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# Study of analgesic and anti-inflammatory potential of hydro-alcoholic extract of *Curcuma angustifolia* leaf

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**Abstract**

The objective of the study was to assess the anti-nociceptive and anti-inflammatory potential of the aqueous extract of *Curcuma angustifolia* leaves. The defatting of leaf powder was achieved by extraction with n-hexane and the de-fatted leaf powder was then extracted with distilled water for collection of maximum amount of water soluble extractives. The extracts were sticky and the aqueous extract was dark brown in color with 14.6% yield. The preliminary phytochemical analysis suggest the presence of saponin glycosides, cardiac glycosides, phenolics, terpenoids, sterols, and flavonoids in the leaves. The total phenolic content of the aqueous extract of *Curcuma angustifolia* was 76.28 ± 0.45 GAE mg/g. The anti-inflammatory activity was assessed using carrageenan induced rat paw edema method while the analgesic action was determined using tail flick method. Ibuprofen at dose of 10 mg/Kg inhibited 80.36% edema after 6h of administration while the CAE 200 and CAE 400 were able to inhibit 46.33% and 51.02% edema formation respectively. The duration of response time in Ibuprofen and *Curcuma angustifolia* was significantly higher as compared to the saline treated animals. The reaction time for CAE 200 and CAE 400 was 6.086 ± 0.089 sec and 6.602 ± 0.126 sec respectively at 60 min post administration while it was 3.038 ± 0.036 sec and 7.946 ± 0.301 sec for vehicle treated group and Ibuprofen respectively at the same time duration.

**Keywords**

Anti-nociceptive, Anti-inflammatory, *Curcuma angustifolia*, Edema, Extract,

## Introduction

Pain is an uncomfortable sensation that usually signals an injury or illness. When tissues are damaged, inflammatory mediators are released, resulting in arteriole dilation that then causes the area to become red and hot. Eventually the endothelium of capillaries and venioles contracts, opening spaces for fluid and cells to escape

into the inflamed area, which causes swelling.[1] Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is characterized by redness, swollen joints, joint pain, its stiffness and loss of joint function.

One of the most challenging works for the pharmaceutical and medical chemists is the search for newer and more potent drugs with little toxic effects. In the developed countries the concept that 'natural' is better than 'chemical' or 'synthetic' has led to the evolution of a thrust in herbal research. Plant-derived natural products are significant as sources of medicinal agents and models for the design of new remedies. Lately, research has been directed to traditional folk medicines as they are generally characterized by high acceptability and good toleration.[2-5]

*Curcuma angustifolia* Roxb. is a plant of the Zingiberaceae family and has nutritional value as a source of starch for Indian foods.[6] The major compounds detected in the essential oil include Eucalyptol, α-Curcumene, Curzerenone, Boldenone, α-Elemenone, Longiverbenone, 14-hydroxy-δ-cadinene.[7]

The literature pointed out several pharmacological and biological actions of *Curcuma angustifolia*. Most of the work was directed to rhizomes or essential oil obtained from the rhizomes of the plant.[7] A very few scientific exploration of the leaf of *Curcuma angustifolia* has been done. The presence of phenolic compounds paves way for a number of bioactivities in plant extracts. Hence it was envisioned to perform hydroalcoholic extraction of *Curcuma angustifolia* leaf and screen the extract for analgesic and anti-inflammatory activities using animal models.

**Material and Methods**

The leaves of *Curcuma angustifolia* were collected from the local places of Bhopal, Madhya Pradesh in the month of February. The authenticated plant leaves were washed with distilled water, dried under shade and powdered using a blender at low speed. The powdered leaves were stored in air tight container until taken for use.

**Extraction of leaves**

The powdered leaves were used for the extraction process. 78 g of powder was evenly packed in the extractor of the soxhlet apparatus and extracted with n-hexane followed by ethanol-water (70:30 v/v) using hot continuous extraction process. The extracts were filtered hot to remove any impurity. The extracts were concentrated using rotary vacuum evaporator and the concentrated extracts were transferred to large evaporating dishes in order to remove the remaining solvents using water bath.[8] The extracts were collected and stored in desiccators to remove the excessive moisture**.** The dried extracts were stored in tight containers for carrying out the testing procedures.

