

# Investigate of Analgesic Activity of Ethanolic Extract of Leaves of *Epiphyllum Oxypetalum*

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**Abstract:** Pain as a sensory modality; represent a symptom for diagnosing various diseases and its associated conditions. Although it acts as a sensory modality in drawing attention to tissue injury, it is associated with poor quality of life and socioeconomic burden to the victim. Conventional synthetic drugs that are used to manage pain are not readily available and are associated with adverse effects. Thus, the use of herbal medicine from medicinal plants is an age-old practice used by many communities to cure diseases. These medicinal plants are known to contain phytochemical compounds capable of relieving pain and healing diseases. *Epiphyllum oxypetalum* is a species of cactus and one of the most commonly grown of the *Epiphyllum* species. It is one of the under-utilized resources available in the tropical regions of the globe and can be used as a substitute for digitalis. The Shoshone Indian tribe calls the night blooming *Cereus* "Pain in the heart" and used it for heart pain. Scanty work was reported on the phytochemical properties of leaf extract and no documented research work was reported on its leaf for analgesic activity. Thus, the present investigation was carried out to access the analgesic activity, phytochemical constituents and analgesic potentials of leaf extracts. Phytochemical analysis of *Epiphyllum oxypetalum* showed the presence of saponins, phenolic compounds, steroids, glycosides, tannins, terpenoids, and resins while reducing sugars, alkaloids, flavonoids, sterols, phlorotannins and acidic compounds were absent.

**Keywords:** Ethanolic Extract, Analgesic Activity, *Epiphyllum Oxypetalum*

## 1. Introduction

The International association for the study of pain defines pain as "unpleasant sensory and emotional experience that is caused by actual or potential tissue damage". The emotional component differs from one person to the other and in the same individual from time to time and it can be classified in several ways, but in therapeutic application into; nociceptive and neuropathic [1]. In the body, Sensory nerve endings are generally found in every part of the body such as the blood vessels, internal organs, muscles, joints, and the skin [2]. Damage caused by the chemical stimuli, mechanical stimuli, and thermal stimuli sensitize nociceptors. When cells are damaged a number of chemical mediators are released which then activate and sensitize nociceptors to other mediators of pain. Major mediators of pain include; Bradykinin, histamine, serotonin and prostaglandins [3]. However, pain is beyond sensation, it comprises of perception and subjective interpretation of the discomfort [4]. The sensation of pain is a sign that something in the body is wrong. Thus it plays an important role in drawing attention to tissue injury from harmful stimuli and reflexes are elicited to protect the injured part of the body [5].

In the brain, pain stimulus is processed and generated impulses are send down the spinal cord following the appropriate nerves and instructs the body to respond, for instance withdrawing your hand from the fire [5]. Peripheral nerves transmit pain stimulus to the spinal cord which then links to the brain. Two types of nerve fibers are involved in this process; slow pain fibers and Fast pain fibres. Transmission of fast pain is through the A $\delta$  fibres to the spinal cord while slow pain fibres are through the C-fibres. Fast pain nerve endings secrete a neurotransmitter called glutamate, which transmits fast pain impulses to the brain in the cortex. Therefore localization of pain in certain part of the body becomes relatively precise [5]. Although pain is beneficial to the immune system. However, it causes a lot of

suffering and discomfort to the victims, lowering the quality of life and therefore need to be managed. To suppress pain, NSAIDs are prescribed all over the world [6,7]. For severe or chronic malignant pain opioids analgesics are drugs of choice [8]. However, prolonged use of these NSAIDs only provides asymptomatic relief and the greatest drawback lies in their toxicity to liver, kidney and gastrointestinal linings [9]. In this regard, herbal medicines from medicinal plants have been employed in complementary and alternative medicine (CAM) for treatment of pain as well as diseases related to these conditions [10]. Traditional medicinal herbs for over centuries have served as a potential source for alternative medicine and the knowledge of herbal medicine has been passed on from generation to generation [11]. Considering that most of the analgesic, anti-malarial and anti-pyretic synthetic drugs such as aspirin, morphine, artemisinin, atropine and chloroquine were derived from the plant products [12].

