



## ANTI INFLAMMATORY, ANALGESIC AND PHYTOCHEMICAL STUDIES OF *CLITORIA TERNATEA* LINN FLOWER EXTRACT

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Article Received on: 14/01/12 Revised on: 21/02/12 Approved for publication: 19/03/12

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### ABSTRACT

*Clitoria ternatea* Linn is a well known bio active plant in the Ayurvedic medicine. The present study evaluates the analgesic and anti-inflammatory activity of *Clitoria ternatea* Linn flower extract. The petroleum ether (60-80°C) extract poses significant anti inflammatory, analgesic properties. The extract is found to be safe even at the dose of 2000 mg/kg body weight by acute toxicity studies. Phytochemical investigation carried out on Petroleum ether(60-80°C) extract reveals the presence of Taraxerol, a pentacyclic triterpenoid which may impart the pharmacological activity of the extract.

**Key words:** *Clitoria ternatea*, Anti inflammatory, Analgesic, Phytochemical investigation, Eddy's hot plate, Taraxerol,

### INTRODUCTION

The traditional Indian system of medicine has a very long term history of usage in a number of diseases or disorders, but lacks safety and efficacy data for development of standardized safe and effective herbal formulations with proven scientific evidence provide an economical alternative in several disease areas.<sup>1</sup>

*Clitoria ternatea* Linn belongs to family Fabaceae. It is a well known plant in the traditional medicine. The juice of the flowers is reportedly used in insect bites and skin diseases, the paste of the flowers is applied to cure infections of eye, and entire plant is used as antidote for snake bites.<sup>2</sup>

The present investigation evaluated the anti-inflammatory, analgesic activities of *Clitoria ternatea* flowers to provide experimental evidence for its traditional use. The petroleum ether extract (60-80°C) prepared from the flowers of *Clitoria ternatea* Linn will be subjected to analgesic, anti inflammatory studies using animal experiment models which was approved by Institutional and Animal Ethical Committee. Phytochemical studies of this extract were also carried out to isolate active constituents.

### MATERIALS AND METHODS

#### Collection and authentication of plant material

The flowers of *Clitoria ternatea* Linn were collected from places around Mangalore and authenticated by Mrs Neoline Pinto, H.O.D, Department of Botany, St. Agnes College, Mangalore and a voucher specimen was deposited in Shreedevi College of pharmacy, Mangalore.

#### Preparation of flower extract

50 g of dried flowers of *Clitoria ternatea* Linn were extracted with Petroleum ether( 60-80° C) by using soxhlet extractor.<sup>3</sup> The extracts were concentrated with the help of vacuum evaporator and kept in the desiccator.

#### Preliminary phytochemical screening

The flower extract prepared were then subjected to qualitative tests for the identification of various plant constituents such as alkaloids, glycosides, phytosterols, saponins, phenolic compounds, tannins, proteins, amino acids and carbohydrates.

### Pharmacological investigation of Petroleum ether (60-80° C) extract

#### Acute toxicity studies

Acute toxicity studies were determined according to OECD guidelines 425<sup>5</sup>. Female albino rats were used for the study. A total of five animals were used. The animals were fasted overnight prior to the experiment and maintained under standard conditions. The dose of 2000mg/kg body weight was administered to the animals and was observed for any behavioral changes.

#### Anti inflammatory activity studies

Anti inflammatory activity studies of *Clitoria ternatea* Linn flower extracts were carried out by carrageenan paw edema method.<sup>6</sup>

Healthy albino rats of either sex were used for the study. The animals were grouped into four of six animals in each group. Group I served as control and was administered orally, 1ml 0.5% tween 80, Group II receives diclofenac sodium, Group III and IV receives the test drugs (200 and 400mg/kg respectively) prepared in 0.5% tween 80. After the administration of the drugs 0.1ml of 1% carageenan in normal saline was injected into the right hind paw under the plantar aponeurosis of all the animals. The volume of hind paw edema was measured by plethysmometer just before and after 3hrs of carageenan administration and data recorded in

#### Table 1

#### Analgesic activity studies

The extract prepared from *Clitoria ternatea* Linn flowers were subjected to analgesic activity studies by Eddy's hot plate method.<sup>7</sup>

The analgesic activity was studied by Eddy's hot plate method. Albino mice of either sex weighed between 25-30 g were selected for study. In this experiment hot plate was maintained at  $55 \pm 5^\circ\text{C}$ . A cut off period 15 sec was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws or jump response which ever appeared first. The basal reaction time was noted before and 30,60,90 and 120 min after the administration of the drugs. The animals were divided into four groups of six animals in each group. Group I served as control (received 1% tween 80, 5ml/kg orally) Group II served as standard and given pentazocine (5mg/kg i.p) body

weight. The other groups (III and IV) received the test drugs and data recorded in Table 2.

#### Phytochemical studies of Petroleum ether(60-80°C) extract

150 gm of silica gel (lab grade) was activated in hot air oven for 1 hour. The petroleum ether (60-80°C) was used to build the silica gel in the glass column. The activated silica gel was charged into the column in small portions with gentle tapping to get uniform bed of adsorbent. The petroleum ether extract (60-80 °C) of *Clitoria ternatea* Linn flowers was adsorbed in small quantities of silica gel and charged into the column.

The column was eluted with various solvents of increasing order of polarity. The elution was monitored by TLC (anisaldehyde reagent and iodine chamber). Each time 5ml was collected and identical eluents were combined and concentrated. The chloroform, petroleum ether (60-80 °C) eluent (30:70) gave a single spot on TLC which were combined, concentrated and evaporated. The eluent after recrystallisation gave colorless needle shaped crystals and designated as SCFP-1. The compound SCFP-1 gave positive test for steroids.

