

## Anti-inflammatory, Antioxidant and Phytochemical properties of *Clematis gouriana* Roxb. ex. DC. Leaves.

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**ABSTRACT:** The ethanolic leaf extract of *Clematis gouriana* Roxb.ex.DC. were assessed for its phytochemical, antioxidant and anti-inflammatory effect in animals. The extract showed a significant inhibition of carrageenan-induced rat paw edema in Wistar rat compared to the standard anti-inflammatory drug indomethican. Antioxidant activity was evaluated through (in vitro) assays such as DPPH radical scavenging assays and total antioxidant capacity by phosphomolybdenum method.

**Key Words:** *Clematis gouriana*, indomethican, Anti-inflammatory, DPPH

### FIGURES AND TABLES



Figure 1: *Clematis gouriana*

### Introduction

Medicinal plants have been identified and used throughout human history. Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. Drugs currently used for management of pain and inflammatory conditions present toxic side effects on chronic administration. This enables herbal medicines to have beneficial pharmacology and attempt are being taken to study promising plants which may lead to develop newer drugs.

*Clematis gouriana* Roxb. (family: Ranunculaceae) is a large climber locally known as bridal bocquet. Leaves are opposite, exstipulate, paniced inflorescence with white flowers, brown stems and are grooved. Traditionally the rhizomes and leaves of clematis gouriana are used as analgesic, abbirative, antibacterial, antipholgestic, anticancer and diuretic agent. Leaves are used for the treatment of blisters and as a poultice for festering wounds and ulcers. In a study of 120 people with gallstones it was found that *Clematis* was 87 percent effective in treating symptoms (Rana et al.,2015). It is also very effecient for healing mastitis – one study showed that with two days of treatment they were totally healed of mastitis. It can be toxic in large doses and should be used under the supervision of an eminent physician. It is mainly used for its curative property for healing wounds, fever and musculo skeletal disorder. However till date no scientific

validation of these properties have been reported. Hence the present study was carried out to scientifically evaluate the anti-inflammatory potential of *Clematis gouriana*.

## Materials and methods

### Plant material

The leaves of *Clematis gouriana* Roxb; were collected from Peringammala. The specimens of the plant were deposited in the Herbarium of JNTBGRI with voucher no:TBGT - 86806 dt 1/03/2016.

### Extraction

Collected leaves were shade dried then again kept in oven for drying. This is then powdered using a mechanical grinder. 40g of powdered plant material was extracted by cold extraction method using solvent ethanol. Obtained crude extracts were evaporated and removed excess ethanol by using a rotary evaporator.

### Experimental Animals

Wistar rats (170 to 275 gm) and Swiss albino mice, (35 to 45 gm), of either sex, were obtained from the animal house of JNTBGRI, Palode. They were grouped and housed in poly acrylic cages (three animals per cage) and maintained under standard laboratory conditions (24 - 28 C, relative humidity 60-70% and 12 hours' dark light cycles). They were fed commercially rat feed (Lipton India Ltd, Mumbai, India) and boiled water (ad libitum). All animals were carried out according to NIH guidelines, after getting the approval of the Institutes Animal Ethics committee.

### Acute toxicity study

Six groups of 2 mice were administered 50, 150, 450, 1400, 2500 and 5000 mg/kg of CG extract respectively, maintaining appropriate controls. All the animals were observed continuously for the first 3 h then 1 h intermittently up to 24 h for behavioral changes like convulsions, hyperactivity, sedation, grooming, loss of righting reflex, epilation, respiratory rate, food and water intake, state of faecal pellets, and mortality. The animals were observed for post treatment toxic symptoms daily for 14 days after treatment.

### Anti-inflammatory activity

#### Carrageenan induced paw oedema

The anti - inflammatory activity was studied in groups of three rats. Oedema was induced according to the method of Neha et al., (2013). Briefly, 0.1ml of 1% carrageenan was injected into the right hind paw, under the planar aponeurosis (carrageenan controls). A similar volume of 0.5% Tween80 was injected into the left hind paw of the animals in one group (vehicle controls). The hind paw volume of the vehicle controls and the carrageenan controls and the drug treatment groups was measured before carrageenan injection plethysmographically and the time course of edema formation was followed for 3 hours. In a separate group of animals, indomethacin (10 mg/kg) was administered orally 30 min before carrageenan injection. The volume of increase of the inflamed paw was estimated by subtracting the volume of the control hind paw. The anti-inflammatory activity of the plant extract was esteemed as the degree of oedema inhibition.

