

Anti-inflammatory activity of Carumbelloside-III, isolated from *Caralluma umbellata*

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ABSTRACT From the dried bark and flower of *Caralluma umbellata*, (Asclepiadaceae) one novel pregnane glycoside named Carumbelloside-III was isolated and their structures was elucidated by spectroscopic experiments, and the pharmacological study of isolated pregnane glycoside from aqueous methanol extract of dried bark and flower were evaluated in male Wistar rats using the paw oedema test with carrageenan. The isolated compound was administered in a dose dependant manner. This showed significant anti-inflammatory activity.

Keywords: *Caralluma Umbellata*; Asclepiadaceae; Pregnane glycoside; Anti-inflammatory activity, Carumbelloside – III

Introduction

The genus *Caralluma* belongs to the family Asclepiadaceae, which comprise 200 genera and 2500 species, which are distributed throughout the world ^{1,2,3}.

Caralluma umbellata Haw. grows wild in dry and arid regions of Chittoor District and several District of Andhra Pradesh, in India. Previously the tribal people of Chittoor District used stem juice warmed and mixed with turmeric powder for stomach disorder and abdominal pains ⁴. *Caralluma umbellata* Haw [Syn: W&A, *Stapelia Umbellata* Roxb, *Carulluma campanulata* N.E.Br] a perennial herb with thick, erect, leafless, branching, succulent, perennial herb. Flower during January and June. Common source in Sri Lanka, Nepal and in India, hilly regions of Orissa, Andhra Pradesh, Tamil Nadu and in Karnataka. Sanskrit name: Dugdhika, Uttamphalini. In Telegu (Tribal) it is known as Kundete kommulu. In Tamil Nadu it is known as Eluman or Elumanpuli ⁵⁻⁸. The medicinal properties of *Caralluma umbellata* have been attributed to glycosides contained therein. The glycosides contained in *Caralluma* belong to pregnane group of glycosides. Some pregnane glycosides are found in *Caralluma* plants includes, but are not limited: Carumbelloside-I, Carum-

belloside-II, Carumbelloside-III, Carumbelloside-IV and Carumbelloside-V. ⁹⁻¹²

Experimental

Collection of plant material

Fresh whole plants of *Caralluma umbellata* (Asclepiadaceae), is the thick, erect, leafless, branching, succulent perennial herb collected from Tirumala hills forests of Tirupati in Chittoor district, A.P, India. The herbarium specimen is available in the Department of Botany, Osmania University, Hyderabad, Andhra Pradesh.

Extraction & isolation

The plant was collected and dried for 21 days and then dried barks and flowers (2.5 kg) are chopped and crushed and passed through sieves (No-60), to made fine free flowing powder. The powder (2 kg) was extracted with methanol in soxhlet extractor. The extract was then concentrated under reduced pressure to afford a dark greenish brown semi solid (950gm). It was dissolved in methanol and water (Methanol 500ml+water 500ml) mixture and absorbed on already extracted plant material (1.5kg). Extracted successively with hexane (pet ether-60 to 80°C, 2.5 L), chloroform (2.5L), ethyl acetate (2.5 L), methanol and water mixture (Methanol 80%+ water 20%) (3L) in

a soxhlet extractor. The extracts then concentrated under reduced pressure and separately examined. In here we concentrated on aqueous methanol extract for the current study.

The (Methanol and water) extract on concentration yielded dark brown semi-solid (85.598gm). The extract contains 0.5% resinous matter. It gave positive to Libermann-Burchard (Red ring form) test, Molisch test (Pink violet color) and in Salkowski test (Red color), response to Shinoda test. Upon TLC examination (Solvent system Ethyl acetate: Methanol: water), showed the presence major spots of our interest and was therefore subjected to column chromatography (850gm of silica gel 230- 400mesh, ACME) and the column was eluted successively with pet ether, Pet-ether : Ethyl acetate, Ethyl acetate : Methanol : Water, respectively Altogether 90 fractions (Each of 1000ml) collected were subjected to perform TLC. The fractions were mixed based on TLC examination. The fractions on concentration gave a greenish white amorphous powder (830mg). Upon TLC examination (Solvent system Ethyl acetate: Methanol) showed presence of one major spot and was therefore subjected to recolumn chromatography over silica (150 gm 230-400 mesh, ACME) eluted successively with pet ether, Pet-ether: Ethyl acetate, Ethyl acetate: Methanol: water respectively. Altogether 40 fractions (Each 250 ml) were collected and concentrated under reduced pressure to get fine buff color solid (752mg). Finally the buff color solid was dissolved in methanol (10ml) then added activated charcoal (5mg) and slightly heated over Hot water Bath. Then the hot solution was vacuum filtered through filter paper (Wattman 40, dia 125mm) and the collected filtrate was concentrated to 10ml under the reduced pressure then cooled the solution up to 0°C to get colorless crystalline solid (690mg). Upon TLC examination of the crystalline compound, showed one major spot. This is designated as compound A

