

# A NOVEL GERMACRANOLIDE SESQUITERPENE LACTONE WITH ANTI-INFLAMMATORY EFFECT FROM *CAPPARIS DECIDUA* (FORSK.)

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## ABSTRACT

Phytochemical investigation of aerial parts of *Capparis decidua* resulted in the isolation of one new compound germacr-3 $\beta$ -ol-7,9-dien-6,14-olide-15-oic acid along with several known compounds. Isolation was done by using column chromatography and ethyl acetate fraction afforded the new compound. The chemical structure of the compound was elucidated with the help of 500/125 MHz NMR using 1D and 2D spectral methods viz. <sup>1</sup>H and <sup>13</sup>C NMR, ESIMS and FABMS aided by IR spectroscopy and then confirmed by mass spectroscopic analysis. Chloroform and methanol extracts have shown potent anti-inflammatory activity evaluated by using carrageenan induced paw edema in Swiss albino rat model. In Sudan this plant may be used as natural source for pharmaceutical purpose designed for anti-inflammatory activity and may provide a new lead or pharmacophore for more potent analogues.

**Keywords:** Germacranolide, Sesquiterpene lactone, *Capparis decidua*, anti-inflammatory.

## 1. INTRODUCTION

The use of traditional medicine is expanding globally. It continues to be used not only for primary health care in developing countries, but also in countries where conventional medicine is predominant in the national health care system<sup>1</sup>. *Capparis decidua* (Family: Capparidaceae) is a xerophytic shrub, found widely in the western parts of India, Pakistan and some of the Asian countries<sup>2</sup>. In Sudan it is widely distributed in desert and semi-desert area of northern and central Sudan especially on sandy soils and in

low rainfall savanna on clays<sup>3</sup>. It is also found in Blue Nile, Upper Nile, western and eastern Sudan besides northern areas of the country<sup>4</sup>. It is known by various names, e.g. Caper (English), Kabbar (Arab), Alcaparro (Spain), Gollaro (Pakistan)<sup>5</sup>. In Sudan it is locally known as Al Tundub<sup>6</sup>. It is used in traditional medicines to cure various illnesses. Phytochemical studies have shown the presence of many beneficial compounds such as spermidine, rutin, quercetin, kaempferol, stigmasterol, campesterol, tocopherols, and carotenoids. Biological studies

reveal important antimicrobial, anti-oxidative, anti-inflammatory, immunomodulatory and antiviral properties<sup>5</sup>.

## 2. MATERIALS AND METHODS

### 2.1. General

In this experiment methanolic extract of aerial parts of *Capparis deciduas* was subjected to normal phase column chromatography for separation of compounds. Various fraction have been collected and purified to get the crystals of pure compound. The chemical structure of the compound was elucidated with the help of 500/125 MHz NMR using 1D and 2D spectral methods viz. <sup>1</sup>H and <sup>13</sup>C NMR, ESIMS and FABMS aided by IR spectroscopy and then confirmed by mass spectroscopic analysis. Chloroform and methanol extracts were evaluated for anti-inflammatory activity using carrageenan induced paw edema in Swiss albino rat model.

### 2.2. Plant material

*Capparis deciduas* (aerial parts) was collected from Shambat, Khartoum north-Sudan. The plant was authenticated at the Medicinal and Aromatic Plant Research Institute (MAPRI), Sudan and voucher specimens deposited in the Herbarium.

### 2.3. Animals

Wistar albino male rats weighing 200-220g were used in this study. The animals were obtained from the Experimental Animal Care Centre, College of Pharmacy, King Saud University, Riyadh. The animals were housed under constant temperature (22 ± 2°C) and light/dark cycle (12/12 h). They were provided with Purina Chow and free access to drinking water *ad libitum*.

### 2.4. Extraction and isolation

Collected plant was dried under shade and then powdered. The powdered plant material (200 grams) was extracted by cold maceration method with sufficient quantity of 80% methanol at room temperature for 48 hour. The process of extraction was repeated twice for the complete extraction. The extract was filtered using Whatman filter paper and the filtrates were concentrated under reduced pressure which afforded 39 g of a concentrated extract.

### 2.5. chromatographic separation from methanol Extract

A portion (30 g) of this extract was fractionated on a normal phase silica gel column eluted with petroleum ether-chloroform and chloroform-ethyl acetate mixtures of increasing polarity to give fractions 1–37. Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions (having same R<sub>f</sub> values) were combined and crystallized. Ethylacetate fractions (fraction 34, 35, 36 and 37) were further purified on preparative thin layer chromatography [dichloromethane:ethylacetate:formic acid (80:30:10)], Three sub-fractions were obtained, two of them were re-subjected to preparative TLC again with other solvent system [benzene:dichloromethan (1:9)] and compound **MW-6** was obtained and recrystallized to get the pure compound.

