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Antimicrobial Benzofuran Derivatives and Chemosystematic Significance of Senecio glaucus L. (Asteraceae)

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Abstract

hytochemical investigation of methylenechloride/methanol (1:1) extract of the air-dried aerial parts of Senecio glaucus L. afforded three benzofuran derivatives (1-3); 1-(6-hydroxy-2-(prop-1-en-2-yl) benzofuran-5-yl) ethanone, 1-1'-(6-hydroxybenzofuran-2,5-diyl) diethanone, and 1-1'-(6-methoxybenzofuran-2,5-diyl) diethanone. The crude extract and isolated compounds (1-3) were screened against S. aureus and E. coli and also against C. albicans and A. flavus. Compound 1 and 2 were more potent than 3 against S. aureus and E. coli, however, the crude extract and isolated compounds (1-3) showed weak antifungal activity against C. albicans and A. flavus. In addition, a biosynthetic pathway of isolated compounds (1-3) was proposed, as well as the chemotaxonomic significance of the reported benzofuran and furanoeremophilane derivatives from S. glaucus compared to those isolated from other Senecio species was also studied.

Keywords: Senecio glaucus; Chemosystematic significance; Biosynthetic pathway; Antimicrobial Benzofuran derivatives

1. Introduction

Medicinal plants are considered a rich source of bioactive phytochemical constituents which have promising pharmacological activities [1-3]. The aromatic plants have been commonly used in pharmaceutical, foods, cosmetic industry due to their characteristic biological activities such as antimicrobial, anticancer, antiviral and many other therapeutic effects [4,5]. *Senecio* is the largest genera of family Asteraceae (Compositae), composed of nearly 3000 species widely distributed over the world [6]. In Egypt, *Senecio* genus is represented by about six species including; *S. aegyptius*, *S. belbeysius*, *S flavus*, *S. glaucus*, and *S. hoggariensis* [7,8]. The Egyptian *Senecio* species is mainly present in the desert valleys, saline and coastal sandy soils, East Mediterranean region, and Sinai which used as sedative, diuretic and emetic [7,9]. Many *Senecio* species have been used traditionally for treatment of various diseases such as, dysentery, infections, conjunctivitis, cough, asthma, rheumatism, bronchitis, cancer and inflammation [10]. The previous phytochemical studies of a number of *Senecio* species have resulted in the isolation of particularly characteristic groups of secondary metabolites including; pyrrolizidines alkaloids (PAs), eremophilane-type sesquiterpenes of the eremophilanolides and furanoeremophilane type [11]. Some of these bioactive phytochemicals have antimicrobial, cytotoxic, anti-inflammatory, antioxidant, phytotoxic, and insect antifeedant activities [11-16]. The toxicity of *Senecio* plants has been proposed due to their furanoeremophilans and PAs content which act as an important part of the defence mechanism of these plants [17]. There are few reports on cacalolides [18], essential oils [19], monoterpenes, sesquiterpenoids [20], diterpenes [21], triterpenes [22], and phenolic constituents [23]. In the present study, the hexane/methylene chloride (1:1) crude extract of *S. glaucus* L., and isolated

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compounds (1-3) (Figure 1) were assessed to antibacterial and antifungal activities. In addition to the chemosystematics significance of *S. glaucus* L., and the proposal biosynthetic pathway of isolated compounds were studied in this paper.

2. Experimental

General experimental procedures

^{1}H NMR (600 MHz, $CDCl_3$), ^{13}C NMR (125 MHz, $CDCl_3$) and the 2D spectra were recorded on a JEOL 500 MHz, Lambda spectrometer, with TMS as an internal standard. ESIMS was recorded on a JEOL SX102A mass spectrometer.

Plant material

The aerial parts of *S. glaucus* were collected, during the flowering stage, September 2019, from Aswan (South of Egypt). A voucher specimen has been deposed at Department of Botany, Aswan University, Egypt.

Extraction and isolation

Air-dried plant material (500 g) was ground and extracted with CH_2Cl_2–MeOH (1:1) at room temperature. The extract was concentrated *in vacuo* to obtain a residue of 80 g. The residue was prefractionated by CC (6 x 120 cm) on silica gel eluting with *n*-hexane (2 L) followed by a gradient of *n*-hexane–CH_2Cl_2 up to 100 % CH_2Cl_2 and CH_2Cl_2–MeOH up to 15 % MeOH (2 L each of the solvent mixture). The *n*-hexane–CH_2Cl_2 (1:1) fraction was subjected to a silica gel column chromatography (2 x 60 cm), eluted with *n*-hexane–CH_2Cl_2, with increasing polarity up to 100 % CH_2Cl_2 to give pure compound 2 (10 mg). The CH_2Cl_2 (100 %) fraction was chromatographed on a Sephadex LH-20 column eluted with *n*-hexane-methylenechloride-methanol (7:4:0.5) to give compounds 1 (10 mg) and 3 (15 mg).

