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Original Article

Phytochemical Investigation, Isolation and Characterization of Coumarins from Aerial Parts and Roots of Tunisian *Pituranthos chloranthus (Apiaceae)*

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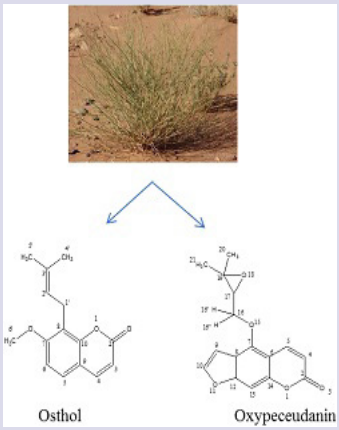
ABSTRACT

Pituranthos chloranthus, commonly known in Arabic as 'Aljen,' is a small aromatic plant which grows spontaneously in North Africa and it is widespread in central and southern Tunisia. This paper is the first report of its kind on the isolation and characterization of certain coumarin derivatives from the extract of the roots and aerial parts of this plant. **Background:** *Pituranthos chloranthus* (Apiaceae) commonly known as 'Aljen' is an endemic Tunisian aromatic plant, largely used in folk medicine. This plant contains bioactive compounds, particularly coumarin derivatives. The objective of the present study was to isolate and characterize some bioactive phytochemical constituents from the extract of the aerial parts and roots of *Pituranthos chloranthus*. **Methods:** Different extracts were subjected to column chromatography and eluted with solvent mixtures of increasing polarity (petroleum ether, ethyl acetate and methanol) to isolate five pure Products. The structure of the isolated compound was established using spectroscopic methods (UV, ^{1}H-NMR, ^{13}C-NMR, DEPT, HMBC, HSQC, COSY), and HRMS. **Results:** Isoimperatorin, osthol and oxypeucedanin were isolated from the *n*-hexane and ethyl acetate extract of the aerial part of the plant. Bergapten and nodakenetin were isolated from the methanolic extract of the roots. **Conclusion:** *Pituranthos chloranthus* contains bergapten, isoimperatorin, nodakenetin, osthol and oxypeucedanin which may be responsible for various pharmacological activities of the plant.

Key words: *Apiaceae*, Bioactive compounds, oxypeucedanin, *Pituranthos chloranthus*.

SUMMARY

- First detection of coumarin derivatives from roots and arial parts of *Pituranthos Chloranthus*.
- This plant contains osthol and oxypeucedanin that were never reported in the genus *Pituranthos*.



PICTORIAL ABSTRACT

Abbreviations used: NMR: Nuclear Magnetic Resonance, HRMS: High Resolution Mass Spectrometry.

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INTRODUCTION

Medicinal and aromatic plants are the principal health care resources for the majority of people all over the world due to their therapeutic potential. According to the World Health Organization, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs.[1] The medicinal value of these plants is due to bioactive phytochemical constituent's action in the human body. Some of the most important bioactive compounds include alkaloids, flavonoids, essential oils, tannins, coumarins and saponins.[2]

Consequently, there is growing scientific interest focused on the recovery of bioactive

secondary metabolites from natural sources due to their beneficial effects on human health.

These compounds can provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity.[3] The genus *Pituranthos* (family *Apiaceae*) includes more than 20 species.[4] Several species of this genus have been used in traditional medicine for the treatment of fevers, hepatitis,[5] asthma, rheumatism,[6,7] diabetes and digestive difficulties.[8] Among them, *P. chloranthus*, commonly known in Arabic as 'Aljen', is a small aromatic plant which grows spontaneously in North Africa and is widespread in central and southern Tunisia. Previous phytochemical investigations report the isolation of flavonoids and glycosides from the aerial parts of *P. chloranthus* such as, Isorhamnetin 3-O- β -glucoside, isorhamnetin 3-O-(6"-O-6-rhamnosyl)- β-glucoside, tamarixetin 3-O-β-glucoside, apigenin 6,8-di-C-β-glucoside (vicenin-2), scopoletin and L-iditol.[9] In addition, the isolation of β-Sitosterol from the chloroform extract of the aerial parts of this plant has been described.[10] However, other phytochemical studies on the genus of *Pituranthos* have shown the presence of coumarin and furanocoumarin.[11,12] To the best of our knowledge, *P. chloranthus* has not been investigated for its coumarin derivatives until now. Therefore, we report here, for the first time, the isolation and the characterization of some coumarin derivatives from the extract of the roots and aerial parts of this plant growing wild in Tunisia.

MATERIALS AND METHODS

Plant material

Different samples of *P. chloranthus* (Coss. and Dur.) (Apiaceae) were collected at the flowering stage in February 2014 from Naffatia region located in southeastern Tunisia.

