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RESEARCH ARTICLE

Cytotoxic Prenyl and Geranyl Coumarins from the Stem Bark of *Casimiroa edulis*

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Abstract: Phytochemical investigation of the methanolic extract of the stem bark of *Casimiroa edulis* afforded four coumarins. Various spectroscopic experiments were used to characterize the isolated coumarins. The structures were identified as auraptene (**K-1**), suberosin (**K-2**), 5-geranyloxypsoralen (bergamottin) (**K-3**), and 8-geranyloxypsoralen (**K-4**), based on the chemical and spectral analysis. Among these compounds, suberosin (**K-2**) and 5-geranyloxypsoralen (bergamottin) (**K-3**) were isolated for the first time from this genus, and auraptene (**K-1**) was isolated from this plant for the first time. Cytotoxicity of pure compound **K-4** and sub-fraction MD-3 was evaluated against HeLa and T47D cell lines and moderate activity was found with an IC₅₀ value in the range 17.4 to 72.33 µg/mL.

Keywords: *Casimiroa edulis*, coumarins, HeLa, spectroscopic experiments, stem bark, T47D.

1. INTRODUCTION

Nature is a good source of potential chemotherapeutic drugs [1]. The isolation process is a key step in discovering new biologically active substances from complex natural extracts [2]. A large number of bioactive compounds are isolated from research studies and screened each year, thus realizing the intrinsic therapeutic potential of natural products, and providing vast resource for further research [3]. Despite significant developments in the extraction and separation techniques, it is still a challenging task to isolate natural products from plants, animals, marine organisms or micro-organisms [4].

Coumarins (2H-1-benzopyran-2-ones) are a class of naturally occurring compounds found in various plants with an extensive pharmacological profile. To date, approximately 1500 coumarin derivatives have been identified from plants [5]. They have been identified as potent anti-inflammatory [6,10], anti-oxidant [7], anti-melanogenic [8], anti-bacterial [9], anti-viral [10], anti-coagulant [11], and cytotoxic agents [12].

A number of coumarins from *Casimiroa* spp. have been found by several researchers. Phellopterin, isopimpinellin,

imperatorin, xanthotoxol, 8-hydroxy-5-methoxypsoralen, 8-[(6,7-dihydroxy-3,7-dimethyl-2-octen-1-yl)oxy]-5-methoxypsoralen, 8-[(4-hydroxy-3-methyl-2-buten-1-yl)oxy]psoralen, 8-[(6,7-dihydroxy-3,7-dimethyl-2-octen-1-yl)oxy]psoralen, 8-geranyloxypsoralen (**K-4**), 8-[(4-hydroxy-3-methyl-2-buten-1-yl)oxy]-5-methoxypsoralen, are reported to belong to the furanocoumarins, a class of chemical compounds produced by the roots, seeds, and leaves of *Casimiroa* spp. Another report has shown that simple coumarins (umbelliferone, esculetin, herniarin) are present in the leaves and seeds of *Casimiroa* spp. Some isolated furanocoumarins and simple coumarins have been reported to show anticoagulant and anti-mutagenic activity. In addition, the structure modification of some furanocoumarins was also reported [13-20].

This paper outlines the isolation and structure elucidation of compounds **K-1** to **K-4** (Figs. 1-4) from the stem bark of *Casimiroa edulis*, as well as the cytotoxicity of sub-fraction MD-3 (section 3.3) and pure compound **K-4**.

2. RESULTS AND DISCUSSION

2.1. Structure Elucidation

Consecutive chromatographic purification of the MeOH soluble fraction of the stem bark of *C. edulis* yielded four compounds. The molecular structures of isolated compounds were identified on the basis of their UV, IR, ¹H NMR, ¹³C NMR, DEPT (Distortionless Enhancement by Polarization

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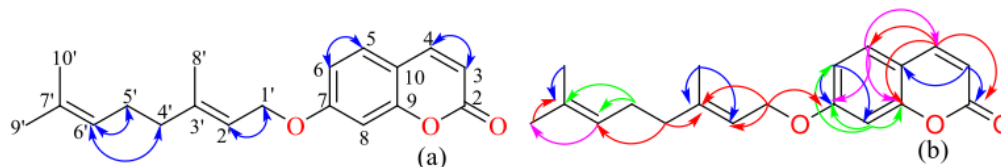


Fig. (1). COSY (a) and HMBC (b) key correlations in compound K-1.

