EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF PETROLEUM SPIRIT EXTRACT OF CARALLUMA UMBELLATA
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ABSTRACT
Prolonged uses of both steroidal and non-steroidal anti-inflammatory drugs are associated with side effects like peptic ulcer formation and some skin allergic reactions. To minimise these side effects we prefer naturally obtained pregnane glycoside namely, Carumbelloside-III. It is one of the phyto chemical constituents of the plant Caralluma umbellata belongs to the family Asclepiadaceae, which is thick, erect, succulent herb found in countries Africa, Spain, Pakistan, Sri Lanka and India. In India it grows in hilly regions of states Orissa, Tamil nadu, Karnataka, Telangana, Andra Pradesh. The telugu name of this plat is “Kundeti kommulu”. From this plant pregnane glycoside Carumbelloside-III was extracted by using solvet petroleum spirit and then isolated. The anti-inflammatory activity of isolated compound was evaluated by the method Egg white-induced rat paw oedema test in albino rats. The isolated pregnane glycoside shows significant anti-inflammatory activity.

Keywords: - Caralluma umbellata; Asclepiadaceae; Pregnanpe glycosides; anti-inflammatory activity; Carumbellosides; paw oedema.

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1. INTRODUCTION
Drugs commonly used in modern medicine for suppression of pain and inflammation like non-steroidal anti-inflammatory drugs and corticosteroids provide only symptomatic relief. Long-term use of these drugs is associated with serious adverse effects.

The use of plant medicines in the treatment of various ailments, including central nervous system disorders is an age long practice. It is important to note that plant medicines are also gaining popularity in developed countries. Herbal medicine is currently enjoying a revival in popularity in the west and in fact it is the primary form of medicine in many parts of the world. With the great reliance on this type of medicine, it becomes pertinent to search for potent, effective and relatively safe plant medicines, as well as scientific validation of the success claims about plants already in use by traditional medicine practitioners in order to enhance their safety and efficacy. These are some of the problems making this alternative healthcare system less acceptable, especially by orthodox medicine practitioners. For instance, inflammation is a coordinated response that protects and heals the host after infection or tissue damage and it involves several molecular cues generated from either host or disease agent (Nathan, 2002).
and non-steroidal anti-inflammatory drugs (NSAIDs), are available for the treatment of various inflammatory disorders. These drugs however, offer only temporary relief and often elicit undesirable side effects. Hence, the investigations of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap and have little side effects. Hence, the search for a new, safe analgesic and anti-inflammatory drug is ongoing.

1.1 Introduction To Inflammation

Inflammation is a tissue reaction to infection, injury, irritation or foreign substance. It is a part of host defense mechanism but when it becomes uncontrolled it is a hopeless condition. Aging is also considered to be an inflammatory response.

1.1.1 Mechanism involved in inflammation:

The inflammatory response is a physiological characteristic of vascular tissue. Increased permeability seen in the inflammatory reaction leads to exudation of fluid rich in plasma proteins, coagulation factors and injured tissues with subsequent edema at the site. Exudation which is a consequence of vascular permeability is considered as major features of acute inflammation. Histamine and other mediators of inflammation increase vascular permeability at various times after injury. There are several tissue factors or mechanisms that are known to be involved in the inflammatory reactions such as release of

- Histamine
- Bradykinin
- Prostaglandins
- 5-Hydroxy tryptamine

Inflammatory produced substances:

There are several substances that causes inflammation and oedema in a few minutes by release of histamine, bradykinine, 5-HT and prostaglandins in the body when injected in dorsum of foot rat. The substances that causes the inflammation such as

- Carrageenan
- Formalin
- Bradykinin
- Histamine
- 5-HT
- Mustard
- Egg white

Carragen induced in paw edema is the most commonly used method in experimental pharmacology. Carrageen is a sulphated polysaccharide obtain from sea weed (Rhodophycae) and by causing the release of histamine, 5HT, bradykinin and prostaglandin, it produces inflammation and edema [1].

Here, we use egg white as inflammatory causing substance.
1.1.2 Anti-inflammatory drugs

Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system. Generally used NSAIDs are Indomethacine, Ibuprofen, Aspirine, Diclofenac sodium, etc.

