrile up to 50 ml and centrifugation at 4000 rpm, for 2 min.

This study also includes a quantitative estimates of the percentage (%) relative content of phenolic components, using the method described by Mohammed et al.<sup>19</sup> The area under the peak for each identified phenolic component was converted into peak area (%), showing the occurrence levels of the identified compounds, that were calculated relative to the total area of all the peaks observed in the LC-chromatogram.

### RESULTS

#### Extraction yield

The percentage of extractable compounds using water and maceration extraction method varied from 3.9% to 4.1% (w/w).

#### Qualitative screening of secondary metabolites

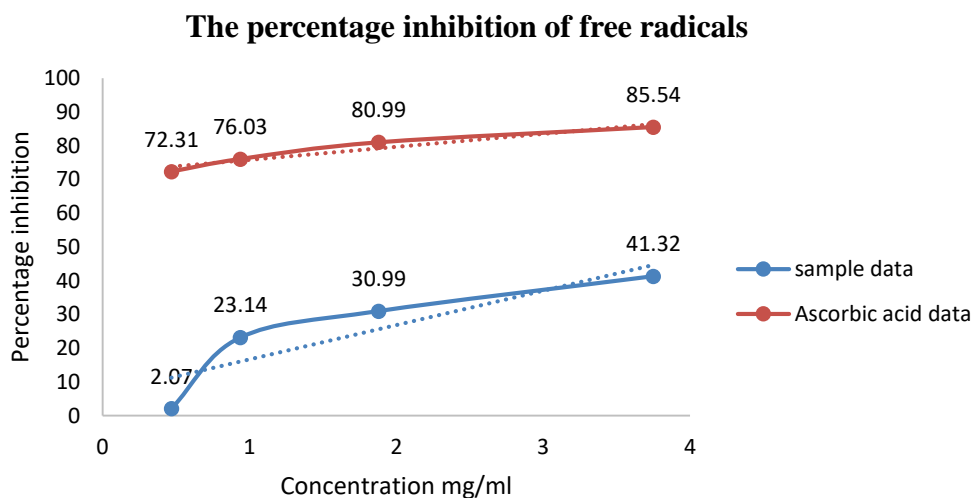
According to the obtained results, *P. setaceum* aqueous leaf extract showed to contain steroids, triterpenoids, tannins, flavonoids, and polyphenols, while the saponins test showed negative result.

#### Antioxidant activity (DPPH Assay) for *P. setaceum* aqueous leaf extract

The antioxidant efficiency, expressed by the free radical inhibition activity, was calculated for serial concentrations of the plant extract, and compared to the standard ascorbic acid (Figure 1). Findings revealed that the plant extract

possesses weak antioxidant activity compared to the standard ascorbic acid. For example, at the higher tested concentration (4.0 mg/ml), the percentage inhibition of the

extract was found to be 41.32% compared to 85.54% for the ascorbic acid at the same tested concentration.



**Figure 1: The %inhibition of free radical activity for serial concentrations of plant extracts and ascorbic acid, using the DPPH assay method.**

#### Identification of phenols using LC/MS-MS analysis

Eight different phenolic components have been detected using the LC/MS-MS analysis, and the integrated natural compounds library. The detected compounds are listed in Table 1. Estimation for the individual percentage

content of the detected compound shows that succinic acid to be the most abundant compound (48%). Other compounds were abundant in the range of (5-9%) relative to the total detected compounds.