Transfer), HSQC (Heteronuclear Single Quantum Coherence), HMBC (Heteronuclear Multiple Bond Coherence), NOESY (Nuclear Overhauser Effect Spectroscopy) data, and in comparison with the data reported by the literature. Detailed assignments of ^1H and ^{13}C NMR for compounds K-1, K-2, K-3, and K-4 are summarized in Tables 1-4.

21 The isolated compound K-1 was obtained as a crystalline solid. Its molecular formula was established as $\text{C}_{19}\text{H}_{22}\text{O}_3$ by HR-FAB-MS at m/z 299.1644 $[\text{M} + \text{H}]^+$. Its melting point was 68-76 $^\circ\text{C}$ and its UV spectrum exhibited the characteristic absorptions of coumarin skeleton at λ_{max} 327, 252, and 220 nm. On the basis of the IR spectrum studied, compound K-1 exhibited the characteristic absorption bands for C-H stretching of methine (3084 cm^{-1}), methylene (2972 cm^{-1}), methyl (2850 cm^{-1}), aromatic C=C (1612 and 1508 cm^{-1}), and α,β -unsaturated- δ -lactone (1726 cm^{-1}). The ^1H NMR spectrum exhibited a distinct pair of doublets at δ_{H} 6.24 (d, 1H, $J = 9.3$ Hz, H-3) and δ_{H} 7.63 (d, 1H, $J = 9.5$ Hz, H-4) due to the presence of coumarin skeleton. It also displayed three aromatic protons at δ_{H} 6.85 (dd, 1H, $J = 8.6$ and 2.4 Hz, H-6), δ_{H} 6.82 (d, 1H, $J = 2.3$ Hz, H-8) and δ_{H} 7.36 (d, 1H, $J = 8.6$ Hz, H-5) revealing the presence of a trisubstituted benzene ring. The ^1H - ^1H COSY (CORrelated Spectroscopy) spectrum showed a correlation between H-3 and H-4 protons, while H-5 proton had a correlation with the H-6 proton. Detailed analysis of the ^1H and ^{13}C NMR spectra, including COSY, HSQC, HMBC, suggested the existence of an extra C_{10} moiety on the coumarin skeleton, which incorporated an oxymethylene, three methyl groups, and two carbon-carbon double bonds. In the ^1H NMR spectrum, three broad triplet signals at δ_{H} 2.13 (br t, 2H, $J = 6.8$ Hz), 5.47 (br t, 1H, $J = 6.6$ Hz), and 5.08 (br t, 1H, $J = 6.7$ Hz) were assigned to H₂-5', H₂-2', and H-6', respectively. One oxymethylene doublet signal at δ_{H} 4.61 (d, 2H, $J = 6.6$ Hz) was assigned to H₂-1', while another broad doublet signal at δ_{H} 2.10 was assigned to H₂-4'. The remaining three methyl singlet signals at δ_{H} 1.76, 1.67, and 1.60 (s, each 3H) were assigned to H₃-8', H₃-9', and H₃-10', respectively. COSY showed that oxymethylene protons H₂-1' were coupled to one methine proton at δ_{H} 5.47 (H-2'), while another methine proton at δ_{H} 5.08 (H-6') was coupled to methylene protons at δ_{H} 2.10 (H₂-4') and 2.13 (H₂-5'). To confirm the linkage position of monoterpene moiety at C-7, ^1H - ^{13}C HMBC correlation between H-1' and C-7 was observed. Based on the above data and previous reports, compound K-1 was identified as auraptene (Fig. 1) [21, 22].