Antimicrobial assay

Modified Kirby-Bauer disc diffusion method

Antimicrobial activity of the tested samples was determined using a modified Kirby-Bauer disc diffusion method [62]. Briefly, 100 µl of the test bacteria/fungi were grown in 10 ml of fresh media until they reached a count of approximately 10^8 cells/ml for bacteria or 10^5 cells/ml for fungi [63]. 100 µl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by disc diffusion method [64, 65]. Plates inoculated with filamentous fungi *as Aspergillus flavus* at 25°C for 48 hours; Gram (+) bacteria as *Staphylococcus aureus,* Gram (-) bacteria as *Escherichia coli*, they were incubated at 35-37°C for 24-48 hours and yeast as *Candida albicans* incubated at 30°C for 24-48 hours and, then the diameters of the inhibition zones were measured in millimetres [62]. Standard discs of Ampicillin (Antibacterial agent), Amphotericin B (Antifungal agent) served as positive controls for antimicrobial activity but filter discs impregnated with 10 µl of solvent (distilled water, chloroform, DMSO) were used as a negative control. Blank paper disks (Schleicher & Schuell, Spain) with a diameter of 8.0 mm were impregnated 10µ of tested concentration of the stock solutions. When a filter paper disc impregnated with a tested chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "Zone of inhibition" or" Clear zone". For the disc diffusion, the zone diameters were measured with slipping callipers of the National Committee for Clinical Laboratory Standards [64]. Agar-based methods such as Etest and disk diffusion can be good alternatives because they are simpler and faster than broth-based methods [66, 67].

The minimal inhibitory concentration assay (MIC)

The minimum concentration of isolated compounds (1-3) at which no growth of microorganisms occurred (MIC), were determined against *S. aureus*, *E. coli*, *A. flavus*, *C. albicans*, strains by using broth dilution method [68]. Nutrient broth and Sabouraud Dextrose broth (100 µl) were distributed into 96-wells plate. Each compound (1-3) was added to the first well followed by 10 serial two-fold dilutions. Bacterial and yeast suspensions were added into all wells except the negative control wells. Plates were incubated at 37 °C for 48 hrs. The minimum inhibitory concentration was determined by visual examination of the culture turbidity.

3. Results and Discussion

Structure elucidation of the isolated compounds

Repetitive chromatographic steps of the methylenechloride/methanol of the aerial part of *Senecio glaucus* L. afforded three benzofuran derivatives (1-3) (Figure 1).

Compound 1 was obtained as a yellowish powder. The low resolution mass spectrum exhibited a $[M+H]^+$ at *m/z* 217, in accord with the molecular formula $C_{13}H_{12}O_3$. The structure of 1 was determined

from careful investigation of the 1D and 2D NMR data. The ^{1}H-NMR spectrum (Table 1) indicated the presence of six singlet signals at δ 6.57, 7.94, 7.04, 2.20, 2.71, and 12.51 for H-3, H-4, H-7, H-11, H-14 and phenolic Hydroxyl group (OH at C-6), respectively. Furthermore, it showed the methylene protons as two singlet signals at δ 5.21 and 5.78 for H-12, which showed correlation in HMQC spectrum with a carbon signal at δ 113.0, C-12. The ^{13}C-NMR data (Table 1) revealed the presence of 13 carbon atoms and their multiplicities (by APT experiment) confirmed the number of atoms of the formula given above. The carbon atoms were assigned as two methyl carbon atoms at δ 19.2, 26.8 (C-11, C-14); one methylene carbon atom at δ 113.0 (C-12); three methine carbon atoms at δ 102.4, 123.4, 99.8 for C-3, C-4 and C-7, respectively; six quaternary carbon atoms at δ 157.6, 132.5, 159.5, 161.2, 121.9, 132.0 for C-2, C-5, C-6, C-8, C-9, and C-10 respectively and one carbonyl carbon atom at δ 204.0 for C-13. Moreover, all proton and carbon signals were determined by ^{1}H-^{1}H COSY, HMQC and HMBC (Table 1). Confirmation of the structure of 1 was given by the results of the 2D-long range heteronuclear correlation (HMBC) analysis (Table 1). The most correlations were observed between H-3 (δ= 6.57, s) with C-8 (δ= 161.2); H-4 (δ= 7.94, s) with C-6 (δ= 159.5); H-11 (δ= 2.20, s) with C-2 (δ= 157.6), C-10 (δ= 132.0) and C-12 (δ= 113.0). Therefore, compound 1 was assigned as 1-(6-hydroxy-2-(prop-1-en-2-yl) benzofuran-5-yl) ethanone, common name Euparin, previously isolated from *Ruscus aculeatus* [24].

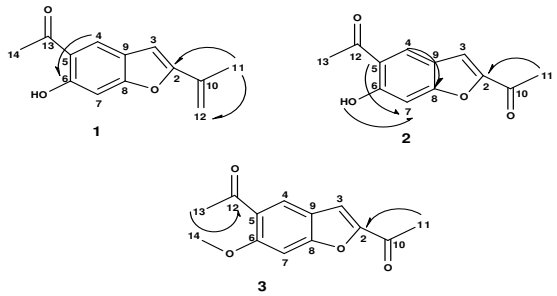
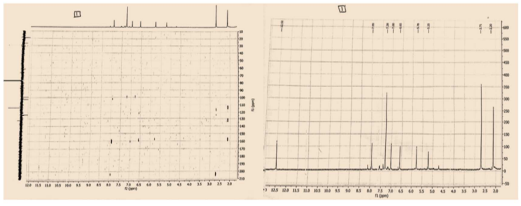
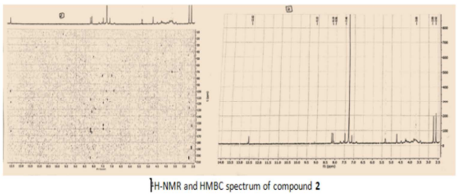


FIGURE 1: Structures with HMBC arrows of the isolated compounds (1-3)



