Evaluation of anti-inflammatory activity of seeds extract of Luffa Cylindrica

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ABSTRACT

The present work emphasizes on screening of the seed oils and other secondary metabolites from Cucurbitaceae seeds. The oil, CU-01, its unsaponifiable matter, CU-02 and the cucurbitacin, CU-03 extracted by Soxhlet method from the seeds of Luffa cylindrica was examined for its acute anti-inflammatory at doses of 1ml, 50mg and 50mg/kg body weight respectively. It was evident that CU-02 significantly inhibited the paw edema formation induced by carrageenan when compared to CU-01 and CU-03. However the percentage of inhibition was lesser when compared to the standards diclofenac sodium gel and flurbiprofen gel. So, by observing the all comparison studies, we concluded that CU-2 is having the maximum potency for the anti-inflammatory activity.

Key words: Luffa cylindrica, Paw oedema, Anti-inflammatory activity, Cucurbitacin-1 (CU-1), Cucurbitacin-2(CU-2), Cucurbitacin-3(CU-3). Flurbiprofen gel, Diclofenac sodium gel.

INTRODUCTION

Inflammation is the complex biological response of vascular tissue to harmful stimuli, such as pathogens, damaged cells or irritants. Inflammatory diseases are major cause of morbidity of the working force throughout world. Inflammatory responses of the body are achieved by the increased movement of plasma and leukocyte from the blood into the insured site. Although inflammatory diseases are the oldest known to mankind, there is no substantial progress in the therapeutic regimen of inflammation in terms of efficacy and safety. Many commercially available products have produced a dramatic symptomatic improvement in inflammatory conditions but cannot arrest the progress of disease poses and all of them shared some undesirable side effects.1

Herbal drugs have been used since ancient times as medicines for the treatment of range of diseases. Medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. A large proportion of the Indian population for their physical and psychological health needs depend upon traditional systems of medicine. Medicinal plants have become the focus of intense study in terms of conservation and as to whether their traditional uses are supported by actual pharmacological effects or merely based on folklore.2-5

The plant Luffa cylindrica (Linn.) M. Roem. Syn. L. aegyptica Mill. Ex Hook. f. belonging to family Cucurbitaceae is commonly called as Rajakoshaataki or Sponge gourd. Vegetable Sponge is a large climber with slender, slightly hairy, furrowed stem, cultivated throughout India. A search of the literature revealed that many of the medicinal or toxic properties were in the area of chemical constituents often referred to as bitter principles,3,5,6 which are present in about 64 species belonging to 30 genera, these principles being found in various parts of the plants, viz fruits (37 species, 9 genera), roots (24 species, 18 genera) and leaves (8 species). Also most of the investigations reported were either from roots, leaves or whole of the aerial part extracts.

During a routine screening of this plant for medicinal activity, the seeds were reported to contain about 35% of crude protein7 showing ananthelmintic, cytotoxic activities8,9 besides as flavanoids10-11. The ethanolic extract of the seeds of L. cylindrica showed more than 80% fungitoxic activity against Helminthosporium oryzae,12,13 whereas the alcoholic extract of the leaves and roots of certain species of Cucurbitaceae showed anti-inflammatory activity similar to phenylbutazone14. The aqueous extracts of L. chinensis fruits significantly lowered bilirubin levels in chlorpromazine induced jaundiced rats15. The alcoholic and other extracts of the whole plant showed definite protection against carbon tetrachloride induced liver injury in rats. The ether extract exerted significant stimulation of isolated guinea pig ileum. In a few reports, work was carried on seeds which again are mostly from the plants belonging to other genera. Even the reports from the genus Luffa showed that there were very few reports on Luffa cylindrica. Although the family has been investigated extensively, it is very obvious that there is little information on seed phyto-constituents. Since many of the Cucurbitaceae produce seeds rich in oil and protein and there is no specific investigation on Luffa cylindrica, we have taken the seed constituents oil of L. cylindrica for the present work16-17.

MATERIALS AND METHODS

The seeds of Luffa cylindrica were obtained from wild source and authenticated (Authentication No.- PARC/ 2008/173) by Prof. P. Jayaraman, Director, National Institute of Herbal Science, Plant Anatomy Research Centre, West Tambaram, Chennai- 600 045. Carrageenan sodium salt 1% w/v were obtained from the S.D.Fine Chem, Ltd, Mumbai. Diclofenac Sodium Gel 1% w/v and Flurbiprofen gel were obtained from the Win Medicare and Knoll – Abbott as gift samples.