Compound K-2 was obtained as a crystalline solid and its molecular formula was found to be $\text{C}_{15}\text{H}_{16}\text{O}_3$, based on HR-FAB-MS (m/z 245.1175 $[\text{M} + \text{H}]^+$). The typical absorption

bands at 323, 251, and 218 nm in the UV spectrum exhibited a coumarin skeleton. The presence of a methoxy group, C-H in conjugation, α,β -unsaturated- δ -lactone, an ethylenic double bond and an aromatic ring was confirmed by the IR spectrum with the absorption bands at 3084, 3055, 2852-2972, 1728, 1612, 1562, and 1508 cm^{-1} . Initial spectral data (UV, IR, ^1H NMR, ^{13}C NMR) analysis showed that compound K-2 had a coumarin skeleton with prenylated moiety. The ^1H NMR spectrum exhibited characteristic signals of the 6,7-disubstituted coumarin, with two *cis*-olefinic protons at δ_{H} 6.23 (d, $J = 9.4$ Hz, H-3) and δ_{H} 7.62 (d, $J = 9.4$ Hz, H-4), and two singlet aromatic protons at δ_{H} 7.18 (s, H-5) and δ_{H} 6.78 (s, H-8). Additional prenylated moiety in compound K-2 exhibited the presence of two sharp singlet peaks at δ_{H} 1.71 (H₃-4') and 1.77 (H₃-5'), a doublet peak at δ_{H} 3.31 (d, $J = 7.3$ Hz, H₂-1'), and a broad triplet at 5.29 (br t, $J = 7.4$ Hz, H-2'). Based on the combinational analysis of ^{13}C NMR and DEPT data, compound K-2 displayed 15 carbon resonances consisting of 6 sp^2 quaternary carbons (including ester carbonyl carbon), 5 sp^2 methine carbons, 2 *gem*-dimethyl carbons, 1 sp^3 methylene carbon, and 1 oxygenated sp^3 carbon. In the ^1H - ^1H COSY experiment, the cross-peaks of H-3 with H-4 and H-1' with H-2' were observed. The proton signals at δ_{H} 3.31 (H₂-1') were correlated with the carbon signals at δ_{C} 127.5 (C-6), 160.7 (C-7), 121.4 (C-2'), 133.6 (C-3') in the HMBC spectrum. In addition, the HMBC cross-peaks of the two methyl signals at δ_{H} 1.71 (H₃-4') and 1.77 (H₃-5') were also correlated with the carbon signals at δ_{C} 121.4 (C-2') and 133.6 (C-3'). A sharp singlet signal at δ_{H} 3.90 indicated the presence of a methoxy group on the aromatic ring. This was supported by the long-range HMBC correlation between the methoxy group (δ_{H} 3.90) and C-7 (160.7). In addition, the NOESY spectrum showed a correlation between the methoxy signal and H-8 proton. The above evidence indicated that compound K-2 was suberosin (7-methoxy-6-prenylcoumarin) (Fig. 2) [11, 23].

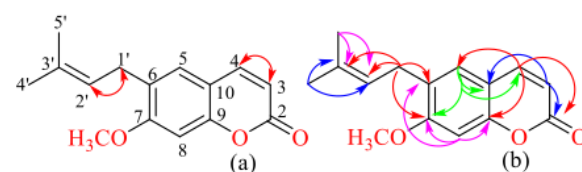


Fig. (2). COSY (a) and HMBC (b) key correlations in compound K-2.

Compound K-3 showed a molecular ion peak at m/z 339.1595 $[\text{M} + \text{H}]^+$ in its HR-FAB-MS corresponding to the

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Table 1. ¹H NMR (600 MHz) and ¹³C NMR (151 MHz) data of K-1 and comparison with the literature.

Nucleus	K-1 (CDCl ₃)		[19]	
	δ _H [m, J (Hz)]	δ _C	δ _H [m, J (Hz)]	δ _C
2	-	161.3	-	161.2
3	6.24 (d, 9.3)	112.9	6.24 (d, 9.6)	112.9
4	7.63 (d, 9.5)	143.4	7.63 (d, 9.6)	143.4
5	7.36 (d, 8.6)	128.6	7.36 (d, 7.2)	128.6
6	6.85 (dd, 8.6, 2.4)	113.2	6.85 (dd, 7.2, 2.0)	113.2
7	-	162.2	-	162.1
8	6.82 (d, 2.3)	101.6	6.82 (d, 2.0)	101.5
9	-	155.9	-	155.8
10	-	112.4	-	112.3
1'	4.61 (d, 6.6)	65.5	4.59 (d, 7.0)	65.4
2'	5.47 (br t, 6.6)	118.4	5.47 (t, 7.0)	118.3
3'	-	142.3	-	142.3
4'	2.10 (br d, 6.9)	39.5	2.13 (m)	39.4
5'	2.13 (br t, 6.8)	26.3	2.15 (m)	26.4
6'	5.08 (br t, 6.7)	123.6	5.08 (q, 7.0)	123.5
7'	-	131.9	-	131.9
8'	1.76 (s)	16.8	1.75 (m)	16.7
9'	1.67 (s)	25.6	1.65 (m)	25.6
10'	1.60 (s)	17.7	1.59 (q, 7.0)	17.6