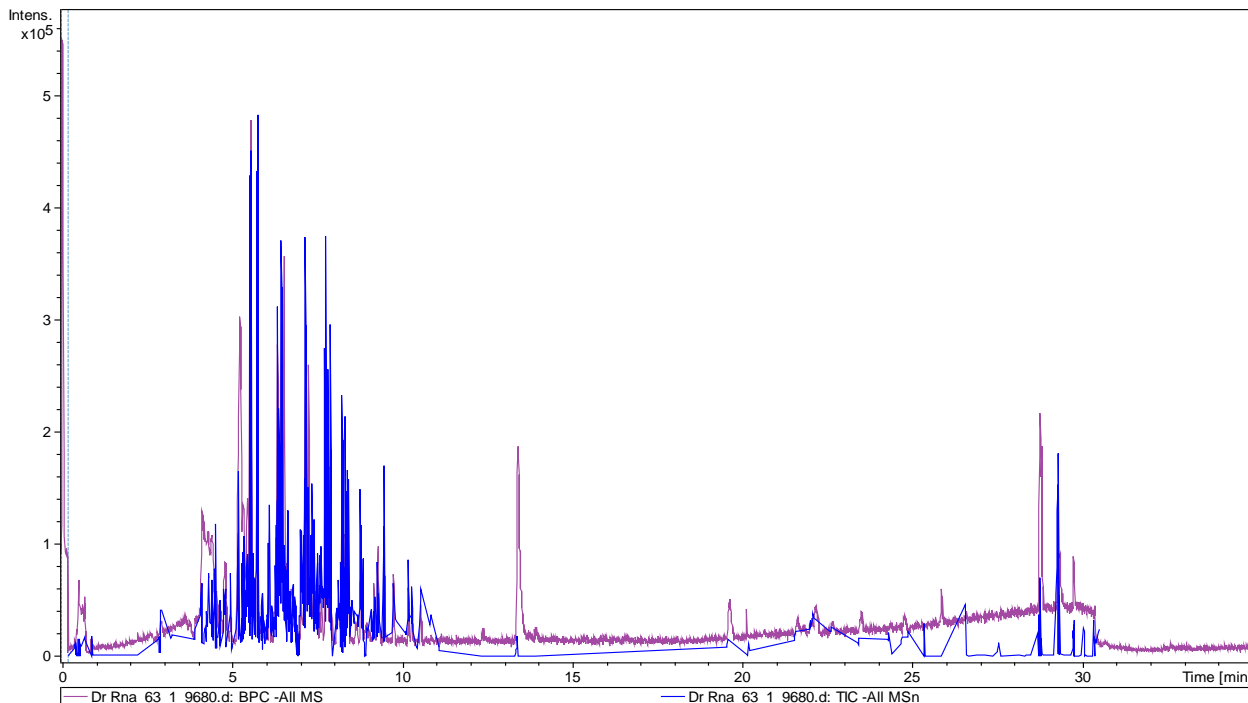
**Table 1: Components detected in *P. setaceum* aqueous leaves extract based on retention time (RT) and Mass (m/z) using LC-MS/MS analysis.**

RT [min]	m/z meas.	M meas.	Name	Molecular Formula	Peak area (%)*
0.98	117.0194	118.0267	Succinic acid	C <sub>4</sub> H <sub>6</sub> O <sub>4</sub>	48%
1.23	137.0246	138.0318	Protocatechuic aldehyde	C <sub>7</sub> H <sub>6</sub> O <sub>3</sub>	5%
1.75	153.0195	154.0268	2,5-Dihydroxybenzoic acid	C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>	8%
3.59	305.0699	306.0772	2,3-Trans-3,4-trans-Leucocyanidin	C <sub>15</sub> H <sub>14</sub> O <sub>7</sub>	6%
4.87	563.1414	564.1486	Apiin	C <sub>26</sub> H <sub>28</sub> O <sub>14</sub>	9%
4.9	447.0928	448.1001	ISO-Orientin	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	9%
5.67	607.1303	608.1376	Apigenin	C <sub>27</sub> H <sub>28</sub> O <sub>16</sub>	7%
10.3	329.0671	330.0743	5,6,4'-Trihydroxy-7,3'-dimethoxyflavone	C <sub>17</sub> H <sub>14</sub> O <sub>7</sub>	8%

\* Relative % (percentages) of the occurrence levels of the identified compounds were calculated in relative to the total area of all the peaks detected in the LC-chromatogram.

Figure 2 shows the total ion chromatogram for all compounds detected in *P. setaceum* aqueous leaves extract. The LC-chromatograms showing peaks and

retention time of each compound detected in the extract are shown in Figure 3. Figure 4 shows the Mass spectra ( $m/z$ ) and fragments for each compound detected in the extract.