Samples were authenticated by use of *Flore de la Tunisie*.[13] After the collection, the fresh vegetable matter was dried in the shade, and the aerial parts were then separated from the roots.

Extraction and isolation of aerial parts

The dried and powdered aerial parts of *P. chloranthus* were extracted successively in *n*hexane, ethyl acetate and ethanol, using the maceration method at room temperature. After 48 hours, the different extracts were filtered and concentrated *in vacuo* to give the corresponding extract. The *n*-hexane and ethyl acetate soluble fractions (4 g) were dissolved in a minimum volume of ethyl acetate and adsorbed into silica gel (Merck, 60-120 mesh). After evaporation of the solvent, each resulting mass was subjected to silica gel column chromatography (CC). The nhexane and ethyl acetate columns were then eluted with different solvents using increasing polarity starting from petroleum ether (100%) and ending with ethyl acetate (100%) to yield respectively 681 and 405 fractions collected on the basis of their TLC profiles. Compound (1) was isolated from the *n*-hexane fractions 144-158 (20% of ethyl acetate and 80% of petroleum ether) and crystallized as a pure compound. The second compound (Compound 2) was also isolated from *n*-hexane fractions 205-210 (30% of ethyl acetate and 70% of petroleum ether).

This fraction was further chromatographed over silica gel column using petroleum ether and ethyl acetate in different ratios to get 60 subfractions. The subfractions 33-45 (10% of ethyl acetate with 90% of petroleum ether) were mixed and recrystallized to obtain Compound (2).

Compound (3) was isolated from ethyl acetate fractions 240-253 (40% of ethyl acetate and 60% of petroleum ether). This fraction was subjected to silica gel column chromatography to yield 45 subfractions in which the subfractions 28-45 afford Compound (3).

Extraction and isolation of roots

Dried and powdered roots of *P. chloranthus* were extracted with methanol using a speed extractor apparatus at 200°C and evaporated using a Rotavapor apparatus at 40°C. The concentrated crude methanol extract obtained after evaporation of the solvent was subjected to silica gel chromatography column using a gradient of a mixture of petroleum ether-ethyl acetate and ethyl acetate-methanol with increasing polarity to give 450 fractions. Fractions 222-274, obtained from a mixture of ethyl acetate-petroleum ether (v/v), were combined based on their TLC profile and further purified by column chromatography over silica gel which was eluted with gradient systems of petroleum ether/ethyl acetate and ethyl acetate/methanol followed by methanol to afford 50 sub-fractions. Among them, subfraction 30-39 (20% of ethyl acetate and 80% of petroleum ether) was crystallized as Compound (4).

Compound (5) was isolated from fractions 392-420 and crystallized as a pure compound.

Instrumentation

NMR spectra were measured using a Bruker Shield 400 MHz instruments. The operating frequencies were 400MHz for acquiring ^{1}H NMR and 100 MHz for ^{13}C NMR spectra.

Samples were measured at 300 K in $CDCl_3$. NMR chemical shifts are given in ppm (δ) and J values are given in Hz. Jeol JMS-700 High Resolution Mass Spectrophotometer (JEOL (Germany) GmbH, Muenchen, Germany) was used for the mass determination. Silica gel 60F254 precoated plates (Merck) were used for the TLC.

RESULTS AND DISCUSSION

From the *n*-hexane extract of the aerial parts of *P. Chloranthus,* two coumarins; isoimperatorin (Figure 1) and osthol (Figure 2) were isolated by silica gel column chromatography while oxypeucedanin (Figure 3) was isolated from the ethyl acetate extract of this plant. Besides the later compounds, two other coumarins; bergapten (Figure 4) and nodakenetin (Figure 5) were isolated from methanolic extract of the roots. The structures of these compounds were elucidated by spectroscopic methods (^{1}H-NMR, ^{13}CNMR, DEPT, HMBC, HSQC, COSY) and HR-MS as well as by comparison of their NMR data with those reported in the literature.[14-20]

Compound (1)

Compound (1) was isolated as yellow crystals. The molecular formula of Compound (1) was determined to be $C_{16}H_{15}O_4$ by HR-MS exhibiting the quasimolecular ion at m/z 271.0965 $[M+H]^+$(Figure 6). The 1H-NMR spectrum of Compound (**1**) showed essentially two proton doublets at δH 6.26 (*J*=9.6 Hz) and δ_H8.18 (*J*=9.6 Hz) characteristic of α-pyrone protons attributed respectively to H-4 and H-5, and a pair of doublets occurring at δ_H6.96 (*J*=2.2 Hz) and δ_H7.60 (*J* = 2.2 Hz) typical of furanic protons