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Table 2. ¹H NMR (600 MHz) and ¹³C NMR (151 MHz) data of K-2 and comparison with the literature.

Nucleus	K-2 (CDCl ₃)		[20]	
	δ _H [m, J (Hz)]	δ _C	δ _H [m, J (Hz)]	δ _C
2	-	161.5	-	161.5
3	6.23 (d, 9.4)	112.8	6.24 (d, 9.6)	112.7
4	7.62 (d, 9.4)	143.6	7.63 (d, 9.6)	143.6
5	7.18 (s)	127.4	7.19 (s)	127.4
6	-	127.5	-	127.5
7	-	160.7	-	160.6
8	6.78 (s)	98.5	6.78 (s)	98.5
9	-	154.5	-	155.4
10	-	111.9	-	111.9
1'	3.31 (d, 7.3)	27.8	3.31 (d, 7.2)	27.8
2'	5.29 (br t, 7.4)	121.4	5.29 (t, 7.2)	121.3
3'	-	133.6	-	133.6
4'	1.71 (s)	25.8	1.71 (s)	25.8
5'	1.77 (s)	17.8	1.78 (s)	17.7
7-OCH ₃	3.90 (s)	55.9	3.91 (s)	55.8

Table 3. ^1H NMR (600 MHz) and ^{13}C NMR (151 MHz) data of K-3 and comparison with the literature.

Nucleus	K-3 (CDCl ₃)		[21]	
	δ_{H} [m, J (Hz)]	δ_{C}	δ_{H} [m, J (Hz)]	δ_{C}
2	-	161.3	-	161.3
3	6.27 (d, 9.8)	112.6	6.25 (d, 9.8)	112.6
4	8.16 (d, 9.7, 0.5)	139.6	8.14 (d, 9.8)	139.6
5	-	149.0	-	149.0
6	-	114.3	-	114.3
7	-	158.1	-	158.2
8	7.16 (s)	94.2	7.13 (s)	94.3
9	-	152.7	-	152.7
10	-	107.6	-	107.6
2'	7.59 (d, 2.4)	144.9	7.57 (d, 2.5)	144.9
3'	6.96 (dd, 2.3, 1.0)	105.0	6.94 (dd, 2.5, 0.9)	105.0
1''	4.95 (d, 6.7)	69.8	4.93 (d, 6.8)	69.8
2''	5.54 (t, 6.9)	118.9	5.51 (m)	118.9
3''	-	143.0	-	143.0
4''	2.10 (m)	39.5	2.08 (m)	39.5
5''	2.10 (m)	26.2	2.08 (m)	26.2
6''	5.06 (m)	123.5	5.05 (m)	123.5
7''	-	132.0	-	132.0
8''	1.69 (s)	16.7	1.67 (s)	16.7
9''	1.68 (s)	25.7	1.66 (s)	25.7
10''	1.60 (s)	17.7	1.58 (s)	17.7

