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Bioassay-directed isolation of two novel antimicrobial coumarin and flavanone from *Acanthus montanus*

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Abstract. This work is geared towards extraction, isolation and characterization of phytocompounds from the ethyl acetate extract of *Acanthus montanus* root. The ethyl acetate extract was obtained through sequential maceration using a nonpolar solvent first before introducing ethyl acetate. The extract was subjected to vacuum liquid chromatography (VLC) for fractionation and phytocompounds were purified through column chromatography. Characterization was done employing infrared spectroscopy (IR) and nuclear magnetic spectroscopy (NMR). The phytocompounds were isolated and characterized as 6,7-dihydroxy-4-methoxycoumarin and 2′-acetoxy-4′,5-dihydroxy-6′-methoxy-3-(2″-hydroxy-6″-oxanyl)-8-(2‴-hydroxy-4‴-oxanyl)flavanone named Acanthusin G with percentage yield of 0.7% and 1.16% respectively. 6,7-dihydroxy-4-methoxycoumarin and Acanthusin G indicated good antimicrobial activities at 200 μg/ml with zone of inhibition range of 18 - 36 mm against fifteen pathogens using agar well diffusion method.

Keywords**:** *Acanthus montanus*; vacuum liquid chromatography; coumarin; flavanone; oxanol; Acanthusin G; pathogens.

1. Introduction

Plants are considered as the major sources of natural products, endowed with phytochemicals of very complex structures with interesting bioactivities [1]. These phytochemicals are phytonutrients or compounds produced by plants and can be found in all parts of the plant which include its flowers, fruits, seeds, leaves, bark and roots. These secondary metabolites in plants do exhibit their activities singularly or in synergy with other phytocompounds present. There are several thousands of phytocompounds in plants and have been used in the pharmaceuticals as antibacterial, antifungal, antiviral, anticholesterol, antitumor and anti-inflammatory agents just to mention a few. These phytochemicals include phenolics, terpenes, lipids, carbohydrates and alkaloids [2].

The phenolics form a large group of these phytochemicals. Flavonoids are polyphenols and make up the largest group of secondary metabolites in plants. They are known for plant pigments and co-pigments which are responsible for the colors which we find in leaves, flowers, fruits, seeds and barks of plants. They are crystalline compounds which are soluble in water and alcohol. They show different colors fluorescence under the UV light. They are classified under the following groups: flavanones, flavones, isoflavones, flavonols, anthocyanidin, chalcones and flavanols. Their activities include antimicrobial, anti-inflammatory, antioxidant, antiviral, anti-allergic, anticancer, antiulcer, antidiabetic, antiplasmodial, antihypertensive, anticonvulsant and preventive effects of most humane diseases [3].

Coumarin and its derivatives are phenolic compounds which are like flavonoids in their structures. Just like flavonoids, they are benzopyrones having the benzopyran moiety, while the flavonoids have the benzopyran-4-one, the coumarins have the benzopyran-2-one. They are readily available in different parts of the plant. Most derivatives of coumarin are phenolic compounds and research are mostly geared towards getting a potent, non-toxic and cost-effective analogues for the treatment of cancer. Coumarins have been shown to exhibit anti-inflammatory and antioxidant, anti-cancer and photochemotherapy, anti-bacterial and anti-fungal, and anti-coagulant properties. They are also used in cosmetic and agrochemical industries [4-6].

Acanthus montanus known as mountain thistle is a popular plant in Nigeria and other tropical parts of the world. It is a tinny perennial plant with oblong to lance shaped dark green leaves. The leaves could be as long as 30 cm with silver marks and wavy margins. It can have a height of up to 180 cm with very aggressive roots [7]. The roots of the plant are used for the treatment of boils, inflammation and arthritis. In our previous studies, results revealed that ethyl acetate extract of the root of *A. montanus* possess good broad spectrum antibacterial and antifungal properties [8]. In continuation of our studies, we report the isolation and characterization of novel antimicrobial benzopyrones (a coumarin and a flavanone) from the ethyl acetate extract of *A. montanus* root that was previously shown to be bioactive.