**Preliminary phytochemical screening**

All the extracts were evaluated by qualitative phytochemical screening in order to identify the type of plant secondary metabolites present in them. The screening was performed for triterpenes/steroids, alkaloids,

glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation was used as analytical responses to these tests.[9]

**Total Phenolic Content**

For total phenolic content determination, 200 µL of sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After at least 30 s (but not exceeding 8 min), 300 µL of 20% $Na_2CO_3$ aqueous solution was added and the mixture allowed to stand for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer.[10] Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the analytical curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

**Determination of Anti-inflammatory and analgesic action**

Healthy Wistar rats of either sex, weighing 180-250g were used for the study. The animal were housed as per standard conditions and faster overnight before the study.

**Acute toxicity study**

A total of three animals were used which received a single oral dose (2000mg/kg) of the hydro-alcoholic extract of *Curcuma angustifolia*. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a period of 14 days. Once daily observations were made for changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, perspiration, urinary incontinence, and defecation) and central nervous system (drowsiness, tremors and convulsion) changes. Mortality, if any, was also observed over the period of 2 weeks.[11]

**Carageenan induced rat paw edema method**

The carageenan induced rat paw edema method was used for evaluating the anti-inflammatory activity of extract whereas the tail flick method was used for determining the analgesic potential.[12] Paw oedema was induced by subcutaneous injection of 0.1mL (1% solution) of Carrageenan into the plantar surface of the right hind paw of the rat. The test sample was administered in dose of 10 mg/kg in different groups of animals, 30 min prior to carrageenan injection. Ibuprofen (10 mg/kg i.p.) was used as a standard anti-inflammatory drug which was administered 30 min prior to carrageenan injection. Animals were divided into 4 groups (n = 6) as follows

Group -- I - Control - treated with vehicle (normal saline)

Group -- II - Standard drug – Ibuprofen

Group – III– *Curcuma angustifolia* hydro-alcoholic extract (CAE) administered in dose of 200 mg/kg.

Group – IV– *Curcuma angustifolia* hydro-alcoholic extract (CAE) administered in dose of 400 mg/kg.

Paw diameters were measured immediately before the administration of the Carrageenan and thereafter at 1, 2, 4 and 6 h using vernier caliper. The results obtained were compared with control group. The percentage inhibition of paw inflammation exhibited by each group was calculated by using following formula:

$$\% \text{ inhibition} = C\text{-}T/\ C \times 100$$

C= Paw diameter (mm) in vehicle treated group (control)

T= Paw diameter (mm) in drug treated group

**Tail flick method**

The analgesic activity was evaluated using tail flick method.[13]

Animals were divided into 4 groups (n = 6) as follows

Group -- I - Control - treated with vehicle (normal saline)

Group -- II - Standard drug – Ibuprofen

Group – III– CAE was administered in dose of 200 mg/kg.

Group – IV- CAE was administered in dose of 400 mg/kg

About 5 cm from the distal end, tail of each rat was immersed in warm water maintained at 50°C. The reaction time (in seconds) was the time taken by the rat to flick its tail due to pain. The first reading was omitted and reaction time was taken as the average of the next two readings. The reaction time was recorded before (0 min) and at 15, 30, 45, and 60 min after the administration of the treatments. The maximum reaction time was fixed at 15 sec to prevent any tail tissue injury. If the reading exceeds 15 sec, it would be considered as maximum analgesia. The maximum possible analgesia (MPA) was calculated as follows:

$$MPA = \frac{\text{Reaction time for treatment} - \text{reaction time for saline}}{15 \text{ sec} - \text{reaction time for saline}} X\ 100$$

**Results and Discussion**

**Extraction Yield**

The defatting of leaf powder was achieved by extraction with n-hexane and the defatted leaf powder was then extracted with ethanol-water (70:30 v/v) for collection of maximum amount of water soluble extractives. The extracts were sticky and the aqueous extract was dark brown in color with 14.6% yield.

**Phytochemical Screening**

The findings of the phytochemical analysis suggest the presence of saponin glycosides, cardiac glycosides, phenolics, terpenoids, sterols, and flavonoids in the leaves (Table 1).

