

## Anti-inflammatory and Antimicrobial Activity of Rhizomes of *Hedychium coronarium*

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### INTRODUCTION

*Hedychium coronarium* Koen is an aromatic herb belonging to the family Zingiberaceae. This ayurvedic medicinal plant is available in all Asian countries like India, China, Japan and Bangladesh.<sup>1</sup> The rhizomes of *Hedychium* are used in the treatment of diabetes, anticancer.<sup>2-5, 24</sup> Extracts, phytochemical constituents and their derivatives are also used as antirheumatic, excitant, febrifuge and tonic. Earlier phytochemical investigations disclosed that the plant comprises diterpenoids and flavonoids and chalcones;<sup>2, 4, 24</sup> the diterpenes like hedychenone, 7-hydroxyhedychenone, coronarin A, B, C & D and isocoronarin D. However the plant is traditionally used in many parts of India, no scientific report is available to validate the folkloric practice. Most of diterpenoids, chalcones, flavonoids and their derivatives have showed extraordinary pharmacological properties from other medicinal plants.<sup>6-28</sup> As a part of our focus on the medicinal plants of India, we investigated the anti-inflammatory and antimicrobial activities of various extracts of *H. coronarium*.

### MATERIALS AND METHODS

#### Plant material

The rhizomes of *H. coronarium* were procured from the botanical garden, Bhopal University, India in July, 2014 and was identified (voucher specimen No. DUH-02) by the taxonomist of the Department of Botany, Bhopal University. Collected plants, after cutting into small pieces, were dried and pulverized into a coarse powder and stored into an air-tight container.

#### Preparation of the extracts

The rhizomes (500 g) of *H. coronarium* were air-dried in shade and finely powdered. These rhizomes were extracted with 70% ethanol till exhaustion, and evaporated under reduced pressure until the complete dryness. The remaining powdered rhizomes were

successively extracted with solvents in increasing polarity: petroleum ether (60-80), chloroform, ethylacetate and ethanol (90%).

#### Phytochemical and TLC screening of the extractives

The various extracts were subjected to preliminary phytochemical screening, TLC screening, was carried out for detection of individual components. Determination of LD<sub>50</sub>: LD<sub>50</sub> of the total ethanol extracts of the rhizomes of *H. coronarium* were estimated according to Spearman and Karber procedure.<sup>29</sup> Thirty male albino mice (25-30 g body weight) were divided into five groups each of six animals. Preliminary experiments were carried out to determine the minimal dose that kills all animals (LD<sub>100</sub>), and the maximal dose that failed to kill any animal. Several doses at equal logarithmic intervals were selected in between these two doses. Each dose was injected in a group of six animals by subcutaneous injection. All groups of animals were observed for 24 hours, and symptoms of toxicity and mortality rates in each group were recorded, and the LD<sub>50</sub> calculated.

#### In vivo anti-inflammatory activity

The anti-inflammatory activity of the total ethanol 70% extract of the rhizomes was determined, in-vivo, by adopting the carrageenan-induced oedema in the hindpaws of rats.<sup>30</sup> Thirty male albino rats, weighing 130-150 g, were divided into 5 groups (each of 6), and orally treated one hour before induction of oedema. Group 1 receiving saline and served as negative control. Groups 2, 3 and 4 were administered the total ethanol extracts of the three organs leaf, flower and root, respectively, at a dose of 50 mg/kg b.wt., each. Group 5 received Indomethacin,<sup>31</sup> as standard anti-inflammatory drug (30 mg/kg b.wt). Induction of oedema was performed by sub-planter injection of 0.1 ml of 1%

Carrageenan,<sup>31</sup> insaline into the pad of experimental animal right paw and 0.1 ml saline in its left hind paw. Four hours after drugs administration, the rats were sacrificed. Both hind paws were excised and weighed separately; the difference in weight between both represents the weight of the oedema.

