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Phytochemical Investigation of *Senecio vulgaris* L. Growing in Egypt

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Abstract

Senecio vulgaris L. is a well known toxic plant, phytochemical investigation of the whole plant afforded the isolation of a new coumarin; 3,7-dihydroxy-5,6 dimethoxycoumarin-7-O-*β*-D-glucoside (1), in addition to the isolation of isorhamnetin-3-O-*β*-D-glucoside (2) and two known alkaloids of common occurrence in genus *Senecio*; senecionine (3) and seneciphylline (4). The isolated compounds were identified by physical and chemical analyses and by intensive investigation of their spectral data. EtOH extract, light petrol and $CHCl_3$ fractions of *S.vulgaris* were found to have cytotoxic activity on colorectal cell line HCT-116 cell line, with IC_{50} values of 19.9, 27.3 and 17.8 µg mL^{-1}, respectively. That indicates the most active cytotoxic fraction is the alkaloid-bearing chloroform fraction. These findings promote the future perspective of *S. vulgaris* as an anticancer agent. The antioxidant activity of EtOH extract, light petrol fraction and $CHCl_3$ fraction was evaluated by DPPH assay, %inhibition was 23.31, 4.677 and 17.598, respectively. Also the ratio between senecionine and seneciophylline in their mixture was determined using ^{1}H-NMR spectrum.

1. Introduction

Genus *Senecio* can be considered one of the largest genera of family Asteraceae (subfamily Asteroideae, tribe Senecionae) as it comprises about 1250 species of annual or perennial herbs, shrubs or small trees which are distributed all over the world, especially in South Africa, Mediterranean floristic region and in temperate areas of Asia and America (Teles et al., 2008; Lotfi et al., 2010).

Senecio vulgaris L. is one of the common Egyptian *Senecio* species. It is known by the common English names: groundsel and common groundsel; and by the vernacular Arabic names: Morrar, Murrar and eshbet salema (Umberto, 2000). It can be considered as a problematic weed as it is rich in toxic pyrrolizidine alkaloids. Many compounds were isolated from it, such as; alkaloids, flavonoids and terpenes (El-Shazly et al., 2002, 2014; Gharbo et al., 1969; Habib, 1981).

Pyrrolizidine alkaloids show either no or low acute toxicity; but they can undergo a 3-step metabolic toxication process leading to alkylating agents which affect the liver and other organs. Metabolic toxication process of pyrrolizidine alkaloids starts first by conversion of PAs into unstable hydroxyl- PAs by cytochrome p-450 monoxygenase enzyme complex in the liver. Then, instability of these hydroxyl PAs leads to dehydration and formation of dehydro-PAs. After that, rearrangement and formation of stabilized carbonium ions by loss of hydroxyl groups or ester functions as hydroxyl or acid anions these ions react with electrophilic parts of proteins, DNA and RNA leading to genotoxicity (Wiedenfeld et al., 2008; Mattocks et al., 1986).