**Table 1. Phytochemical screening of *C. angustifolia* leaf extract**

| Chemical Tests | Standard Observation | Inference |
|---|---|---|
| **Test for Alkaloids** | | |
| *Mayer's reagent* | cream colour precipitate | - |
| *Hager's reagent* | yellow colour precipitate | - |
| *Wagner's reagent* | reddish brown precipitate | - |
| *Dragendorff's reagent* | reddish brown precipitate | - |
| **Test for Glycosides** | | |
| *Froth test* | Frothing is seen | + |
| *Kedde's Test* | Blue or violet color | - |
| *Bontrager's Test* | Rose pink or red color in the ammonical layer not found | - |
| *Keller-Kiliani* | No color in acetic acid layer | ++ |
| **Test for Phenols/Tannins** | | |
| *Ferric chloride* | Blue green color | ++ |
| *Gelatin Solution* | White precipitate | - |
| *Alkaline reagent test* | Yellow to red precipitate | ++ |
| *Vanillin HCl test* | Purplish red color | ++ |
| **Test for Flavonoids** | | |
| *Shinoda test* | red color | ++ |

| | | |
|---|---|---|
| *Alkaline reagent test* | Yellow color that turns red on acidification | ++ |
| *Zinc HCl reductino test* | red color | ++ |
| **Test for Proteins** | | |
| *Ninhydrin Test* | Voilet color | ++ |
| **Test for Sterols/triterpenoids** | | |
| *Salkowski Test* | Yellow color in lower layer | ++ |

**Total Phenolic content**

The aqueous extract of *Curcuma angustifolia* was evaluated for quantifying the total phenolic content content. Standard curve of gallic acid was calculated and plotted in distilled water for determining absorption data. The results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method. The total phenolic content in extracts, expressed as gallic acid equivalents. The total phenolic content of the aqueous extract of *Curcuma angustifolia* was 76.28 ± 0.45 GAE mg/g.

**Acute Toxicity Study**

The acute toxicity test was performed by using the dried ethylacetate extract at concentration of 2000 mg/kg to the test animal, administered orally. No animal died and hence the dose of upto 2000 mg/Kg was considered to be safe. As none of the animals died, the $LD_{50}$ was considered to be more than 2000 mg/Kg and any dose less than 2000 mg/Kg would be considered for evaluation of wound healing action.

**Evaluation of analgesic and anti-inflammatory action**

Table 2 shows the effect of *Curcuma angustifolia* extract and standard drug as compared to the normal saline control at different hours in carrageenan-induced rat paw edema model using vernier caliper. Ibuprofen at dose of 10 mg/Kg inhibited 80.36% edema after 6h of administration while the CAE 200 and CAE 400 were able to inhibit 46.33% and 51.02% edema formation respectively.

This suggests comparatively significant anti-inflammatory potential in the aqueous extract of *Curcuma angustifolia* leaf at both low and high doses.

**Table 2. Effect of *C. angustifolia* extracts on rat paw edema**

| Group | Change in Paw thickness (mm) | | | |
|---|---|---|---|---|
| | 1h | 2h | 4h | 6h |
| Normal Saline | 1.472 ± 0.192 | 3.182 ± 0.036 | 3.798 ± 0.061 | 4.002 ± 0.047 |
| Ibuprofen | 0.484 ± 0.005 | 0.91 ± 0.016 | 0.958 ± 0.008 | 0.786 ± 0.030 |
| CAE 200 | 1.402 ± 0.061 | 2.478 ± 0.098 | 2.564 ± 0.067 | 2.148 ± 0.030 |
| CAE 400 | 1.284 ± 0.021 | 2.052 ± 0.086 | 2.23 ± 0.029 | 1.96 ± 0.030 |

Values are mean ± SD (n = 6)

Carrageenan-induced acute inflammation is one of the most suitable test procedures to screen anti-inflammatory agents. As shown in the table, *Curcuma angustifolia* extract was not able to inhibit edema significantly in the early hours but was able to inhibit edema considerably at 6h. The anti-inflammatory effect of *C. angustifolia* was less as compared to Ibuprofen (Figure 1).

Carrageenan-induced paw edema model in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis.[14] Therefore, it can be inferred that the inhibitory effect of *Curcuma angustifolia* on carrageenan-induced inflammation may be due to inhibition of the enzyme cyclo-oxygenase leading to inhibition of prostaglandin synthesis.