2. Experimental

2.1. Materials and reagents

All solvents used for maceration, vacuum liquid chromatography and column chromatography were properly redistilled. The solvents are *n*-hexane, ethyl acetate (EtOAc) and ethanol. Other reagents used were

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hydrochloric acid (HCl) 37.5% (May and Baker), dimethyl sulfoxide 98.0% (Sigma-Aldrich), concentrated sulfuric acid (Sigma-Aldrich), acetic anhydride (Eastman Chemical), glacial acetic acid, (Eastman Chemical), ferric chloride (Sukha chemical), and potassium iodide (Silverline Chemical). Ceftriaxone and Terbinafine are well known antibiotic and antifungal used as standard drugs for the antimicrobial assays conducted in this study.

Microbes used in this research were previously characterized bacterial and fungal clinical isolates obtained as stock culture from the Department of Microbiology, Federal Medical Centre, Owerri, Imo State, Nigeria. The bacteria are *Salmonella typhi, Salmonella paratyphi, Pseudomonas aeruginosa, Shigella sonniea, Shigella dysenteriae, Escherichia coli, Staphylococcus aureus* and *Streptococcus pyogenes*. While *Candida albicans, Saccharomyces cerevisiae, Rhizopus oligastus, Aspergillus flavus, Aspergillus fumigates, Fusa equisetti* and *Aspergillus niger* were fungi used for this study.

2.2. Extraction

Acanthus montanus roots were harvested from Choba, in Obio-Akpor Local Government Area of Rivers State, Nigeria. The plant was identified by Dr. Chimezie Ekeke of the herbarium of the University of Port Harcourt, Rivers State, Nigeria. The roots were washed, air dried and pulverized. Pulverized root (1.5 kg) was completely defatted in an aspirator bottle using *n*-hexane before introducing ethyl acetate and allowed to stand for 48 hours with daily stirring of the content. The ethyl acetate extract obtained was concentrated using a rotary evaporator and allowed to air dry before been used for isolation.

2.3. Isolation

The ethyl acetate extract of the roots of *Acanthus montanus* was selected for isolation because of the observed antimicrobial activities and its phytochemical composition [8]. Ethyl acetate extract (11.32 g) was mixed with 15 g of silica gel (70-230 mesh ASTM) to obtain a light brown powder. A sintered glass funnel (9.8 cm x 10.4 cm) and separatory funnel were clamped to a retort stand and a hose connected from the vacuum pump to the vacuum liquid chromatography (VLC) set up. TLC grade silica gel (100 g) was introduced into the sintered glass and was evenly distributed to the height of 3.5 cm. Treated sand was spread evenly on top of the silica gel to protect the column bed and the prepared extract introduced unto the column, making a total height of 4.5 cm. Gradient elution using different combinations of *n*-hexane, ethyl acetate and ethanol was used and fractions of 200 ml each collected. A total of 39 fractions were obtained from the VLC. Fractions were monitored using thin layer chromatography [9, 10]. Fractions 6-10 and 13-16 obtained as combined fractions from VLC were subjected to purification through column chromatography using glass columns of 80 cm by 2.3 cm and 70 cm by 1.5 cm (length by diameter respectively). The method used in packing the column was wet packing and *n*-hexane-ethyl acetate was used in different ratios as the solvent system. Silica gel (60-200 mesh ASTM) was mixed with 100% *n*-hexane in a beaker forming a slurry and gradually poured into the column using a glass funnel with the effluent tap opened while tapping the column for an even distribution.

Fractions 6-10 which eluted with *n*-hexane - ethyl acetate (8:2 and 7:3) from VLC had a light brown color after solvent evaporation. Silica gel (4 g, 60-200 mesh ASTM) was added to 0.76 g of the crystals and properly mixed to obtain a light brown powder. Silica gel (30 g, 60-200 mesh ASTM) slurry was poured into the column and treated sand was evenly distributed on top of the silica gel while the prepared fraction was gradually introduced into the column. The column was eluted using *n*-hexane - EtOAc (9:1, 8.5:1.5, 8:2, 7.5:2.5 and 7:3). A total of 135 fractions of 20 ml each were collected and monitored using TLC. while fractions 56-68 (which showed similar spot on TLC) eluted with *n*-hexane - EtOAc (7.5:2.5) were combined and solvent evaporated to give 80 mg of compound F.