Preparation of Extract18,19

Dried seeds of Luffa cylindrica were extracted by continuous hot percolation method using solvents of increasing polarity from petroleum ether to ethanol. The crude extracts obtained by using the different solvents such as petroleum ether, benzene, chloroform and alcohol were subjected to preliminary phytochemical screening for the presence of bioactive constituents. Later the promising extracts namely petroleum ether and benzene extracts were subjected to column chromatography on silica gel and the various fractions screened for significant anti-inflammatory properties by carrageenan-induced paw oedema in rats. The spectral data of the obtained extract having the isolated compounds spectral data were mentioned in the table no:-01. The oil characteristics of cucurbita seeds were determined by using the different tests and the

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results are shown table no:02.

### Animals

Albino (Wister) rats 150–250g of either sex were used. The animals were kept in the standard polypropylene cages and provided with food and water ad libitum. The animals were acclimatized for a period of 14 days prior to performing the experiments. The experimental protocols were approved by Institutional Animal Ethics Committee (Regn No: 1220/a/08/CPCSEA).

### Acute Toxicity Study

Acute toxicity study was performed according to the OECD-423 guidelines 7. Albino (Wister) rats of either sex were used. The animals were fasted for 4 h, but allowed free access to water throughout. The fasted mice were divided into different groups of six animals each. The Extract was administered orally at a dose of 50mg/kg. The control animals received a similar volume of 2% (v/v) aqueous Tween 80 solution. Mortality in each group was observed for 7 days. As no mortality was observed, the procedure was repeated at doses 50, 100, 200 and 300 mg/kg.

### Carrageenan Induced Paw Edema

Anti-inflammatory activity was evaluated using the carrageenan induced rat paw oedema technique. Albino rats of Wistar strain (150–250g) were conditioned at 20–25°C and maintained on standard pellet and water ad libitum. They were kept in 12/12 h light/dark cycle. After 12h of fast the rats were divided into five groups of six each. Group I served as control group and received Tween 80 (5 ml/kg) of 2% w/v, orally. Group II served as standard group and received Diclofenac Sodium gel. Group III, IV, V animals received isolated compounds of CU-1, CU-2, CU-3 at a dose of 50kg as a fine suspension in 2% v/v aqueous Tween 80 solution orally. All Albino rats were injected with 0.1 ml of 1% w/v carrageen solution in normal saline into the sub plantar tissue of the left hind paw of the rat, after one hour of respective drug samples given to the respective groups of rats. The paw volume was measured before and at 30 minutes, 1, 2, 3, 4, and 5 hour after the injection of carrageenan by the volume displacement method using a mercury column connected to pressure transducer. The output from transducer was led to a four-channel polygraph (Polyrite, Recorders and Medicare Systems, Ambala), amplified and recorded by a pen recorder (Omniscribe, Digital Electronics, Mumbai). The edema volume was determined and expressed as percentage swelling and, compared with the edema volume of 2% (v/v) aqueous Tween 80 solution. Mortality in each group was noted. The animals were acclimatized for a period of 14 days prior to performing the experiments. The experimental protocols were approved by Institutional Animal Ethics Committee (Regn No: 1220/a/08/CPCSEA).

### Statistical analysis

The experimental data were calculated as mean ±SEM, evaluated by unpaired one way anova test. The Values of p<0.001 were considered as statistically significant.

### Table No. 01 Spectral data of the isolated compounds of Luffa cylindrica seeds

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Drug (50mg/kg)</th>
<th>Paw volume in ml ± SEM and percentage of inhibition</th>
<th>0 hour</th>
<th>0.5 hour</th>
<th>1 hour</th>
<th>2 hour</th>
<th>3 hour</th>
<th>4 hour</th>
<th>5 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.372±0.034</td>
<td>0.241±0.034</td>
<td>0.543±0.034</td>
<td>0.621±0.034</td>
<td>0.754±0.034</td>
<td>0.821±0.034</td>
<td>0.897±0.034</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>0.371±0.034</td>
<td>0.286±0.034</td>
<td>0.323±0.034</td>
<td>0.275±0.034</td>
<td>0.284±0.034</td>
<td>0.204±0.034</td>
<td>0.185±0.034</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Cu – 01 (1ml/kg)</td>
<td>0.372±0.034</td>
<td>0.334±0.034</td>
<td>0.396±0.034</td>
<td>0.385±0.034</td>
<td>0.412±0.034</td>
<td>0.362±0.034</td>
<td>0.315±0.034</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Cu – 02 (50mg/kg body weight)</td>
<td>0.373±0.034</td>
<td>0.291±0.034</td>
<td>0.356±0.034</td>
<td>0.332±0.034</td>
<td>0.334±0.034</td>
<td>0.276±0.034</td>
<td>0.244±0.034</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Cu – 3 (crystals) (50mg/kg)</td>
<td>0.371±0.034</td>
<td>0.346±0.034</td>
<td>0.415±0.034</td>
<td>0.407±0.034</td>
<td>0.432±0.034</td>
<td>0.374±0.034</td>
<td>0.356±0.034</td>
<td></td>
</tr>
</tbody>
</table>