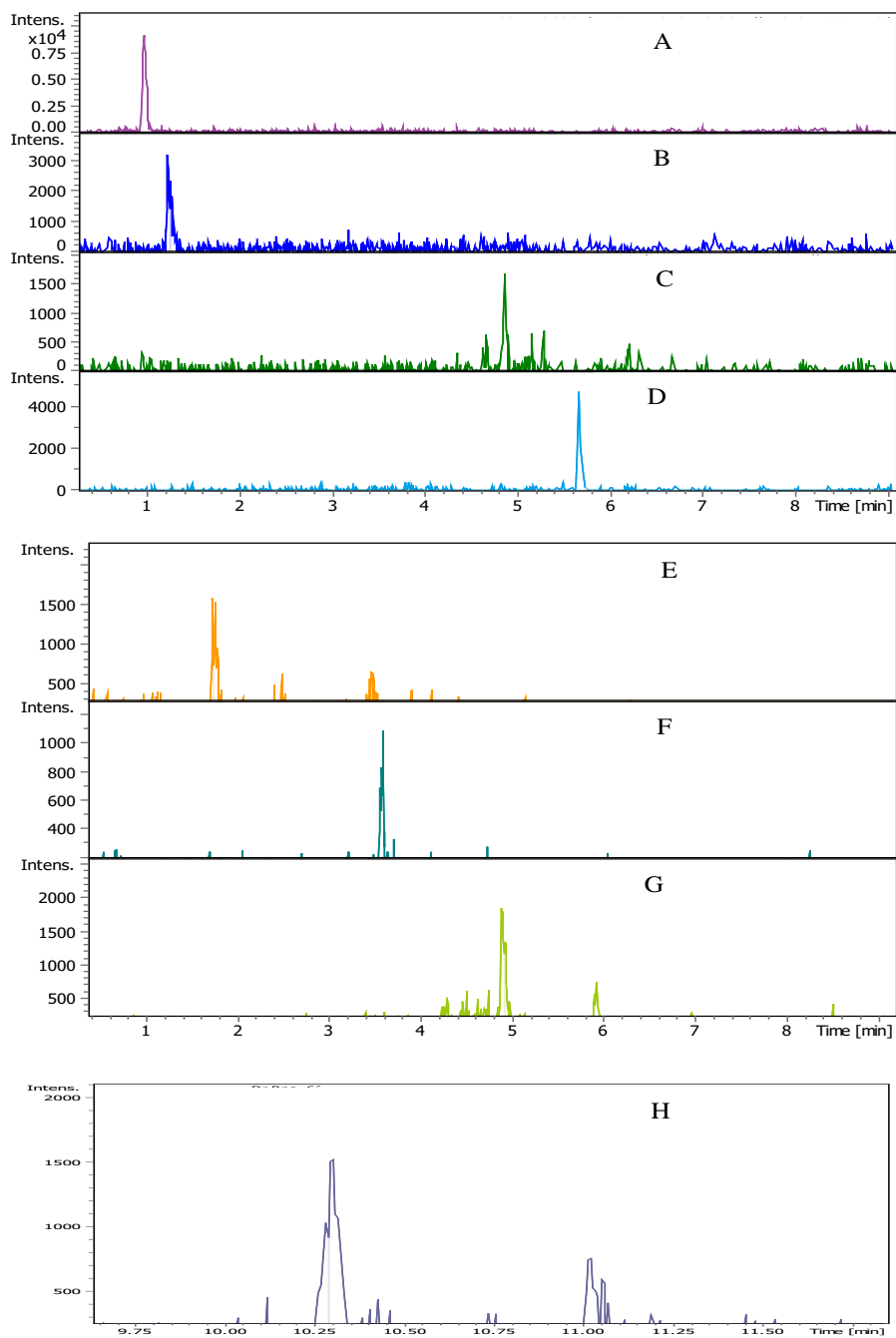
**Figure 2. Total ion chromatogram for all compounds detected in *P. setaceum* aqueous leaves extract.**