(Carumbelloside-III). It gave red color in Libermann-Burchard test, and gave violet color in Molisch test but no response to Shinoda test, indicating compound it to be steroidal glycoside. Carumbelloside – III was identified by extensive spectral study, molecular modeling methods and literature review^{13, 14, 15,}

Anti-inflammatory activity study of Carumbelloside-III

The anti-inflammatory activity of the test compounds was evaluated in Wistar rats employing the method of Winter *et al*^{16 17, 18,} Male Wistar rats were used for the study. Animals were fasted overnight and were divided into control, standard and test groups, each containing 6 animals. The test compounds were administered to the animals in the test group at the dose of 10mg/kg, 20mg/kg and 40mg/kg by oral route. Animals in the standard group received Indomethacin at dose of 10mg/kg by oral route. All test and standard compounds were administered as 1% gum acacia suspension. Rats in control group received the vehicle solution without drugs. One hour after drugs administration, rats in all groups were challenged with 0.1 ml of 1% Carrageenan in sub plantar region of left hind paw. A zero hour paw volume was measured for the rats using digital Plethysmo meter (Ugo Basile, Italy Model 7150) before the administration of Carrageenan for all groups. Paw volumes were again measured at 30-minute interval for 3hour. The mean paw oedema value for test group being compared with its mean value for control group.

Statistical analysis

All results were expressed as \pm S.E.M. The differences between experimental groups were compared by one way ANOVA (Control vs. treatment, Bonferroni's method) (Using Jindal Scientific Sigmastat Statistical software, Version1.0) and were considered statistically significant when $P < 0.005$

Result

In Pharmacological studies carrageenan induced rat paw oedema is used in the search for the new anti-inflammatory drug

and compared with that of Indomethacin anti-inflammatory drug utilizing oedema model.

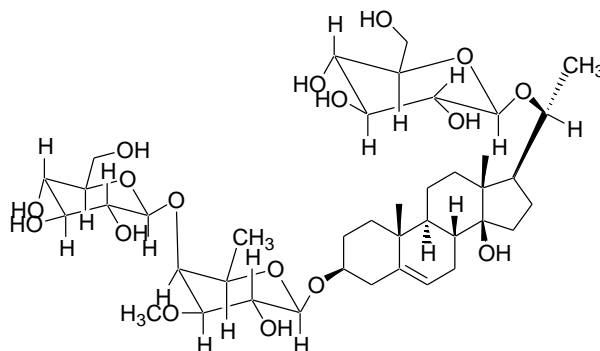


Figure 1: Chemical structure of Carumbelloside-III

Discussion

The anti inflammatory activity of Carumbelloside-III of *Caralluma umbellata* Haw (Asclepiadaceae) was evaluated by Carrageenan induced rat paw oedema method and the result showed in Table-1. The isolated compound was tested at three different doses level 10mg/kg, 20mg/kg and

40mg/kg in a dose dependant manner. As shown in Table-1, the Carumbelloside-III, showed maximum inhibitions, 58.4% at the dose of 40mg/kg after 3 hours of drug treatment in Carrageenan induced paw oedema, where as standard drug showed 66.1% of inhibition.

Table 1: Effect of Carumbelloside-III on Carrageenan induced paw

Treatment	Dose (mg/kg)	Change in Paw oedema mean	% of inhibition
Control	0	0.736±0.070	-
Indomethacin	10	0.320±0.030	66.1
Carumbelloside- III	10	0.452±0.040	38.6
Carumbelloside-III	20	0.408±0.030	43.8
Carumbelloside-III	40	0.350±0.030	58.4

Values are mean ±S.E.M (n=6). Experimental groups were compared with control p<0.005.

The compound up to a dose of 60mg/kg did not produce any toxicity symptoms. The results obtained complimented the earlier investigation that Carumbelloside-I^{19, 20}. The major constituents of the *Caralluma umbellata* have no significant anti-inflammatory activity but have the potent antinociceptive activity. From current study of the isolated compound (Carumbelloside-

III) from *Caralluma umbellata* showed the significant anti-inflammatory activity on Carrageenan induced rat paw oedema method.

Conclusion

In view of our interest in the chemical constituents of indigenous medicinal plants, the chemical examination of the dried bark

and flower of *Caralluma umbellata* has now been undertaken. The present pharmacological study was undertaken to evaluate the possible anti-inflammatory properties of the isolated pregnane glycoside named Carumbelloside-III from the aqueous

methanol extract from *Caralluma umbellata*. The study was therefore aimed at investigation the anti-inflammatory activity of stem bark and flower extract with a view to justifying the use of the plant in the treatment of inflammatory diseases,^{21, 22.}

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