molecular formula, $\text{C}_{21}\text{H}_{22}\text{O}_4$, and was obtained in an oily form. The UV signals of compound **K-3** in MeOH at 309, 258, 249 nm suggested the furanocoumarin skeleton for the molecule. Its IR spectrum exhibited absorption peaks due to the presence of an ester carbonyl group (1735 cm^{-1}), olefinic groups and aromatic ring (1624 , 1606 , 1577 , and 1544 cm^{-1}). ^{13}C NMR of compound **K-3** included 21 carbon signals, comprising 3 methyl carbon signals, 2 adjacent methylene carbon signals, 1 oxymethylene carbon signal, 7 methine carbon signals, and 8 quaternary carbon signals, which were classified by DEPT and HSQC experiments. Its ^1H NMR spectrum had a doublet at δ_{H} 6.27 (d, 1H, $J = 9.8$ Hz) and a double doublet at 8.16 (dd, 1H, $J = 9.7$ and 0.5 Hz) assigned to H-3 and H-4, respectively, and a furan doublet at 7.59 (d, 1H, $J = 2.4$ Hz) and double doublet at 6.96 (dd, 1H, $J = 2.3$, 1.0 Hz) assigned to H-2' and H-3', respectively, as well as a singlet at 7.16 (s, 1H, H-8). Five-bond coupling constants (5J) were observed for H-4 (dd, $^3J_{3,4} = 9.7$, $^5J_{4,8} = 0.5$ Hz) and H-3' (dd, $^3J_{2,3'} = 2.3$, $^5J_{3',8} = 1.0$ Hz). The above information and previous data indicated that compound **K-3** was a furanocoumarin. Long-range HMBC correlations from H-3 to C-2,10, H-4 to C-2,9, H-8 to C-6,7,9,10, and from H-2',3'

to C-6,7 further confirmed the presence of the furanocoumarin moiety. The ^1H NMR spectrum of compound **K-3** showed an additional geranyl group on the furanocoumarin skeleton as compared with compound **K-1**. The existence of geranyl group was indicated by the presence of two multiplet signals at δ_{H} 2.10 (H₂-4'',5'') and 5.06 (H₂-6''), an oxymethylene doublet signal at δ_{H} 4.95 (H₂-1''), another triplet signal at δ_{H} 5.54 (H-2''), and three methyl singlet signals at δ_{H} 1.69, 1.68 and 1.60, assigned to H₃-8'', H₃-9'', and H₃-10'', respectively. The observed ^1H - ^{13}C HMBC correlation between H-1'' and C-5 confirmed the linkage position of the geranyl moiety at C-5. Detailed HMBC and COSY correlations of compound **K-3** are shown in Fig. (3). Therefore, the structure of compound **K-3** was determined to be 5-geranyloxypsoralein (bergamottin) [24, 25].

Compound **K-4** was obtained as a yellow crystalline solid. Its molecular formula was determined as $\text{C}_{21}\text{H}_{22}\text{O}_4$ by HR-FAB-MS (m/z 339.1590 [$\text{M} + \text{H}$]⁺). The UV spectrum showed absorption maxima at 249 and 298 nm. The IR spectrum showed the characteristic absorption bands at ν_{max} 1585, 1625, 1702, and 1720 cm^{-1} indicating the presence of olefinic moieties, aromatic ring, and ester carbonyl function.

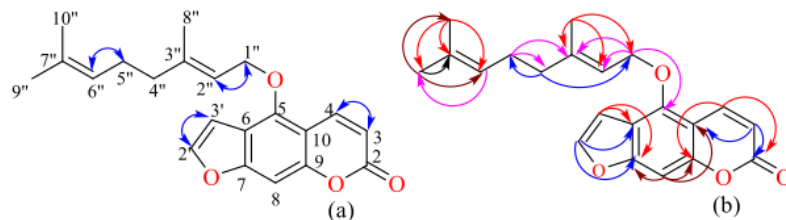


Fig. (3). COSY (a) and HMBC (b) key correlations in compound K-3.

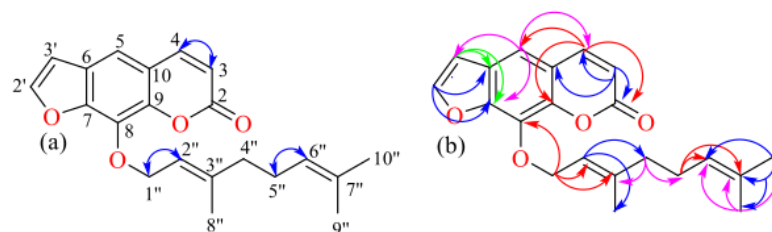


Fig. (4). COSY (a) and HMBC (b) key correlations in compound K-4.

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Table 4. ¹H NMR (600 MHz) and ¹³C NMR (151 MHz) data of K-4 and comparison with the literature.