1.2 INTRODUCTION OF PLANT

![Caralluma umbellata](image)

**Fig. 1:** Caralluma umbellata

1.2.1 TAXONOMICAL CLASSIFICATION

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliate</td>
</tr>
<tr>
<td>Order</td>
<td>Gentianales</td>
</tr>
<tr>
<td>Family</td>
<td>Asclepiadaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Caralluma</td>
</tr>
<tr>
<td>Species</td>
<td>C.umbellata</td>
</tr>
</tbody>
</table>

1.2.2 Morphology

- **Field Tips**
  
  Stems and branches angled with watery latex. Leaves along the angles, caducous, leaving scars. Flowers foul smelling.
Flower
In terminal umbel, purple with cross stripes. Flowering with a peak from March-April.

Fruit
A cylindrical follicle, green, with small dark spot, hooked at apex, paired. Seed flattened, broadly margined, with silky white hairs [2].

Leaf apices
Acuminate

Leaf bases
Attenuate

Leaf shapes
Asymmetric

Leaf Types
Simple

Habit
An erect succulent herb.

1.2.3 Geographical Sources
Caralluma is found in Africa, Spain, Saudi ArabiaMiddle East, Pakistan and India. In India Occasionally, it was found in Hilly Regions of Orissa, Andhra Pradesh, Tamil Nadu, Telangana and in Karnataka.
In Andhra Pradesh it is found in Tirumala hills of chitoor district and other districts.

1.2.4 Growth
Caralluma umbellata Haw belonging to the Asclepiadaceae family. It is a thick, erect, leafless, branching, succulent thorny perennial herb. It grows in Hilly Regions of Orissa, Andhra Pradesh, Telangana, Tamil Nadu and in Karnataka in India.

1.2.5 Etymology & Common Names
The Etymology of ‘Caralluma’ was derived from the Arabian word ‘qarhal-luhum’ meaning wound in the Flesh or abscess. Various medicinal uses of caralluma spices, have been document in Arabic and Indian traditional medicine including treatment of diabetic, inflammation, stomach disorders, cancer and in many other diseases [3].
Caralluma umbellate have different local names in different regions.

Sanskrit Name       : Dugdhika, Uttamphalini.
Telugu Name         : Kundete kommulu.
Tamil Name          : Eluman, Elumanpuli, Kallimulayan
Kolli Hills Name    : Chirukalli. (Malabar Tribes)
. MATERIALS & METHODS

Anti-inflammatory activity of petroleum spirit extract of *Caralluma umbellate* done on albino rats. This research would need different materials and equipments.

**Fig.2: Materials used**

2.1 Materials

2.1.1 Collection Of Plant Material

Fresh whole plant of *Caralluma umbellata* (*Asclepiadaceae*), is the thick, erect, leafless, branching, succulent perennial herb collected from local regions Anantapur district, A.P, India. The herbarium specimen is available in the Department of Botany, Sri Krishna Devaraya University, Ananthapuramu, Andhra Pradesh.

2.1.2. Part Used

All parts (stem, flowers, roots & bark) of the plant Caralluma umbellata are have medicinal importance. Different compounds are isolated from the different parts. Here, based on the part used different pregnanes glycosides are isolated. In this experiment the plant used and respectable pregnane glycoside was evaluated. Preganes glycosides, sapanoins, flavanoids, triterpenoids, flavones glycosides, steroidal glycosides are the phyto chemical constituents, which are present in CarallumaUmbellata Haw.
From the whole plant *Caralluma umbellata* (Asclepiadaceae) different types of pregnane glycosides are extracted. Pregnan glycosides (Carumbelloside-I, Carumbelloside-II, Carumbelloside-III & Carumbelloside-IV) are the one of the phyto chemical constituents of the plant *Caralluma umbellate* [4-6]

By using different solvents the following pregnane glycosides are extracted from different plant parts.

**Table No. 1** Pregnan glycosides from different parts of plant

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Solvent used</th>
<th>Identified compound</th>
<th>Isolated compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>whole plant</td>
<td>Hexane</td>
<td>Carumbelloside-I</td>
<td>3-O-β-D-glucopyranosyl (1-6)-β-D-glucopyranol-3β, 14β-dihydroxy pregn-5-en-20-one</td>
</tr>
<tr>
<td>Stems &amp; roots</td>
<td>Tolune/Ethanol acetat / n-Butanol</td>
<td>Carumbelloside-II</td>
<td>3-O-β-Dglucopyranosyl-3β, 14β-dihydroxy pregn-5-ene-20-one</td>
</tr>
<tr>
<td>Stem, Bark &amp; flowers</td>
<td>Petroleum spirit/chloroform</td>
<td>Carumbelloside- III</td>
<td>carallumagenin 3-O-β-D-glucopyranosyl(1-4)-β-D-digitalopyranoside-20-O-β-Dglucopyranoside</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Ethyl acetate/n-Butanol</td>
<td>Carumbelloside-IV</td>
<td>carallumagenin 3-O-β-D-glucopyranosyl(1-4)-β-D-digitalopyranoside-20-O-β-Dglucopyranoside</td>
</tr>
</tbody>
</table>

