

Phytochemical Screening of Different Parts of *Prangos Platychlaena* Boiss by Liquid-Chromatography Tandem Mass Spectrometry (LC-MS/MS)

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Doi: 10.23918/eajse.v7i2p30

Abstract: Liquid-chromatography tandem mass spectrometry (LC-MS/MS) is a hyphenated analytical technique that combines the separating power of liquid chromatography with mass analysis capabilities. The aim of present study to identify and characterize different chemical compounds present in different (roots, leaves, stems and flowers fractions) parts of *Prangos platychlaena* Boiss. (family: Apiaceae) by LC-MS/MS method. The LC-MS/MS analysis for identification and characterization of phytochemicals in different extracts were performed, and that leads identification of five new compounds first time in *P. platychlaena* plant and they were psoralen, bergapten, 5,8-dimethoxy psoralen, isoimperatorin and pthalic acid mono-2-ethylhexyl ester. The identification and characterization of phytochemicals were performed on the basis on mass spectroscopy data and literature reports. The LC-MS/MS method was found simple and fast for identification and characterization of phytochemicals in different extracts of *P. platychlaena*.

Keywords: *Prangos Platychlaena*, Apiaceae, LC-MS/MS, Psoralen, Bergapten, 5, 8-Dimethoxy Psoralen, Isoimperatorin

1. Introduction

The genus of *Prangos* has 30 species distributed from the Mediterranean to central Asia, 14 of them are found in Turkey while seven of them are found in the different areas of the Kurdistan region of Iraq (Hamad et al., 2017). *Prangos platychlaena* Boiss. (family: Apiaceae) abundantly found in the mountains of Halgurd, Kurdistan region, Iraq. *P. platychlaena* has been reported as potent antioxidant plant, and traditionally used as carminative, and diuretic. *P. platychlaena* also traditionally used in the

Received: October 12, 2021

Accepted: December 10, 2021

Rahman, J.K., & Jaff, D.M.A., Mehmet Ozdemir, M., Ahamad, J., Hussain, F.H.S. (2021). Phytochemical Screening of Different Parts of *Prangos Platychlaena* Boiss by Liquid-Chromatography Tandem Mass Spectrometry (LC-MS/MS). *Eurasian Journal of Science and Engineering*, 7(2), 30-38.

treatment of burning and wounds, kidney, and urinary disorders (Massumia et al., 2007; Kafash-Farkhad et al., 2013; Sharma et al., 2013; Mottaghipisheh et al., 2020). *P. platychnaena* contains several types of secondary plants metabolites such as essential oils, coumarins, and polyphenols etc. (Rahman & Jaff, 2020). The main chemical components in *P. platychnaena* essential oils were (*E*)- β -ocimene (25.93%), bornyl acetate (24.58%) and α -pinene (5.84%) in leaves; and bornyl acetate (25.49%), (*E*)- β -ocimene (22.94%) and α -pinene (9.5%) in the stem, and while in the flowers (*E*)- β -ocimene (28.5%), bornyl acetate (24.18%) and γ -terpinene (14.15%) as major compounds (Rahman et al., 2020). *Prangos* sp. have been reported to have several bioactivities such as antioxidant (Ahmed et al., 2011; Cesur et al., 2017), antibacterial (Nosrati et al., 2018; Rahman et al., 2020; Uzel et al., 2006), anticancer (Farooq et al., 2014), antiviral (Shokoohinia et al., 2014), and also has wound healing potential (Yousefi et al., 2017).

Isolation of natural compounds by column chromatography is tedious process and requires more time and solvents (Naquvi et al., 2020; Ahamad et al., 2014). Identification and characterization of phytochemicals by IR, NMR and Mass spectroscopy is difficult and expensive (Mir et al., 2020; Ahamad et al., 2013). That's new technologies with simultaneous isolation, identification and characterization is the need of day. LC-MS/MS provides simultaneous isolation, identification and characterization of natural compounds with high precision and accuracy (Ahamad et al., 2020). The current research based upon this concept, that simultaneous isolation, identification and characterization of phytochemicals from different parts (roots, leaves, stems and flower extract) of *P. platychnaena*.