### 2.6. Homogeneity of the fractions

The fractions collected were subjected to thin layer chromatography (TLC) to check homogeneity of various fractions. Chromatographically identical fractions (having same R<sub>f</sub> values) were combined together and concentrated. They were then crystallized with suitable solvent system.

### 2.7. Anti-inflammatory Activity

The anti-inflammatory activity was studied using the paw oedema model induced by 1% carrageenan, administered at volume of 0.1 ml/animal into the subplantar region of the right hindpaw of the rat<sup>7</sup>. The volume of the paw was measured by the removal of the water column using a Ugo-Basil plethysmometer, at the time 0 and the intervals of 1,2 and 3h immediately after the subplantar injection of carrageenan. The animals were divided into ten groups of six each. In group 1, animals (control) received normal saline while group 2 to 4 animals received 50, 100 and 200 mg/kg dose of methanolic extract of aerial parts of *Capparis decidua*, group 5 to 7 animals received 50, 100 and 200 mg/kg dose of chloroform extract of aerial parts of *Capparis deciduas* and animals of group 8 to 10 received 50, 100 and 200 mg/kg dose of dichloromethane extract of aerial parts of *Capparis deciduas*.

The volume of formed oedema was then calculated using the following formula:

$$\text{Percentage inhibition} = \frac{(\text{Ct} - \text{Co})_{\text{control}} - (\text{Ct} - \text{Co})_{\text{treated}}}{(\text{Ct} - \text{Co})_{\text{control}}} \times 100$$

Where Co is volume of oedema in control group and Ct is volume of oedema in test group. Net oedema volumes formed two hours following injection of Carrageenan were used to calculate the influence of the extracts on the induced oedema. Pretreatment time is 60 minutes.

### 3. RESULTS AND DISCUSSION

Compound MW-6 was obtained as a colorless mass. The TLC chromatogram using mobile phase ethyl acetate: acetone [8:2] was showed one pure compound ( $R_f$  value 0.80) which give violet color with vanillin sulphuric acid. Its molecular formula  $C_{15}H_{20}O_5$  was deduced ( $m/z$  279.0  $[M-H]^+$ ). The planar structure of MW-6 was established by analysis of the NMR spectral data.

$^1H$ -NMR spectrum showed the presence of one proton double doublets at 4.23 ( $J = 14.5$ ,  $14.5Hz$ ) and proton broad multiplets signal at  $\delta$  3.37 ( $w_{1/2}=19.5$  Hz) were ascribed to oxygenated methine assigned to  $H_{6\alpha}$  and  $H_{3\alpha}$  proton respectively. Two doublets signals at  $\delta$  7.73 ( $J=3.0$  Hz) and  $\delta$  7.73( $J=3.0$  Hz) due to olefinic protons assigned to  $H_9$  and  $H_8$ . Two multiplets signal at  $\delta$  1.70 and  $\delta$  1.40 ascribed to methylated methine  $H_{12}$  and  $H_{4\alpha}$  proton respectively.  $^1H$ -NMR data also showed multiplets signals attributed to methylene protons at  $\delta$  1.91,  $\delta$  1.28,  $\delta$  1.37,  $\delta$  1.39 and  $\delta$  1.37 assigned to  $H_1$ ,  $H_{2\beta}$ ,  $H_{2\alpha}$ ,  $H_{5\beta}$  and  $H_{5\alpha}$  respectively. Two doublets signal at  $\delta$  0.98 ( $J=7.5$  Hz) and  $\delta$  0.94 ( $J=7.5$  Hz) ascribed to methyl  $H_{13}$  and  $H_{11}$  proton.

The  $^{13}C$  NMR spectrum displayed 15 carbon signals. The signals in the  $^{13}C$  spectrum suggested the presence of four quaternary carbons including one carbonyl group at  $\delta$

180.38 assigned to  $C_{15}$ , two carbons contributed in double bond at  $\delta$  133.62 and 133.60 assigned to  $C_7$  and  $C_{10}$  respectively, one lactone carbonyl group at  $\delta$  169.35 assigned to  $C_{14}$ .  $^{13}C$ -NMR data also showed intense signals attributed to six methine signals at  $\delta$  69.10,  $\delta$  72.01(attached to oxygen),  $\delta$  30.15,  $\delta$  40.21 and  $\delta$  132.41,  $\delta$  129.88 (olefinic proton) assigned to  $C_3$ ,  $C_6$ ,  $C_4$ ,  $C_{12}$ ,  $C_8$  and  $C_9$  respectively, three methylene signals at  $\delta$  31.46,  $\delta$  24.04 and  $\delta$  24.22 assigned to  $C_1$ ,  $C_2$  and  $C_5$  respectively, two methyl groups at  $\delta$  11.42 and  $\delta$  14.41 assigned to  $C_{11}$  and  $C_{13}$  respectively.