2.2. Extraction Procedure

The whole fresh plant (7kg) was collected and dried for 14 days and then dried plant material are chopped and crushed and passed through sieves (No-40), to made fine free flowing powder. The powder was extracted with Petroleum spirit in soxhlet extractor for 7 days at room temperature. The extract was filtered and concentrated under reduced pressure to afford a dark greenish brown semi solid [7,8]. To concentrate, 2 liter of water was added and extracted successively with petroleum spirit (Carumbelloside-III). After evaporation of solvent, the extracts were subjected to flash silica gel to yield the respect pregnane glycoside compound. The extract (18.3gm) was subjected to silica gel chromatography using same solvent as the eluent and fraction of 50 ml each were collected. Upon TLC examination (Solvent system), showed the presence major spots of our interest and was therefore subjected to re chromatography using same system to purify the compound. The structure of the compound elucidated and identified by extensive spectroscopic methods and molecular modelling[9].
2.3. Animals
Anti-inflammatory activity study, young Swiss-albino rats of either sex, weighing 125 to 200 gm, taken from the Animal house of Balaji College of pharmacy, Anantapur. The animals were kept at separate shelve in the same animal house of pharmacy discipline, Balaji college of pharmacy, for adaptation under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 ± 2.0 °C and with light-dark cycle). All rats were provided standard feed (Lipton India, Bangalore) and had free access to tap water. The experimental met the national guidelines on the proper care and use of animals. The CPCSEA (1563/PO/a/11) approved the experimental protocol.

2.4. Anti-inflammatory activity by egg white-induced paw edema

Anti-inflammatory activity of *C.umbellata* was evaluated in albino rats employing the method of egg white-induced paw edema as previously described by Winter et al. (1962) [10,11]. Animals were fasted overnight before the experiment started.

The rats were divided into three groups (control, standard and test), each group consisting of 6 animals as follows:

**Control Group:** Rats were treated with saline without drug.

**Test Group:** Rats treated with *test compound petroleum spirit extract (Carumbelloside-III)* at the dose of 10mg/kg, 20mg/kg and 40mg/kg as a single dose intra-peritoneally.

**Standard Group:** Rats treated with the standard drug indomethacin (10 mg/kg) by intra-peritontial route.

One hour after drugs administration, rats in all groups were challenged with 0.1 ml of egg white in sub plantar region of left hind paw using a glass syringe.

A zero hour paw volume was measured for the rats using digital Plethysmo meter (UgoBasile, Italy Model 7150) before the administration of Egg white for all groups. Paw volumes were again measured at 30-minute interval for 3 hour. The mean paw edema value for test group being compared with its mean value for control group.
**Fig. 3:** Inflammation of Egg white

**Fig. 4:** Egg white induced paw edema (left leg)
2.5. Statistical Analysis
The data obtained were expressed as mean ± SEM. Statistical analysis were performed by one way analysis of variance (ANOVA) followed by student’s test. At 95% confidence interval, P values < 0.001 were considered significant (table-1).

3. RESULT & DISCUSSIONS
3.1. Result

Table.No. 2 Anti-inflammatory activity of petroleum spirit extract of Caralluma umbellata

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Diameter Of Right Paw (mm)</th>
<th>Diameter Of Left Paw (mm)</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 Min</td>
<td>30 Min</td>
<td>60 Min</td>
</tr>
<tr>
<td>Control (Untreated)</td>
<td>0</td>
<td>25.33 ± 0.877</td>
<td>39 ± 1.00</td>
<td>37 ± 0.578</td>
</tr>
<tr>
<td>Standard (Indomethacin)</td>
<td>10 mg/kg</td>
<td>23.67 ± 0.334</td>
<td>37.33 ± 0.663</td>
<td>33.67 ± 0.878</td>
</tr>
<tr>
<td>Test (Carumbelloside-III)</td>
<td>10 mg/kg</td>
<td>23.67 ± 0.334</td>
<td>38.33 ± 2.725</td>
<td>34.67 ± 3.377</td>
</tr>
<tr>
<td></td>
<td>20 mg/kg</td>
<td>23.67 ± 0.334</td>
<td>37.9 ± 2.845</td>
<td>34.43 ± 3.654</td>
</tr>
<tr>
<td></td>
<td>40 mg/kg</td>
<td>23.67 ± 0.334</td>
<td>38.02 ± 2.255</td>
<td>33.67 ± 2.736</td>
</tr>
</tbody>
</table>