VLC Fractions 13-16 (0.44 g) eluted with *n*-hexane - EtOAc (6:4) was a brown solid after solvent evaporation. Silica gel (5 g, 60-200 mesh ASTM) was added to 0.44 g of the fraction and properly mixed to obtain a light brown powder. Silica gel (30 g) slurry was poured into the glass column using a glass funnel. Treated sand was evenly distributed on top of the silica gel and the prepared fraction was gradually introduced into the column using a glass funnel. The column was eluted using *n*-hexane - EtOAc (9.5:0.5, 9:1, 8:2, 7.5:2.5, 7:3, 6:4, 1:1, 4:6 and 3:7) and monitored with TLC. A total of 158 fractions of 20 ml each was collected. Fractions 73-120 (eluted with *n*-hexane - EtOAc (7:3, 6:4 and 1:1)) which showed similar spot on TLC, were combined and solvent evaporated to give 131 mg of compound G.

Compounds F and G were subjected to spectroscopic and melting point analyses.

2.4. Characterization

Infrared spectra were obtained from IR spectrometer of Agilent Technologies Cary 630 FTIR in the range 4000-400 cm^{-1} using the transmittance method. Potassium bromide pellets were prepared and used for the IR spectroscopic analysis of the isolated crystals. Nuclear magnetic resonance (NMR) data were acquired using the NMR spectrometer of Agilent Technologies 400 MHz Premium +AR. One dimension and two-dimension experiments were used for structural elucidation. Compounds F and G were dissolved in deuterated chloroform for NMR experiments. Silica gel precoated aluminum plates (Merck F_{254}) were used to perform thin layer chromatography. Detections were done under UV light at the wavelength of 254 nm. Further detections were done using a spray reagent (H_2SO_4-MeOH, 1:10) followed by heating at 105 °C for 3 minutes.

2.5. Bioassay

The isolated compounds (F and G) were screened for antimicrobial activity against some pathogens using standard procedures where agar well diffusion method was employed [11, 12]. The bacterial isolates were reconfirmed for purity and viability using selective media (mannitol salt agar and eosin methylene blue

agar). Molten agar was introduced into Petri dishes and allowed to solidify. The bacteria were introduced unto the sterile Petri dishes containing agar and sterile spreader was used to evenly spread the isolates. A cup borer was used to make 5 mm diameter holes in each dish uniformly and isolated compounds (200 and 100 µg/ml) were introduced into the holes aseptically and allowed to stand for one hour for pre-diffusion. Also previously prepared standard antibiotic (ceftriaxone, 30 µg/ml) was aseptically introduced into one of the holes in the Petri dishes. The Petri dishes were labelled and incubated at 37 °C for 24 hours. After 24 hours, zones of inhibition were measured and recorded in millimeters. Zones of inhibition less than 6 mm were considered insignificant.

For the antifungal assay, potato dextrose agar medium was used. The organisms were collected from the cultures previously incubated for 48 hours and were aseptically introduced into sterile Petri dishes. Molten agar was also aseptically introduced into the Petri dishes and the content allowed to solidify. A cup borer was used to make uniform holes (5 mm) on the Petri dishes. The isolated compounds (200 and 100 µg/ml) and standard antifungal (terbinafine, 30 µg/ml) were aseptically introduced into the holes and allowed to stand for one hour for pre-diffusion and incubated at 37 °C for 48 hours. After 48 hours, zones of inhibition were measured and recorded in millimeters.

