Original Research

Phytochemical screening and structure elucidation of isolated compounds from *Convolvulus dorycnium* plant originated from west of Libya

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Abstract

Plants are an ancient source of medicine due to presence of bioactive molecules of various compounds in their different parts. *Convolvulus dorycnium* plant belongs to the family of wind plants (*convolvulaceae*). The greatest variety of *Convolvulus* plants are broadly distributed over the world and was found in Western and Central Asia, Mediterranean, Macaronesia, East Africa and Arabia. The plant C. dorycnium was collected from Tarhuna in Sidi Said area of Libya in May 2013. Plants belonging to Convolvulus genus contain various phytochemicals profiles. The focus of this study is on the phytochemical analysis of *convolvulus dorycnium* including phytochemical screening, extraction, isolation and characterisation. The phytochemical screening reveals the presence of flavonoids, tannins, saponins, steroids, carbohydrate and coumarin in ethyl acetate and methanolic extract. Column chromatography and column chromatography and TLC were used to fractionate the ethyl acetate extract and H-NMR was used to elucidate the isolated compounds. Some compounds were isolated from the aerial parts of the plant; scopoletin, which is a phenolic coumarin (1) (7-hydroxy-6-methoxy coumarin), caffeic acid (2) (3,4-dihydroxycinnamic acid) and, ferrulic acid (3) (4-hydroxy-3-methoxy cinnamic acid) which are phenolic acids. The chemical constituents present in the extract have been reported to possess many biological activities.

Keywords: *Convolvulus dorycnium, elucidation, Libyan plant, NMR*

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Introduction

Medicinal plants of 2103 species belonging to 856 genera and 155 families have been documented in Libya, and the life form distribution among Libyan plants is represented by a high proportion of herbs (annual to perennial) and a low number of woody (tree and shrub) species [1]. This has an important influence on the distribution of plants and their utilization by the society [2, 3]. Medicinal herbs have been in use for thousands of years and were renowned for their effectiveness in many diseases. The natural herbs are very effective in boosting the immune system, increasing the body resistance to infections, healing the allergies and raising and renewing the body vitality [4, 5]. Convolvulaceae is a family of plants from the order of Solanales covering over 1,880 species in 57 types. They are found on all continents except for circumpolar areas. *Convolvulus* (convolere) is a genus of the Convolvulaceae family (bindweed) which is one of the medicinally and economically important family, including about 250 species of flowering plants, present as trees, shrubs and herbs [7]. The greatest variety of *Convolvulus* plants has been found in Western and Central Asia, Mediterranean, Macaronesia, East Africa and Arabia. Some common species of this genus include *Convolvulus lineatus* L, *Convolvulus althaeoides* L. *Convolvulus pilosellifolius desr* [6], *Convolvulus prostratus* Forssk [7] and *Convolvulus arvensis* L [8, 9]. Plants belonging to *Convolvulus* genus contain various phytochemicals profiles including

flavonoids, steroids, terpenoids, carbohydrate, amino acids [9], anthraquinones [10], anthocyanidins, phenylpropanoids, coumarins, lignans, resins, tannins, saponins, alkaloids, lipids, essential [9] and caffeoylquinic acid derivatives [11]. The convolulus species exhibit interesting biological properties such as an antiulcerogenic [12], *C. arvensis* and *C. pilosellifolius* inhibited tumor growth and *C. prostratus* against Alzheimer's disease [17] and treat stress-induced neurodegeneration, while *C. prostratus* for preventing aluminum-induced neurotoxicity [6, 12]. The plant *C. dorycnium* might have similar thus far not well-studied phytochemical and biological activities. Phytochemical screening was started to isolate and identify form the aerial parts of *C. dorycnium* the main active constituents and to investigate their phytochemical profile.

Materials and Methods

Plant materials

The aerial parts of the plant *C. dorycnium* were collected from Tarhuna city (Sidi Said area), Libya during May 2018. The plant was authenticated by Mohammed Abu Hadra, plant taxonomist at the National Herbarium, Department of Botany, Faculty of Science, University of Tripoli, Tripoli, Libya. A voucher specimen (D6845205) was deposited at Libyan National Herbarium, Faculty of Science, University of Tripoli, Tripoli, Libya. The plant material of the aerial part was air-dried, reduced to powder and kept in tightly closed dark glass container at room temperature.