The DEPT 90 spectrum of MW-6 showed the signals at  $\delta$  133.60 ( $C_7$ ) and  $\delta$  72.01 ( $C_6$ ) which had not appeared at the spectrum of  $^{13}C$ -NMR. The  $^1H$ - $^1H$  Cosy spectrum of MW-6 showed correlations of  $H_{11}$  and  $H_4$ . The HMBC spectrum of MW-6 exhibited interactions of  $H_{11}$  ( $\delta$  0.94) with  $C_2$  ( $\delta$  24.02) by four bond correlation and  $C_5$  ( $\delta$  24.22) by three bond correlation.  $H_{13}$  ( $\delta$  0.98) correlated with  $C_{12}$  ( $\delta$  40.21) by two bond correlation.  $H_2$  ( $\delta$  1.37) and  $H_5$  ( $\delta$  1.37) with  $C_4$  ( $\delta$  30.15) by three and two bond correlation respectively and  $H_4$  ( $\delta$  1.40) with  $C_1$  ( $\delta$  31.46) by four bond correlation. The GC chromatogram exhibited one peak at retention time (Rt) 42.688 min. On the bases of these evidences the structure of MW-6 has been established as Germacr-3 $\beta$ -ol-7,9-dien-6,14-olide-15-oic acid.

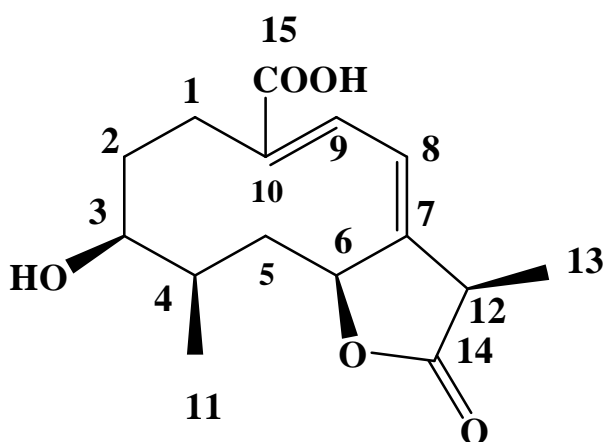
**$^1H$  and  $^{13}C$  NMR spectral data of compound MW-6**

Position	Proton	$^1H$ NMR ( $w_{1/2}$ , $J$ in Hz)	$^{13}C$ NMR
1	$H_1$	1.91 m	31.46
2	$H_{2\alpha}$	1.37 m	24.04
	$H_{2\beta}$	1.28 m	
3	$H_{3\alpha}$	3.37 brm ( $w_{1/2}=19.5$ )	69.10
4	$H_{4\alpha}$	1.40 m	30.15
5	$H_{5\alpha}$	1.37 m	24.22
	$H_{5\beta}$	1.39 m	
6	$H_{6\alpha}$	4.23 dd ( $J=14.4$ )	72.01
7	-	-	133.60
8	$H_8$	7.64 d ( $J=3.0$ )	132.41
9	$H_9$	7.73 d ( $J=3.0$ )	129.88

10	-	-	133.62
11	H <sub>11</sub>	0.94 d (J=7.5)	11.42
12	H <sub>12</sub>	1.70 m	4021
13	H <sub>13</sub>	0.98 d (J=7.5)	14.41
14	-	-	169.35
15	-	-	180.38

Methanolic and chloroform extract of *Capparis decidua* showed good antiedematous effect and exhibited (64.0%) and (65.0%) percentage inhibition respectively indicating its good ability to inhibit the inflammatory mediators, while

dichloromethane extract did not possess anti-inflammatory activity. The effective dose for this anti-inflammatory activity was found to be 200 mg/kg.



Germacr-3 $\beta$ -ol-7,9-dien-6,14-olide-15-oic acid

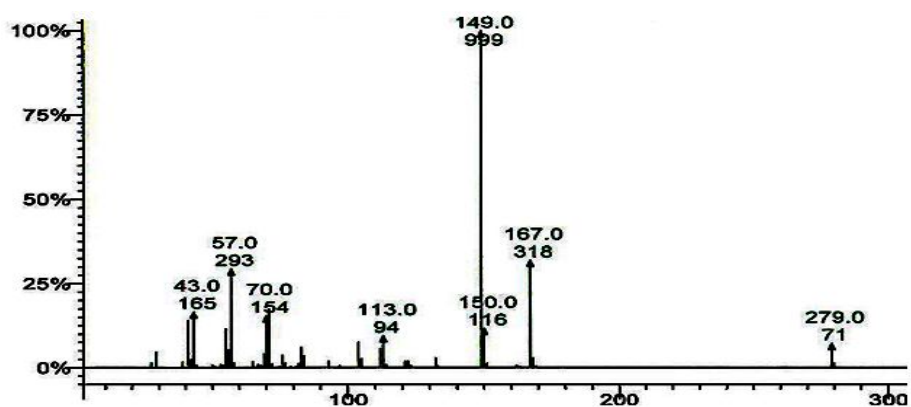


Fig. 2: Mass-spectrum of compound MW-6

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