3. Results and discussion

3.1. Isolated compounds

Compound F (80 mg) was light yellowish crystals with a melting point of 235 °C. The yield was 0.7% with R_f value of 0.625 using mobile phase of 60% ethyl acetate in *n*-hexane (Table 1). All IR data were applied accordingly. IR absorption frequencies for compound F (Table 2) showed aromatic ring stretch at 1610 cm^{-1}, aromatic C-H stretch at 3007 cm^{-1} and aromatic combination band at 1993 cm^{-1} [13]. There was also a carbonyl band at 1824 cm^{-1} and a phenol OH stretch at 3524 cm^{-1}. A methoxy C-H stretch was also seen at 2893 cm^{-1}. The ^{13}C-NMR data of compound F (Table 3) shows ten carbon signals including one ester carbonyl carbon signal at δ = 169.9 (C-2) and eight signals δ = 112.1 (C-3), 146.2 (C-4), 114.2 (C-5), 121.0 (C-6), 115.3 (C-7), 125.1 (C-8), 132.2 (C-9), 150.7 (C-10) identified as aromatic carbon signals. From the ^{1}H-NMR data of compound F (Table 3), three aromatic proton signals at δ = 7.54 (H-3), 6.96 (H-5) and 7.71 (H-8) were identified. Proton signals at δ = 6.87 (H-^{a}O) and 8.01 (H-^{b}O) were not attached to any carbon in HSQC data therefore were assigned as hydroxy protons. The structure of compound F (Figure 1) was determined using ^{1}H-NMR and ^{13}C-NMR aided by connectivity observed in HSQC and verified by HMBC experiments. The signal at δ = 169.9 (C-2) is reported as ester carbonyl carbon signal [14]. The connectivity observed in HMBC (Figure 1) shows that the signal at δ = 169.9 had a strong correlation with δ = 7.54 (H-3) and 7.71 (H-8) (Figure 1), confirming the position of the ester carbonyl carbon in the structure. Long range correlation as seen in HBMC between δ = 148.2 (C-4) and 6.05 (H-11) confirmed the attachment of the methoxy carbon to C-4 while the correlation between the aromatic carbon δ = 121.0 (C-6) and 6.96 (H-5) also confirmed the position of one of the hydroxy groups in the structure (Figure 1). Compound F (Figure 1) was confirmed as 6,7-dihydroxy-4-methoxycoumarin after due comparison with the coumarin isolated from the roots of *Clausena anisata* [14]. Coumarins have been shown to exhibit anti-inflammatory and antioxidant, anticancer and photochemotherapy, antibacterial, antifungal and anticoagulant properties [4-6]. They are also used in cosmetic and agrochemical industries [15].

Table 1. Physical properties of compounds F and G

Property	Isolated compound F	Isolated compound G
Type	Crystalline	Crystalline
Color	Light yellow	Brown
Weight (mg)	80	131
% Yield	0.7	1.16
Melting Point (°C)	235	257
Polarity/ Solubility	Semi-polar/Soluble in chloroform	Semi-polar/Soluble in chloroform
R_f value / TLC Mobile Phase	0.625/6:4 EtOAc:*n*-Hexane	0.375/8:2 EtOAc:*n*-Hexane
Spot under UV light	Purple	Purple
Spot after spray reagent	Pink	Nil

Table 2. IR data of compound F

Absorption wavenumber (cm^{-1})	Assigned vibration
1610	Aromatic ring stretch
1824	Carbonyl C=O stretch
3007	Aromatic C-H stretch
3524	Phenol OH stretch
1993	Aromatic combination band
2893	Methoxy C-H stretch

Table 3. ^{13}C and ^{1}H chemical shift data of compound F in $CDCl_3$

Position	δ ^{13}C	δ ^{1}H
2	169.9	-
3	112.1	7.54
4	146.2	-
5	114.2	6.96
6	121.0	-
7	115.3	-
8	125.1	7.71
9	132.2	-
10	150.7	-
11	56.10	6.05
^{a}O		6.87
^{b}O		8.01

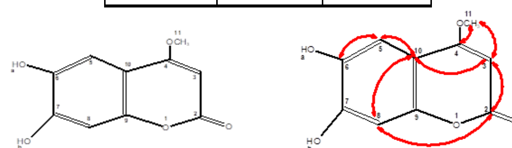

Figure 1. Structure and numbering of compound F (6,7-dihydroxy-4-methoxycoumarin) used in this report. HMBC correlations are indicated with red arrows.

Compound G (131 mg) was brownish needlelike crystals with a melting point of 257 °C. It had a yield of