In this context, our present work has focused on the

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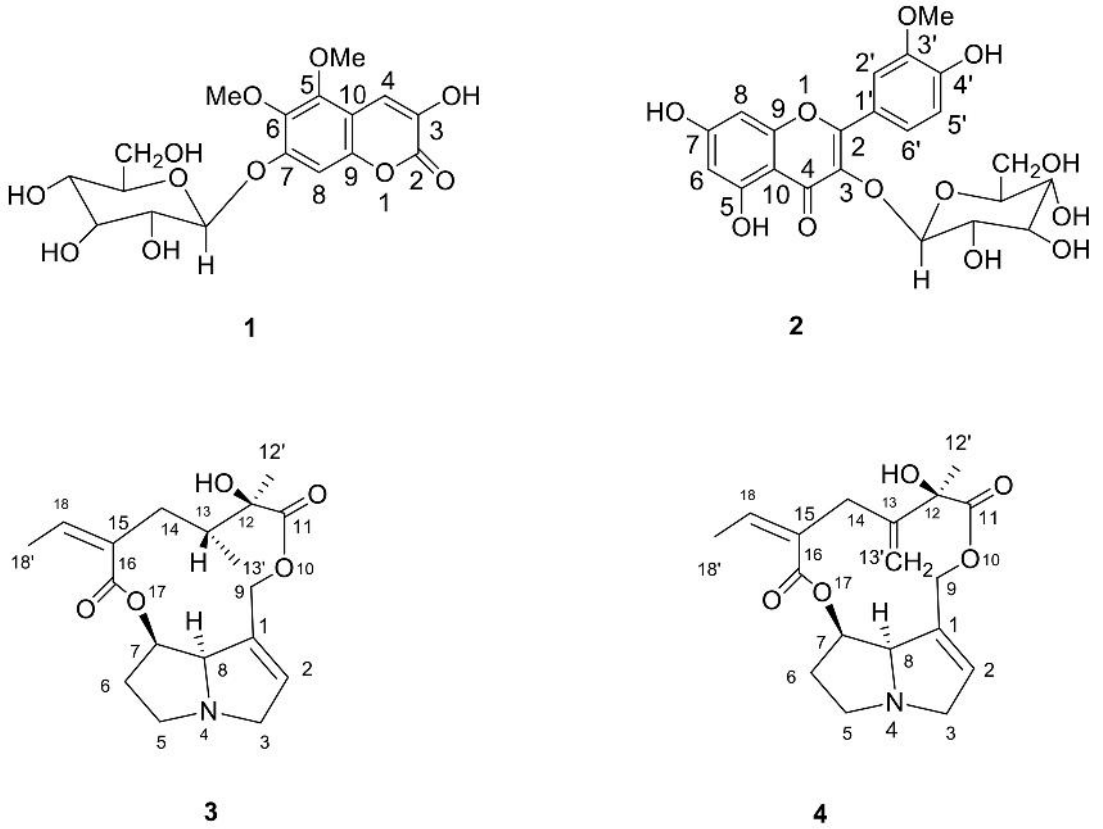

Figure 1: Structures of compounds (1-4)

phytochemical study of *Senecio vulgaris* L., which includes isolation of four compounds (**1-4**) their structures are shown in **Figure 1**; as well as evaluation of the cytotoxic activity of different fractions against colorectal cell line HCT-116 and their free radical scavenging activity using DPPH assay.

2. Materials and Methods

2.1. Plant materials

Senecio vulgaris L. was collected in March 2013 (flowering stage) from El-Shalalat garden in Alexandria, Egypt and identified at Plant Taxonomy Department, Faculty of Science, Alexandria University, Egypt. A voucher sample (No Sv169) has been kept in the herbarium of Faculty of Science, Alexandria University.

2.2. General experimental procedures

Precoated TLC plates (silica gel 60 F254) with adsorbent layer thickness 0.25 mm plates, E-Merck, Dramstadt, Germany. and Silica gel Merck, Kieselgel 60, 0.063-0.20 μm for column chromatography, E-Merck, Dramstadt, Germany, rotary evaporator; BUCHI Rotavapor R-200 was used for evaporation of solvents under reduced pressure, melting point was measured using Sturat SMP heating stage microscope, United Kingdom, UV measurements was done using Pye Unicam SP8-100 UV/VIS spectrophotometer and TLC plates were examined under Duo-UV lamp, Desage, Heidelberg, Germany. ^{1}H-NMR (500 MHz), ^{13}C-NMR (125MHz), DEPT-135, and 2D-NMR spectra were recorded using the residual solvent signal as an internal standard on a Varian AS 400 NMR spectrometer in National Center for Natural Products Research, School of Pharmacy, University of Mississippi.

2.3. Extraction and isolation

The aerial parts of *S.vulgaris* (4 kg) were extracted with 95% MeOH at room temperature and filtered. The filtrate was evaporated under reduced pressure to give a MeOH extract (300 g), which was divided into two equal portions, one of them (150 g) was suspended in water (300 mL) and then successively partitioned with light petrol, CH_2Cl_2, EtOAc and *n*-BuOH, respectively. The other portion (150 g) was suspected to acid-base fractionation.

2.4. Column chromatography of ethyl acetate fraction

Isolation of compounds **1** and **2** from EtOAc fraction: the EtOAc soluble fraction (7 g) was chromatographed

on a silica gel column, (300 g, 4 cm X 70 cm). The elution was performed using $CHCl_3$: MeOH mixtures with gradual increase in polarity.
Fractions (35% MeOH in $CHCl_3$) showed a major violet spot at R_f 0.52 ($CHCl_3$: MeOH, 8:2). Twenty-five mg of the residue left after removal of the solvent was subjected to pTLC on fluorescent silica gel plates using ($CHCl_3$: MeOH, 8:2) as a developing system. The plate was visualized by UV light and ammonia vapor showing a major sky blue florescent zone. The zone was scraped off and eluted using a mixture of $CHCl_3$: MeOH (1:2) to yield 16 mg of white amorphous powder (compound **1**)**.**

Fractions (15% MeOH in $CHCl_3$) showed one major yellow spot at R_f 0.65 ($CHCl_3$: MeOH, 9:1). Crystallization from methanol yielded 50 mg of yellow crystals (compound **2**).