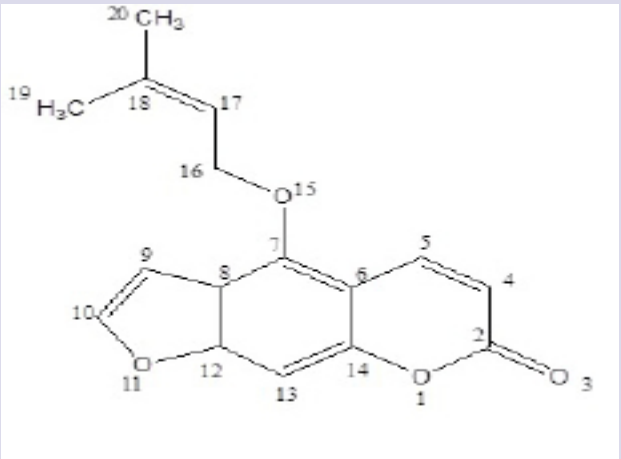

Figure 1: Chemical structure of Isoimperatorin

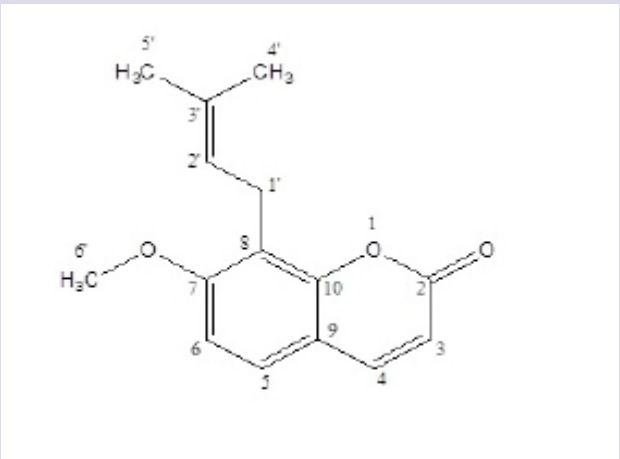

Figure 2: Chemical structure of Osthol

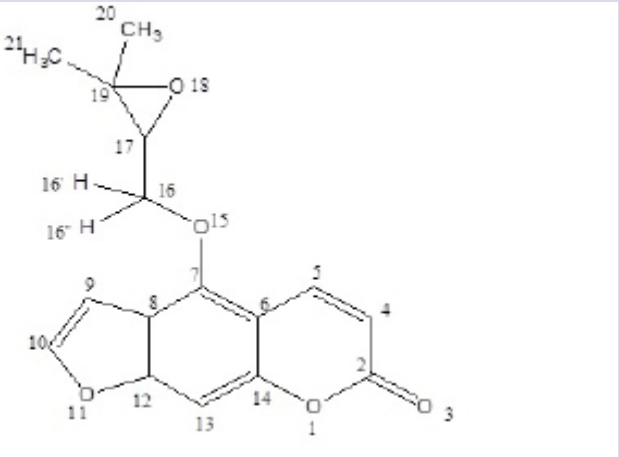

Figure 3: Chemical structure of Oxypeucedanin

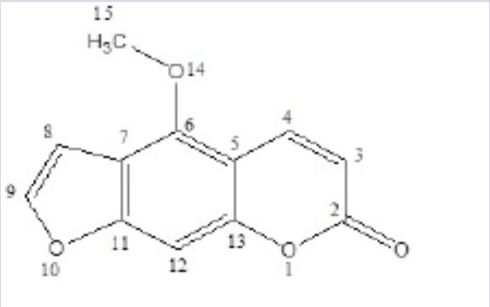

Figure 4: Chemical structure of Bergapten

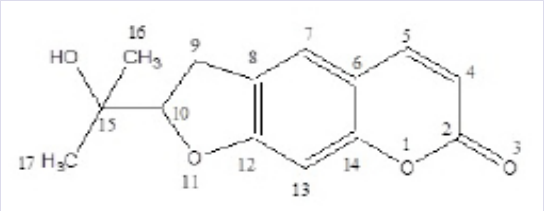

Figure 5: Chemical structure of Nodakenetin

assignable to H-9 and H-10, respectively (Figure 7). The presence of a singlet proton at δ_H7.16 ppm can be attributed to the proton attached to carbon atom number 13. The signal 17, which appears at δ_H5.54 ppm can be assigned to the olefinic proton attached to carbon atom 17. In addition, the spectrum showed a proton doublet at δ_H4.93 ppm attributed the oxymethylene proton. The signals 19 (singlet) and 20 (singlet), appearing respectively at 1.80 ppm and 1.70 ppm, are due to the isopentenyl group protons attached to carbon atoms 19 and 20. These attributions were confirmed by ^{13}CNMR spectrum and DEPT 135 which displayed a total of 16 atoms of carbon confirming the structure of this compound. The NMR data of this compound was compared favorably to isoimperatorin (1) published in the literature with a molecular formula of $C_{16}H^{15}O^4$.[14] In addition, a survey of the literature showed that this compound has been isolated from the shoots[4] and the roots[15] of *Pituranthos triradiatus.* However, this is the first time that this compound was isolated from P. *Chloranthus*.