### Antioxidant studies

#### DPPH based free radical scavenging activity method

The free radical scavenging activities of *Clematis gouriana* extract (CGE) was carried out using DPPH based free radical scavenging activity method (Blois et al., 1958). To 2ml of 0.2 Mm DPPH, 0.2 ml of CGE in methanol at varying concentrations (25, 50, 100, 150, 200 µg/ml) was added. Simultaneously a control was prepared without adding the extract. The reaction mixture was mixed well and incubated for 20 minutes in the dark at 28 C. Then the scavenging activity of each concentration of CGE was determined by measuring the absorbance (abs) of the decolorized solution at 515 nm using a spectrophotometer against the control solution.

#### Total antioxidant activity using phosphomolybdenum method

The total antioxidant capacity of *C.gouriana* extract (CGE) was obtained by phosphomolybdenum method (Prieto et al., 1999). 0.2 ml of CGE solution (200 µg/ml) in the respective solvent was mixed with 2 ml of reagent solution (6 M sulphuric acid , 28 mM sodium phosphate , 4 mM ammonium molybdate ). The reaction mixture was incubated at 95 c for 90 min. The blank solution contained 2 ml of reagent solution and the approximate volume of same solvent used for the sample. The absorbance of the solution was measured 695 nm against blank. The antioxidant capacity of CGE was evaluated as equivalents of ascorbic acid.

### Phytochemical analysis

Preliminary phytochemical tests were carried on the extracts to detect the various constituents present in them (H. Wagner, 1993; J.B Harborne, 1998; Gahan, 1984).

### Detection of Alkaloids

To a few ml of filtrate, few drops of Wagner's reagent are added by the side of the test-tube. A reddish brown precipitate confirms the presence of alkaloids.

### **Detection of Flavonoids**

Shinoda's test

The extract (50 mg) was dissolved in 5ml of alcohol and few fragments of magnesium ribbon and concentrated HCL acid drop wise added. Presence of flavonol glycosides was inferred by the development of pink to crimson colour

### **Detection of Phenols**

The methanol extract is treated with magnesium turnings followed by concentrated HCL acid which is added in drops. The appearance pink scarlet /intense red/green to blue colour indicates the presence of phenols.

### **Detection of Tannins**

About 0.5g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride (10mg in 10ml) was added and observed for brownish green or a blue black colorization.

### **Detection of Coumarins**

To the test solution add a few ml of alcoholic sodium hydroxide solution. The appearance of intense yellow colour on addition of con. HCL acid indicates its presence of Coumarin.

### **Detection of Steroids**

Fehling's test

1ml of filtrate was treated with each 1ml of fehling's solution A and B and boiled in a water bath. A reddish precipitate was obtained which shows the presence of sugar.

### **Detection of Oils**

A small quantity of extract was pressed between two filter paper oil stain on the paper indicates the presence of fixed oils.

### **Detection of Saponins**

50 mg of extract is diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. The formation of 2 cm layer of foam indicates the presence of saponin.

### **Detection of Anthraquinones**

0.5g of the extract was boiled with 10 ml of Conc. sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipetted into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour changes.

### **Detection of Aminoacids**

To 2ml of extract add 2drops of ninhydrin (10 mg of ninhydrin in 200 ml of acetone). Purple colour indicates the presence of amino acids.

### **Detection of Proteins**

Biuret test

An aliquot of 2ml of filtrate was treated with one drop of 2% copper sulphate solution. To this 1ml of ethanol (95%) was added, followed by excess of KOH pellets. Pink colour in the ethanolic layer indicates the proteins.

### **Estimation of total phenolic content**

Total phenolic content (TPC) of CGE extract was determined according to the method described by Lachman *et al.* (2000). 0.5 ml of each extract, 2.5 ml Folin-Ciocalteu reagent, 2 ml of 7.5% (w/v) sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) were mixed. The mixture was incubated at room temperature for 30 min. The absorbance was read using UV-Vis spectrophotometer at 743 nm. Each analysis was performed in triplicates and the values were expressed in mean  $\pm$  standard deviation. The results were expressed as mg GAE (gallic acid equivalents)/g ethanolic extract.