Preliminary phytochemical screening

All the extracts were qualitatively examined for their phytochemicals by standard procedure in literature. The chemical screenings were carried out for detection of phytochemicals, including; coumarins, anthraquinones, tannins, flavonoids, saponins, carbohydrates, steroids and alkaloids compounds [13].

Extraction and isolation

The dried and grinded plant material of *convolvulus dorycnium* (400 gm) was hot extracted using soxhlet apparatus using hexane, ethyl acetate (EA) and methanol (M), respectively for 72 hrs. After filtration and concentration, the residues of the methanolic extract were dissolved in water (600 ml).

The resulting solution was extracted successively with ethyl acetate and n-butanol, filtered and concentrated under reduced pressure (rotatory evaporator, Heidolph) led to the ethyl acetate extract (4.27 gm) and n-butanol extract (4.9 gm). A number of chromatographic techniques were applied for isolation of compounds from the crude extracts. Each extract was passed through many necessary separation processes to isolate the pure compounds.

Column chromatography

The column was packed with sephadex gel LH-20 (Megalla 1983) and the system eluted with gradient solvent system. The hexene and ethyl acetate extract were chromatographed over sephadex (LH-20) and eluted with 5% hexane in $CHCL_3$, followed by $CHCL_3$, then the polarity changed gradually from 5%, 10%, 20% and 50% CH_3OH, finished with 100% CH_3OH. The fractions were collected and analyzed by thin layer chromatography (TLC).

Thin layer chromatography

Two types of TLC were used; analytical TLC (pre-coated silica gel 60 PF 254, 0.2 nm thick, preparative chromatography (glass silica gel, 0.5-1 mm thick self-prepared) and ethyl acetate- hexane 5:95 was used for elution. The separated compounds on TLC was detected under UV light at 254 nm and 366 nm and confirmed by anisaldeyde sulfuric acid spray reagent [14].

Spectroscopic techniques

1D and 2D experiments were used to detect the type of the compounds in the extract fractions as well as to elucidate the structure of pure compounds. The NMR data were obtained on JEOL Eclipse (400 MHz). Deuterated chloroform ($CDCl_3$) and methanol (CH_3OD) were used appropriately for dissolving the samples. Mass spectrometry (MS) technique was also used to identify the compounds present in the samples by measuring the mass-to-charge ratio and abundance of gas-phase ions.

Results

An extraction procedure was carried out by using soxhlet and different residue was obtained based on the polarity of solvent which were used. The percentage of yield was calculated for each residue as shown in **Table 1.**

Table 1: The yields of plant extractions

Plant sample	Hexane extract		Ethyl acetate extract		Methanol Extract	
Weight (g)	Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)
400	5.31	1.33	5.94	1.49	12	3

Phytochemical screening for the main constituents

The phytochemical screening of the extracts of *C. dorycinum* revealed the presence of flavonoids, tannins, saponins, steroids, carbohydrate and coumarin in ethyl acetate and methanolic extract, while, anthrquinones was absent in all the extract. Only steroids and carbohydrates were present in hexene extract (**Table 2**).

Table 2: the results of phytochemical screening of hexane, ethyl acetate, and methanol extracts of *convolvulus dorycnium*

Phyto constituents	Tests preformed	Findings		
		Hexene extract	Ethyl acetae extarct	Methanol extract
Carbohydrate	Fehling test	-	+	+
Saponins	Foam test	-	+	+
Anthraquinone glycosids	Borntrager's test	-	-	-
Steroids	Salkowski 's test	+	+	+
Flavonoids	Shibita's test	-	+	+
Alkaloids	Mayer's test Dragendroff's test	-	+	+
Coumarin	UV test	-	+	+

Structure elucidation of isolated compounds

The compounds, coumarin: scopoletin **(1)** and phenolic compound; caffeic acid (2**)** and ferulic acid (3**)** were isolated from *C. dorycinum* and coded as NC1 and NC2, respectively.