2.5. Column chromatography of chloroform fraction

Isolation of compounds **3** and **4** from $CHCl_3$ fraction: the residue left after evaporation of $CHCl_3$ fraction obtained by the acid-base treatment (8 g) was fractionated over a silica gel column (350 g, 4 cm X 70 cm). The elution was performed using $CHCl_3$: MeOH mixtures with gradual increase in polarity.

Fractions (30% MeOH in $CHCl_3$) showed one major violet spot when sprayed with anisaldehyde /H_2SO_4 followed by heating in the oven (150^{o}C) for 2 min and orange color when sprayed by Dragendorff‘s reagent. After NMR analysis of the residue obtained by crystallization, it was found to be a mixture of two alkaloids, so 40 mg of the residue was subjected to pTLC on fluorescent silica gel plates using ($CHCl_3$:MeOH:NH_3, 8:2:0.2) as a developing system. The plate was visualized by UV showing two zones with R_f 0.65 and 0.64. Both zones was scraped off and eluted with a mixture of ($CHCl_3$: MeOH, 1:2) to yield 6 mg of **3** and 11 mg of **4**, respectively.

2.5. Cytotoxicity evaluation using viability test

The cytotoxic activity of EtOH extract, light petrol and $CHCl_3$ fractions of *S.vulgaris* were evaluated by using viability assay (Skekhan et al.,1990) using vinblastin as a reference compound against colorectal carcinoma cell line (HCT-116 cell line).

2.6. Free radical scavenging activity screening using DPPH assay

The free radical scavenging activity of ethanol extract, petroleum ether and chloroform fractions were evaluated using DPPH assay (Bondet et al., 1997) using ascorbic acid as a reference.

2.7. Determination of the ratio between senecionine and seneciphylline using ^{1}H NMR data

Senecionine and seneciphylline are ubiquitous coexisting alkaloid-pair among many *Senecio* species (Habib., 1980; Segall et al., 1983). In the present work, determination of the ratio of these two alkaloids in the studied plant was attempted by ^{1}H-NMR evidence; referring to the integration of their H-13’ signal(s) in ^{1}H-NMR spectrum of their mixture.

2.8. Early investigation of Senecio toxicity in rats

Early investigation of the toxicity with *senecio* plant was done by detecting its characteristic key elements in the stool of the rat that was fed with powdered *senecio* plant at a dose lower than the lethal dose.

3. Results and discussion

Four compounds were isolated from *S.vulgaris*; a new coumarin; 3,7-dihydroxy-5,6 dimethoxycoumarin-7-*O*-*β*-D-glucoside (**1**) and a known flavone: isorhamnetin-3-*O*-*β*-D-glucoside (**2**) from the EtOAc fraction and two known alkaloids of common occurrence in genus *Senecio*; senecionine (**3**) and seneciphylline (**4**) from the $CHCl_3$ fraction .

Compound **1** was isolated as a white amorphous powder; the mass spectrometry suggested the molecular formula to be $C_{17}H_{20}O_{11}$ (m/z 400.12). In thin-layer chromatography, it was detected using UV lamp at 366 nm as a sky blue fluorescing spot which intensified upon exposure to ammonia vapor. ^{1}H-NMR spectrum (**Table 2**) showed two signals at δ_H 7.77 (1H, br.s) and at 7.66 (1H, br.s) corresponding to H-4 and H-8, respectively (Alqasomy et al., 2013).

Observation of broad singlet peaks for both H-4 and H-8 was due to long range coupling between both protons and indicated unsubstitution of the corresponding carbons (Hammoda et al., 2008). The absence of the typical AB system for H-3 and H-4 indicated a 3- substituted coumarin, this substitution was proved to be with oxygenated substituents due to downfield shift of C-3 (δ_C 141.0) (Cussans et al., 1975).

