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Antiviral Furanosesquiterpenes from *Commiphora erythraea*

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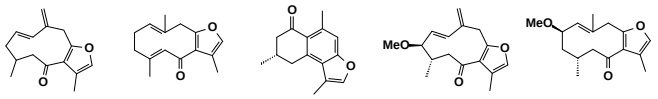
The crude methanolic extract obtained from *C. erythraea* resin was chromatographed on silica gel with solvent of increasing polarity. The extract and fractions were evaluated for cytotoxicity and antiviral activity [parainfluenza type 3 virus (PIV3)] by plaque forming units (PFU) reduction assay using HEp-2 cells (human larynx epidermoid carcinoma cell line). From the active fraction, five compounds were isolated and tested. Only two of these showed anti-PIV3 activity with a selectivity index (SI) of 66.6 and 17.5, respectively. Both the compounds are furanosesquiterpenoids.

Keywords: *Commiphora erythraea* (Burseraceae), Furanodienone, Myrrhone, Parainfluenza type 3 virus, HEp-2 cells, Antiviral activity.

In infants and young children, parainfluenza viruses are the most common cause of lower respiratory tract infections, after respiratory syncytial virus (RSV) and possibly human metapneumovirus (hMPV). Among viral pathogens, different members of the Paramyxoviridae family, including parainfluenza type 3 virus (PIV 3), have been recognized as a major cause of such infections [1]. While upper respiratory infections (URIs) are very frequent, but seldom life-threatening, lower respiratory infections (LRIs) are the leading contributors to acute respiratory infections (ARI) mortality [2]. Reinfection with any of the parainfluenza viruses can occur throughout life, usually resulting in mild upper respiratory infections. The screening of plants for compounds with a viral inhibitory activity could potentially help in identifying new active molecules [3]. In the present paper we describe the antiviral activity of the crude methanolic extract of *Commiphora erythraea* resin and the isolation of two antivirally active furanosesquiterpenoids.

Commiphora erythraea (Ehrenb.) Engl. (Burseraceae) is a small tree widely distributed in southern Arabia, Somalia, Ethiopia and Kenya. Its resin (commonly called Agarsu) is traditionally used on livestock against ticks, and in humans to treat eye infections, malaria and against snake venom poisoning. Many *Commiphora* spp. are characterized by the presence of furanosesquiterpenoids [4]. Despite the wide traditional uses, reports about *C. erythraea* are very few. Recently, the composition of its essential oil has been described [5] and its radical scavenging and anti-inflammatory activities reported [6].

The methanolic extract of *C. erythraea* resin, obtained in yields of 61% (w/w), showed antiviral activity against parainfluenza virus type 3 in a HEp-2 cells plaque forming units (PFU) reduction assay. Fractionation on silica gel of this extract led to two fractions (M1 and M2). Only M2 was shown to be active (Table 1). In order to investigate the role of major components in the observed activity, we further purified the M2 fraction and compounds **1-5** were isolated and were confirmed to be 1,10(15)-furanogermacra-dien-6-one (**1**), 1(10),4-furanodien-6-one (**2**), myrrhone (**3**), rel-3R-methoxy-4S-furanogermacra-1E,10(15)-dien-6-one (**4**), and rel-2R-methoxy-4R-furanogermacra-1(10)E-en-6-one (**5**), by NMR spectral data and comparison with literature values [4].



1 2 3 4 5

Figure 1: Isolated and tested compounds from *C. erythraea* resin methanolic extract.

Table 1: Anti-PIV 3 activities of extract, fractions and isolated compounds in HEp-2 cells by the plaque forming units (PFU) reduction assay.[a]

Compds	% PFU inhibition[a]
Methanolic extract (M)	74.5±1.82
M1	9.7±0.34
M2	33.4±0.86
1	53.2±0.99
2	31.9±0.59
3	58.1±1.50
4	7.2±1.41
5	20.7±0.78
DMSO	0.0
Ribavirin	80.2±0.62

[a]Results are the mean of four determinations ±SD.

The antiviral activities of the isolated compounds were evaluated against PIV-3 and results are reported in Table 1. With the exception of compound **4**, all the compounds possessed *in vitro* antiviral activity, but only furanodienone (**2**) and myrrhone (**3**) showed significant anti-PIV3 activity with IC_{50} values of 11.7 µg/mL and 22.3 µg/mL, respectively (Table 2). Compound **2** with a SI of 66.6 was shown to be a good antiviral agent. It is worthy of emphasis that the compounds were not cytotoxic at the tested doses. Furanodienone (**2**) is commonly present in *Commiphora* and *Curcuma* extracts and is known to possess insecticidal [7], antimicrobial [8], analgesic [9] and anti-inflammatory [10] activities. As far as we know, this is the first report about the antiviral activity of compounds **2** and **3**.