Nucleus	K-4 (CDCl ₃)		[22]	
	¹ H δ _H [m, J (Hz)]	δ _C	¹ H δ _H [m, J (Hz)]	δ _C
2	-	160.5	-	160.5
3	6.37 (d, 9.3)	114.7	6.34 (d, 9.6)	114.7
4	7.76 (d, 9.5)	144.3	7.74 (d, 9.5)	144.3
5	7.36 (s)	113.2	7.34 (s)	113.2
6	-	125.8	-	125.8
7	-	148.8	-	148.7
8	-	131.6	-	131.5
9	-	143.9	-	143.9
10	-	116.5	-	116.4
2'	7.69 (d, 2.2)	146.6	7.66 (d, 2.2)	146.6
3'	6.81 (d, 2.2)	106.7	6.79 (d, 2.3)	106.7
1''	5.04 (d, 7.1)	70.1	5.01 (d, 7.2)	70.1
2''	5.60 (br t, 7.6)	119.4	5.57 (t, 7.1)	119.4
3''	-	143.1	-	143.2
4''	2.01 (s)	39.6	1.98 (s)	39.5
5''	2.02 (s)	26.4	1.99 (s)	26.3
6''	5.01 (m)	123.8	4.98 (m)	123.7
7''	-	131.7	-	131.7
8''	1.70 (s)	16.5	1.67 (s)	16.5
9''	1.64 (s)	25.6	1.61 (s)	25.6
10''	1.57 (s)	17.6	1.54 (s)	17.6

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The ^1H and ^{13}C NMR spectra of compound **K-4** were very similar to that of compound **K-3**, with the exception of the side-chain substitution. The ^1H NMR spectrum exhibited an easily distinguishable pair of doublets at δ_{H} 6.37 (d, 1H, $J = 9.3$ Hz, H-3) and δ_{H} 7.76 (d, 1H, $J = 9.5$ Hz, H-4). It also displayed two furan doublets at δ_{H} 7.69 and 6.81 (each d 1H, $J = 2.2$ Hz, H-2' and H-3' respectively), and an aromatic singlet at δ_{H} 7.36 (s, 1H, H-5). The ^1H - ^1H COSY showed a correlation between H-3 and H-4. Long-range HMBC correlations were observed from H-3 to C-2,4,10, H-4 to C-2,5,9, H-5 to C-3',4,7 and H-2',3' to C-6,7. Analysis of the ^1H and ^{13}C NMR spectra, including COSY, HSQC, HMBC, suggested the presence of an additional C_{10} moiety (one geranyl group) on the furanocoumarin skeleton, which comprised of oxymethylene, three methyl, and two carbon-carbon double bonds. In the ^1H NMR spectrum, one multiplet signal at δ_{H} 5.01 (m, 1H) was assigned to H-6'', and two methylene singlet protons at 2.01 and 2.02 (each s 2H) were assigned to H₂-4'',5''. One oxymethylene doublet signal at δ_{H} 5.04 (d, 2H, $J = 7.1$ Hz) was assigned to H₂-1'', while one broad triplet signal at δ_{H} 5.60 was assigned to H-2''. The remaining three methyl singlet signals at δ_{H} 1.70, 1.64, and 1.57 (s, each 3H) were assigned to H₃-8'', H₃-9'', and H₃-10'', respectively. COSY showed that oxymethylene protons H-1'' were coupled to one methine proton δ_{H} 5.60 (H-2''), while another methine proton at δ_{H} 5.04 (H-1'') was coupled to two methylene protons at δ_{H} 2.01 and 2.02 (H₂-4'',5''). In compound **K-4**, the position of monoterpene moiety (a geranyl group) at C-8 was confirmed by the ^1H - ^{13}C HMBC correlation between H₂-1'' and C-8. All the protons and carbons were allocated with the help of 2D NMR data analysis, such as COSY, DEPT, HSQC and HMBC. Thus, the structure of compound **K-4** was determined as shown in Fig. (4) and identified as 8-geranyloxypсорalen [26].