### **Estimation of total flavonoid content**

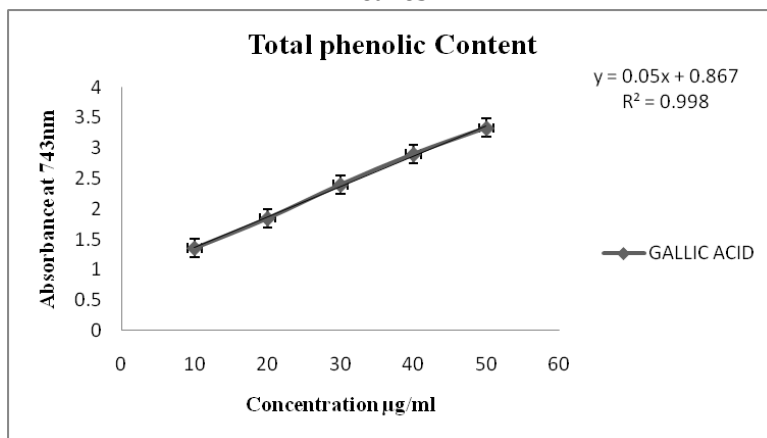
The total flavonoid content (TFC) of CGE extract was determined according to the Aluminum chloride colorimetric method described by Chang *et al.* (2002). The plant extract (0.5 ml) was mixed with 1.5 ml of methanol, 0.1 ml of 10% Aluminum chloride hexahydrate ( $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ), 0.1 ml of 1 M potassium acetate ( $\text{CH}_3\text{CO}_2\text{K}$ ), and 2.8 ml of distilled water. After incubation at room temperature for 30 min. the absorbance of the solution was measured at 415 nm. Each analysis was performed in triplicates and the values were expressed in mean  $\pm$  standard deviation. The results were expressed as mg RE (rutin equivalents)/g ethanolic extracts.

## **Results**

### **Quantification of total phenolic content in ethanolic extract of *Clematis gouriana* Roxb. Leaves:**

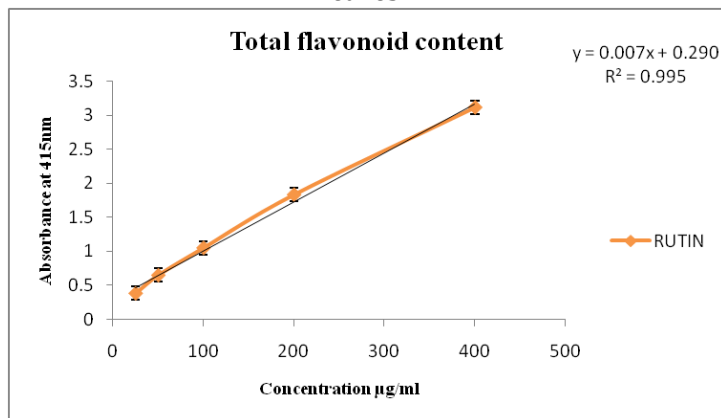
From the standard calibration curve, total phenolic content was found to be 63.2 mg GAE/g of Ethanolic extract(graph1).

Graph 1: Quantification of total phenolic content in ethanolic extract of *Clematis gouriana* Roxb. Leaves:



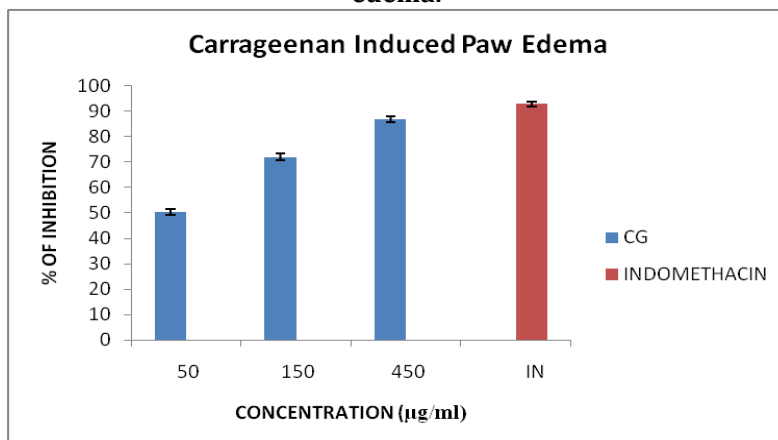
**Quantification of total flavonoid content in ethanolic extract of *Clematis gouriana* (CG) Roxb. leaves:**  
 From the standard calibration curve, total flavonoid content was found to be 99.28 mg GAE/g of Ethanolic extract (graph 2).