The two signals at δ_C 61.9 and 62.3 along with their proton signals at δ_H 3.59 (3H, s) and 3.64 (3H, s) indicated the presence of 2 methoxy groups (Hammoda

Table (1): Free radical scavenging activity screening using DPPH assay

Extract/fraction	IC_{50}, µg mL^{-1}	Extract/fraction	% Inhibition
Ethanol	19.9	**Ethanol**	23.31
Petroleum ether	27.3	**Petroleum ether**	4.677
Chloroform	17.8	**Methylene chloride**	17.598

et al., 2008). The location of the two methoxy groups at C-5 and C-6 was deduced from ^{13}C NMR spectrum as their chemical shifts above 60 ppm indicating that they are both ortho-flanked (Kaysar et al., 1995).

NMR spectra showed the sugar moiety to be one glucose unit with anomeric proton at δ_H 5.20 (1H, d) along with its anomeric carbon at δ_C103.1 (Godecke et al., 2005).

The site of glycosylation was deduced from UV spectrum, as it was not strongly modified in alkaline medium (NaOAc) indicating the absence of a free hydroxylated C-7 (Alqasomy et al., 2013; Girard et al., 2005), thus the location of the sugar moiety should be at C-7. The *β* configuration of glucose was indicated from the chemical shift and *J*–value of its anomeric proton (She et al., 2009). The structure was confirmed as 3,7-dihydroxy-5,6 dimethoxycoumarin-7-*O*-*β*-D-glucoside by 2D-NMR analyses.

Compound **2** was isolated as yellow crystals with MP 215°C. In thin-layer chromatography, its spot gave a yellow color upon exposure to ammonia vapor and identified as isorhamnetin-3-*O*-*β*-D-glucoside.

Compound **3** and **4** were isolated as white powder. In thin- layer chromatography, both compounds gave orange spot upon spraying with Dragendorrf's reagent indicating its alkaloidal nature, identified as senecionine and seneciphylline.

The cytotoxic activity of EtOH extract, light petrol and $CHCl_3$ fractions of *S.vulgaris* indicated that the most active cytotoxic fraction is the alkaloid-bearing chloroform fraction. These findings promote the future perspective of pyrrolizidine alkaloids as anticancer agent.

Free radical scavenging activity screening using DPPH assay results showed %inhibition at values of 23.31%, 4.677% and 17.598%, respectively (**Table1**).

In senecionine C-13' is a methyl group, giving a three protons which observed at δ_H 0.90 (3H, d, *J*=5 Hz) with integration value of 3.05 ; whereas in seneciphylline C-13' is terminal methylene group that gives two well separated signals at δ_H 5.34 and δ_H 5.00 with integration values of 2.82 and 2.39,

Table (2): NMR data of compound (1)

#	δH, ppm	δC, ppm
2	----------	163.0 (s)
3	----------	141.0 (s)
4	7.77 (1H,*br.s*)	114.6 (d)
5	----------	155.3 (s)
6	----------	136.4 (s)
7	----------	151.1 (s)
8	7.66 (1H, *br.s*)	109.6 (d)
9	----------	148.1 (s)
10	----------	112.2 (s)
Glucose 1'	5.20 (1H, *d*, *J*=7 Hz)	103.1 (d)
2'	3.24-3.29 (5H)	74.9 (d)
3'		77.8 (d)
4'		71.3 (d)
5'		77.6 (d)
6'		64.0 (t)
5-OCH_3	3.59 (3H, *s*)	61.9 (q)
6-OCH_3	3.64 (3H, *s*)	62.3 (q)

respectively (Roeder et al., 1992). That means in case of senecionine the one proton (CH_3) is approximately equivalent to one, but in case of seneciphylline each proton (CH) is approximately equivalent to two, so the ratio of senecionine to seneciphylline is 1:2.

Early investigation of the toxicity with *Senecio* plant was done by detecting its characteristic key elements in the stool of the rat that was fed with powdered *Senecio* plant at a dose lower than the lethal dose.

4. Conclusion

A flavonoid and a new coumarin were isolated from ethyl acetate fraction and two pyrrolizidine alkaloids were isolated from chloroform fraction of the aerial parts of *Senecio vulgaris* L. Cytotoxic and free radical scavenging activities of different fractions were evaluated.

5. Conflict of interest

The authors report no declaration of conflict of interest.

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