Compound (2)

This compound was obtained as white crystal. The HR-MS showed a molecular ion peak at m/z 245.1167 [M+H]+ in agreement with the proposed structure of the known prenylated coumarin, osthol, with the molecular formula $C_{15}H_{16}O_3$ (Figure 8). The ^{1}H NMR spectrum of this compound showed two proton doublets at δH 6.24 (*J*=9.5 Hz) and 6.82 (*J*=8 Hz), characteristic of the H-3 and H-6 of the isolated compound (Figure 9). The presence of further two proton doublets at δ_H 7.3 (*J*=8 Hz) and 7.6 (*J*=9.5 Hz) indicated the presence of H-4 and H-3 in the ring of coumarin. The ^{1}H NMR spectrum also displayed a proton singlet at δ_H3.92 ppm (s) characteristic of a methoxy group (H6') and a multiplet appeared at δ_H 5.24 ppm (J=7.3 Hz) which can be assigned to the olefinic proton attached to carbon atom 2'. In the ^{1}H NMR spectrum, a doublet at 3.54 ppm (J=7.3 Hz) can be attributed to the proton attached to carbon atom 1'. However, the singlets that appear at 1.84 ppm and 1.66 ppm are

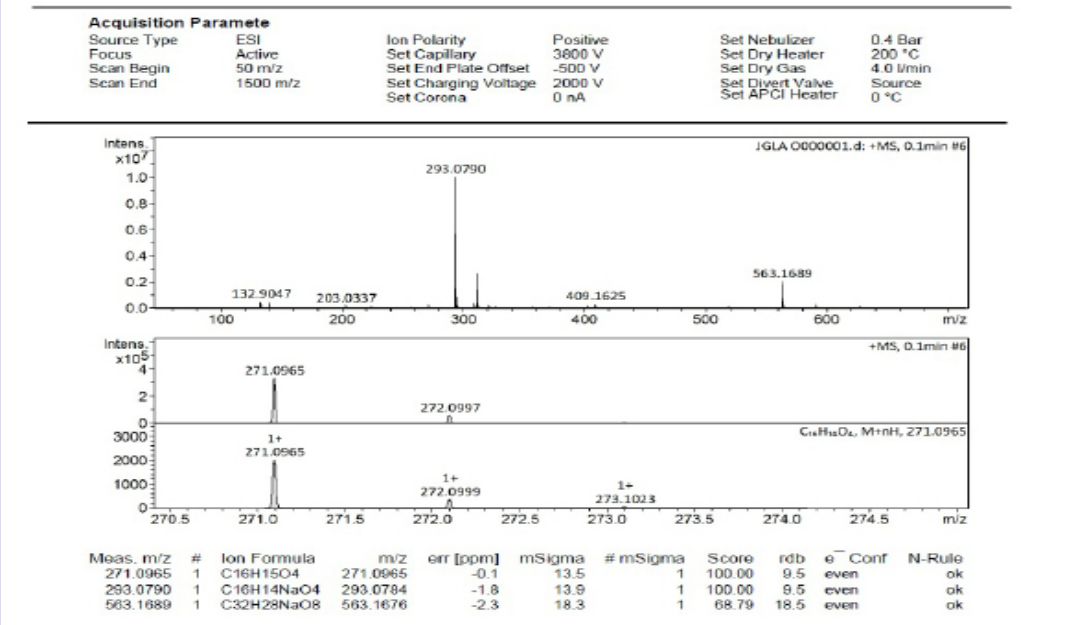

Figure 6: HRMS spectrum of compound (1)