1.16% and R_f value of 0.375 using a mobile phase of 80% ethyl acetate in *n*-hexane (Table 1). All IR data were considered during characterization. IR absorption frequencies for compound G (Table 4) indicated the presence of aromatic ring stretch at 1614 cm^{-1}, aromatic C–H stretch at 3121 cm^{-1} and aromatic combination band at 1882 cm^{-1}. There was also phenolic C-O stretch at 1208 cm^{-1} and a phenolic OH band at 1388 cm^{-1}. Methoxy C-H stretch was seen at 2851 cm^{-1} and a normal hydroxy stretch at 3357 cm^{-1}. Twenty-eight carbons were identified from the ^{13}C-NMR data (Table 5) of compound G; these include two carbonyl carbons signals at δ = 178.5 (C-4) and 170.5 (C-8′). From the ^{1}H-NMR data (Table 5), proton signals at δ = 7.55 (H-6), 6.88 (H-7), 7.35 (H-3′) and 7.56 (H-5′) were identified as aromatic protons. The ^{1}H-NMR and ^{13}C-NMR assignments for compound G aided by HSQC data and verified with other two dimension NMR experiments (HMBC and COSY) showed that compound G had no quaternary carbon, two primary carbons, δ = 22.9 (C-9′) and 56.4 (C-7′); six secondary carbons, δ = 28.7 (C-3″), 24.5 (C-4″), 33.7 (C-5″), 29.7 (C-3‴), 18.2 (C-5‴), 19.9 (C-6‴); and two tertiary carbons, δ = 31.9 (C-3) and 17.6 (C-4‴). Protons at δ = 7.24 (H-^{a}O), 6.25 (H-^{b}O), 3.40 (H-^{c}O) and 3.80 (H-^{d}O) were not attached to carbons in the HSQC experiment. Structure of compound G (Figures 2 and 3) was determined using ^{1}H-NMR and ^{13}C-NMR experiments aided by connectivity observed in HSQC, HMBC and COSY experiments. Carbon signals at δ = 67.4 (C-2), 31.9 (C-3),178.5 (C-4),119.9 (C-5),116.8 (C-6), 114.9 (C-7), 121.4 (C-8),149.6 (C-9), 143.2 (C-10), 127.4 (C-1′), 139.9 (C-2′), 107.2 (C-3′), 120.5 (C-4′), 124.5 (C-5′), 146.7 (C-6′) (Table 5) indicated the presence of a flavanone skeleton**.** These flavanone signals are similar to the NMR signals of flavanones isolated from *Chromolaena tacotama* [16-18]. Long range connectivity in HMBC (Figure 2) shows that the carbonyl carbon signal at δ = 170.5 (C-8′) is correlated to 7.35 (H-3′) and 2.03 (H-9′) confirming the position of the acetoxy group in the structure while the signal at 146.7 (C-6′) is correlated to 3.93 (H-7′), confirming the position of the methoxy group. The flavanone was characterized as 2′-acetoxy-4′,5-dihydroxy-6′-methoxyflavanone [18]. Signals at δ = 83.4 (C-2″), 28.7 (C-3″), 24.5 (C-4″), 33.7 (C-5″) and 14.1 (C-6″) were identified as oxanyl while a second oxanyl was observed with carbon signals, δ = 62.2 (C-2‴), 29.7 (C-3‴), 17.6 (C-4‴), 18.2 (C-5‴) and 19.9 (C-6‴). From COSY experiment (Figure 3), the hydroxy group at δ = 3.40 (H-^{c}O) is correlated to δ = 4.39 (H-2″) of the first oxanyl while δ = 3.80 (H-^{d}O) is correlated to δ4.45 (H-2‴) of the second oxanyl. The first oxanyl was identified as 2″-hydroxy-6″-oxanyl while the second oxanyl was identified as 2‴-hydroxy-4‴-oxynyl. The position of 2″-hydroxy-6″-oxanyl was confirmed from the correlation of carbonyl carbon at δ = 178.5 (C-4) to 2.33 (H-5″) in HMBC (Figure 2) and the correlation between δ = 1.29 (H-3) and 0.85 (H-6″) in COSY (Figure 3) while the position of 2‴-hydroxy-4‴-oxynyl was confirmed from the correlation between δ = 4.10 (H-2) and 1.23 (H-4‴), 6.88 (H-7) and 1.98 (H-5‴) from COSY (Figure 3) and HMBC (Figure 2). Compound G (Figures 2 and 3) was characterized as 2′-acetoxy-4′,5-dihydroxy-6′-methoxy-3-(2″-hydroxy-6″-oxanyl)-8-(2‴-hydroxy-4‴-oxanyl)flavanone and named Acanthusin G. Flavanones which belong to the group of flavonoids are the least available in plant sources. They are documented for several biological and antimicrobial activities. They are good antioxidants, destroying free radicals which antagonize the human body, anti-inflammatory effect, anticancer and antitumor effect, antimutagenic, and atherosclerosis / coronary heart disease effect [19-22].