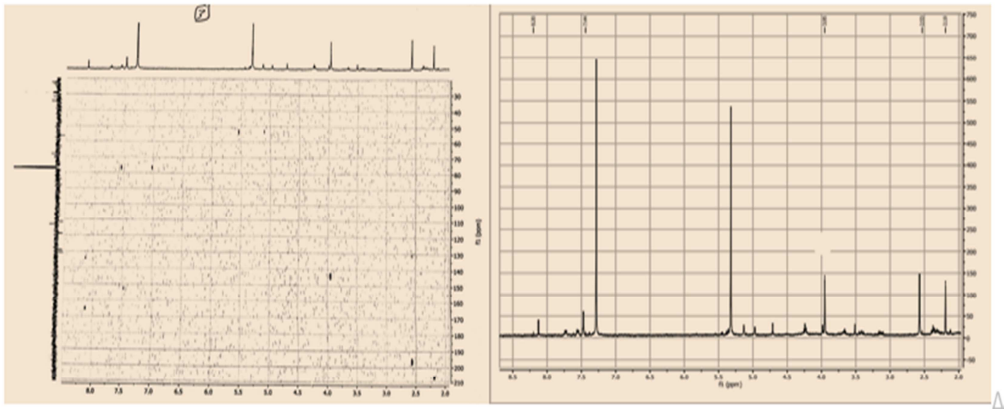
^{1}H-NMR and HMBC spectrum of compound **1**

Compound 2 was isolated as a yellowish powder and its mass spectrum showed the molecular ion peak $[M+H]^{+}$ at *m/z* = 219, established the elemental composition as $C_{12}H_{10}O_4$. Again, the spectral data were indicative of a phenolic 2,5,6-trisubstitued benzofuran containing two acetyl groups, one of them was ortho to the phenolic hydroxyl which appeared as a singlet signal at δ= 12.55. The ^{1}H-NMR spectrum (Table 1) indicated the presence of the two methyls as two singlet signals at δ= 2.61 and 2.75 for H-11 and H-13, respectively. Also, showed the aromatic protons as three singlet signals at δ= 7.11, 8.18 and 7.49 for H-3, H-4 and H-7, respectively. The structure of 2 was determined from careful investigation of the 1D and 2D NMR data. Confirmation of the structure of 2 was given by the results of HMBC (Table 1). Clear correlations were observed between H-4 (δ 8.18) with C-8 (δ 160.0); H-7 (δ 7.49) with C-5 (δ 121.0), C-6 (δ 162.0), C-8 (δ 160.0); H-11 (δ 2.61) with C-2 (δ 154.0), C-10 (δ 189.0); OH (δ 12.55) with C-5 (δ 121.0), C-6 (δ 162.0), and C-7 (δ 102.0). Therefore, compound 2 was assigned as 1-1'-(6-hydroxybenzofuran-2,5-diyl) diethanone, common name Euparone, previously isolated from *Ruscus aculeatus* L [24].



^{1}H-NMR and HMBC spectrum of compound **2**

Compound 3 was obtained as a white powder and its mass spectrum revealed the presence of the molecular ion $[M+H]^{+}$ at *m/z* = 233, in accord with the molecular formula $C_{13}H_{12}O_4$. Again, the spectral data were indicative of a phenolic 2,5,6-trisustituted benzfuran containing two acetyl groups, one of them was ortho to the methoxyl group which appeared as a singlet signal at δ= 3.96. The ^{1}H-NMR spectrum (Table 1) showed two singlet signals appeared at δ= 2.56 and 2.19 for H-11 and H-13, respectively. Moreover, all proton and carbon signals were determined by ^{1}H-^{1}H COSY, HMQC and HMBC (Table 1). Confirmation of the structure of 3 was given by (HMBC) analysis (Table 1). Strong correlations were observed between H-13 (δ= 2.19) with C-12 (δ= 208.0); H-11 (δ= 2.56) with C-10 (δ= 199.0). From above data, Compound 3 was assigned as 1-1'-(6-methoxybenzofuran-2,5-diyl) diethanone, common name Euparone methyl ether, previously isolated from *Ruscus aculeatus* L [24].



¹H- NMR and HMBC spectrum of compound **3**

TABLE 1: ^{1}HNMR (600 MHz, $CDCl_3$) and ^{13}CNMR (125 MHz, $CDCl_3$) spectral data of isolated compounds (1-3)

	1		2		3	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	-	157.6	-	154.0	-	147.8
3	6.57, s	99.8	7.11, s	103.0	7.47, s	103.9
4	7.94, s	123.4	8.18, s	121.8	8.13, s	121.4
5	-	132.5	-	121.0	-	118.9
6	-	159.5	-	162.0	-	156.0
7	7.04, s	102.4	7.49, s	102.0	7.47, s	99.7
8	-	161.2	-	160.0	-	165.0
9	-	121.9	-	116.0	-	116.4
10	-	132.0	-	189.0	-	199.0
11	2.20. s	19.2	2.61, s	26.20	2.56	26.44
12	5.21, s 5.78, s	113.0	-	205.0	-	208.0
13		204.0	2.75, s	26.10	2.19, s	30.00
14	2.71, s	26.8	-	-	3.96, s	57.00