Characterization of NC1 as Scopoletin

NC1 was separated from ethyl acetate extract of *C. dorycinum* plant by fractionation over sephadex LH-20 column and finally purified by using PTLC. On TLC, the fraction showed blue fluorescence activity in UV light under 254 and 336 nm and yellow florescence color on heating after spraying with anisaldhyde sulfuric acid reagent was detected. The HRESI-MS showed $[M+H]^+$ at m/z 192 for the molecular formula $C_{10}H_8O_4$. The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$, **Table 3**) was typical of 6, 7 dioxygenated substituted coumarin.

The ^{1}H-NMR spectrum (**Figure 1**) showed four aromatic protons (δH 6.27, 7.60, 6.83 and 6.91 ppm) were two singlets at δ 6.83 and 6.91 ppm which were explained by a 5, 8-disubstitution, two doublets with coupling constant of 9.50 Hz at δ 6.27 and 7.60 ppm, which were assigned as H-2 and H-3, respectively, characteristic for coumarins and one methoxy group (–OCH_3) singlet (δH 3.90 ppm).

Based on the spectroscopic data and reference comparation, the NC1 was proposed to be scopoletin in R_1= OCH_3 and R_2 = OH.

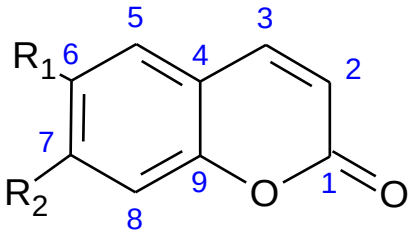
Characterization of NC2 as a mixture of caffeic acid and cinnamates

NC2 was separated from ethyl acetate extract of *C. dorycinum* plant by fractionating over sephadex LH20 column and finally purified by using PTLC. On TLC, the NC2 showed blue florescence in UV light under 254 and 336 nm, and yellow color on heating after spraying with anisaldhyde sulfuric acid reagent was obtained.

The HRESI-MS showed $[M+H]^+$ at m/z 180 for the molecular formula $C_9H_8O_4$ and $[M+H]^+$ at m/z 194 for the molecular formula $C_{10}H_{10}O_4$, respectively. The ^{1}H-NMR spectrum (400 MHz,$CDCl_3$, **Table 4**) was typical of 3, 4 dioxygenated substituted cinnamic acid.

Table 3: The ^{1}H-NMR chemical shift for NC1

Position	COMP δH Mult., JHz	REF 1 δH Mult., JHZ (Zhang, 2011)
1	-	-
2	6.27(d,9.50)	6.21(d,9.3)
3	7.59(d,9.50)	7.92(d,9.3)
4	-	-
5	6.84 (S)	7.21(S)
6	-	-
7	-	-
8	6.91(S)	6.78(S)
9	-	-
R1	3.93(S)	3.87(S)



(1)

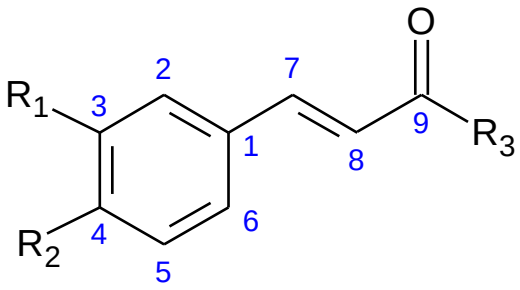
R_1= OCH_3 R_2 = OH

Figure 1: The chemical structure of scopoletin.

The ^{1}H-NMR spectrum (**Figure 2**) showed two coupled doublets (J=8.0 Hz) at δ 6.85 and 6.98, which were explained by a 5,6-disubstitution and a broad singlet at δ 7.50 for H-2 in the aromatic region indicated the presence of a trisubstituted aromatic ring in the molecule. The 1H-NMR spectrum also displayed two doublets (J=15.0 Hz) at δ 7.50 (H-7) and 6.20 (H-8).