Table 4. IR data of compound G (Acanthusin G)

Absorption frequency (cm^{-1})	Assigned vibration
1208	Phenol C-O Stretch
1388	Phenol OH band
1614	Aromatic ring stretch
1882	Aromatic combination band
2851	Methoxy C-H stretch
3121	Aromatic C-H stretch
3357	Normal OH stretch

Table 5. ^{13}C and ^{1}H chemical shift data of compound G (Acanthusin G) in $CDCl_3$

Position	δ ^{13}C	δ ^{1}H
2	67.4	4.10 (1H)
3	31.9	1.29 (1H)
4	178.5	-
5	119.9	-
6	116.8	7.55 (1H)
7	114.9	6.88
8	121.4	-
9	149.6	-
10	143.2	-
1′	127.4	
2′	139.9	-
3′	107.2	7.35 (1H)
4′	120.5	-
5′	124.5	7.56 (1H)
6′	146.7	-
7′	56.4	3.93 (3H)
8′	170.5	-
9′	22.9	2.03 (3H)
2″	83.4	4.39 (1H)
3″	28.7	1.23 (1H), 1.29 (1H)
4″	24.5	1.67 (1H), 1.60 (1H)
5″	33.7	2.33 (1H), 2.36 (1H)
6″	14.1	0.85 (1H)
2‴	62.2	4.45 (1H)
3‴	29.7	2.75 (1H), 2.53 (1H)
4‴	17.6	1.23 (1H)
5‴	18.2	1.98 (1H), 1.88 (1H)
6‴	19.9	0.97 (1H), 0.84 (1H)
^{a}O		7.24
^{b}O		6.25
^{c}O		3.40
^{d}O		3.80

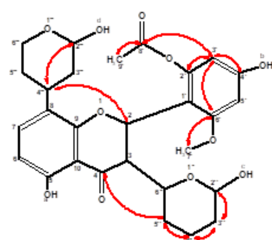

Figure 2. Structure and numbering of compound G (Acanthusin G) used in this report. HMBC correlations are indicated with red arrows.

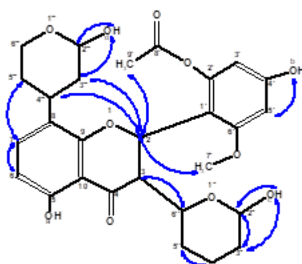

Figure 3. Structure and numbering of compound G used in this report. COSY correlations are indicated with blue arrows.

3.2. Antimicrobial activity of compounds F and G

Compound F indicated concentration-dependent activities against all the test organisms (Table 6). At 200 µg/ml the zone of inhibition (ZOI) ranged from 24 mm to 34 mm as *S. paratyphi* was the most sensitive with 34 mm ZOI while *P. aeruginosa* with 24 mm ZOI was the least sensitive to compound F. Compound G (Table 6) also showed dose-dependent activities against all test bacteria, with their ZOI ranging from 22 mm to 32 mm at 200 µg/ml. *S. paratyphi*, *S. aureus* and *S. pyogenes* were highly susceptible to compound G with 32 mm ZOI while *E. coli* had the least sensitivity with 24 mm ZOI.

The two isolated benzopyrones (compounds F and G) showed concentration-dependent antifungal activities which were similar with those exhibited by the standard drug (Table 7). Compound F was active against the test fungi with ZOI that ranged from 18 mm to 34 mm at 200 µg/ml. *S. cerevisiae* and *A. niger* showed the highest sensitivity with 34 mm ZOI while *C. albicans* and *F. equisetti* had the least sensitivity with 18 mm ZOI at 200 µg/ml. Compound G showed activity against the fungi with ZOI that ranged from 16 mm to 36 mm at 200 µg/ml. *R. oligastus* and *A. niger* had the highest susceptibility with 36 mm ZOI while *F. equisetti* had the least susceptibility with 16 mm ZOI. Hence compound G (Acanthusin G) showed slightly better antifungal activity compared to compound F (6,7-dihydroxy-4-methoxycoumarin).

Compound F is a novel polyhydroxy methoxy compound (6,7-dihydroxy-4-methoxycoumarin) which has not been reported in literature. However similar molecules such as fraxetin (7,8-dihydroxy-6-methoxycoumarin) and 6,7-dihydroxy-8-methoxycoumarin were reported to have antitumor, antidiabetic and antioxidant properties [23, 24]. Compound G (Acanthusin G) is a novel flavanone (2′-acetoxy-4′,5-dihydroxy-6′-methoxy-3-(2″-hydroxy-6″-oxanyl)-8-(2‴-hydroxy-4‴-oxanyl)flavanone). Flavanones had been reported to have antioxidant, anti-inflammatory, antitumor and antimutagenic properties [20-22, 25].