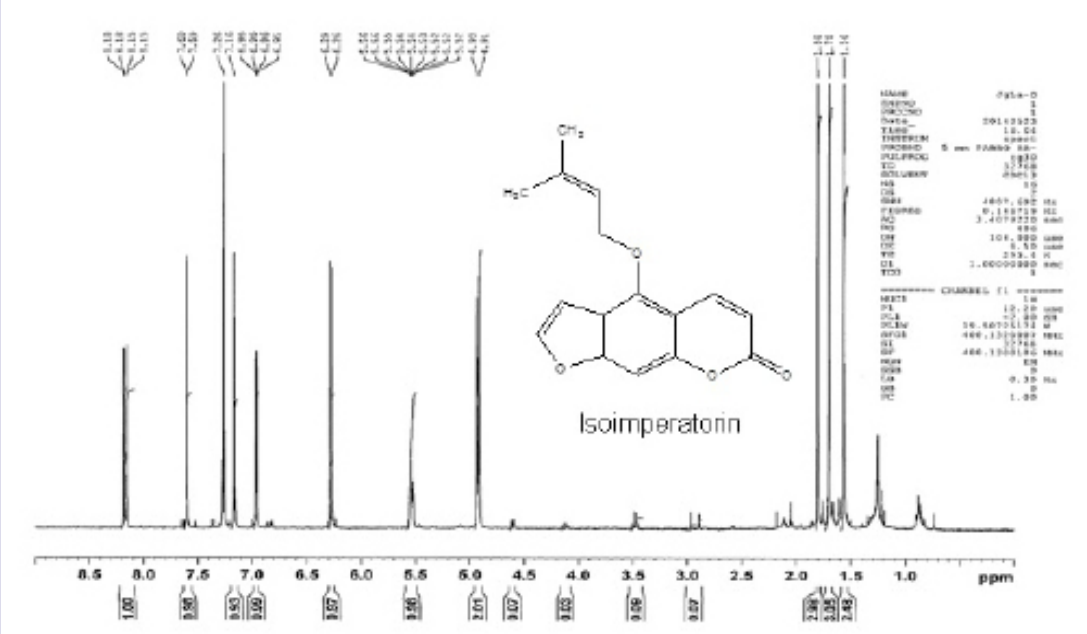

Figure 7: $_1$H NMR spectrum of compound (1)

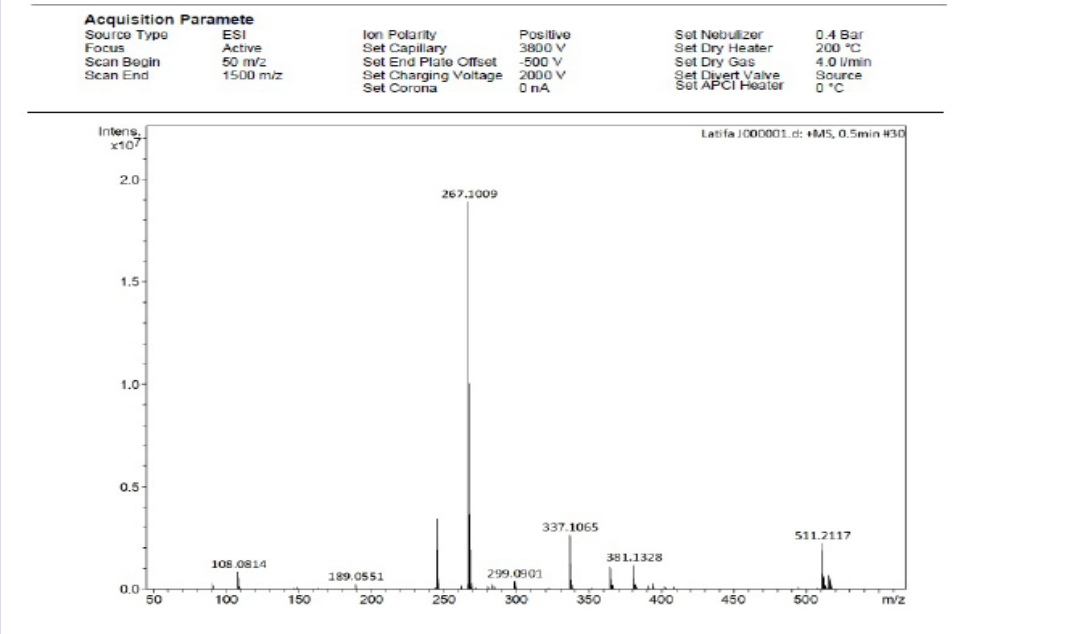

Figure 8: HRMS spectrum of compound (2)

due to the isopentenyl group protons attached to carbon atoms 4' and 5', respectively. All these NMR data and those of the literature[16] led to identifying Compound (2) as Osthol with a molecular formula of $C_{15}H_{16}O_3$ which was isolated, for the first time from the *Pituranthos* genus.

Compound (3)

Compound (3) was obtained as a white powder. The HR-MS gave a molecular ion peak at *m/z* 287.0923, corresponding to a molecular formula C16H14O6 (Figure 10). The ^{1}H-NMR spectrum shows two AB systems at 8.20, 6.34 ppm (^{1}H, d, J = 9.8 Hz, H-5, H-4, respectively) and at 7.61, 6.95 ppm (^{1}H each, d, J = 2.2 Hz, H-10, H-9), which are characteristic of the furanocoumarin skeleton (Figure 11). The signal 13, appearing at 7.20 ppm, can be attributed to the methine proton attached to carbon atom 13. The signals H16' (δ_H4.42) and H16" (δ_H 4.62) arising from the methylene protons have almost the same chemical shift values of 4.46–4.42 ppm (doublet) and 4.62–4.58ppm (doublet), respectively. In fact, these protons have the same direct atomic neighbors or a similar chemical environment of methylene group 16.