2.2. Cytotoxicity of Compound K-4 and Sub-Fraction MD-3 against HeLa and T47D Cell Lines

Pure compound **K-4** and sub-fraction MD-3 (see section 3.3) were evaluated for their cytotoxicity against HeLa (Henrietta Lacks cervical carcinoma) and T47D (human hormone-dependent breast cancer) cell lines by the MTT [2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-2H-tetrazolium bromide] method. According to Table 5, pure compound **K-4** showed moderate activity [12, 27] against T47D cell line with the IC₅₀ (half maximal Inhibitory Concentration) value of 72.33 $\mu\text{g}/\text{mL}$; sub-fraction MD-3 also showed moderate activity [10, 24] against both HeLa and T47D cell lines with IC₅₀ values of 56.40 and 17.40 $\mu\text{g}/\text{mL}$, respectively.

Table 5. Cytotoxicity of pure compound **K-4** and sub-fraction MD-3.

Sample	IC ₅₀ , $\mu\text{g}/\text{mL}$	
	HeLa	T47D
K-4	148.93	72.33
MD-3	56.40	17.40

2.3. Discussion

Coumarins were originally discovered in Tonka Bean and are known to be present in around 150 species, extending to approximately 30 distinct families, including Rutaceae, Umbelliferae, Clusiaceae (Guttiferae), Oleaceae, Nyctaginaceae and Apiaceae. Natural coumarins are primarily divided into six different kinds according to the chemical compound composition. They are simple coumarins, furanocoumarins, dihydrofuranocoumarins, pyranocoumarins (linear and angular types), phenylcoumarins, and bicoumarins [28]. Natural and synthetic coumarins are known to have a wide range of pharmacological properties [29-31]. 8-Geranyloxypсорalen (**K-4**) was previously isolated from various plant sources and demonstrated nematocidal activity (against *Bursaphelenchus xylophilus* and *Panagrellus redivivus*), high anti-microbial effect (against *Staphylococcus epidermidis* and *Candida kefyr*), weak anti-oxidant activity [DPPH (DiPhenylPicryl Hydrazyl) assay], cytotoxic effect on McCoy cell line, and weak anti-fungal activity (against yeast *Malassezia globosa*) [32, 33]. Auraptene (**K-1**) was isolated from *Cleome viscosa* (L.), *Citrus junos*, *Ferula* spp., *Zosima absinthifolia*, and showed no anti-microbial activity, weak cytotoxicity, neuro-protective effects, allelopathic effects, and anti-carcinogenic properties [34-37]. Bergamottin (**K-3**), a geranyloxyfuranocoumarin, is the most abundant in nature. It showed a significant dose-dependent inhibitory effect on human liver cancer line (HepG2), human leukemia cell line (HL-60), and human gastric cancer cell line (BGC-823) [38, 39]. Suberosin (**K-2**) is a bioactive and useful secondary metabolite. It was previously isolated from *Arracacia toluensis* var. *multifida*, *Ferulago* spp. and showed anti-mycobacterial, anti-coagulant, anti-diabetic, and anti-cancer activities [40-43]. This research described the isolation and identification of methoxyprenylcoumarin **K-2**, geranyloxyfuranocoumarin **K-1**, and geranyloxyfuranocoumarins **K-3,4** from Myanmar Rutaceae. Moreover, the cytotoxicity of compound **K-4** and sub-fraction MD-3 against HeLa and T47D cell lines was investigated. The results showed moderate cytotoxicity effect on the tested substances.

3. EXPERIMENTAL

3.1. General

Melting points were determined by the Fisher-Johns melting point apparatus. UV spectra were recorded on the UV-Vis Shimadzu spectrometer. FTIR-8400 spectrophotometer was used for measuring IR spectra. The NMR spectra were recorded at 600 MHz for ^1H and 151 MHz for ^{13}C on the Bruker Avance III spectrometer. Chemical shift values were determined on a δ (ppm) scale and tetramethylsilane (TMS) was used as an internal standard. The kind of carbon (CH_3 , CH_2 , and CH) was confirmed by DEPT experiments. HR-FAB-MS was measured on a JEOL JMS HX-110 mass spectrometer. Purification of the compounds was performed by using vacuum liquid chromatography (VLC) on silica gel 60 (Merck, 63–230 μm), and column chromatography (CC) on silica gel 60 (Merck, 0.043–0.063). Analytical thin-layer chromatography (TLC) was performed on pre-coated Kieselgel silica gel 60 F₂₅₄ plates (Merck, Germany). Preparative thin-layer chromatography (PTLC) was carried out on silica

gel 60 F₂₅₄ glass plates (Merck, 20 × 20 cm, 0.25 mm). The compound bands were scraped off and washed with 100% chloroform.