Proposed biosynthetic pathway of the isolated compounds

Generally, dehydrotremetone (13) is considered the main precursor of bezofuran derivatives in Asteraceae [25]. The biosynthesis of (13) is originated via isoprenylation of para-hydroxyacetophenone (8) (Figure 2) [25]. Compound (8) has been derived from condensation of four units of acetyl-CoA (1) via Claisen condensation reaction to form polyketide-type intermediate (2) (Figure 2) [26,27]. On the other hand, phenylalanine which derived from deoxyxylulose phosphate pathway has been published as another precursor to form (8) [28,29]. An intermediate (9) is originated via electrophilic substitution of the aromatic nucleus of (8) with dimethylallyl diphosphate (DMAPP) or isopentenyl diphosphate (IPP) which obtained either from mevalonate or deoxyxylulose phosphate pathway (Figure 2) [25,27]. However, a dihydrofuran ring system in (11) is originated as a result of intermediary formation of the epoxide (10) then, dehydrotremetone (13) is formed by dehydration of (12) which produced by alcoholic hydroxyl group attack at C-3 of (11) (Figure 2). Moreover, hydroxylation of (13) by phenolic hydroxyl group attack at C-6 to afford isolated compound (1) which converted to intermediates 14 and 15 by oxidation of exomethylene group at C-13 into alcoholic hydroxyl

group in (16) (Figure 2). The hydroxylation and oxidation reactions in plants catalyzed by cytochromes P450 enzyme (CYP) and in presence of O2 and NADPH [30]. In addition, the hydroxyl group of intermediate 15 is oxidized to ketonic one, forming compound (2) which subsequently converted to compound (3) by methylation of phenolic hydroxyl function group (Figure 2).

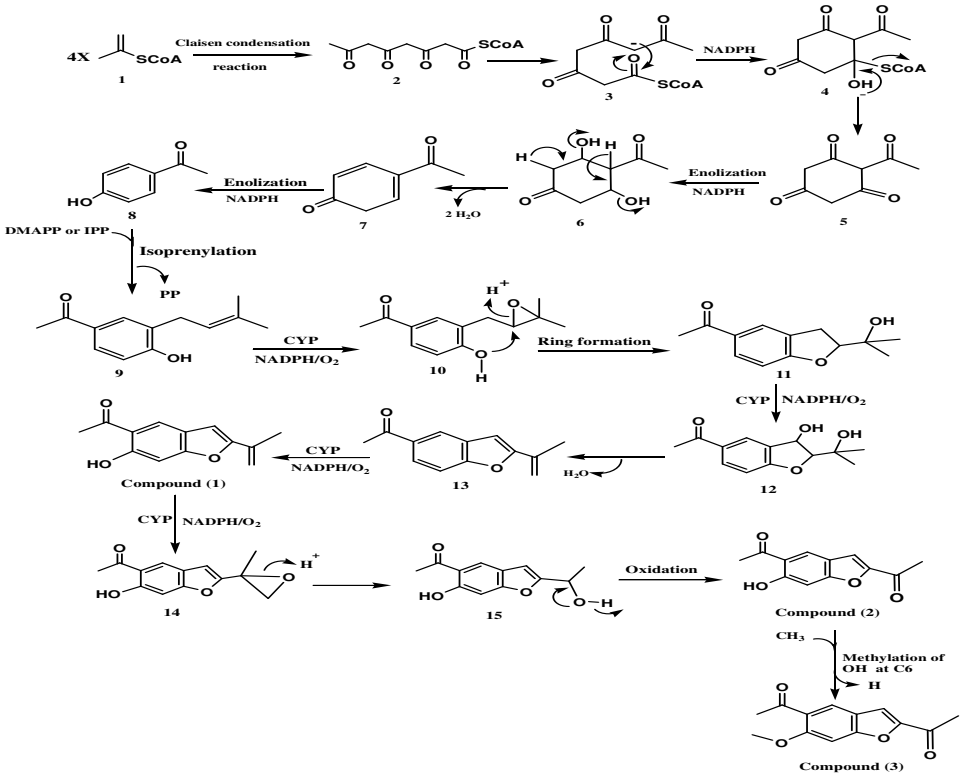


FIGURE 2: Biosynthesis of the isolated compounds (1-3)

Antimicrobial activity

A large number of plant extracts and their isolated compounds have been shown to possess potential antimicrobial activity. In the present study, the hexane / CH_2Cl_2 (1:1) crude extract and isolated compounds (1-3) of *S. glaucus* were assessed for antibacterial activity against two different strains of bacteria; *S. aureus* and *E. coli* and also against two strains of fungi; *Aspergillus flavus* and *Candida albicans*. The results showed that the tested compounds inhibited the growth of both stains with inhibition zone ranging from 10-14 mm (Table 2). The highest antibacterial activity against *S. aureus* and *E. coli* was observed with both compounds 2 and 3, showing equal inhibition zone 14 mm, however they exhibited lower activity against *E. coli* compared with *S. aureus* with inhibition zone 12 and 10 mm respectively (Table 2). In contrast, compound 1 showed weak antibacterial activity with inhibition zone 10 mm against both *S. aureus* and *E. coli*, however the Hexane/CH_2Cl_2 crude extract showed weak activity toward *S. aureus* and *E. coli* with inhibition zone 2 and 3 mm, respectively (Table 2). For both compounds 1 and 2, *S. aureus* was more sensitive with minimum inhibitory concentration (MIC) value 4.1 µl, compared to 3 with MIC value 7.5 µl. however, *E. coli* showed more sensitivity toward 1 with MIC, 5.5 µl, compared to 2 and 3 with MIC value 7.5 µl (Table 2). On the other hand, the antifungal activity of the Hexane/CH_2Cl_2 crude extract and isolated compounds (1-3) showed weak activity against *C. albicans* and *A. flavus* with inhibition zone ranging from 2-5 mm (Table 2).

In the present study, compound 1 has the same chemical structure of 2, except the presence of ketonic group at C-12 in 2 instead of exomethylene

group at C-13 in 1 (Figure 1). *S. aureus* showed the same sensitivity with 1 and 2, but *E. coli* was more sensitive to 1 comparing with 2, demonstrating that the presence of $\Delta^{12,13}$ double bond in 1 is favourable to enhance the antibacterial activity and sensitivity (Figure 1, Table 2). Interestingly, compound 2 was more active than 3 against *S. aureus* and *E. coli* showed the same sensitivity toward 2 and 3 (Table 2). These results can be attributed to the presence of free phenolic hydroxyl group at C-6 in 2 instead of methoxyl group in 3 is essential to enhance the sensitivity of *S. aureus* toward 2 more than 3 which is considered as methoxylated analogue of 2 (Figure 1).

