Mass spectra analysis of quinoline alkaloids detected in *Sauuda fruticosa* leads to novel biosynthesis pathway of quinoline alkaloids

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Abstract

Alkaloids reported to have a wide range of biological activity moreover, the biological effects of alkaloids depends on the type of the alkaloids and since the phytochemical screening studies on *Suaeda fruticosa* indicates the presence of alkaloids but they did not determine the types of the alkaloids present on this plant. Consequently, the LC-MS/MS analysis of the alkaloidal part of *Suaeda fruticosa* leave extarct reveals the presence of six quinoline alkaloids beside that mass spectra analysis of the present quinoline alkaloids leads to suggest a new biosynthetic pathway of quinoline alkaloids.

1.introduction

The coastal area of Saudi Arabia is rich in halophytes. One of these halophytes is *Suaeda fruticosa* which belongs to the Chenopodiaceae family and represents 23 genera one of them is suaeda genus. Six species of suaeda genus are found in Saudi Arabia(Alfarhan *et. al.* 2015; Nouri *et. al.* 2017; Oueslati *et. al.*2012 and Petropoulos *et. al.*2018)

Suaeda fruticosa is a shrub or small tree 1-2 m tall. The leaves are linear, glabrous, succulent, green, up to 2.5 cm long, 1-2 mm wide, and flattened on both sides. Flowers are in axillary clusters, sessile, small, and unisexual. Perianth fleshy is up to 1 mm long. Male flowers are larger than female flowers(Alfarhan *et. al.* 2015).

The phytochemical screening of *Suaeda fruticosa* indicates the presence of phenolics, flavonoids, tannins, alkaloids, saponins, proanthocyanins and carotenes(Mzoughi *et. al.* 2018 and Qasim *et. al.* 2017). Alkaloids are one of natural product classes present in *Suaeda fruticosa* which may have a good biological activity(Debnath *et. al.* 2018).Unfortionately, there is no study which describes the isolation and charaterization of alkaloids in *Suaeda fruticosa*.

Also the type of alkaloids present in *Suaeda fruticosa* is still unknown. The most important alkaloid type for the treatment of sub-tropical area diseases is quinoline alkaloids(Shang *et. al.* 2018 and Debnath *et. al.* 2018).

Quinoline alkaloids have attention from early times since the isolation of quinine from the bark of the Cinchona tree in 1820, which is used for malaria treatment(Shang *et. al.* 2018 and Debnath *et. al.* 2018).Quinoline alkaloids have a wide biological activity spectrum such as anti-malaria and anti-cancer applications(Nainwal *et. al.* 2019). Quinoline alkaloids can be divided into several subgroups according to their chemical structure but our focus here will be on simple quinoline alkaloids which consist of benzene ring fused with pyridine ring and different substitution patterns(Solomon *et. al.* 2011). There are two reported boisynthetic pathways for simple quinoline alkaloids: the first one is through anthralic acid and the other one is through tryptophane(Mann, 2005; de Souza *et. al.* 2003 and Sultana, 2013).

In this study LC-ESI-QTOF-MS/MS was used to analyse quinoline alkaloids in *Suaeda fruticosa* . In addition, the quinoline alkaloids biosythetic pathway was described.

2. Results

The LC-ESI-QTOF-MS/MS analysis of the alkaloidal part of *Suaeda fruticosa* extract revealed the presence of six oxygenated simple quinoline alkaloids (Fig. 1). The qualitative and quantitative analysis data for quinoline alkaloids in dry plants are described in table 1.

Table1: retention time, name of quinoline alkaloids, molecular mass found and calculated, main mass fragments, mass error in ppm, uncertainty in mass found , concntration of compounds in extratc and concentration of compounds in dry plants