Graph 2: Quantification of total flavonoid content in ethanolic extract of *Clematis gouriana* (CG) Roxb. leaves:



**Effect of ethanolic extract of *Clematis gouriana* (CG) leaves on carrageenan induced rat paw edema:**  
*Clematis gouriana* leaf ethanolic extract (CG) at the doses of 150 and 450 mg/kg significantly inhibited the carrageenan induced rat paw edema in rats. At 150 mg/kg dose, there was 70.04 % inhibition and at 450 mg/kg dose 85.45% inhibition, at three hours after carrageenan injection. Indomethacin (10 mg/kg) produced 92.72% inhibition of edema formation(graph 3).

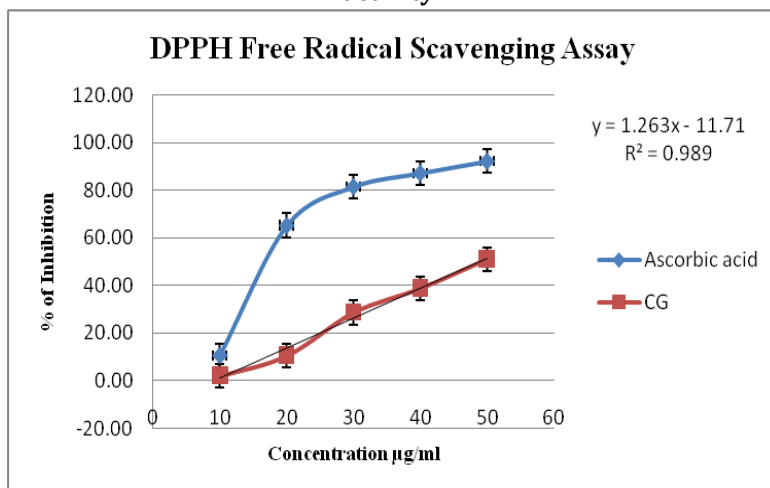
Graph 3: Effect of ethanolic extract of *Clematis gouriana* (CG) leaves on carrageenan induced rat paw edema:



**Effect of ethanolic extract *Clematis gouriana* (CG) leaves in DPPH radical scavenging activity:**

The leaf ethanolic extract of *Clematis gouriana* (CG) at the dose of 50µg/ml exhibited the significant free radical scavenging activity (51.63%) of DPPH (1, 1 - diphenyl -2- picrylhydrazyl). The CG extract exhibited an IC 50 at a concentration of 48.97µg/ml(graph 4)

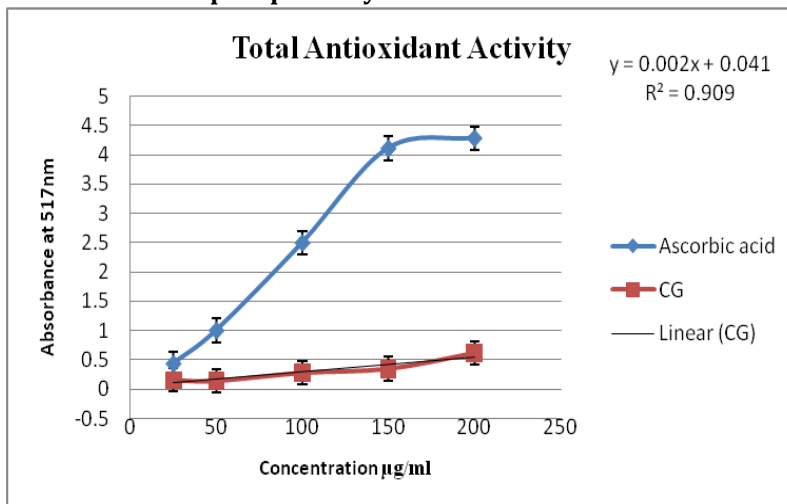
**Graph 4: Effect of ethanolic extract of *Clematis gouriana* (CG) leaves in DPPH radical scavenging activity:**



**Measurement of total antioxidant property of *Clematis gouriana* extracts (CGE) by phosphomolybdenum method.**

The total antioxidant activity of CGE at a concentration of 200µg/ml was found to be equivalent to the activity produced by 163.85 µg/ml ascorbic acid( graph 5).