2. Material and Methods

2.1 Plant Material

The different plant parts (roots, leaves, stems and flowers) of *Prangos platychnaena* Boiss were collected during May -July 2017 from the Halgurd mountain in Kurdistan region of Iraq, at the altitude of 2170 m. The plant samples were authenticated by Dr. Abdulah Shukur, a plant taxonomist at the Department of Biology, College of Education, Salahaddin University, based upon morphological features reported by Ghazanfar and Edmondson, (2013) (Figure 1).

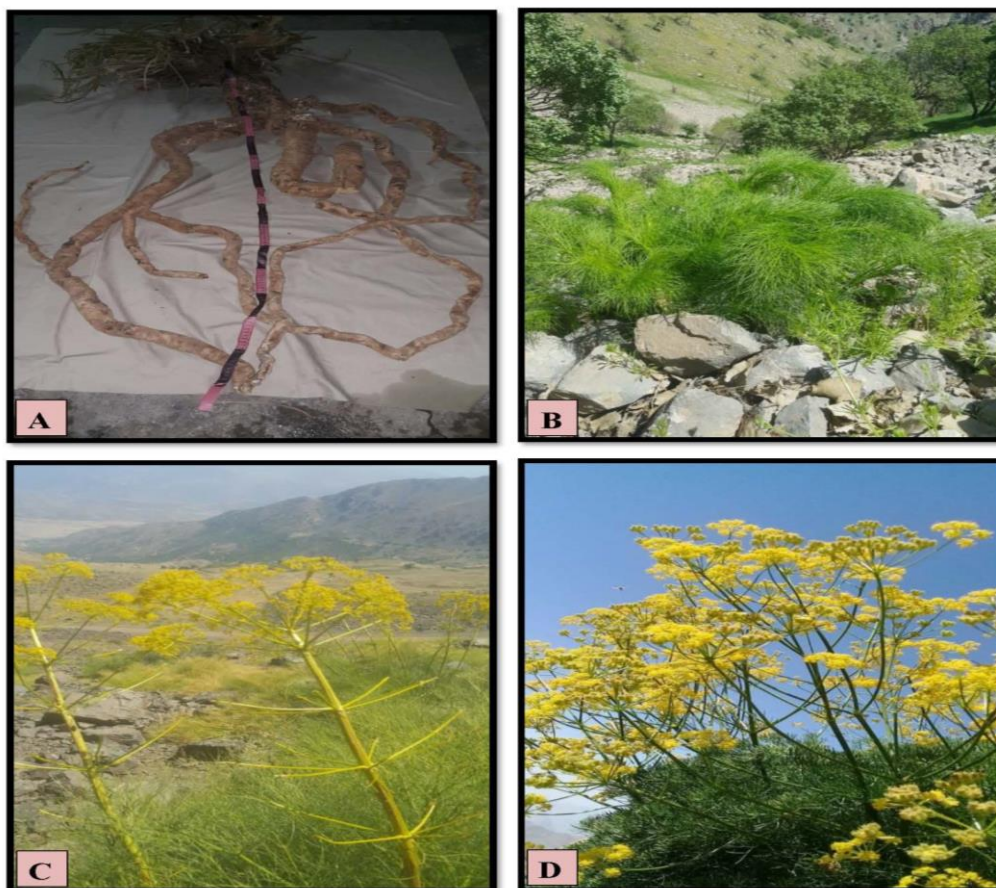


Figure 1: Different plant parts of *P. platychlaena* Boiss A: Root; B: Leaves; C: Stem; D: Flowers

3. Extraction of Plant Material

The different plant parts (root, leaves, stem and flowers) of the *P. platychlaena* Boiss were collected, cleaned and shed dried at the room temperature near 38-40 °C until to gain constant of weight and grinding by grinders slowly, and were passed through a sieve to obtain fine powder after that were stored in the special bottle. Dry powder of each plant part (10 g) was extracted by using a solvent (100 ml), with increasing polarity in the order of petroleum ether (40-60°C), ethanol (99.9%) and distilled water, for three days at room temperature and repeated for three times with stirring a regular interval. The extracts solution were filtered through double layer of muslin cloth and Whatman no.1 filter paper and concentrated using rotary evaporator to obtain the crude extract (Yeo et al., 2014).