Signal 17, which appears at 3.24 ppm, can be assigned to the proton attached to carbon atom 17. The signals 20 (singlet) and 21 (singlet) that appear at 1.41 ppm and 1.33 ppm are due to the methyl group protons attached to carbon atoms 20 and 21, respectively. These attributions were confirmed in the DEPT 135 and ^{13}C-NMR spectrum which displayed a total of 16 carbon resonances. It showed eleven carbon resonances for the furanocoumarin nucleus, as described for oxypeucedanin,[17] and five

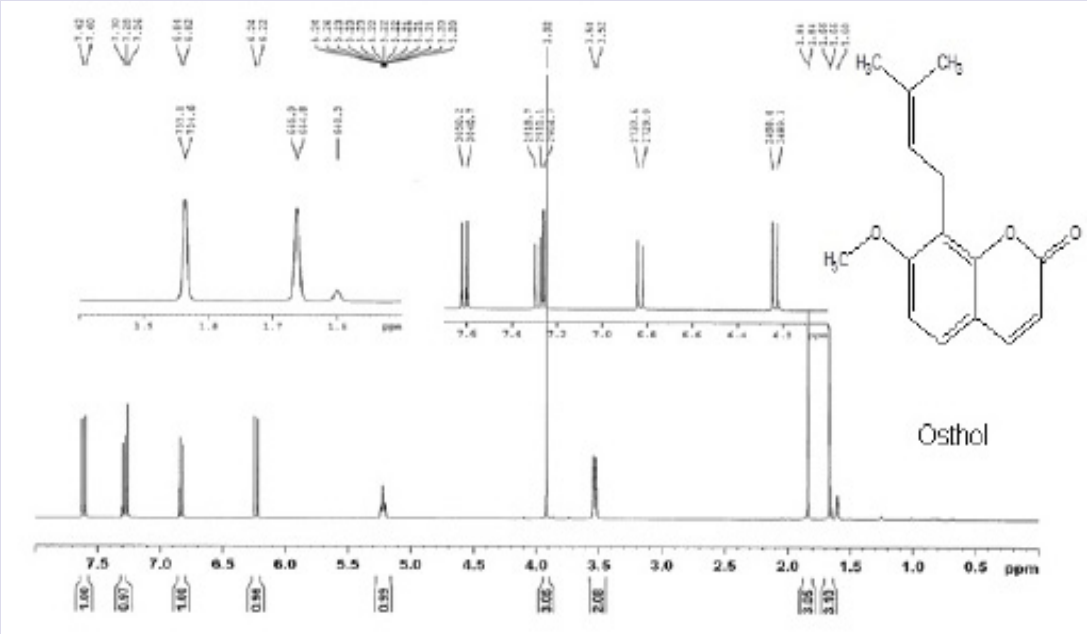

Figure 9: $_1$H NMR spectrum of compound (2)

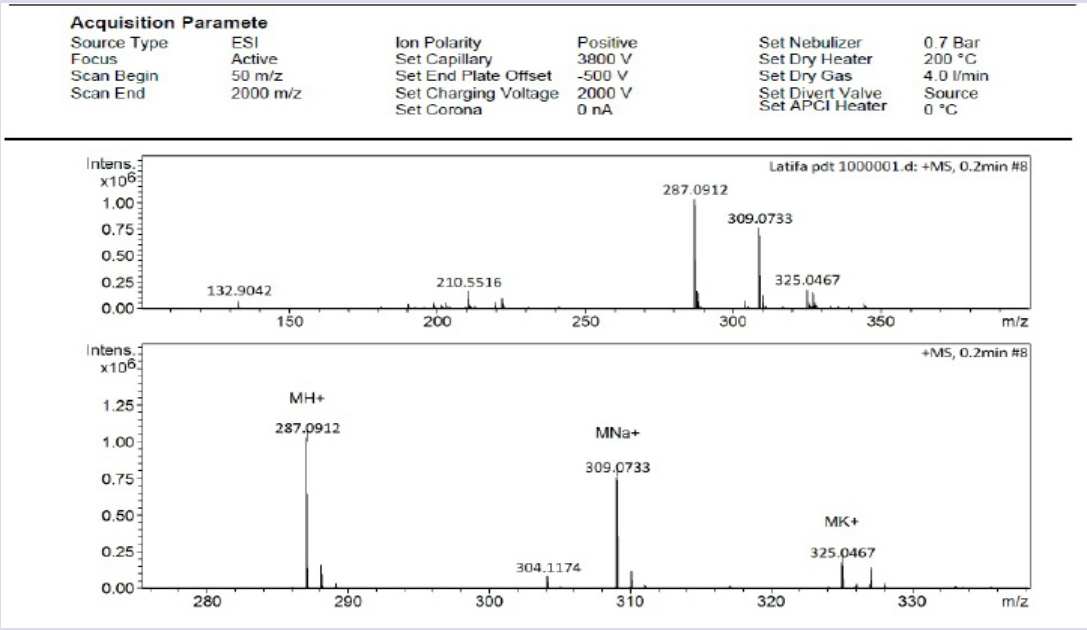

Figure 10: HRMS spectrum of compound (3)