rt	Compound Name	Compound formula	(M+H) found from ms	(M+H) found for ms/ms, Intensity (%)	(M+H) Calculated	Mass error (ppm)	Uncertainty in m/z	Conc. Of compound in extract (ppm)	Conc. Of compound in dry plant (ppm)	ref
1.64	3-hydroxyquinoline	C ₉ H ₇ NO	146.0602	146.0602(100%), 128.0497(5%),117.0574(10%), 104.0484(7%), 91.0548(50%), 77.0392(50%)	146.0611	-6.1618	±0.0009	934.5	5.4	Felczak <i>et. al.</i> 2016
1.83	6-Hydroxyquinoline	C ₉ H ₇ NO	146.0602	146.0602(100%), 128.0520(10%), 118.0635(10%) , 91.0543(75%), 77.0388(50%)	146.0611	-6.1618	±0.0009	224.6	1.3	
5.64	3-hydroxy-2-quinolinone	C ₉ H ₇ NO ₂	162.0550	162.0550(10%), 147.0462(10%), 144.0443(30%), 118.0660(80%), 116.0506(100%), 91.0535(60%), 89.0379(30%), 79.0544(25%)	162.0561	-6.1707	±0.0010			
5.31	Quinoline-2,3-oxide	C ₉ H ₅ NO	144.0451	144.0451(60%), 116.0497(80%),89.0388(100%)	144.0455	-2.7769	±0.0004			
5.31	3-Hydroxy-2-methoxyquinoline	C ₁₀ H ₉ NO ₂	176.0706	176.0706(20%),158.0609, (10%), 144.0456, (100%), 119.0783(10%),117.0575(60%), 116.0494(80%), 106.0727(5%), 89.0387, 60%.	176.0717	-6.2475	±0.0011	48.5	0.3	
5.65	2,3-Dimethoxyquinoline	C ₁₁ H ₁₁ NO ₂	190.0863	162.0552(10%), 144.0450(60%), 118.0652(100%), 117.0575(38%), 116.0487(40%), 91.0540(100%), 89.0391(35%)	190.0874	-5.7868	±0.0011	132.9	0.8	

Also, mass spectra analysis of the detected quinoline alkaloids leads to suggest a new biosynthetic pathway(scheme 1 and 2).

Table 2: LC gradient parameters

Time(min)	Flow Rate (mL/min)	A Conc.	B Conc.
00.0	0.7	90.0	10.0
7.00	0.7	2.00	98.0
8.50	0.7	2.00	98.0
8.60	0.7	90	10.0
9.50	Stop		

3. Discussion

3.1. Mass spectra analysis of detected quinoline alkaloids:

Two types of oxygenated quinoline alkaloids were detected in *Suaeda fruticosa* extract. One is oxygenated in one position while the other is oxygenated in two positions.

3.1.1. Mass spectra analysis of quinoline alkaloids oxygenated at one possiton:

From this type two quinoline alkaloids were detected. 3-hydroxyquinoline (1) and 6-hydroxyquinoline (2). Their mass spectra (Fig. 2) illustrated a similarity and the only difference was the relative intensity of M-28 peak and this exclude the probapility of 2,4 and 8 oxygenated quinoline[15].

Also, M-55 peak appeared at high intensity compared with 5 and 7 oxygenated quinoline, so the possible oxygenation positions are 3 and 6 (Kaczmarek and Steinegger, 1958). The difference between 3-hydroxy and 6-hydroxy is the presence of mass peak at 104.0484 which indicates the loss of H₂O followed by loss of C₂. This is only possible for 3-hydroxyquinoline. Also, the retention time indicates a very low difference in polarity between the two compounds, therefore 3-hydroxy is more polar than 6-hydroxy. The mass fragmentation pattern of the two compounds was given in Fig. 3.

3.1.2. Mass spectra analysis of quinoline alkaloids oxygenated at two positions:

Four quinoline alkaloids were detected to be oxygenated at two positions but the only two possible positions were 2 and 3. Fig. 4 represents the mass spectra of the four compounds. The main peaks in all compounds are 144.0450, 116.0494 and 89.0387. Figure 5 shows the structure of these peaks and how they are formed. On the other hand, the characteristic fragmentation in compounds 3 and 5 was due to the loss of H₂O [M-18] and this indicates the presence of hydroxyl group in both compounds. In addition, some characteristic peaks were found to prove the position of substituents.

For compound 3, three characteristic peaks proved the proposed structure of the compound. The first peak appeared at m/z=147.0462 which results from NH loss and this indicates an amid group in the compound. The other peaks at m/z=118.0660 and m/z=91.0555 indicate the presence of α -hydroxy to carbonyl group.

In compound 5, the appearance of two peaks at m/z=119.0783 and 106.0727 gives an evidence for the proposed structure as explained in fig. 6.

Two main peaks proving the proposed structure for compound 6. The first one [M-C₂H₄] at m/z=162.0552 indicates that the two methoxy groups are adjacent and this peak was followed by the loss of CO₂ to give a peak at m/z=118.0652 like compound 3, after that the loss of HCN. The other peak at m/z=146.0937 which due to [M-CO₂] reveals a methoxy group at position 2 to allow rearrangement and the loss of CO₂ as described in Fig. 7.

3.2. Biosynthetic pathway for the detected quinoline alkaloids

LC-MS/MS analysis of *Suaeda fruticosa* showed the presence of quinoline alkaloids, anthranilic acid and indole and the absence of tryptophane. Moreover, all detected quinoline alkaloids do not have hydroxyl group at position 4 which indicates that the biosynthetic pathway of quinoline alkaloids in *Suaeda fruticosa* is different from the reported one in other plants. The presence of 3-hydroxyquinoline and indole supports the hypothesis of suggested biosynthetic pathway shown in scheme 1. Since this pathway was reported for indole, the only difference is in the cyclization mechanism in the last step in indole to give 5 membered ring and 3-phosphoglyceraldehyde but in the suggested pathway gives 3-hydroxyquinoline and phosphoacetaldehyde.