3.2. Plant Material

The fresh stem bark of *C. edulis* Llave et Lex was collected in August 2016 from Taunggyi (Shan State), Myanmar and identified by Prof. Dr. Aye Aye Win Kyi, Department of Botany, University of Taunggyi. A voucher specimen (BT001289) has been deposited at the Department of Chemistry, University of Taunggyi.

3.3. Extraction and Isolation

The stem bark of *C. edulis* was cut into small pieces and air-dried at room temperature. The dried samples (1000 g) were extracted with MeOH (3000 mL) for 28 days. The whole extract was filtered and the solution was evaporated under vacuum. MeOH crude extract (250 g) was obtained. After that, the MeOH extract was partitioned successively with hexane and MeOH. The hexane soluble fraction (13 g) and MeOH soluble fraction (50 g) were obtained. Each fraction was evaporated under pressure by using a rotary evaporator. The MeOH extract (50 g) was subjected to VLC eluting with a gradient solvent system hexane/EtOAc (100:0-60:40, 0:100, v/v) to give fractions, A to F. Fraction B (300 mg) was separated by silica gel CC with hexane/EtOAc (100:0 to 96:4) to give compound **K-3** (9.1 mg) and sub-fraction MD-3. MD-3 (120 mg) was further purified by PTLC using 80% chloroform in hexane as an eluent to yield compounds **K-1** (7.2 mg) and **K-2** (2.3 mg). Fraction C (1 g) was separated by in silica gel CC using a gradient solvent of hexane/EtOAc (9:1 – 7:3) to obtain compound **K-4** (30 mg).

3.4. MTT Assay

The cytotoxicity of the isolated compound **K-4** and fraction MD-3 on HeLa and T47D cells was measured by using the MTT assay *in vitro*. The isolated samples were dissolved in DMSO (100 µL) to obtain various concentrations. Each sample was prepared in triplicate. The final DMSO concentration was adjusted to <0.1 %. Doxorubicin was used as a positive control. The media control solution consisted of the media culture and the cell control solutions consisted of the culture and cell media. The cells were prepared by following the method for cell subculture. Enough amount of HeLa cell and T47D cell (213.4 × 10⁴ cells / well and 167 × 10⁴ cell/well) solution was prepared and 100 µL was placed in each 96-well plate. The plates containing the cancer cells were treated with species under study and incubated for 24 h. After 24 h incubation, the cells were washed and treated with 100 µL of MTT per well. Plates were incubated at 37 °C in a 5% CO₂ atmosphere for 4 h, and 0.1 mL of the extraction buffer (10% sodium dodecyl sulfate in 0.01% HCl) was added. After overnight incubation at 37 °C, the absorbance was measured at 595 nm using an ELISA (Enzyme-Linked Immuno Sorbent Assay) reader and was compared with the control cultures without compound. To determine cell viability, percent viability was calculated by the formula

$$\% \text{ Viability} = \frac{A_t - A_{mc}}{A_{ng} - A_{mc}} \times 100$$

where A_t = absorbance of treatment, A_{mc} = absorbance of media control, A_{nc} = absorbance of the negative control. IC₅₀ values of the tested sample were calculated from the concentration v/s percentage of cell viability inhibition curves.

CONCLUSION

Four coumarins, auraptene (**K-1**), suberosin (**K-2**), bergamottin (**K-3**), and 8-geranyloxypsoralen (**K-4**), were obtained from the stem bark of *C. edulis*. Among these compounds, suberosin (**K-2**) and bergamottin (**K-3**) were isolated for the first time from this genus and auraptene (**K-1**) was isolated from this plant for the first time. 8-Geranyloxypsoralen (**K-4**) and sub-fraction MD-3 were tested for their cytotoxicity against HeLa and T47D cell lines. The results demonstrated that they had moderate activity against HeLa and T47D cell lines with IC₅₀ values in the range of 17.4 to 72.33 µg/mL.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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