additional signals arising from carbons at the sidechain that accounted for: 2 methyl groups (δ_C 19.15 and 24.73), one oxymethylene (δ_C 72.45), one oxymethine (δ_C 61.23) and a quaternary oxygenated carbon (58.48 ppm). In the HMBC spectrum, the oxymethylene proton signals H16' (δ_H 4.42) and H16" (δH 4.62) correlated with the proton signal at δ_H 6.96 (H-9). Consequently the oxymethylene was in the same side of H9 which was connected to C-9. These data confirmed the structure of (**3**) as oxypeucedanin with a molecular formula of $C_{16}H_{14}O_6$.[18] To the best of our knowledge, this compound has been isolated for the first time from the *Pituranthos* genus.

Compound (4)

Compound (4) was obtained as white needle-like crystals. The ^{13}C-NMR spectrum exhibited 12 carbon resonances including five methines, one methoxy, one carbonyl and five quaternary carbons. The 1H-NMR spectrum showed two proton doublets at δ_H 6.28 (*J*=9.6 Hz) and δ_H 8.16 (*J*=9.6 Hz), characteristic of α-pyrone protons assignable to H-3 and H-4 respectively, and a pair of doublets occurring at δ_H 7.03 (*J*=2.2 Hz) and δ_H 7.61 (*J*=2.2 Hz), typical of furanic protons assignable to H-8' and H-9 respectively (Figure 12). The spectrum further showed a proton singlet at δH 7.37 and a methoxy signal at δ_H 4.42, assignable to C-15 of the fu-

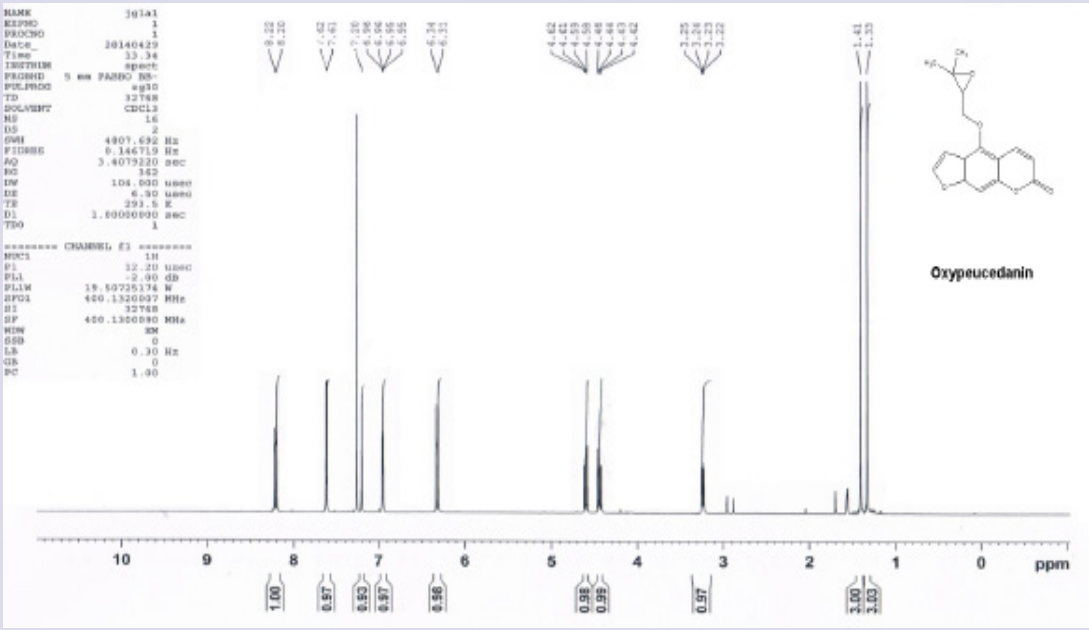

Figure 11: HRMS spectrum of compound (3)

