PHYTOCHEMICAL STUDY OF
HALOXYLON SALICORNICUM
(FAM. CHENOPODIACEAE )
GROWING IN SAUDI ARABIA

By:

Mosa M. Qasheesh
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1. Introduction:

_Haloxylon salicornicium_ (Moq.) Bunge ex Bioss. belongs to the family Chenopodiaceae, which has 120 genera and more than 1300 species [1]. They are worldwide distributed especially in desert and semidesert areas in soils containing much salt [2, 3]. The plants are herbs, shrubs, subshrubs and rarely small trees [3]. The family is characterized by the presence of betacyanin, plant with C4 photosynthesis and CAM (Crassulacean acid metabolism) accumulating organic acids [1].

The genus _Haloxylon_ Bunge (Incl. _Hammada_) comprises of about 25 species [3]. It is distributed from Western Mediterranean region to Arabia, Iran, Mangolia, Burma and Southwest of China [3]. In Saudi Arabia, two species are found, which are _H. persicum_ Bunge ex Boiss & Buhse. and _H. salicornicum_ Bunge ex Boiss. [4].

Two species of the genus were recorded in the literature to have folkloric uses. _H. salicornicum_ is reported to be used for diabetes [5], as antiseptic and anti-inflammatory [6]. In Oman the stems of this species are used as a mordant for dyeing wool in traditional weaving [1]. In addition, _Hammada scoparia_ Pomel (= _Haloxylon articulatum_) is used to treat eye disorders [7].

On the other hand, few species of the genus _Haloxylon_ (seven species) have been chemically investigated, which resulted in the isolation of the several alkaloids belonging to mainly seven classes of alkaloids. These classes are:

1- Aliphatic quaternary alkaloids

Betaine chloride (1) is the only aliphatic quaternary alkaloid that has been isolated from _Haloxylon salicornicum_ [8] and _H. persicum_ [9].

2- Pyridine alkaloids

Six pyridine alkaloids have been isolated from _Haloxylon_ species. Piperidine (2), anabasine (3), aldotripterideine (4), haloxine (5) and halosaline (6) were isolated from _H. salicornicum_ [8]. In the other hand, anabasine (3) and nicotine (7) were isolated from _H. persicum_ [10].

3- Indole alkaloids

Tryptamine (8) and dipterine (9) are the only two indole alkaloids that have been isolated from _Hammada articulata_ ssp. _scoparia_ [11] (= _Haloxylon articulatum_). Dipterine (9) was also reported from _Hammada leptoclada_ [12].

4- Isoquinoline alkaloids

The six isoquinoline alkaloids N-methylisosalsoline (10), carneine (11), isosalsoleline (12), salsolidine (13), dehydrosalsolidine (14) and isosalsolidine (15) have also been isolated from _Hammada articulata_ ssp. _scoparia_ [11] (= _Haloxylon articulatum_). Alkaloids 10, 11 and 13 were also reported from _H. articulatum_ [13].
5- Isoquinolone alkaloids

One isoquinolone alkaloids namely, N-methylcorydaldine (16) has been reported from Hammada articulata ssp. scoparia [11] \(\{=\)Haloxylon articulatum\}.  

6- \(\beta\)-Carboline alkaloids

Four \(\beta\)-carboline alkaloids have been reported from Haloxylon species. Tetrahydroharman (17) was isolated from Hammada articulata ssp. scoparia [11] and Hammada leptoclada [12]. While, leptocladine (18) and 3-methyl-1,2,3,4-tetrahydro-\(\beta\)-carboline (19) were isolated from H. leptoclada [12] and 2-methyl-1,2,3,4-tetrahydro-\(\beta\)-carboline (20) was only isolated from H. articulatum [13].

7- Phenylethylamine alkaloids

Oxedrine (21), tyramine (22) and N-methyltyramine (23) are the phenylethylamine alkaloids that have been isolated from H. salicornicum [8, 14].

