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PHYTOECDYSTEROIDS AND ANTIBACTERIAL ACTIVITY OF THE PLANT *Coronaria flos-cuculi*

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Plants of the genus *Coronaria* (Carophyllaceae) are potential producers of ecdysteroids [1]. It was shown previously that a representative of this genus, *C. coreacea*, contains 20-hydroxyecdysone and viticosterone E [2].

We investigated the ecdysteroid composition of *C. flos-cuculi* L. that was introduced to the Botanical Garden of the Academy of Sciences of the Republic of Uzbekistan. According to TLC of the methanol extract, the plant contains at least 11 ecdysteroids, 3 of them in large amounts. Air-dried aerial part of *C. flos-cuculi* (5.5 kg) was extracted at room temperature with MeOH (20 L). The MeOH extract was condensed and diluted with twice the volume of water. Traces of MeOH were distilled off. The solution was extracted with $CHCl_3$ (3 L) to remove hydrophobic compounds. The purified aqueous layer was thoroughly extracted with *n*-butanol (2 L). Solvent was evaporated in vacuo to afford the butanol fraction (185 g). Column chromatography of the butanol fraction over silica gel with elution by $CHCl_3$:CH_3OH (9:1) isolated a chromatographically homogeneous fraction (140 mg) consisting of a mixture of two ecdysteroids **1** and **2**. The fractions were separated by HPLC (RP-HPLC) in an ACE C_{18} semipreparative column (150 × 9.4 mm) with elution by CH_3CN:*i*-PrOH (5:2) and trifluoroacetic acid (0.1%, 17:83 v/v) at 1 mL/min. The mixture of edcysteroids was separated in a Zorbax-SIL (duPont) HPLC column (250 × 4.6 mm) with elution by cyclohexane:*i*-PrOH:H_2O (100:30:1.5) at 1 mL/min. The resulting ecdysteroids were dissolved in the minimum volume of MeOH. Mass spectra were recorded in a Jeol JMS-700 mass spectrometer using ammonia. Retention times and mass spectra of the ecdysteroids identified them as α-ecdysone (**1**), $C_{27}H_{44}O_6$ [3], and taxisterone (**2**), $C_{27}H_{44}O_6$ [4].

Continued elution of the column by the same system produced two fractions containing minor amounts of ecdysteroids **3** and **5**. These ecdysteroids were separated by HPLC and identified by comparison of mass spectra with those of polypodine B (**3**), $C_{27}H_{44}O_8$ [5, 6], and 20,26-dihydroxyecdysone (**5**), $C_{27}H_{44}O_8$ [7, 8].

Further elution of the column isolated pure 2-deoxyecdysterone (**4**, 2.42 g, 0.044% of air-dried plant weight), $C_{27}H_{44}O_6$, mp 254-256°C (CH_3OH:H_2O), identical to an authentic sample [9].

Switching to $CHCl_3$:CH_3OH (4:1) produced 20-hydroxyecdysone (**6**, 12.65 g, 0.173%), $C_{27}H_{44}O_7$, mp 241-242°C (acetone), identical to an authentic sample using TLC and mixed melting point [10]. All isolated ecdysteroids, except 20-hydroxyecdysone (**6**), were observed in this plant for the first time.

Antibacterial activity. We studied the antibacterial activity of this plant in addition to the chemical composition. The test subjects were the $CHCl_3$, CH_3OH, and C_2H_5OH extracts of *C. flos-cuculi* and test cultures of various physiological groups of bacteria, *Klebsiella oxitoca* 6653, *K. pneumoniae* 40602, *Citrobacter freundii* 82073, *Acinetobacter haumanii* 60649, *Staphylococcus aureus* MRSA16, and *Micrococcus luteus*, obtained from the Microbiology Department of Manchester University, Great Britain; *Escherichia coli* NCTC9001, *Pseudomanas aeruginosa* NCTC6749, *Staphylococcus epidermis* NCTC7944, *Enterococcus faecalis* NCTC775, *K. aerogenes* NCTC8172, and *Proteus rettgerri* NCIMB9570, obtained from the National Collection of Great Britain (NCTC); and bacterial strains *E. hormaechei* T2, *A. faecalis* T3, *E. hormaechei* T10, *Acinetobacter* sp. T16, *P. agglomerans* T26, *B. cereus* T80, *P. aeruginosa* T145, and *Staphylococcus saprophyticus* T415, obtained from the Biology Department, National University of Uzbekistan.

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TABLE 1. *In vitro* Antibacterial Activity of *C. flos-cuculi* Extracts

Bacterial strain	Extract		
	ethanol	methanol	chloroform
Alcaligenes faecalis T3	-	+	+
A. haumanii 60649	-	-	-
Acinetobacter sp. T16	-	-	+
B. cereus T80	-	-	-
Citrobacter freundii 82073	+	+	+
Enterococcus faecalis NCTC775	-	-	-
E. coli NCTC9001	+	+	+
E. hormaechei T2	+	-	+
E. hormaechei T10	+	+	+
Klebsiella aerogenes NCTC8172	-	+	+
K. pneumoniae 40602	-	-	-
K. oxitoca 6653	-	+	+
Micrococcus luteus	+	+	+
Pantoea agglomerans T26	-	+	+
Pseudomonas aeruginosa NCTC6749	-	+	+
P. aureginosa T145	-	-	-
Proteus rettgerri NCIMB9570	+	+	+
Staphylococcus aureus MRSA16	-	-	+
S. epidermis NCTC7944	-	+	+
S. saprophyticus T415	-	-	+

The $CHCl_3$, CH_3OH, and C_2H_5OH extracts were tested against the pathogenic microorganisms. The antibacterial activity of the extracts was studied using the disk diffusion test [11]. Microorganisms were cultivated on agar dishes at 30°C overnight in Mueller—Hinton medium (Oxoid). The suspension (100 μL) contained 10^8 CFU of bacteria per mL. Sterile filter disks (5 mm diameter) were soaked with extract solution (20 μL, 5 mg/mL) and placed on the surface of the innoculated Petri dishes. The dishes were incubated at 37°C for 24 h. The antibacterial activity was estimated by measuring the inhibition zone formed around the disks. We used five disks in each Petri dish. Each test was carried out in triplicate [12].

The CH_3OH and C_2H_5OH extracts of the plant inhibited the growth of 8 gram-negative (*K. oxitoca*, *E. coli*, *P. rettgerri*, *C. freundii*, *P. aeruginosa*, *A. faecalis*, *E. hormaechei*, and *P. agglomerans*) and 4 gram-positive (*B. cereus*, *S. epidermis*, *M. luteus*, and *K. aerogenes*) bacteria species (Table 1). The C_2H_5OH extract exhibited more weak action in comparing with the CH_3OH extract against the pathogenic bacteria. The $CHCl_3$ extract exhibited antimicrobial activity of a broader spectrum where it was also active against *S. aureus*, *Acinetobacter* sp., and *S. saprophyticus*. Thus, the microbiological investigations indicate that various extracts of *C. flos-cuculi* have antimicrobial activity against various bacterial species. We note especially that the $CHCl_3$ extract is most interesting from a microbiological viewpoint, possibly because of the presence in it of ecdysteroid derivatives. It is known from the literature that slightly polar acylated ecdysteroids possess antimicrobial activity [13].

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