Table 4: The ^{1}H-NMR chemical shift for NC2

Position	COMP δH Mult., JHZ	REF 1 δH Mult., JHZ (Sun et al., 2006)
1	-	-
2	7.05 (brs)	7.10 (brs)
3	-	-
4	-	-
5	6.85(d,8.0)	6.81(d,8.0)
6	6.98(d,8.0)	7.03(d,8.0)
7	7.54(d,15.0)	7.51(d,15.0)
8	6.23(d,15.0)	6.35(d,15.0)



(2) R_1=OH R_2=OH R_3=OH---------- Caffeic acid
(3) R_1=OCH_3 R_2=OH R_3=OH----------Ferulic acid

Figure 2: The chemical structures of caffeic acid and ferulic acid.

The large value of coupling constant indicated the presence of Trans–disubstituted ethylene moiety in the molecule.

Based on the spectroscopic data and reference comparation, the NC2 was proposed to be a mixture of caffeic acid (2) (R1=OH, R_2=OH and R_3=OH) and ferulic acid (3) (R_1=OCH_3, R_2=OH & R_3=OH).

Discussion

Phytochemical analysis of the hexene, ethyl acetate and methanol extracts of *C. dorycnium* reveal presence flavonoids, tannins, saponins, steroids, carbohydrate and coumarin in ethyl acetate and methanolic extract, while steroids was only constituent present in hexane extract. The present results are similar to those reported previously in the literature. The chemical constituents present in the extract have been reported to possess many biological activities [15]. The sephadex gel column chromatography of ethyl acetate extract of *C. dorycinum* with gradient solvent system afforded compounds NC1 and NC2. Isolation and purification of ethyl acetate extract arial part of *C. dorycinum* yields phytochemicals as coumarins NC1 (scopoletin) and phenolic compounds NC2 (caffeic acid and ferulic acid) using three simple isolation techniques. Starting with sample extraction followed by fractionation with organic solvent. The fraction underwent one-time separation with column chromatography using a mixture of hexene, chloroform and methanol gradient from non-polar to polar and finally purified with preparative TLC. When comparing the published literature with all the ^{1}H-NMR values for compounds isolated from *C. dorycinum*, match with slight differences based on the different type of instruments used. Molecular ion peak was observed in the mass spectrum of 1 at showed $[M+H]^+$ at m/z 192 for the molecular formula $C_{10}H_8O_4$. The ^{1}H-NMR spectrum [400 MHz] showed four aromatic protons (δH 6.27, 7.60, 6.83, and 6.91 ppm) were two singlets at δ 6.83 and 6.91 ppm, which was typical of 6, 7 dioxygenated substituted coumarin. The identity was established as 7- hydroxy-6-methoxycoumarin (scopoletin) by comparison with literature data [15-17).

NC2 showed $[M+H]^+$ at m/z 180 for the molecular formula $C_9H_8O_4$ and $[M+H]^+$ at m/z 194 for the molecular formula $C_{10}H_{10}O_4$, respectively. The ^{1}H-NMR spectrum showed two coupled doublets (J = 8.0 Hz) at δ 6.85 and 6.98, which were explained by a 5, 6-disubstitution and a broad singlet at δ 7.50 for H-2 in the aromatic region indicated the presence of a trisubstituted aromatic ring in the molecule.

The 1H-NMR spectrum also displayed two doublets (J = 15.0 Hz) at δ 7.50 (H-7) and 6.20 (H-8). Based on the spectroscopic data and reference comparison, the NC2 was proposed to be a mixture of caffeic acid (R_1=OH, R_2=OH and R_3=OH) and ferulic acid (R_1=OCH_3, R_2=OH and R_3=OH). Also, other compounds in NC1 and NC2 can contribute. While the roots and flowers of *C. dorycnium* have been reported to contain convoldorine, a phenolic metabolite methyl 3-(3-caffeoyl-1, 2-dihydroxycyclobutyl)-3-hydroxypropanoate and in addition dorycnic acid, scopolin and scopolitin [18, 19]. Thus, the results obtained in this study indicated that the roots, flower and aerial parts probably differ in the chemical composition.

Conclusion

The present study provides that the plant has some important phytochemicals. Its arial part contained scopoletin, caffeic acid and ferulic acid. Those isolated compounds can present potential pharmacological activity, which recommended for future investigation.

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Conflict of Interest

All authors declare no conflict of interest.

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