The antimicrobial activities of the isolated compounds were similar with those exhibited by the standard drugs against the same organisms (Tables 6 and 7). It is therefore safe to conclude that compound F (6,7-dihydroxy-4-methoxycoumarin) and compound G (Acanthusin G) may treat diseases caused by all test organisms except those caused by *C. albicans* and *F. equisetti*.

Table 6. Comparative antibacterial activities of compounds F and G

Bacterium	Zone of inhibition (mm)				
	Compound F (µg/ml)		Compound G (µg/ml)		Standard antibiotic (Ceftriaxone)
	200	100	200	100	
S. typhi	32±1	20±2	28±0	18±0	32±0
S. paratyphi	34±2	23±2	32±2	21±1	32±0
P. aeruginosa	24±1	13±1	28±1	18±1	29±0
S. sonniea	30±1	19±1	30±1	19±0	34±0
S. dysenteriae	31±0	22±0	31±1	24±2	28±0
E. coli	29±0	20±0	24±0	12±0	30±0
S. aureus	26±0	16±0	32±0	21±1	32±1
S. pyogenes	28±0	18±0	32±0	22±1	36±1

Table 7. Comparative antifungal activities of compounds F and G

Fungus	Zone of inhibition (mm)				
	Compound F (µg/ml)		Compound G (µg/ml)		Standard Antibiotic (Terbinafine)
	200	100	200	100	
C. albicans	18±0	0	28±0	10±0	18±0
S. cerevisiae	34±1	11±1	32±1	12±1	30±0
R. oligastus	33±1	12±0	36±2	12±0	23±0
A. flavus	32±2	14±1	30±0	11±0	26±0
A. fumigates	30±1	10±0	30±0	10±0	28±1
F. equisetti	18±0	0	16±0	0	20±0
A. niger	34±2	13±2	36±1	10±1	24±0

4. Conclusions

Two novel bioactive benzopyrones (6,7-dihydroxy-4-methoxycoumarin and Acanthusin G) were isolated from the ethyl acetate extract of the roots of *Acanthus montanus*. These compounds have previously not been isolated from *Acanthus montanus* and have also not been reported in literature. 6,7-dihydroxy-4-methoxycoumarin and Acanthusin G are broad spectrum antimicrobials.

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Conflict of interest

Authors declare that they have no competing interest.

References

[1]. L. Dang, E.J. VanDamme, Toxic proteins in plants, Phytochemistry 117 (2015) 51-64.

[2]. A. Crozier, M.N. Clifford, H. Ashihara, Plant Secondary Metabolites, pp. 1-24, 47-136, Blackwell Publishing Ltd, Oxford, UK (2006).

[3]. H. Zhang, Y. Cheng, X. Luo, Y. Duan, Protective effect of procyanidins extracted from lotus seed pod on immune function injury induced by extremely low frequency electromagnetic field, Journal of Biomedicine and Pharmacotherapy 82 (2016) 364-372.

[4]. K.N. Venugopala, V. Rashmi, B. Odhav, Review of natural coumarin lead compounds for their pharmacological activity, Biomedical Research International 2013 (2013) 1-14.

[5]. J. Jin, H. Li, P. Li, L. Xing, X. Huang, J. Zhang, X. Zhou, W. Qin, C. He, D. Zhao, H. Chu, Y. Ma, H. Cao, Pharmacological advances of coumarin and its derivatives, Indian Journal of Pharmaceutical Sciences 85 (2023) 565-580.

[6]. J.R. Hoult, M. Paya, Pharmacological and biochemical actions of simple coumarins: Natural products with therapeutic potential, Journal of General Pharmacology 27 (1996) 713-722.

[7]. L.C. Tapsell, I. Hemphill, I. Cotiac, C.S. Patch, D.R. Sullivan, Health benefits of herbs and spices: The past, the present, the future, Medicinal Journal of Australia 185 (2006) 54-24.

[8]. G.I. Ndukwe, O.F. Okoronkwo, I.R. Jack, Phytochemical contents and in vitro pathogenic microbial growth inhibitory activities of *Acanthus montanus* root and leaf extracts, Nigerian Journal of Chemical Research 28 (2023) 128-141.

[9]. A. Oluah, A.I. Oputa, G.I. Ndukwe, G.K. Fekarurhobo, Application of vacuum liquid chromatography in the separation of secondary metabolite of *Baphia nitida* Lodd. Stem, Journal of Chemical Society of Nigeria 45 (2020) 220-225.