4. LC-MS/MS Analysis

The petroleum ether crude extracts of different plant parts and standards compounds were subjected to LC-MS/MS analysis (Agilent 1290 Infinity LC system coupled to Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with dual ESI source). The column C18 (2.1 mm x 150 mm, 3.5-micron length of the column, 10 m) and UV spectrophotometric detector was used. As eluent, 0.1% formic acid in water and 0.1% formic acid in acetonitrile was used at a flow-rate of 0.5 ml/min and the sample injection volume of 1.0 µl. In order to identify the compounds in plant extracts, several standards of phytochemical compounds in database of library Malaysia were used.

5. Results and Discussion

The results of LC-MS/MS analysis of different parts (root, leaf, stem, and flower extract) of *P. platychna* were presented in Table 1 and Figure 2. The LC-MS/MS chromatogram of root, leaf, stem and flower extract of *P. platychna* showed different peak, Rt, Mass, amount and chemical formula of the compounds. Based on the comparison of the peak with the standard compounds, five peaks identified as bergapten, psoralen, 5,8-dimethoxy psoralen (isopimpinellin), isoimperatorin and phthalic acid mono-2-ethylhexyl ester (Figure 2). These above chemical compounds were recorded for the first time in this species, except isoimperatorin was recorded by other worker (Ulubelen et al., 1995), while other peaks refer to the different chemical compounds which do not match with the available database obtained in the University Technology of Malaysia (UTM) in Malaysia, those results were shown in Table 1 and Figure 3-5. From Table 1, it is evident that bergapten is present in leaves (2.15%) and flowers (1.77%), and not detected in roots and stems. Psoralen is detected in only in flowers (3.81%) and absent in other parts of the *P. platychna*. Isopimpinellin also reported only in flowers (1.85%) and not detected in other parts of plant. Exceptionally isoimperatorin in stem was reported to contain maximum amount with 49.50% and flowers with 71.10%. Phthalic acid mono-2-ethylhexyl ester were reported in leaves (6.49%) and flowers (9.75%) extracts of *P. platychna*. The *P. platychna* flower extract contains maximum chemical compounds. The plants show pharmacological actions due to presence of secondary plant metabolites. The results of the present study show flowers contain maximum phytochemical, so it proves beneficial potential of this plants lies in flowers mainly.

Table 1: The contents phytochemicals in different parts of *P. platychna* determined by LC-MS/MS method

Name of compounds	Chemical formula	Mass	Root		Leaves		Stem		Flowers	
			R _t	%	R _t	%	R _t	%	R _t	%
Bergapten	C ₁₂ H ₈ O ₄	216.04	-	-	1.54	2.15	-	-	12.04	1.77
Psoralen	C ₁₁ H ₆ O ₃	186.03	-	-	-	-	-	-	11.37	3.81
Isopimpinellin	C ₁₃ H ₁₀ O ₅	246.052	-	-	-	-	-	-	12.25	1.85
Isoimperatorin	C ₁₆ H ₁₄ O ₄	270.088	-	-	-	-	14.7	49.5	14.74	71.1
Phthalic acid mono-2-ethylhexyl ester	C ₁₆ H ₂₂ O ₄	278.152	-	-	16.7	6.49	-	-	16.77	9.75