### RESULTS AND DISCUSSION

Pharmacological study on Luffa cylindrica was carried out on the basis of traditional literature that the plant has anti-inflammatory activity. The extracts were selected for the present study, on the basis of increased yield obtained through soxhlet extraction methods using different solvent systems based on the polarity of the solvent system. The obtained extracts were subjected for column chromatography and other analytical studies for the isolation of active constituents such as CU-1, CU-2, CU-3. Spectral data of the isolated compounds of Luffa cylindrica seeds was mentioned in the table no: - 01 and oil characteristics of Cucurbita seeds were also shown in table no: - 02. Three compounds were isolated, characterized and named as CU-01, CU-02, and CU-03. CU-01 is oil both in physical appearance and by chemical analysis. The characteristics of this oil have been compared with pumpkin oil. This oil has shown more unsaturation and less acid value.

### Table No. 02 Oil characteristics of Cucurbita seeds

<table>
<thead>
<tr>
<th>Assay</th>
<th>Pumpkin seeds</th>
<th>L. cylindrica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Value</td>
<td>0.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Iodine Value</td>
<td>103.2</td>
<td>121.5</td>
</tr>
<tr>
<td>Saponification Value</td>
<td>199.3</td>
<td>194.8</td>
</tr>
<tr>
<td>Unspoilifiable Matter</td>
<td>0.67</td>
<td>0.65</td>
</tr>
<tr>
<td>R.M. Value</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Refractive Index</td>
<td>1.4616</td>
<td>1.4662</td>
</tr>
</tbody>
</table>

### Table No. 03 Anti-inflammatory Activity of Luffa cylindrica seeds

The effect of Luffa cylindrica seed constituents on carrageenan-induced oedema are shown in Table 03 and 04. The sterols or related compounds of the oil.

The best anti-inflammatory activity of the isolated compound was again compared with the marketed available flurbiprofen gel. The above mentioned procedure was followed for the determination of potency of the isolated compound of CU-2. Again rats were divided into three groups, each group containing six animals. A control group of rats received orally 5ml of the normal saline solution. The reference standard group received flurbiprofen gel on paw. It was applied gently to the affected area 3-4 times. Similarly the test group received orally 50mg/kg CU-2. Then above mentioned procedure was followled.

The experimental data were calculated as mean ±SEM, evaluated by unpaired one way anova test. The Values of p<0.001 were considered as statistically significant.
From the above, it was evident that CU - 01 and CU – 03 showed moderate inhibition of inflammation in doses of 1 ml and 50 mg/kg body weight respectively. The anti-inflammatory effect of CU-02 was significant at doses of 50mg/kg body weight but was less than that of standard. The anti-inflammatory activity of different constituents of Luffa cylindrica, were graphically also represented in the graph no: - 01 and 02.

CONCLUSION

Earlier reports revealed that the anti-inflammatory activity was due to iso plumbagin and various other phytoconstituents of the plant. These were reported only in the alcoholic extract of a few Luffa species that too in other genera. Against this background, we have directed our work towards seed oils and extracts. Later these oils will be screened for their nutritive utility for human and domestic animals. The three isolated compounds were evaluated for anti-inflammatory activity by using carrageenan induced paw oedema method comparing with marketed available diclofenac sodium gel. Among this, the best anti-inflammatory activity by using carrageenan induced paw oedema method with marketed available diclofenac sodium gel. In this work CU-2 having the higher percentage of inhibition when compared with CU-1, CU-3 and lesser percentage of inhibition available flurbiprofen gel. So in this work CU-2 having the higher percentage of inhibition when compared with CU-1 and CU-3 and lesser percentage of inhibition available flurbiprofen gel.

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