*Epiphyllum oxypetalum* is a species of cactus and one of the most cultivated species in the genus. It is a variety of night blooming *Cereus*. *Oxypetalum* (Lat.) = with acute petals, refers to the acute petals of this species. *Epiphyllum oxypetalum* was the most commonly grown of the *Epiphyllum* species, and it is known under several common names including Night-blooming *Cereus*, Dutchman's Pipe, Queen of the Night, Wijaya Kusuma (Indonesian), Nishagandhi in Hindi and Marathi (Table 1). [13]

**Table 1:** Taxonomy of *Epiphyllum Oxypetalum*

| Taxonomy      |                |
|---------------|----------------|
| Kingdom       | Plantae        |
| Sub Kingdom   | Tracheobionta  |
| Superdivision | Spermatophyta  |
| Division      | Magnoliophyta  |
| Class         | Magnoliopsida  |
| Order         | Caryophyllales |
| Family        | Cactaceae      |

Volume 8 Issue 2, February 2019

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|               |                              |
|---------------|------------------------------|
| Genus         | <i>Epiphyllum</i>            |
| Species       | <i>E. oxypetalum</i>         |
| Binomial name | <i>Epiphyllum oxypetalum</i> |

The plant kingdom is a large reservoir of pharmacologically active molecules and a large number of plant-derived medicines now commercially available. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The bioactive compounds obtained from medicinal plants have been used to treat various ailments caused by microorganisms. The most important of these bioactive principles are alkaloids, flavonoids, phenolic compounds and tannins that may be evolved in plants as self-defence against pests and pathogens.

## 2. Material Methods

The leaves sample were collected from NUZVID town, Indian country during the months of January-march, a period in which the herbalist believed the medicinal plant had its utmost healing capacity to cure the named ailments. This process of collection of the plant sample was done with the guidance of a local herbalist. A voucher specimen consisting of fresh twigs with leaves was availed to an acknowledged taxonomist for botanical authentication. Sample leaves were then sorted, cut into smaller pieces and cleaned with clean water and then air dried under shade for two weeks. Chopped leaves samples were packed into paper enveloped, where they were milled into a fine powder using an electrical mill.

### Extraction

For extraction, 1 litres of ethanol was used to soak 60 g of the powdered leaves sample for 48 hours with occasional shaking within the first 10 hours to mix the sample uniformly on an hourly basis. Whatman's filter paper No.1 was used to filter the mixture. The filtrate was concentrated to dryness using the hot air oven at a temperature of 41°C. The concentrates were kept in sealed containers at a low temperature until use in bio screening experiments.

## 3. Experimental Design

### Laboratory animals

Swiss albino mice weighing between 19-24 g were utilized for screening analgesic potential of ethanolic leaf extract of *E.oxypetalum* Standard laboratory cages were used to handle the experimental animals in the animal house at Chalapathi Institute of pharmaceutical sciences. During the entire period of carrying out the research, all the laboratory animals were kept at ambient room temperature for 12 hours darkness followed by 12 hours light cycles. Rodent pellets were used to feed the experimental animals and allowed access to

water *ad libitum*. Rules and regulation for handling experimental animals in the study were adhered to [14]

### Drugs and chemicals

E.Oxypetalm leaf extract was dissolved in tween 80 immediately before used orally, glacial acetic acid diluted in distilled water to provide 0.06% solution for intraperitoneal injection, pentazocine and normal saline.

The mice were divided into three groups containing six animals ( $n = 6$ ) in each group (control, standard, and test group). The test drug Epiphyllum oxypetalum 4 mg/kg and normal saline 25 ml/kg was administered orally 2 h prior. Standard drug pentazocine 10 mg/kg was administered intraperitoneal 15 min prior to the experiment. Significant analgesia of pentazocine occurs between 15 and 30 min.

- Group 1: Normal saline - 25 ml/kg (oral)
- Group 2: Pentazocine - 10 mg/kg (intra-peritoneal)
- Group 3: leaf extract - 4 mg/kg (oral).

### Analgesic activity

#### Eddy's hot plate

Mice weighing 20-30 g were used. mice were placed on the hot plate, which consists of the electrically heated surface. The temperature of the hot plate was maintained at 55°C. Responses such as jumping, withdrawal of the paws and licking of the paws were observed. The time period (latency period) when animals were placed and until responses occur was recorded by the stopwatch. Ethanolic leaf extract was administered orally and latency period was recorded after 0, 30, 60, 90 and 120 min. These values were compared with the standard drug pentazocine and control normal saline. This model evaluates the central pain.[15]

## 4. Results and Discussion

Animal tests of analgesic drugs commonly measure nociception and involve testing the reaction of an animal to painful stimuli. In the present study the thermal test was selected because of several advantages including the sensitivity to strong analgesics and limited tissue damage.

The Eddy's hot plate method involves spinal reflexes and is regarded as one of the most suitable methods for studying the involvement of centrally acting analgesics. Drugs that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase.