Treatment with the petroleum spirit extract of Cralluma umbellate (Carumbelloside-III) induced anti-inflammatory effect with the highest effect at 40 mg/kg as compared to the effect of the conventional drug indomethacin. The extract showed maximum inhibition of 54.64% at dose of 40 mg/kg body weight after 180 min of drug treatment in Egg white-induced paw edema as compared to 62.4% inhibition after 180 min of indomethacin treatment. The results indicate also that the petroleum spirit extract of C.umbellata has a significant anti-inflammatory effect against Egg white-induced paw edema. Interestingly, the anti-inflammatory effect of petroleum spirit extract of C.umbellata shows approximately equal response compared with that of the conventional drug indomethacin. Indomethacin, a non-steroidal anti-inflammatory drug (NSAID) is commonly used for the treatment of inflammation. Indomethacin reduces inflammation and swelling by inhibiting prostaglandin synthesis or production (Ojewole, 2006). The extract caused a marked inhibition (54.64%) at a dose of 40 mg/kg after 180 min of drug treatment in the Egg white-induced paw edema model. The results of the present study suggest that the C.umbellata used probably produced its anti-inflammatory effect by inhibiting the release, synthesis and/or production of inflammatory mediators, including polypeptide (kinins), prostaglandin and so forth, like indomethacin.
Fig.5: Morphological representations of rat paw after sub-plantar administration of petroleum spirit extract of C.umbellata complete adjuvant indicated production of inflammation (A) normal rat (B) control rat (C) Indomethacin (10 mg/kg) treated rat (D) Carumbelloside-III (40 mg/kg) treated rat

3.2. Discussion

The petroleum spirit extract of Caralluma umbellata (Asclepiadaceae) is recommended for treating pain and inflammation in folk medicine. Chemical investigations of Caralluma umbellata resulted in the isolation of the pregnane glycosides known as Carumbelloside-III one of the phyto constituent of the plant. The anti-inflammatory activity of Carumbelloside-III was evaluated in Egg white-induced induced rat paw edema method. Chemically induced vascular permeability can causes an immediate reaction and its inhibitions suggests that the administration of test formulation petroleum spirit extract of Caralluma umbellata may effectively suppress the exudative phase of acute inflammation induced by undiluted fresh egg white. Carumbelloside-III was administered in three different doses level 10mg/kg, 20mg/kg and 40mg/kg in a dose dependant manner. As shown in Table.2, the Carumbelloside-III, showed maximum inhibitions, 54.64% at the dose of 40mg/kg after 3 hours of drug treatment in Egg white-induced paw edema, whereas standard drug showed 62.4% of inhibition.
The compound up to a dose of 80mg/kg/i.p. did not produce any toxicity symptoms. From current study of the isolated compound Carumbelloside-III from *Caralluma umbellata* showed the significant anti-inflammatory activity.

### 4. CONCLUSION

In view of our interest in the chemical constituents of indigenous medicinal plants, the chemical examination of the dried whole plant 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-II from the Petroleum spirit extract from *Caralluma umbellata*. Determination of anti-inflammatory activity is based on plethysmographic measurement of oedema produced by sub planar injection of egg white in the hind paw of rat. The increase in oedema in animals treated with standard drug (Indomethacin) and test formulation were compared with increase in oedema of untreated control animals at constant intervals of 0, 30, 60, 120 and 180 mins. Thus percentage inhibition of oedema at known intervals in treated animals was used for the purpose of calculating percent inhibition of oedema of control. The present study revealed that the test formulation showed better anti-inflammatory activity. The maximum activity was observed during 2nd and 3rd h, and the results are significant and are comparable to standard indomethacin. The anti-inflammatory activity may be due to the inhibition of release of histamine, serotonin and kinins in the first three hour after the injection of Egg white, and this also retarded the release of prostaglandin-like substance in 2-3 hrs, showing anti-inflammatory potential of test formulation. The above obtained evidences for the anti-inflammatory study verify the objective of the present study. These findings justify that the plant *Caralluma umbellata* used traditionally for the treatment of pain and inflammatory conditions.

### 5. REFERENCES