TABLE 2: Antimicrobial activity of the crude extract and isolated compounds (1-3)

Sample	Gram-positive	Gram-negative	Yeast	Mold
	S. aureus	*E. coli*	*A. flavus*	*C. albicans*
Compound 1	**14 (4.1)**	**12 (5.5)**	**3**	**4**
Compound 2	**14 (4.1)**	**10 (7.5)**	**2**	**3**
Compound 3	**10 (7.5)**	**10 (7.5)**	**2**	**3**
Hexane/CH_2Cl_2 crude extract	**3**	**2**	**3**	**5**
Ampicillin^R	**21**	**25**	**-**	**-**
Amphotericin^R	**-**	**-**	**17**	**21**

Inhibition zones values are in mm including the well diameters.
Minimum inhibitory concentration MIC values, in parentheses, are expressed in µl ml^{-1}
No inhibition zones were observed in negative control.
^{R}: Reference compound for positive control

Chemosystematics significance

Recently, several natural furan containing metabolites were isolated mainly from plants [31], oils [32], and marine products [33]. Many biological investigations showed different beneficial effects of furan derivatives on human health as widely employed as antimicrobial, anti-tumor, anti-inflammatory, anticonvulsant, antihyperglycemic, etc [34-37]. The bioactive benzofuran and furanoeremophilane derivatives were isolated from *Senecio* genus showed wide range of pharmacological activities as anti-inflammatory and antimicrobial effects [34, 36]. The furanoeremophilane derivatives were isolated from about 24 species and reported as 14-Hydroxycacalol methyl ether from *S. othonnae* [38]; cacalohastine from *S. canescens* [39]; *β*-Angeloyloxy-8*β*-hydroxy-10*β*-H-eremophilanolide and 2*β*-angeloyloxy-8*β*-hydoxy-10*β*-H-eremophilanolide from *S. alatus* [40]; 6*β*-[Isobutyryloxy]furanoeremophil-1[10]-en-9-one from *S. pseudoorientalis* [40];1b,10b-Epoxy-6b-hydroxyfuranoeremophilan-9-one from *S. smithii* [41]; [3Z]-Implexin from *S. implexus* [41]; 4*α*-hydroxy-6*β*-angeloxy-10*β*-acetoxy-9-oxofuranoeremo- philane from *S. fistulosus* [42]; 6*β*-[2-Methylbutyryloxy]-furanoeremophilan-1-one and 6α-Methoxyeuryopsin from *S. auricula* [43]; 13-acetoxycacalol methyl ether from *S. picardae* [44]; 1-Oxocacalol methyl ether from *S. fuertesii* [44]; lα-angeloxy-6*β*-isobutyroxy-9-oxo-l0*α*H-furanoeremophilane from both *S. patagonicus* and *S. chilensis* [45]; 6b-[Propionyloxy]-10aH-furanoeremophilan-9-one from *S. pachyphyllos* [46]; Cacalohastine from *S. canescens* [47]; 6*β*-isobutyryloxy-1[10]-furanoeremophilene, 6*β*-isobutyryloxy-1-oxo-10*α*-furanoeremophilene from *S. boissieri* [48]; furanoligularenone from *S. flavus* [49]; 6*β*-[Isobutyryloxy] furanoeremophil-1[10]-ene, 6*β*-[Isobutyryloxy]-10aH-furanoeremophilan-1-one from *S. boissieri* [50]; 14-isovaleryloxy-1,2-dehydrocacalol methyl ether from *S. madagascariensis* [51]; 13-hydroxy-14-oxocacalohastine from *S. barba-johannis* [52]; 6-hydroxyeuryopsin and 6- acetyloxyeuryopsin from *S. toluccanus* [53] ; 10H-9-oxofuranoeremophilane from *S. filaginoides* [54] ; 1*α*-angeloyloxy-6*β*-acetoxy-10*β*-hydroxy-9-oxo-furanoeremophilane,1α-hydroxy-3α-angeloyloxy-10*α*H-9-oxofuranoeremo philane from *S. fistulosus* [55]; 10*α*H-furanoeremophil-1-one from *S. filaginoides* [56]; 2*β*-(angeloyloxy)furanoeremophilane, 2*β*-{[(Z)-2-hydroxymethylbut-2-enoyl] oxy} furanoeremo-philane from *S. gracilifous* [57]. However, the benzo- furan derivatives were isolated from only about 4 species including the present one as 2-[3-pentenyl]-3,7-dimethylbenzofuran-1,4-dione, l-hydroxy-2-[3- pentenyl]-3,7-dimethylbenzofuran, l-methoxy-2-[3- pentenyl]-3,7-dimethylbenzofuran from *S. virgaureus* [58]; 2-isopropylidene-3-oxo-5-acetal-6-hydroxy-benzodihydrofuran from *S. nelumbifolius* [59]; 3,7-dimethoxy-2-isopropyl-5-acetylbenzofuran, 3,7-dimethoxy-2,5-diacetylbenzofuran,7-methoxy-2α-iso- propyl-3-one-5-acetylbenzofuran,7-methoxy-2*α*-iso- propyl-2*β*-hydroxy-3-ketoacetylbenzofurane, 7-meth- oxy-2*α*-

isopropyl-2β-acethoxy-3-oxo-5-acetyl benzo- furane from *S. subcandidus* [60]; 2,3-dihydro-3β-hydroxyeuparin 3-*O*-glucopyranoside from *S. glaucus* [61]. In addition, three benzofuran metabolites were also isolated from *S. glaucus* in the present study. The previous data revealed that benzofurans isolated from *S. glaucus* is similar to those reported from *S. nelumbifolius* which are classified 2, 5, 6 trisubstituted benzofuran derivatives, however *S. virgaureus* and *S. subcandidus* are characterized by the generation of methoxylated and methylated benzofurans derivatives [58, 60].