#### RESULTS

##### Preliminary phytochemical screening

The preliminary phytochemical investigation showed the presence of Steroids, Triterpenoids, Saponins, Resins, Tannins and Starch in the petroleum ether (60-80°C) extract.

##### Acute toxicity studies

The acute toxicity study showed that Petroleum ether (60-80°C) flower extract of *Clitoria ternatea* Linn was found to be safe at the dose of 2000 mg/kg bodyweight. On the basis of the study doses 200 and 400 mg/kg were selected for further pharmacological evaluation.

##### Anti-inflammatory, analgesic activity studies

The anti-inflammatory, analgesic studies of Petroleum ether extract (60-80°C) from the flowers of *Clitoria ternatea* Linn showed that it exhibited significant anti-inflammatory activity at both the dose levels (200 and 400 mg/kg body weight)( $P < 0.01$ ) while the analgesic activity was exhibited at the dose level of 400mg/kg bodyweight. ( $P < 0.01$ ).

##### Phytochemical studies of Petroleum ether (60-80°C) extract

##### Characterization of compound SCFP-1

Colour: Colorless

R<sub>f</sub> value: 0.51(Chloroform, Petroleum ether (60-80°C, 6:4)

Melting point: 283°C -285°C

##### Spectral characterization

##### I.R spectra

3344 (broad -OH), 2925, 2849 (C-H stretching in CH<sub>3</sub> and CH<sub>2</sub>)

1736 C=C- stretch, 1460,1472 C-H deformation in CH<sub>3</sub>,1377 C-H deformation in gem dimethyl, 1016 C-O stretch in alcohol 1016C-H bend out of plane.

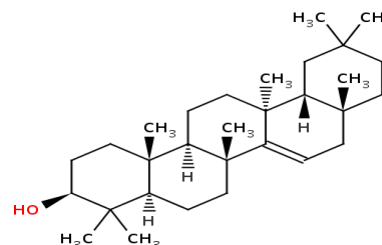
##### Mass spectra

The Mass spectrum exhibited a molecular ion peak m/z 426.

##### H<sup>1</sup> NMR

1. Triplet observed at  $\delta$  5.3 indicating the presence of =CH proton at C<sub>15</sub> carbon atom

2. Peaks observed at  $\delta$  1.9 and  $\delta$  2.3 with integral value 2.3 and 1.4 indicating CH<sub>2</sub> protons at C<sub>2</sub> and C<sub>16</sub>.
  3. Peaks at  $\delta$  0.8 and  $\delta$  0.9 with integral 8.64 and 4.2 indicating four methyl group present at C<sub>4</sub> and C<sub>20</sub>.
  4. Peaks observed in the region 1.2-1.6 with total integral 33 indicating presence of CH<sub>2</sub> protons of remaining carbon atom.
- The spectral data of compound SCFP-1 found to be in good agreement with that of Taraxerol.



#### DISCUSSION

The mechanism involved in the genesis of the carrageenan induced edema can cause the release of prostaglandins and kinnins, among other substances<sup>8</sup>. The three important aspects of inflammation that render themselves readily to measurement are erythema (local vasodilation), edema (increased capillary permeability)

and granulation time.<sup>9</sup> The present study revealed that the test drug shows significant protection against carrageenan induced paw edema. The analgesic activity was observed at a higher dose level. The phytochemical investigation carried out on petroleum ether extract (60-80°C) results in the isolation of Taraxerol a pentacyclic triterpenoid. The characterization was done by using spectral and other analytical data. The presence of Taraxerol may impart the anti-inflammatory, analgesic property of this extract. Hence the present study validates the use of *Clitoria ternatea* Linn flowers as an analgesic, anti-inflammatory agent in the traditional medicine.

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Table 1: Anti-inflammatory activity of *Clitoria ternatea* Linn flower extracts

Groups	Treatment	Paw volume (diff) After 3 hrs (in ml)	% inhibition
I	Negative control (carrageenan)	0.29 ± 0.002	0
II	Diclofenac sodium (50mg/kg)	0.18 ± 0.003**	38
III	P.E 200	0.25 ± 0.002**	14
IV	P.E 400	0.23 ± 0.005**	21

ANOVA followed by Dunnet's multiple comparison test used. All Values are expressed as mean ± SEM, N=6.

\*\*p < 0.01, when compared to negative control.

Abbreviations: P.E – Petroleum Ether extract (60-80 °c)

**Table2: Analgesic activity of *Clitorea ternatea* Linn flower extracts**

Groups	Treatment (n=6)	0 minutes	30 minutes	60 minutes	90 minutes	120 minutes
I	Control (Tween-80)	2.48 ± 0.09	2.69±0.04	2.59±0.11	2.78±0.06	2.50±0.01
II	Pentazocine (5m g/kg)	2.50±0.04	6.98±0.21**	8.48±0.20**	8.98±0.09**	8.31±0.11**
III	P.E (200mg/kg)	2.62±0.06	2.72±0.05	2.75±0.08	2.63±0.13	2.96±0.02
IV	(400mg/kg)	2.61±0.0	5.21±0.04**	7.08±0.03**	7.2±0.06**	7.38±0.14**

ANOVA followed by Dunnet's multiple comparison test used. All Values are expressed as mean ±SEM, N=6, \*\*p<0.01, when compared to control.  
Abbreviations: P.E –Petroleum Ether extract (60-80°c)

Source of support: Nil, Conflict of interest: None Declared