An increase in reaction time is generally considered as an important parameter of analgesic activity in Heat conduction method. In these models, increase in stress tolerance capacity of the animals indicates the possible involvement of a higher centre. It is therefore thought that the analgesic effect of *Swertia chirayita* seen in this study may involve central activity.

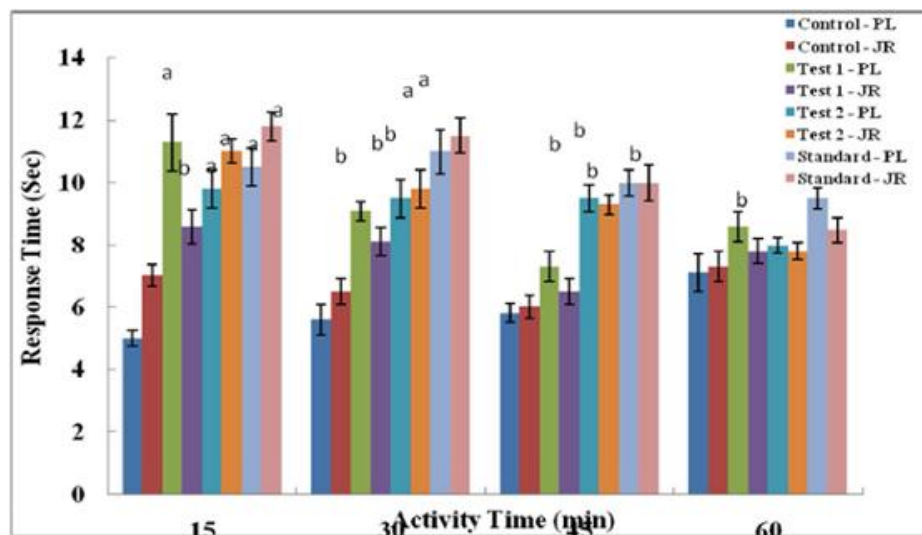
**Table 2:** Mean analgesic activity duration time in model mean and standard deviations

| Drug/Treatment | Time (minutes)  |                 |                 |                 |                 |                 |                 |                 |
|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | 0               |                 | 15              |                 | 30              |                 | 60              |                 |
|                | PL              | JR              | PL              | JR              | PL              | JR              | PL              | JR              |
| Control        | 5.6±1.36        | 3.8±1.47        | 4.8±1.47        | 4.3±2.10        | 3.8±0.40        | 1.5±0.54        | 2.5±0.34        | 0.6±0.24        |
| Standard       | 1.5±0.54        | 1.3±1.03        | 2.1±0.75        | 0.6±0.51        | 3.3±1.21        | 1.1±0.40        | 3.8±1.47        | 1.6±0.81        |
| Test 1         | <b>0.6±0.51</b> | <b>0.2±0.19</b> | <b>1.1±0.40</b> | <b>0.2±0.19</b> | <b>2±0.63</b>   | <b>0.3±0.51</b> | <b>1.6±0.33</b> | <b>0.6±0.81</b> |
| Test 2         | <b>0.2±0.41</b> | <b>0.2±0.19</b> | <b>0.1±0.39</b> | <b>0.1±0.39</b> | <b>0.3±0.51</b> | <b>0.3±0.51</b> | <b>0.1±0.40</b> | <b>0.2±0.41</b> |

Values are expressed in terms of \*  $p < 0.1$  stastically highly significant as compared with control and standards group.

Thus, the latency period of ethanolic extract was significantly ( $P < 0.05$ ) good when compared to control at

time period 30-60 min, whereas the latency period of the standard was more significant ( $P < 0.05$ ) when compared to test drug at all-time intervals of experimentation

**Figure 1:** Graphical presentation of Analgesic activity by Tail flick method

Each group N=6 represents mean  $\pm$  SEM, a =  $P < 0.01$  Vs Control group, b =  $P < 0.05$  Vs Control group, c =  $P < 0.001$  Vs Control

## 5. Conclusion

The present experimental findings suggest that *Epiphyllum oxypetalum* is a promising analgesic drug and will be able to replace synthetic analgesic drug. Further study is needed to explore the exact mechanism of the ethanolic extract for its activity and hence it is necessary to evaluate its analgesic activity on human being in clinical conditions.

## 6. Acknowledgement

We are thankful to head of the department of pharmacology, NARENDRA BABU A, Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, for permitting to carry out the analgesic activity in her institution.

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