[10]. G.I. Ndukwe, A. Oluah, G.K. Fekarurhobo, Isolation of an isoflavonoid and a terpenoid from the heartwood of *Baphia nitida* Lodd. (Camwood), Ovidius University Annals of Chemistry 31 (2020) 5-8.

[11]. G.I. Ndukwe, C.M. Ojinnaka, A.O. Oyedeji, N. Nxasana, T. Apalata, Antibacterial activity of the fruit of *Napoleoneae imperialis* P. Beauv., Journal of Innovation and Research Health Sciences and Biotechnology 1 (2015) 1-11.

[12]. G.I. Ndukwe, S.Y. Garba, E.A. Adelakun, Activity-guided isolation and antimicrobial assay of a flavonol from *Mitracarpus verticillatus* (Schumach. & Thonn.) Vatke, IOSR Journal of Applied Chemistry 9 (2016) 118-131.

[13]. B.C. Smith, The benzene fingers, Part 1: Overtone and combination bands, Spectroscopy 31 (2016) 30-34.

[14]. D. Tamene, M. Endale, Antibacterial activity of coumarins and carbazole alkaloid from roots of *Clausena anisate*, Advances in Pharmacological and Pharmaceutical Sciences 3 (2019) 1-8.

[15]. J.H. Fentem, J.R. Fry, Species differences in the metabolism and hepatotoxicity of coumarin, Journal of Comparative Biochemistry and Physiology 104 (1993) 1-8.

[16]. A.P. Rauter, M. Ennis, K. Hellwich, B.J. Herold, D. Horton, G.P. Moss, I. Schomburg, Nomenclature of flavonoids (IUPAC Recommendation 2017), Journal of Pure and Applied Chemistry 90 (2018) 1429-1486.

[17]. I. Raphael, Natural products: A laboratory guide, Second Edition. Academic Press, London, pp. 1-19, 127-139 (1991).

[18]. R.D. Torrenegra-Guerrero, L. Bautista-Bautista, J. Rodriguez-Mayusa, G. Mendez-Callejas, NMR spectroscopy and antioxidant activity of flavanones and flavones isolated from *Chromolaena tacotana* (klatt), Pharmacology Online 3 (2020) 444-452.

[19]. B.S. Patil, G.K. Jayaprakasha, K.N. Chidambara-Murthy, A. Vikram, Bioactive compounds: Historical perspectives, opportunities and challenges, Journal of Agricultural and Food Chemistry 57 (2009) 8142-8160.

[20]. K. Sakata, Y. Hirose, Z. Qiao, T. Tanaka, H. Mori, Inhibition of inducible isoforms of cyclooxygenase and nitric oxide synthase by flavonoid hesperidin in mouse macrophage cell line, Cancer Letters 199 (2003) 139-145.

[21]. K. Gao, S. Henning, Y. Niu, A.A. Youssefian, N. P. Seeram, A. Xu, D. Heber, The citrus flavonoid naringenin stimulates DNA repair in prostate cancer cells, Journal of Nutritional Biochemistry 17 (2006) 89-95.

[22]. M. Lee, S.S. Moon, S.E. Lee, S.H. Bok, T. Jeong, Y.B. Park, M.S. Choi, Naringin 7-0-cetyl ether as inhibitor of HMG-CoA reductase and modulator of plasma and hepatic lipids in high cholesterol fed rats, Bioorganic and Medicinal Chemistry 11 (2003) 393-398.

[23]. S. Ren, Y. Xing, C. Wang, F. Jiang, G. Liu, Z. Li, T. Jiang, Y. Zhu, D. Piao, Fraxetin inhibits the growth of colon adenocarcinoma cells via the Janus kinase 2/signal transducer and activator of transcription 3 signaling pathway, The International Journal of Biochemistry and Cell Biology 125 (2020) 105777-105778.

[24]. R. Murali, S. Srinivasan, N. Ashokkumar, Antihyperglycemic effect of Fraxetin on hepatic key enzymes of carbohydrate metabolism in streptozotocin-induced diabetic rats, Biochimie 95 (2013) 1848-1854.

[25]. S. Aranganathan, J. Panneer-Selvam, N. Sangeetha, N. Nalini, Modulatory efficacy of hesperidin (citrus flavanone) on xenobiotic-metabolizing enzymes during 1,2-dimethylhydrazine induced colon carcinogenesis, Chemico-Biological Interactions 180 (2009) 254-261.

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