Besides alkaloids, the genus was found to contain coumarins and six of them have been isolated from H. salicornicum [15]. These coumarins were identified as scopoletin (24), scopolin (25), umbelliferone (26), xanthotoxol (27), isooxyimperatorin (28) and esculetin (29). Additionally, the 5-hydroxy-3-methoxy-4\(H\)-pyran-4-one (30) and fucosterol (31) compounds were isolated from H. salicornicum [16, 17]. On the other hand, the flavonoid quercetin (32), its glycoside quercetin-7-O-rhamnoside (33) where isolated from H. salicornicum [14] and isorhamnetin-3-O-\(\beta\)-D-xylopyranosyl-(1\(^{''''}\)\(\rightarrow\)3\(^{''''}\))-\(\alpha\)-L-rhamnopyranosyl-(1\(^{'''}\)\(\rightarrow\)6\(^{''}\))-\(\beta\)-D-galactopyranoside (34), isorhamnetin-3-O-\(\beta\)-D-apiofuranosyl-(1\(^{'''}\)\(\rightarrow\)2\(^{''}\))[\(\alpha\)-L-rhamnopyranosyl-(1\(^{''''}\)\(\rightarrow\)6\(^{''}\)]-\(\beta\)-D-galactopyranoside (35), isorhamnetin-3-O-\(\alpha\)-L-rhamnopyranosyl-(1\(^{''''}\)\(\rightarrow\)2\(^{''}\))[\(\alpha\)-L-rhamnopyranosyl-(1\(^{''''}\)\(\rightarrow\)6\(^{''}\)]-\(\beta\)-D-galactopyranoside (36) [7] and isorhamnetin-3-O-\(\beta\)-D-robinobioside (37) [11] were reported from Hammada scoparia and Hammada articulata ssp. scoparia, respectively. Furthermore, the volatile oil of H. schmittiana was also analyzed and revealed the presence of hydrocarbons, mono- and sesquiterpenes [18].

The crude extracts from certain Haloxylon species were biologically evaluated. The ethanol extract of H. salicornicum was found to have antidiabetic [5] and anticoagulant activity in experimental animals [15]. The aqueous extract of Hammada scoparia has been found to show anticancer and antiplasmodial [19] and larvicidal activity [20]. Furthermore, the volatile oil of H. schmittiana was also studied and showed to exhibit antimicrobial activities against Bacillus subtilis and Staphylococcus aureus [18].

2. Research Objectives:

H. salicornicum is a branched shrub grows up to 1 m height. The new branches are green, succulent while the older ones are yellowish white to silvery white [4]. The plant is known locally as Rimth and it is widely distributed through out the Kingdom.
The qualitative phytochemical analysis of the aerial parts of the plant revealed the presence of alkaloids, cardiac glycosides, anthraquinones, flavonoids, saponins, coumarins, sterols, tannins, volatile oils and volatile bases [5].

Based on the above mentioned information, phytochemical study of both aerial and root parts of the local *H. salicornicum* will be carried out.

2.1. Rational:

1. No Phytochemical study was performed on the Saudi plant.
2. No work has been done on the root part of the genus *Haloxylon*
3. The results obtained from the study will be compared with the literature data.

2.2. Specific aim :

Isolation and structure identification of the major constituents of *H. salicornicum*.

3. Materials and Methods:

3.1. Plant material

*H. salicornicum* Bunge ex Boiss. was collected in Oct. 2004 from Mozahimiah (South West of Riyadh) and identified by Dr. M. Atiqur Rahman, College of Pharmacy, King Saud University. A voucher specimen (# 14778 ) was deposited at the herbarium of the College of Pharmacy , KSU .

3.2. Extraction procedures

The plant (both aerial and root parts) will be separated, then dried, ground to powder and each part will be subsequently extracted with pet. ether, chloroform and then with 20% aqueous methanol . The aq. methanol extract will be evaporate *in vacuo* and then dissolved in water . The aqueous layer will be further fractionated using solvents of different polarities (see Scheme1).