## DISCUSSION

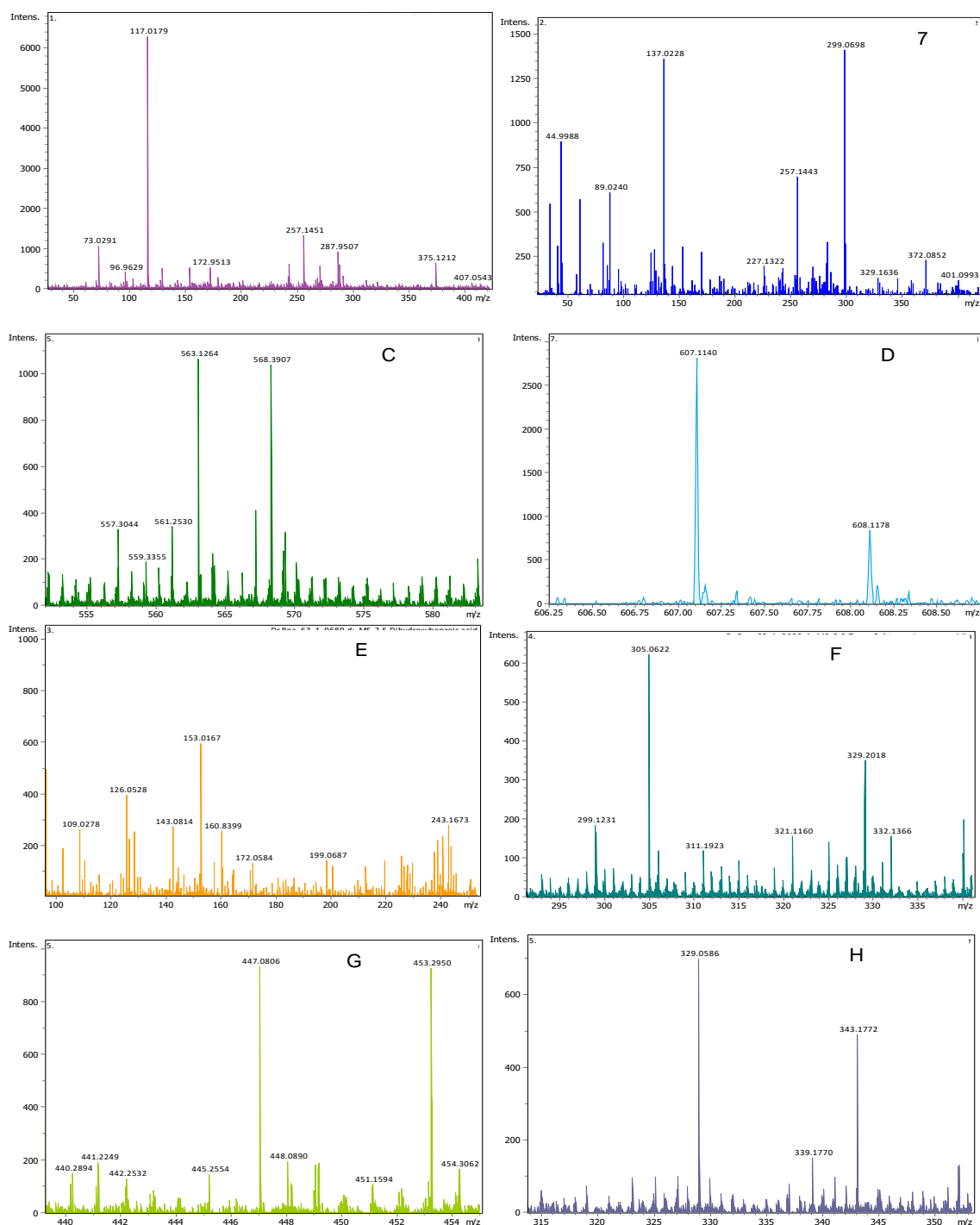
Fountain grass, *P. setaceum*, is highly reputed as an ornamental plant, in addition to its contribution to the ecosystem.<sup>4,9</sup> However, the medicinal activity has not been thoroughly investigated, with limited studies are available on this species.<sup>10, 11, 12</sup> Mostly, the biological activity of a plant extract is attributed to the secondary metabolite content and the antioxidant activity<sup>19</sup>. Therefore, the current study aimed to screen the content of secondary metabolite, phenolic composition, and the antioxidant activity of the *P. setaceum* extract, and to compare it with the content of similar groups among the other related plant species.

In this study, the obtained phytochemical screening results revealed the presence of different bioactive

metabolites in the aqueous extract of the Jordanian fountain grass leaves. These include water-soluble polyphenols, flavonoids, polysteroids, triterpenoids, and tannins. The test failed to detect the presence of saponins. These findings were expected, as previous studies investigated the related plant species; *P. purpureum* phytochemical composition revealed the presence of tannins (67.9%), alkaloids (lunamarine 26.21%), phenols (1.16%), and flavonoids (rutin 2.10%, anthocyanin 0.06%, catechin 2.49%, and kampferol 0.09%). Similarly, seven phenolic compounds (Trans-cinnamic, protocatechic, hydroxybenzoic acids, Gentisic, gallic, caffeic, and p-Coumaric) and two flavonoids (quercetin and catechin) were found in the species *P. glaucum* oil extract.<sup>12</sup>



**Figure 3.** The LC-chromatograms showing peaks and retention time of each compound detected in *P. setaceum* aqueous leaves extract; (A) Succinic acid, (B) Protocatechuic aldehyde, (C) Apiin, (D) Apigenin, (E) 2,5-Dihydroxybenzoic acid, (F) 2,3-Trans-3,4-trans-Leucocyanidin, (G) ISO-Orientin, (H) 5,6,4'-Trihydroxy-7,3'-dimethoxyflavone.