## RESULTS

### Antimicrobial activity

The antimicrobial potential of the total ethanol and successive extractives of rhizomes of *H. coronarium* was evaluated against selected bacterial and fungal strains by applying the agar dilution method, adopting the method of Clinical Laboratory Standards Institute.<sup>32</sup> Nine organisms were used: *Bacillus subtilis*, *Escherichia coli*, *Mycobacterium phlei*, *Listeria innocua* "LMG 2710", *Enterococcus faecalis*, *Staphylococcus aureus* "Non-pathogenic LMG 3242", *Staphylococcus aureus* "Pathogenic LMG3240", *Staphylococcus aureus* "Lab. Strain" and *Candida albicans*. The tested organisms were grown on Sabouraud dextrose agar (SAB Agar) and *Candida* identification agar (FLUKA) at 35°C for 24 hrs. Three colonies were suspended in 5 ml saline, then standardized at 530 nm, and the suspension was adjusted to 0.5 McFarland standard and then diluted 10-fold with saline to give organism suspension of 1×10<sup>6</sup> to 5×10<sup>6</sup> CFU/ml. This suspension was then further diluted by putting 1 ml suspension + 9 ml saline to give a final suspension volume of 1×10<sup>5</sup> to 5×10<sup>5</sup> CFU/ml. A multiple inoculator was used to inoculate the prepared agar-antifungal plates. A 100 µl (i.e. 10<sup>4</sup> CFU) of the prepared inoculums were put in the well of multi-inoculator, where each inoculation time by multi-inoculator gave about 10 µl of prepared inoculums to the plate (i.e. 10<sup>3</sup> CFU). DMSO was used as negative control plate. Each experiment was performed in duplicates. All plates were incubated at 35°C for 48 hrs. Results were recorded in terms of MIC, which is the lowest concentration of antimicrobial/antifungal agent causing almost complete inhibition of growth or giving no visible growth.

### In vivo anti-inflammatory activity

The ethyl acetate extract of the leaf and ethanol extract of the root exhibited the highest growth inhibitory activity (MIC, 50 µg/ml) against *Mycobacterium phlei*. Concerning *Bacillus subtilis*, its susceptibility was chiefly to the ethanol extract of the root (MIC, 100 µg/ml). The chloroform extractives of the rhizomes showed, on the other hand, a significant activity against *Listeria innocua*

(MIC, 200 µg/ml). The chloroform extract of both the flowers and roots moderately inhibited the growth of *Staphylococcus aureus* "Non-pathogenic LMG 3242" (MIC, 200 µg/ml), while *Candida albicans* was mainly susceptible to the ethyl acetate and ethanol extracts of the root (MIC, 200 µg/ml).

## DISCUSSION

The present study was conducted to investigate the bioactivities of a plant used in folk medicine, in order to evaluate the scientific basis of its activity in rheumatic pain and diarrhea. It was reported that the antimicrobial, antioxidant and anti-inflammatory activities are related to the presence of phenolic compounds. Inhibition of leukocyte chemotaxis may be involved in the anti-inflammatory action of diterpenoids, and that one of the anti-inflammatory actions of diterpenoids is the prevention of the production of oxygen free-radicals by leukocytes. Also, related to the antimicrobial activity with the presence of phenolic compounds. Among literature, the chloroform extract is effective as antibacterial, which is in agreement with our finding as most of the chloroform fraction of the three organs are active as antibacterial with MIC ranging from 50-200 µg/ml. Investigation of the activity of plant against non-tuberculous mycobacteria; *Mycobacterium phlei* were reported previously using ciprofloxacin and doxycycline as standards and by comparing these results with *H. coronarium* fractions; *Hedychium* has more activity than other extract and less than the standards. It is, however, important that further studies on isolated pure compounds of *H. coronarium* should be carried out.

## Ethics

All animal procedures were performed upon approval from the Ethics Committee of animal safety department, and in accordance with the recommendations of the proper care and use of laboratory animals.

## CONCLUSION

The data represented in this study demonstrate that the use of *H. coronarium* may lower the risk of microbial infections and exert an anti-inflammatory activity, probably due to the presence of diterpenes. The use of extracts is recommended to achieve health benefits rather than pure isolates due to the synergistic and additive effects of their components.

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