3.3. Isolation process

Every fraction from each part (aerial and root parts) will be subjected to different possible type of column chromatography to isolate the major constituents. These columns will be packed with either silica or reversed phase such as C-18 silica and eluted with solvent systems of suitable polarities. In addition, centrifugal preparative TLC (chromatron) and preparative TLC will also be utilized to isolate the compounds.

3.4. Structure Determination

The purified compounds will be subjected to various physical and spectroscopic methods. Physical methods such as of melting point determination (mp) will be performed using Electrothermal instrument. In addition, specific
rotation measurements \(\{[\alpha]_D\}\) will be recorded on a Perkin-Elemer 242 MC Polarimeter. The carbon, hydrogen and nitrogen analysis will also be performed to get the molecular weight of the compound.

For the spectroscopic methods, the following techniques will be used:
1. Ultraviolet (UV) spectroscopy will be utilized using Hewlett-Packard HP-845 UV-Vis spectrophotometer to record the UV spectra of isolated pure compounds and determine their chromophore groups.
2. Fourier Transform Infrared (FTIR) spectra will be obtained for pure compounds on a Nicolet Impact 410 spectrophotometer. This technique will provide the main functional groups available in the compounds.
3. Nuclear Magnetic Resonance (NMR) spectroscopy: Every pure compound will be subjected to 1D \(^1\text{H}\) and \(^{13}\text{C}\) NMR experiments to determine the numbers of protons and carbons available in that compound, respectively. In addition, 2D NMR experiment such as Correlated Spectroscopy (COSY), Heteronuclear Single Quantum Correlation (HSQC) and Heteronuclear Multible Bond Correlation (HMBC) will be performed to confirm the structure of the isolated compounds.
4. Mass Spectrometry (MS) will be used to determine the molecular weight and hence the molecular formula of the isolated compounds.

Furthermore, chemical modifications to the isolated compounds might be used, if needed, to help in the elucidation of the structure of the compounds.
Betaine chloride (1)  Pipridine (2)  Anabasine (3)

Aldotripiperideine (4)  Haloxine (5)  Halosaline (6)

Nicotine (7)  Tryptamine (8)  Dipterine (9)

N-Methylisosalsoline (10)  Carnegie (11)  Isosaline (12)

Salsolidine (13)  Dehydrosalsolidine (14)  Isosaline (15)
N-Methylcorydalidine (16)
Tetrahydroharman (17)
Leptocladine (18)

3-Methyl-1,2,3,4-tetrahydro-β-carboline (19)
2-Methyl-1,2,3,4-tetrahydro-β-carboline (20)

Oxdrine (21)
Tyramine (22)
N-Methytyramine (23)

Scopoletin (24)
Scopolin (25)
Umbelliferne (26)

Xanthotoxol (27)
Isooxyimperatorin (28)

5-Hydroxy-3-methoxy-4H-pyran-4-one (30)
\[ R = -\beta-D-xylopyranosyl-(1'\prime\prime\prime \rightarrow 3'\prime\prime\prime)[\alpha-L-rhamnopyranosyl-(1'' \rightarrow 6'\prime\prime)]-\beta-D-galactopyranoside \ (34) \]

\[ R = -\beta-D-apiofuranosyl-(1'' \rightarrow 2''\prime)[\alpha-L-rhamnopyranosyl-(1''\prime \rightarrow 6'\prime\prime)]-\beta-D-galactopyranoside \ (35) \]

\[ R = -\alpha-L-rhamnopyranosyl-(1'' \rightarrow 2''\prime)[\alpha-L-rhamnopyranosyl-(1''\prime \rightarrow 6'\prime\prime)]-\beta-D-galactopyranoside \ (36) \]

\[ R = -\beta-D-robinobioside \ (37) \]
Scheme 1: Extraction Procedures
4. References:

19) P. Sathiyaamoorthy, H. Lugasi-Evgi, P. Schlesinger, I. Kedar, J. Gopas, Y. Pollack, A. Golan-Goldhirsh. Screening for Cytotoxic and Antimalarial activities in...