**Figure 4.** MS/MS spectra showing m/z and fragments of each compound detected in *P. setaceum* aqueous leaves extract; (A) Succinic acid, (B) Protocatechuic aldehyde, (C) Apiin, (D) Apigenin, (E) 2,5-Dihydroxybenzoic acid, (F) 2,3-Trans-3,4-trans-Leucocyanidin, (G) ISO-Orientin, (H) 5,6,4'-Trihydroxy-7,3'-dimethoxyflavone.

The free radical scavenging activity of the tested plant extract revealed a weak antioxidant activity compared to the reference ascorbic acid.<sup>18</sup> These results were explained by the findings of the phenolic compounds analysis using the LC/MS-MS, showed the presence of only 8 compounds of these biologically active phenolic compounds, which are widely known for contributing to the antioxidant activity for plants extracts. Similarly, the antioxidant tests using the DPPH or others examining the extract of *P. glaucum* had shown to possess low antioxidant activities, which was influenced by the various ways in which the plant was processed.<sup>12</sup> These findings were explained as when the plant was being exposed to increasingly high temperatures, the flavonoid content of the plant decreases, resulted in a reduction in the antioxidant activity.

In this study, investigating the polyphenol content of the plant extracts, eight metabolites with well-known biological and medicinal activities were identified using the LC/MS-MS in the aqueous extract. Of those the most abundant was succinic acid, is a dicarboxylic acid with a conventional use as an intermediate for variable pharmaceuticals and other consumer products.<sup>20, 21, 22</sup>

The phenolic acid, protocatechuic acid, has a well-known antioxidant activity and was found to treat and/or prevent activities against a multiplicity of disorders, showed activity against bacterial, fungal, and viral infections. Furthermore, the hypotensive, hypolipidemic, and bronchodilation activities were elucidated by several *in vitro* studies, in addition to antispasmodic properties.<sup>23</sup>

A synthetic water-soluble formula of 2,5-hydroxybenzoic acid (2,5-DHBA) and gelatin has a promising antiviral activity by inhibiting the adsorption of alpha-herpesviruses to cells.<sup>24</sup> The metabolite 2,3-Trans-3,4-trans-Leucocyanidin is an antioxidant and serves as a precursor to proanthocyanidin biosynthesis.<sup>25</sup> Apigin is a widely distributed natural flavonoid with reported anti-inflammatory and immunomodulatory activities.<sup>26</sup> A

potential antidiabetic activity for iso-orientin has been reported in *silico* and animal model studies.<sup>27</sup> A health-promoting effect of apigenin was reported after several *in vivo* studies. It has beneficial effects on hypoglycemia, memory enhancement, sleep aid, and anticancer activities.<sup>28</sup> Apigenin was reported as a potential chemotherapeutic agent.<sup>29</sup> In addition, its derivative “genistein” is an isoflavone, isolated previously from soybean, has a chemopreventive effect at low therapeutic doses, with a pharmacological effect at high administered doses.<sup>30</sup>

The eighth detected metabolite, 5,6,4'-Trihydroxy-7,3'-dimethoxyflavone is not an exception. A pharmacological study revealed its potent antioxidant and anti-inflammatory activities. The metabolite reduces the production of nitric acid and cytokine and interferes with nuclear factor- $\kappa$ B translocation and mitogen-activated protein kinase pathways.<sup>31</sup> The revealed antioxidant activity of the current extract matches the expectations, where multiple plants showed free radical scavenger activities in variable efficacies.<sup>32-34</sup>

## CONCLUSION

The LC-MS/MS analysis of *P. setaceum* aqueous leaves extract reveals the presence of a limited number of phenolic compounds. These findings would explain the weak antioxidant activity observed for the extract. Further research is required to identify the other (non-phenolic) secondary metabolites, observed using the screening tests, which would enhance our understanding of potential uses of this plant species for agricultural, ecological, or medical applications.