Where, (-): not detected; and (%): percentage of amount

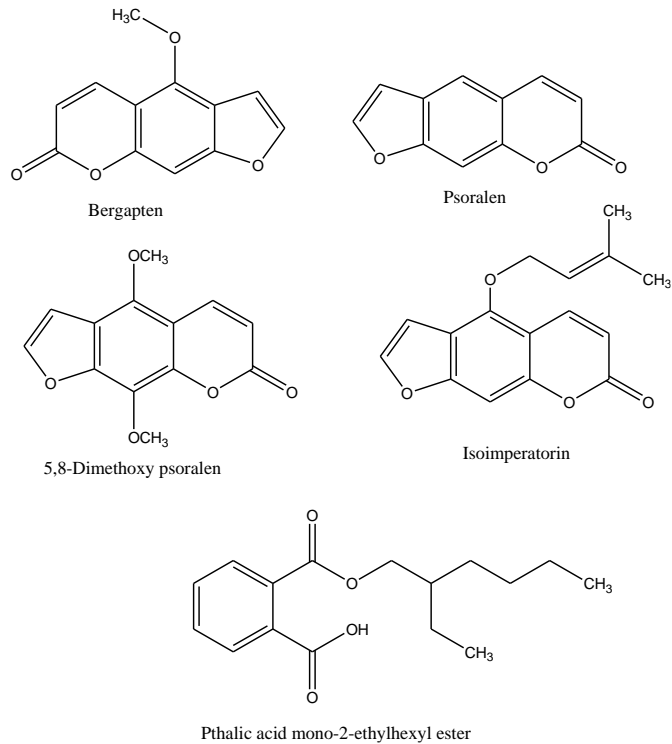


Figure 2: Major phytochemicals identified and characterized by LC-MS/MS from different parts of *P. platychaena*