It is noteworthy that 3-hydroxyquinoline is a precursor for all other quinoline alkaloids present in *Suaeda fruticosa* as shown in scheme 2 and all steps are common steps in plants which include oxidation, hydration and methylation.

4. Conclusion

In this study six quinoline alkaloids were detected in the leaves of *Suaeda fruticosa* by using LC-MS/MS analysis beside that a new biosynthesis pathway for quinoline alkaloids were suggested in light of the component of leaves of *Suaeda fruticosa*.

5. Materials And Methods

All chemicals were purchased from Sigma-Aldrich.

5.2. Plant material

The fresh leaves of *Suaeda fruticosa* collected from Al-Berk beach at (18.334391, 41.463814) Asser region, Saudi Arabia on 21 July 2017. The plant material was identified in biology department of King Khalid University.

For crude extract preparation 200 grams of fresh leaves were washed with distilled water and dried in the shade for 15 days then ground via grinder. The powder was soaked in petroleum ether for 10 days for defatting then extracted thoroughly with ethanol four

times each time needs 7 days later .

The ethanol extract was filtered using filter paper and concentrated to dryness under reduced pressure using a rotary evaporator at 37⁰c (Ikia -Germany). The concentrated crude extract weight 24g, which represent (6%)of the plant weight.

5.3. Preparation of alkaloidal extract

The ethanol residue was dissolved in 5% HCl until the PH = 2 of the solution and filtered, the precipitate which contains neutral material was kept for further fractionation, and the filtrate which provides the basic material was basify using NH₄OH solution, and the PH of the solution becomes around 8. After that, the solution was extracted with chloroform 50 mL three times the chloroform layer was evaporated to get 0.246 g of crude alkaloids which represent 0.123% of the dry plant.

5.4. LC-MS/MS for Plant Extracts

Analyses were performed on SCIEX X500R QTOF system (includes UPLC-MS/MS (Woodlands Central Indus. Estate., Singapore)). The separation was performed using Phenomenex Kinetex 2.6 μ m Phenyl-Hexyl 100 A (50 $\times$ 4.6 mm, Phenomenex, Madrid Avenue, Torrance, CA, USA). The mobile phase consists of phase A (10 mM ammonium formate in water) and phase B (0.05% formic acid in methanol). A variable gradient flow rate was used, which is described in Table 2. A positive non-targeted mode was used for the analyte.

Table 2: LC gradient parameters

Time(min)	Flow Rate (mL/min)	A Conc.	B Conc.
00.0	0.7	90.0	10.0
7.00	0.7	2.00	98.0
8.50	0.7	2.00	98.0
8.60	0.7	90	10.0
9.50	Stop		

6. Declarations

6.1. Ethics approval and consent to participate

Not applicable

6.2. Consent for publication

Not applicable

6.3. Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

6.4. Competing interests

The authors declare that they have no competing interests

6.5. Funding

Self-funded by the authors

6.6. Author Contribution: Experiments and the writing of the manuscript were carried out in contribution of all authors. All authors read and approved the final manuscript.

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Schemes

Scheme 1: Biosynthesis pathway of 3-hydroxyquinoline

Scheme 2: Biosynthesis pathway of compounds 3,4,5 and 6

Figures

3-Hydroxyquinoline (1)

6-Hydroxyquinoline (2)

3-Hydroxy-2-quinolinone (3)

Quinoline-2,3-oxide (4)

3-Hydroxy-2-methoxyquinoline (5)

2,3-Dimethoxyquinoline (6)

Figure 1

Structures of detected oxygenated quinoline alkaloids

Figure 2

a) mass spectrum of 3-hydroxyquinoline (1) b) mass spectrum of 6-hydroxyquinoline

Figure 3

Mass fragmentation of a) 3-hydroxyquinoline (1) b) 6-hydroxyquinoline

Figure 4

mass spectrum of a) 3-hydroxy-2-quinolinone (b) quinoline-2,3-oxide (c) 3-hydroxy-2-methoxyquinoline (d) 2,3-dimethoxyquinoline

Figure 5

Mass fragmentation of a) 3-hydroxy-2-quinolinone (b) quinoline-2,3-oxide (c) 3-hydroxy-2-methoxyquinoline (d) 2,3-dimethoxyquinoline

Figure 6

Rearengment in compound 5

Figure 7

Rearengment in compound 6