4. Conflicts of Interest

The authors declare no conflict of interest.

5. Acknowledgments

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6. References

[1] Abu-Darwish MS and Thomas E. Medicinal plants from near east for cancer therapy. Frontiers in pharmacology. 9, 1-17 (2018).

[2] El-Shemy HA, Aboul-Enein AM, Aboul-Enein KM and Fujita K. Willow leaves' extracts contain anti-tumor agents effective against three cell types. *PLoS One*. 2,178-182 (2007).

[3] Vickers A. Botanical medicines for the treatment of cancer: rationale, overview of current data, and methodological considerations for phase I and II trials. *Cancer Invest.* 20, 1069-1079 (2002).

[4] Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. *J. Agric. Food. Chem.* 536, 1841-1856 (2005).

[5] Kalemba D, Kunicka A. Antibacterial and Antifungal Properties of Essential Oils. *Curr. Med. Chem.*10, 813-829 (2003).

[6] Nori-Shargh D, Raftari S, Deyhimi F. Analysis of the essential oil of *Senecio vernalis* Waldst. & Kit. from Iran. *Flavour Fragr. J.* 23, 357-359 (2008).

[7] Boulos L. Flora of Egypt, Al Hadara Publishing, Cairo, Egypt, 3, (2002).

[8] Boulos L. Flora of Egypt. Checklist All-Hadara Publishing, Cairo, Egypt, (2009).

[9] Eissa T, Palomino O, Carretero M, Gomez-Serranillos M. Ethnopharmacological study of medicinal plants used in the treatment of CNS disorders in Sinai Peninsula. Egypt, *J Ethnopharmacol*. 151, 317-332 (2014).

[10] The Encyclopedia of Traditional Chinese Medicine Science and Technology Press, Shanghai, 2955, (1977).

[11] Portero AG, González-Coloma A, Reina M, Díaz CE. Plant-defensive sesquiterpenoids from *Senecio* species with biopesticide potential. *Phytochem. Rev*. 11, 391-403 (2012).

[12] Reina M, Santana O, Domínguez DM, Villarroel L, Fajardo V, López-Rodríguez M, González-Coloma A. Defensive Sesquiterpenes from *Senecio candidans* and *S. magellanicus*, and Their Structure Activity Relationships. Chem. *Biodivers*. 9, 625-643 (2012).

[13] Ruiz-Vásquez L, Reina M, López-Rodríguez M, Giménez C, Cabrera R, Cuadra P, Fajardo V, González-Coloma A. Sesquiterpenes, flavonoids, shikimic acid derivatives and pyrrolizidine alkaloids from *Senecio kingii* Hook. *Phytochemistry* 117, 245-253 (2015).

[14] Ruiz-Vásquez L, Olmeda AS, Zuñiga G, Villarroel L. Insect Antifeedant and Ixodicidal Compounds from *Senecio adenotrichius*. *Chem. Biodivers.* 2017, 14,1-9 (2017).

[15] Ruiz-Vásquez L, Ruiz Mesia L, Reina-Artiles M, López-Rodríguez M, González-Platas J, Giménez C, Cabrera R, González-Coloma A. Benzofurans, benzoic acid derivatives, diterpenes and pyrrolizidine alkaloids from Peruvian *Senecio*. *Phytochem. Lett.* 28, 47-54 (2018)

[16] Agullo-Ortuño MT, Diaz CE, Gonzalez-Coloma A, Reina M. Structure-Dependent Cytotoxic Effects of Eremophilanolide Sesquiterpenes. *Nat. Prod. Commun*. 12, 663-665 (2017).

[17] Burgueño-Tapia E, Joseph-Nathan P. Cacalolides from *Senecio barba-johannis*. *Magn. Reson. Chem.* 41, 386-390 (2003).

[18] Burgueno- Tapia E, Bucio MA, Rivera A, Joseph-Burgueno-Tapia E, Hernandez LR, Resendiz-Villalobos AY, Joseph-Nathan P. Conformational evaluation and detailed ^{1}H and ^{13}C NMR assignments of eremophilanolides. *Magn. Reson. Chem* 42, 887-892 (2004).

[19] Zhao Y, Wang L, Yu-Fangand C, Man-Li H. Chemical and Pharmacological Research on Plants from the Genus *Senecio*. *Chem. Biodivers.* 8, 13-72 (2011).

[20] Bohlmann F, Zdero C, Jakupovic J, Misra LN, Benarjee S, Singh P, Baruah RN, Metwally MA, Schmeda-Hirschmann G, Vincent LPD, King RM, Robinson H. Eremophilane derivatives and other constituents from *Senecio* species. *Phytochemistry* 24, 1249-1253 (1985).

[21] Dong-Liang C, Xiao-Ping C, Jie-Kai C, Roeder E. Diterpene glycosides from *Senecio rufus*. *Phytochemistry* 32,151-153 (1992).

[22] Torres P, Ayala J, Grande C, Macıas MJ, Grande M. Furanoeremophilanes and a bakkenolide from *Senecio auricula* var. major. *Phytochemistry* 47, 57-61 (1998).