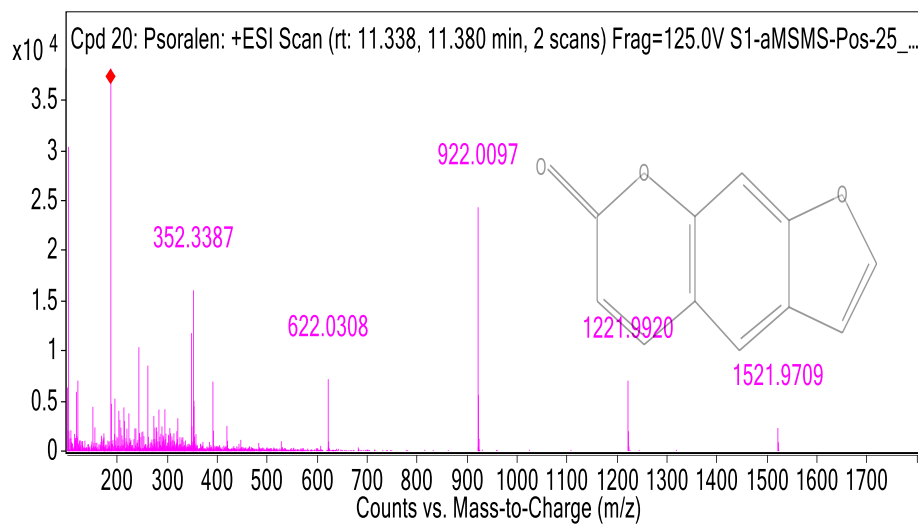


Figure 3: MS spectrum of Psoralen

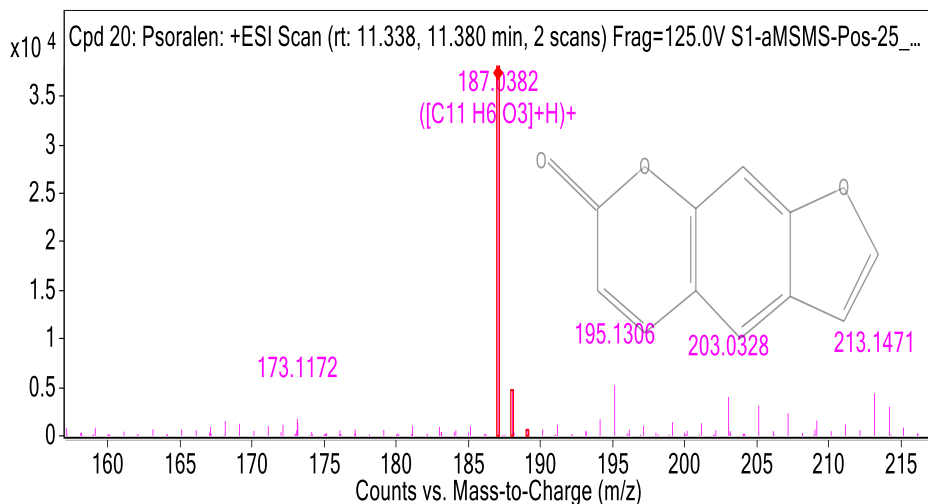


Figure 4: MS zoomed spectrum of Psoralen

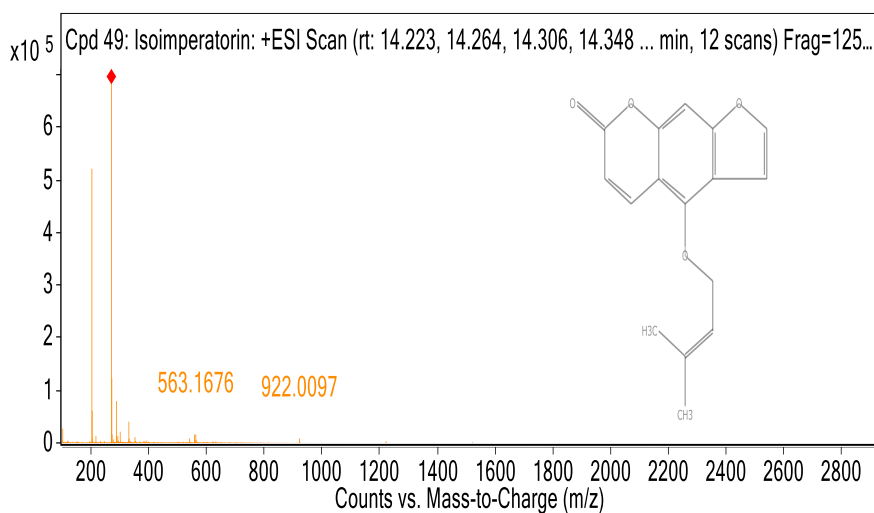


Figure 5: MS spectrum of Isoimperatorin

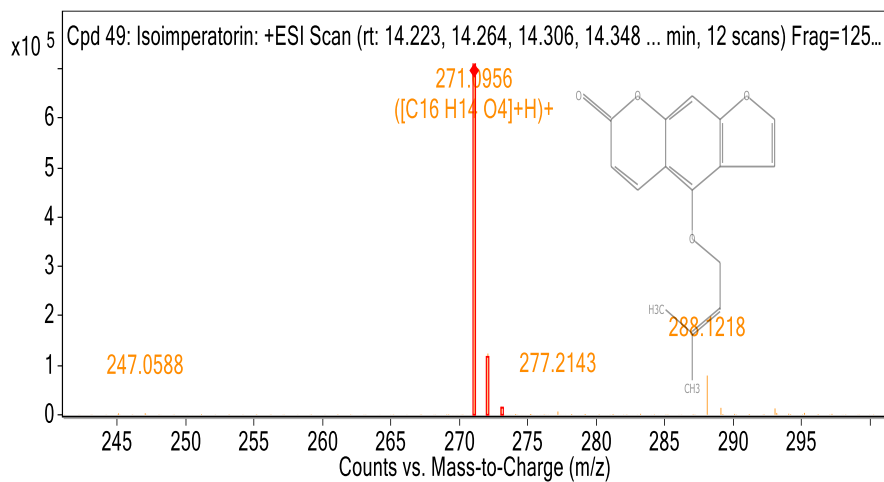


Figure 6: MS zoomed spectrum of Isoimperatorin

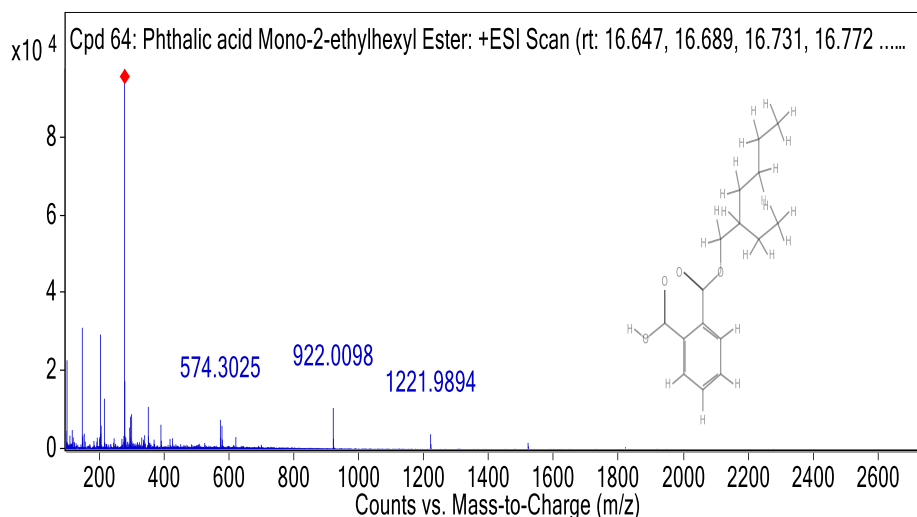


Figure 7: MS spectrum of Phthalic acid mono-2-ethylhexyl ester

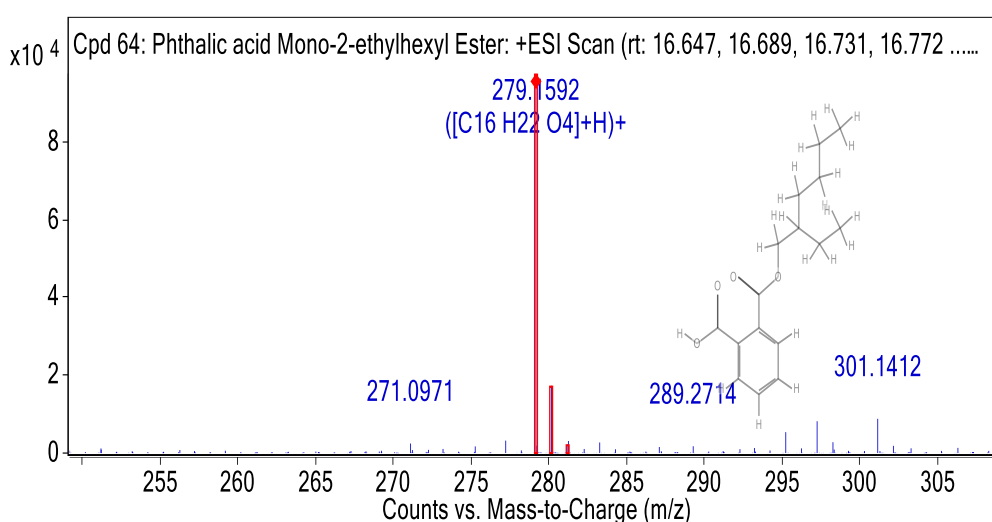


Figure 8: MS zoomed spectrum of Phthalic acid mono-2-ethylhexyl ester

Conclusion

LC-MS/MS method for identification and characterization of phytochemicals in different extracts were performed successfully and that leads identification of five new compounds first time in *P. platychna* plant and they are psoralen, bergapten, 5,8-dimethoxy psoralen, isoimperatorin and phthalic acid mono-2-ethylhexyl ester. The LC-MS/MS analysis showed that flower extract of *P. platychna* showed maximum content of these chemical compounds. The present study explores successfully chemical composition of *P. platychna*.

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