**Graph 5: Effect of ethanolic extract of *Clematis gouriana* (CG) leaves in total antioxidant activity by phosphomolybdenum method:**



**Phytochemical analysis shows (table 1)**

Table 1: phytochemical analysis shows

Compounds	ethanolic extract of <i>Clematis gouriana</i> (leaves )
Alkaloids	+
Flavonoids	+
Phenols	+
Tannins	+
Carbohydrates	+
Saponins	+

Anthraquinones	-
Coumarin glycosides	+
Proteins	-
Amino acids	-
Steroids	+
Fixed oil	+

+ indicates presence of compound and - indicates absence of compound.

#### Acute toxicity study:

Result of acute toxicity study showed that there was no mortality or any significant change in the behavior of the mice after the administration of *Clematis gouriana* (CG) in graded dose drug showed the nontoxic effect of for 24 hours. (table 2)

Table 2: Acute toxicity studies shows

Parameters	Cage side observation
Mortality	Nil
Writhing	Nil
Saliva Erection	Normal
Pain	Nil
Pilo Excretion	Nil
Food taken	Normal
Tremors	Nil
Straub tail reaction	Nil

#### Discussion

Medicinal plants and their various products are extensively utilized not only in traditional systems of medicines but also modern medicine throughout the world. Despite the various uses of *Clematis* in traditional medicine, there is inadequate information about the anti-inflammatory activity of this plant. This study deals to substantiate the traditional knowledge about the anti-inflammatory activity of the plant used in traditional remedies and studies. In the present study, we have investigated the anti-inflammatory, antioxidant, acute toxicity and phytochemical studies were also carried out. From the preliminary phytochemical screening it can be concluded that the ethanolic extract of CG has potential secondary metabolites may be reason for the potent anti-inflammatory activity of the plant. From the preliminary phytochemical screening it can be concluded that the ethanolic extract of CG has potential secondary metabolites may be reason for the potent anti-inflammatory activity of the plant.

The outstanding feature of the polyphenolic compounds (phenol, flavonoid and tannins) is their ability to block specific enzymes that cause inflammation and also modify prostaglandin pathways and thereby protect platelets from clumping. They protect plant from oxidative damage and they play the same role in humans protecting the tissues from oxidative decay, there by acting as antioxidants (Pushpangadhan, 2011) infectious and inflammatory diseases are among those polyphenols play an important role in antioxidant system in plants. The ethanolic extract of *C.gouriana* has been shown to possess the potent phenolic content. According to the present investigation, the high contents of these phytochemicals in *C. gouriana* can explain its high radical scavenging activity. Total antioxidant capacity of the different extracts of *C. gouriana* was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid ( $y = 0.002x + 0.041$ ;  $R^2 = 0.997$ ). Ethanol extract of *C.gouriana* was found to possess the highest total antioxidant capacity. It was however observed that the ethanolic extract possesses significant total antioxidant capacity equivalent to 163.85 mg/g ascorbic acid at higher concentration. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species (Oktay *et al.*, 2003).

In the toxicity study, no mortality occurred for 14 days with the three doses of CG tested. The results of acute toxicity study revealed that no mortality was recorded for the oral administration of graded doses of ethanolic extract of CG for all the doses after 24 hrs of administration to the animal.

The anti-oedematous property of CG may be due to the inhibition of proinflammatory mediators, free radical scavenging, or membrane stabilizing effects. The synergistic action of the phytochemicals present in CG especially high flavonoid content could be the reason for the proposed anti-inflammatory effects. However; detailed studies are warranted to decipher the exact nature and mechanism of action of the phytochemicals responsible for the therapeutic effects.

## Conclusion

The present investigation thus thrown light on potent anti-inflammatory potential of *Clematis gouriana* thus substantiating the traditional claim. The study also explore the beneficial effect of *Clematis gouriana* as it is a safe and efficient drug for inflammation.

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