[23] Juarez BE, Mendiondo ME, Seeligmann P. Flavonoids from leaves and flowers of *Liabum polymnioides* and *L. candidum* (Asteraceae). Chemotaxonomical significance. *Biochem. Syst. Ecol.* 23, 335-336 (1995).

[24] Mahmoud AE, Norman JD, Maynard WQ, Joseph EK, David JS, Paul LS. Euparone, a new benzofuran from *Ruscus aculeatus* L. *J Pharm Sci.* 63,1623-1924 (1974).

[25] Bohlmann F and Grenz M. Neue Isopentenyl-acetophenon-Derivate aus *Helianthella uniflora*. *Chem. Ber.*103, 90-96 (1970).

[26] Lin TJ, Ramstad E, Heinstein P. *In vivo* biosynthesis of isopentenylacetophenones in *Eupatorium rugosum. Phytochemistry* 13, 1809-1815 (1974).

[27] Lin TJ and Heinstein P. *In vitro* biosynthesis of isopentenylacetophenones in *Eupatorium rugosum*. *Phytochemistry* 13, 1817-1823 (1974).

[28] Siebertz R, Proksch P, Wray V, Witte L. Accumulation and biosynthesis of benzofurans in root cultures of *Eupatorium cannabinum*. *Phytochemistry* 28, 789-793 (1989).

[29] Monir H and Proksch P. Yeast extract induced accumulation of benzofurans in cell suspension cultures of *Ageratina adenophora*. *Phytochemistry* 28, 2999-3002 (1989).

[30] Guo J, Zhou YJ, Hillwig ML, Shen Y, Yang L, Wang Y, Zhang X, Liu W, Peters RJ, Chen X. CYP76AH1 catalyzes turnover of miltiradiene in tanshinones biosynthesis and enables heterologous production of ferruginol in yeasts. *Proc. Natl. Acad. Sci.* 110, 12108-12113 (2013).

[31] Hannemann K, Puchta V, Simon E, Ziegler H, Ziegler G, Spiteller G. The common occurrence of furan fatty acids in plants. *Lipids* 24, 296-298 (1989).

[32] Kim DK and Kwak JH. A furan derivative from *Cornus officinalis*. *Arch Pharm Res*. 21(6),787-789 (1989).

[33] Ishii K, Okajima H, Koyamatsu T, Okada Y, Watanabe H. The composition of furan fatty acids in the crayfish. *Lipids* 23, 1988; 694-700 (1988). doi:10.1007/bf02535671.

[34] Koca M, Servi S, Kirilmis C, Ahmedzade M, Kazaz C, Özbek B, Ötük G. Synthesis and antimicrobial activity of some novel derivatives of benzofuran: part 1. Synthesis and antimicrobial activity of (benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl) ketoxime derivatives. *Eur J Med Chem.* 40, 1351-1358 (2005).

[35] Kirilmis C, Ahmedzade M, Servi S, Koca M, Kizirgil A, Kazaz C. Synthesis and antimicrobial activity of some novel derivatives of benzofuran: Part 2. The synthesis and antimicrobial activity of some novel 1-(1-benzofuran-2-yl)-2-mesitylethanone derivatives. *Eur J Med Chem.* 43, 300-308 (2008). doi:10. 1016/ j. ejmech. 2007.03.023.

[36] Kirsch G, Abdelwahab AB, Chaimbault P. Natural and synthetic coumarins with effects on inflammation. *Molecules* 21, 1322-1334 (2016). doi:10.3390/ molecules21101322.

[37] Chand K, Rajeshwari HA, Hiremathad A, Singh M, Ameli M, Rangapp S, Keria S. A review on antioxidant potential of bioactive heterocycle benzofuran: Natural and synthetic derivatives. *Pharmacol Rep.* 69, 281-295 (2017). doi: 10.1016/j. pharep.2016.11.007

[38] Bohlmann F and Zdero C. Inhaltsstoffe damerikanischer *Senecio arten*. *Phytochemistry* 16, 135-136 (1977).

[39] Bohlmann F and Zdero C. New cacalol derivatives from *Roldana heterogama*. *Phytochemistry* 17, 565-566 (1978).

[40] Bohlmann F and Ziesche J. Eremophilane derivatives from *Senecio* species. *Phytochemisry* 19, 2681-2684 (1980).

[41] Bohlmann F, Zdero C, King RM, Robinson H. Furanoeremophilanes from *Senecio smithii*. *Phytochemistry* 20, 2389-2391 (1981).

[42] Bohlmann F, Zdero C, Jakupovic J, Misra LN, Benarjee S, Singh P, Baruah RN, Metwally MA, Schmeda-Hirschmann G, Vincent LPD, King RM, Robinson H. Eremophilane derivatives and other constituents from *Senecio* species. *Phytochemistry* 24,1249-1253 (1985).

[43] Bohlmann F, Zdero C, Jakupovic J, Grenz M, Castro V, King RM, Robinson H, Leszek PDV. Further pyrrolizidine alkaloids and furoeremophilanes from *Senecio* species. *Phytochemistry* 25,1151-1159 (1986).

[44] Bohlmann F, Dupre S, Nordenstam B. Cacalol derivatives from Dominican *Senecio* species. *Phytochemistry* 29, 3163-3165 (1990).

[45] Viuarroel L and Torres R. 9-Oxo-10αH-furanoeremophilanes from *Senecio chilensis* and *Senecio patagonicus*. *J. Nat. Prod.* 54,588-590 (1991).