**Author contribution:** All authors contributed to the research works and approved the submitted manuscript.

**Conflict of interest:** All authors declare no conflict of interest.

**Data availability:** Data is available from corresponding authors upon reasonable requests.



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## نشاط مضادات الأكسدة والفحص الكيميائي النباتي وتوصيف LC / MS-MS لمحتوى البوليفينول في الموائل الأردنية لمستخلص الأوراق المائية *Pennisetum setaceum*

ليديا كمال الهلسه<sup>1\*</sup>، ريم عيسى<sup>2</sup>، رنا سعيد<sup>3</sup>، روان السحيمات<sup>1</sup>

<sup>1</sup> قسم الكيمياء الصيدلانية، كلية الصيدلة، جامعة مؤتة، الأردن

<sup>2</sup> قسم الصيدلة الأساسية، كلية الصيدلة، جامعة الشرق الأوسط، الأردن

<sup>3</sup> مركز الأبحاث الدوائية، كلية الصيدلة، جامعة عمان الأهلية، الأردن

### ملخص

**الخلفية:** *Pennisetum setaceum* هو نبات سهل النمو وقابل للتكيف بدرجة كبيرة ويتميز بالسيقان الجميلة والأوراق الملونة. لذلك، تم استخدام هذا النوع كوسيلة خضراء في الحفاظ على التوازن البيئي واستعادته وتطوير التنوع البيولوجي. بالإضافة إلى ذلك، تم التحقق من بعض الاستخدامات الطبية المختلفة لهذا النبات. تم سابقاً إجراء بعض الأبحاث البسيطة لاستكشاف النشاط المضاد للأكسدة والتركيب الكيميائي النباتي لأوراق هذا النبات.

**الأهداف:** يهدف هذا البحث إلى تقييم التركيب الكيميائي النباتي وفعالية مضادات الأكسدة للموئل الأردني لنبات *P. setaceum*. المنهجية: تم تحضير المستخلص المائي للأوراق عن طريق النقع. أجريت اختبارات تحليلية لتحديد المستقبلات الثانوية النشطة بيولوجياً والناتجة عن الأيض الثانوي باستخدام إجراءات مرجعية. تم تحديد نشاط مضاد الأكسدة للجذور الحرة للمستخلص باستخدام مقايصة DPPH (2-Diphenyl-1-picrylhydrazyl) ومقارنتها بحمض الأسكوربيك. تم إجراء تحليل LC-MS/MS مع التركيز على المحتوى الفينولي للمستخلص.

**النتائج:** كشفت الاختبارات التحليلية عن وجود للمستيريدييات، ترايثيرينويدات، قلويدات، العفص، الفلافونويد، والبوليفينول، في حين لم يلاحظ وجود الصابونينات. عند تركيز 4 ملغ/مل، كان نشاط مضاد الأكسدة للجذور الحرة للمستخلص 41.32%. فقط، مقارنة بـ 85.54% لحمض الأسكوربيك. كشف تحليل LC-MS/MS عن وجود ثمانية مركبات فينولية مختلفة: حمض السكسينيك، وألدهيد البروتوكاتيكويك، وحمض 2،5-ثنائي هيدروكسي بنزويك، و3،2-ترانس-4،3-ترانس-ليوكوسيانيندين، وأبين، وإيزو-أورينتتين، وأبيجينين، و4،6،5-ثلاثي هيدروكسي-7،3-ديميثوكسي فلافون.

**الخلاصة:** وجود عدد محدود من المركبات الفينولية في مستخلص *P. setaceum* قد يفسر ضعف نشاطه المضاد للأكسدة. هناك حاجة إلى مزيد من البحث لتحديد المستقبلات الثانوية الأخرى (غير الفينولية)، والتي من شأنها أن تعزز فهمنا لدور هذا النوع النباتي في التطبيقات الزراعية أو البيئية أو الطبية.

**الكلمات الدالة:** كروماتوغرافي، سبكتروسكوبي، مضادات أكسدة، نواتج طبيعية ثانوية، *P. setaceum*، الفحص الكيميائي النباتي.

\* المؤلف المراسل: ليديا كمال الهلسه

[drhalaseh@mutah.edu.jo](mailto:drhalaseh@mutah.edu.jo)

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