Table 2: Antiviral activities of compounds **2** and **3** in HEp-2 cells by the plaque forming units (PFU) reduction assay.[a]

Compds	IC_{50} (μg/mL)[a]	CC_{50} (μg/mL)[a]	SI
2	11.7±0.48	780±1.27	66.6
3	22.3±0.67	390±1.75	17.5
Ribavirin	5.1±0.48	780±0.87	152.9

[a]Results are the mean of 4 determinations ± SD at a concentration of 25 μg/mL.

Experimental

Plant material: The resin of *Commiphora erythraea* (Agarsu grade I), commercialized by Agarsu Liben Cooperative and imported into Italy by IPO association (www.ipoassociazione.org), was studied. A voucher specimen (# MCM-1) of the resin (Agarsu grade I) is deposited at the Dipartimento di Chimica e Tecnologia del Farmaco - Sez. Chimica Organica, Università di Perugia.

***Extraction and isolation*:** The ground resin (30 g) was extracted by maceration at room temperature for 16 h with 250 mL of methanol. The suspension was filtered under vacuum and the solvent removed under reduced pressure leading to 19.2 g of extract (M) that was evaluated for cytotoxicity and antiviral activity. Silica gel CC was carried out on 3.80 g of the extract (M) obtaining 2 fractions: M1 (elution with *n*-hexane; 0.90 g) and M2 (elution with Et_2O; 2.62 g). The antiviral active fraction M2 was further purified on a silica gel column (CH_2Cl_2-EtOAc 49:1) leading to 6 fractions: M2-1 (0.34 g), M2-2 (0.97 g), M2-3 (0.28 g), M2-4 (0.21 g), M2-5 (0.37 g) and M2-6 (0.38 g). Fractions M2-2, M2-4 and M2-5 contained pure **2**, **4** and **5**, respectively.

Fraction M2-1 was subject to silica CC. Elution with *n*-hexane-EtOAc (24:1) gave 4 fractions: M2-1-1 (30 mg), M2-1-2 (90 mg), M2-1-3 (0.14 g) and M2-1-4 (52 mg). Spectral analysis confirmed that fraction M2-1-1 was constituted of compound **1** and fraction M2-1-4 of pure **2**. Fraction M2-1-3 was a mixture of **1** and **2**. Fraction M2-3 was further purified by silica gel CC. Elution with CH_2Cl_2 gave fraction M2-3-1 (60 mg), constituted of pure **2**, M2-3-2 (100 mg), which was a mixture of **2** and **5**, and M2-3-4, which was pure myrrhone (**3**) (80 mg).

Cells and viruses: HEp-2 cells (human larynx epidermoid carcinoma cell line) were obtained from Vircell, Santa Fé, Spain. Cells were grown in Iscove Modified Dulbecco Medium (IMDM, Invitrogen, San Giuliano Milanese, Italy) supplemented with 10% fetal bovine serum (Invitrogen) (complete medium). The strain of parainfluenza type 3 virus used in the study was isolated from a clinical specimen in the Laboratory of Clinical Microbiology of the S. Maria della Misericordia Hospital of Perugia (Italy).

Antiviral activity and cytotoxicity assays: The antiviral activities of the extract, fractions and isolated compounds were evaluated by the plaque forming units (PFU) reduction assay [11]. Briefly, cell monolayers were prepared by seeding 5 x 10^4 HEp-2 cells in 1 mL of complete medium into 24 well tissue culture plates (Corning Glass Works, Corning, N.Y.) and then incubating at 37°C in 5% CO_2. After 48 h incubation, the culture medium was discarded and the monolayers were infected with 100 PFU/well of PIV3 in the presence of 10 μL of different concentrations of the samples or ribavirin, or 10 μL of diluent (0.1% DMSO). After a 2 h incubation, 1 mL/well of solid overlay medium (IMDM containing 5% FBS and 0.5% agarose) was added. Plates were incubated at 37°C in 5% CO_2 for 48 h, then cultures were drained, the monolayers fixed in methanol, and stained with 1% crystal violet solution (100 μL/well). After extensive washing the PFU were enumerated and the inhibitory effect of the different compounds was calculated by the following formula:

$$[1 - (\text{PFU treated} / \text{PFU diluent})] \times 100$$

The minimal concentrations of each product required to reduce the plaque number by 50% (IC_{50}) were extrapolated from the dose-response curves [12]. To test for cytotoxicity, HEp-2 monolayers were grown in 96 well microtiter plates and exposed to serial dilutions of the different compounds starting at 2.5 mg/mL, or 0.1% DMSO as control. Treated cells were incubated at 37°C for 72 h. Monolayers were examined microscopically for assessment of either changes in cell morphology or visible toxic effects (obvious cellular damage or lysis), the minimal concentration of each product causing a 50% cytopathic effect (CC_{50}) was recorded and, when appropriate, the selectivity index (SI) was calculated as CC_{50} / IC_{50}.

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