[46] Ahmed M and Niemeyer H.-M. Furoeremophilanes and related compounds from

Senecio pachyphyllos. Phytochemistry 30, 2078-2080 (1991.

[47] Abdo S, de Bernardi M, Marinoni G, Mellerio G, Samaniego S, Vidarit G, Vita FP. Furanoeremophilanes and other constituents from *Senecio canescens*. *Phytochemistry* 31, 3937-1941 (1992).

[48] Torres P, Ayala J, Grande C, Macıas MJ, Grande M. Furanoeremophilanes and a bakkenolide from *Senecio auricula* var. major. *Phytochemistry* 47, 57-61 (1998).

[49] Torresa P, Ayala J, Grande C, Anaya J and Grande M. Furanoeremophilane derivatives from *Senecio flavus*. *Phytochemistry* 52, 1507-1513 (1999).

[50] Torres P, Grande C, Anaya J, Grande M. Secondary metabolites from *Senecio minutus* and *Senecio boissieri*: a new jacaranone derivative. *Fitoterapia* 71, 91-93, (2000).

[51] Burgueno-Tapia E, Bucio MA, Rivera A and Joseph- Nathan P. Cacalolides from *Senecio madagascariensis*. J. *Nat. Prod* 64, 518-521 (2001).

[52] Burgueno-Tapia E, Bucio MA, Rivera A, Joseph-Burgueno-Tapia E, Hernandez LR, Resendiz-Villalobos AY, Joseph-Nathan P. Conformational evaluation and detailed ^{1}H and ^{13}C NMR assignments of eremophilanolides. *Magn. Reson. Chem* 42, 887-892 (2004).

[53] Burgueno-Tapia E, Hernandez-Carlos B, Joseph Nathan P. DFT, solution, and crystal conformation of eremophilanolides. *J. Mol. Struct*. 825, 115-123 (2006).

[54] Arancibia L, Naspi C, Pucci G, Arce M. Biological activity of a furanoeremophilane isolated from *Senecio filaginoides* var. *filaginoides.* Bol. *Latinoam Caribe Plant Med Arom*át. 12, 18-23 (2013).

[55] Ruiz-Vásquez L, Reina M, Fajardo V, López M, González-Coloma A. Insect Antifeedant Components of *Senecio fistulosus* var. *fistulosus*—Hualtata. *Plants* 8,176-187 (2019).

[56] Van Baren CM, González SB, Bandoni AL, Di Leo Lira P, Bucio MA, Hernández-Barragán A, Joseph-Nathan P. GC-FID-MS and X-ray Diffraction for the Detailed Evaluation of the Volatiles From *Senecio filaginoides*.. *Nat. Prod. Commun.* 15, 1-7 (2020).

[57] Jameel S, Kaur L, Bhat AS, Malik AF, Bhat AK. Neuroprotective activity of natural products isolated from *Senecio graciliflorus*. DC against corticosterone-induced impairment in SH-SY5Y cells. Naunyn-Schmiedeb. *Arch. Pharmacol.* 394, 2389-2399 (2021).

[58] Jia ZJ and Chen HM. Benzofurano sesquiterpenes from *Ligularia virgaurea*. *Phytochemistry* 30, 3132-3134 (1991).

[59] Zhao Y, Jia ZJ,Yang L. Sinapyl alcohol derivatives and other constituents from *Ligularia nelumbifolia. Phytochemistry* 37, 1149-1152 (1994).

[60] Ruiz-Vásquez L, Ruiz Mesia L, Reina-Artiles M, López-Rodríguez M, González-Platas J, Giménez C, Cabrera R, González-Coloma A. Benzofurans, benzoic acid derivatives, diterpenes and pyrrolizidine alkaloids from Peruvian *Senecio*. *Phytochem. Lett*. 28, 47-54 (2018).

[61] Zaher AM, Sultan R, Ramadan T, Amro A. New antimicrobial and cytotoxic benzofuran glucoside from *Senecio glaucus* L. *Nat. Prod. Res.* 36, 136-141(2020). doi: 10.1080/14786419.2020.1768089.

[62] Bauer AW, Kirby WM, Sherris C, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 45,493-496 (1966).

[63] Pfaller MA, Burmeister L, Bartlett MA, Rinaldi MG. Multicenter evaluation of four methods of yeast inoculum preparation. *J. Clin. Microbiol.* 26, 1437–1441 (1988).

[64] National Committee for Clinical Laboratory Standards (NCCLS). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7- A3. National Committee for Clinical Laboratory Standards, 1993, Villanova, Pa.

[65] National Committee for Clinical Laboratory Standards (NCCLS). Performance 1997, vol. 41, antimicrobial susceptibility of Flavobacteria.

[66] Matar MJ, Ostrosky-Zeichner L, Paetznick VL, Rodriguez JR, Chen E, Rex JH. Correlation between E-test, disk diffusion, and microdilution methods for antifungal susceptibility testing of fluconazole and voriconazole, Rex. *Antimicrob. Agents Chemother.* 47, 1647–1651 (2003).

[67] Liebowitz LD, Ashbee HR, Evans EGV, Chong Y, Mallatova N, Zaidi M, Gibbs D. A two year global evaluation of the susceptibility of Candida species to fluconazole by disk diffusion. Diagn. *Microbiol. Infect. Dis.* 4, 27–33 (2001).

[68] Loizzo MR, Statti GA, Tundis R, Conforti F, Bonesi M, Autelitano G, Houghton PJ, Miljkovic-Brake A, Menichini F. Antibacterial and antifungal activity of *Senecio inaequidens* DC. and *Senecio vulgaris* L. *Phytother Res.* 18, 777-779 (2004).