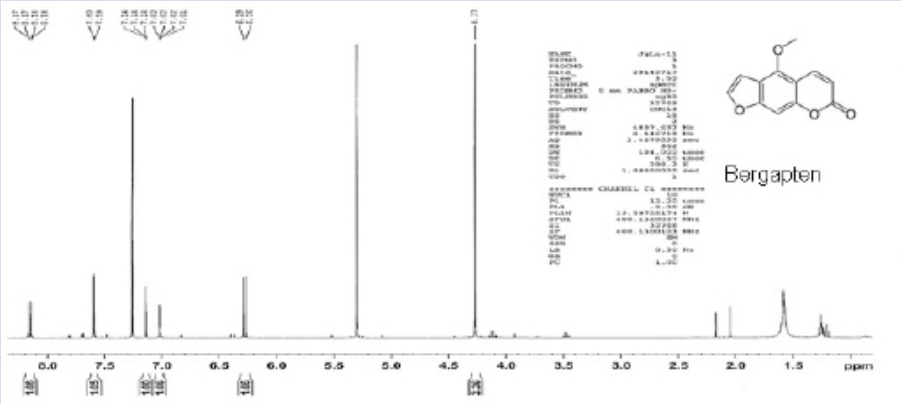

Figure 12: $_1$H NMR spectrum of compound (4)

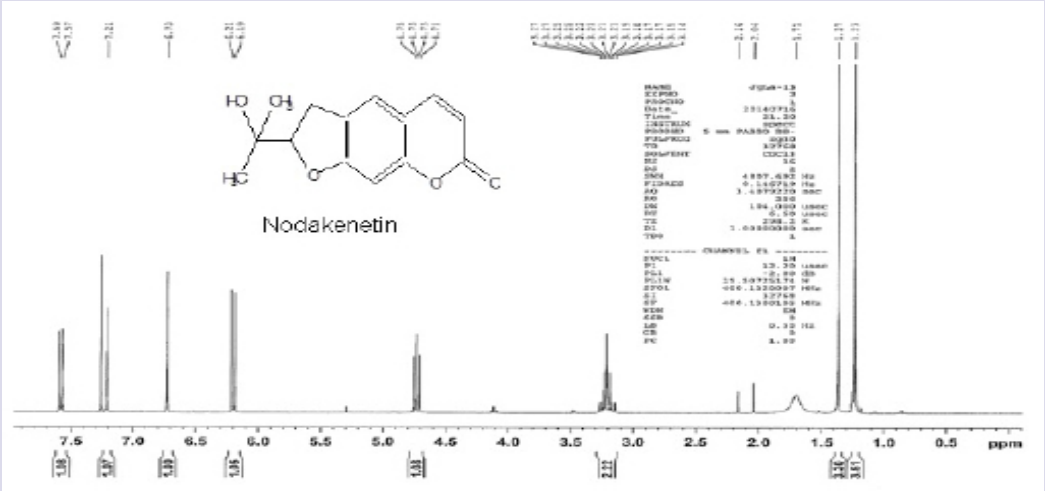

Figure 13: $_1$H NMR spectrum of compound (5)

ranocoumarin structure. Compared to the published data, the structure of (**4**) was identified as bergapten[19] with a molecular formula of $C_{12}H_8O_4$. This compound has been isolated for the first time from *Pituranthos chloranthus*.

Compound (5)

Compound (5) was isolated as colorless plates. HR-MS showed a quasi-molecular ion peak at m/z 247 $[M+H]_+$ in accordance with the molecular formula $C_{16}H_{14}O_5$. The ^{13}C-NMR spectrum exhibited 14 carbon resonances including two methyl, one methylene, five methines, one carbonyl and five quaternary carbons. The ^{1}H NMR spectrum exhibited two doublets at δ_H 6.21 and 7.62 characteristic to the two olefinic protons of the coumarin moiety (H4 and H5 respectively). Two aromatic protons appeared as a doublet at δ_H 6.75 and 7.24, assignable to H7 and H13 respectively, and a one-proton triplet at δ_H4.74 was assigned to the methine proton coupled to the adjacent methylene group which itself appeared as a doublet at δ_H 3.24 (Figure 13). Two uncoupled methyl groups resonated as two singulets at δ_H 1.26 and 1.39.

Therefore, according to these findings and compared to previously reported data,[20] compound (5) was identified as nodakenetin which was isolated for the first time from *Pituranthos chloranthus*.

CONCLUSION

In conclusion, five compounds (bergapten, isoimperatorin, nodakenetin, osthol and oxypeucedanin) were isolated from the aerial parts and the roots of *P. chloranthus*. Among them, osthol and oxypeucedanin are reported for the first time from the genus *Pituranthos*.

However, the literature review revealed that osthol has many biological activities, including the prevention of atherosclerosis and the suppression of hepatic lipids,[21] as well as antitumor[22] and anti-inflammatory activities.[23] As this is the first attempt of any phytochemical investigation from *P. chloranthus* further isolation and purification of other fractions of this plant is recommended, which could yield some novel and bioactive compounds.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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