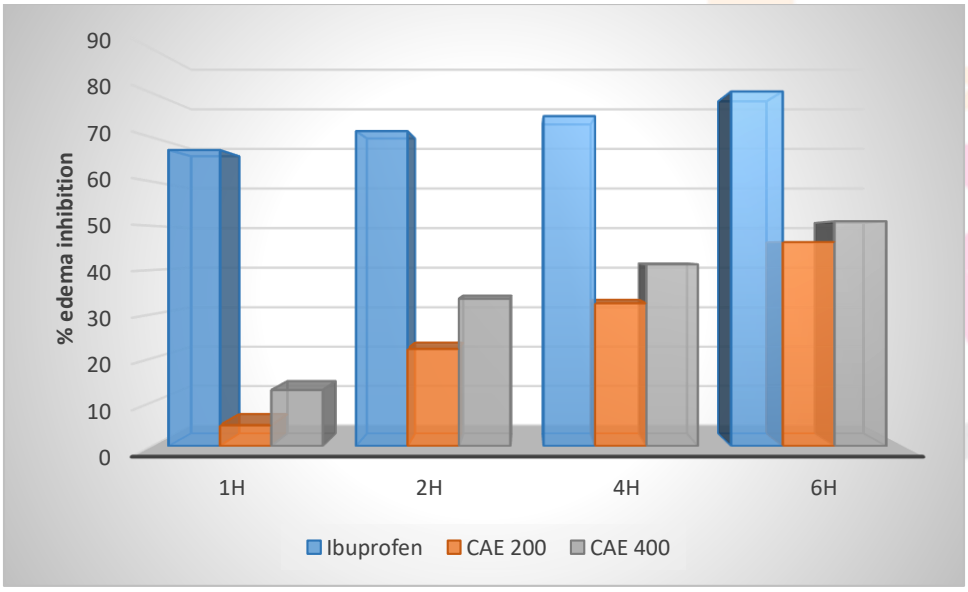


**Figure 1. Comparison of anti-inflammatory effect of ibuprofen and *C. angustifolia***

The results of analgesic activity of *C. angustifilia* extract by tail flick method are shown in Table 3. Rats treated with normal saline (vehicle control) did not exhibit any significant difference in the response time on tail-flick throughout the 60 min duration of observation.

The duration of response time in Ibuprofen and *Curcuma angustifolia* was significantly higher as compared to the saline treated animals. The reaction time for CAE 200 and CAE 400 was 6.086 ± 0.089 sec and 6.602 ± 0.126 sec respectively at 60 min post administration while it was 3.038 ± 0.036 sec and 7.946 ± 0.301 sec for vehicle treated group and Ibuprofen respectively at the same time duration.

**Table 3. Effect of *C. angustifolia* extracts on tail flick response**

| Group | Response Time in seconds | | | | |
|---|---|---|---|---|---|
| | **0 min** | **15 min** | **30 min** | **45 min** | **60 min** |
| Vehicle Control | 3.038 ± 0.036 | 3.25 ± 0.049 | 3.478 ± 0.050 | 3.404 ± 0.058 | 3.34 ± 0.045 |
| Ibuprofen | 4.174 ± 0.117 | 5.606 ± 0.274 | 6.206 ± 0.123 | 6.786 ± 0.105 | 7.946 ± 0.301 |
| CAE 200 | 3.552 ± 0.163 | 4.546 ± 0.426 | 5.796 ± 0.119 | 6.002 ± 0.094 | 6.06 ± 0.117 |
| CAE 400 | 3.612 ± 0.123 | 4.806 ± 0.135 | 6.004 ± 0.159 | 6.220 ± 0.235 | 6.560 ± 0.204 |

Values are mean ± SD (n = 6)

Analgesics are drugs that act on peripheral or central nervous system to selectively relieve pain without significantly altering consciousness.[15] The animal model used for screening of analgesic activity in this study is pain-state model using thermal stimuli which includes tail-flick method. The tail-flick method is known to mediate a spinal reflex to a nociceptive stimulus.[16] Ibuprofen was used as the reference drug, which is considered as mild analgesic. The tail-flick method is based on the observation that morphine-like compounds are selectively able to prolong the response time of typical tail-withdrawal in rats in response to pain stimulus. Analgesic drugs which are centrally acting elevate pain threshold of animals towards heat and pressure.[17] Therefore, the analgesic effect of *Curcuma angustifolia* on this pain-state model indicates that it might be centrally acting.

## Conclusion

The present work was undertaken with an objective to establish the anti-inflammatory action of leaf extract of *Curcuma angustifolia*. The inhibition of rat paw was used to establish the anti-inflammatory action while the change in tail flick response was used to determine the analgesic action. The presence of antioxidant property could be responsible for the anti-inflammatory action of the plant. Fractionation and isolation of the components from extracts would be carried out in future to ascertain the responsible phytochemicals for the anti-inflammatory and analgesic action.

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