# ANTI-INFLAMMATORY ACTIVITY OF THE ETHANOLIC EXTRACT OF TAKIP KUHOL (*Centella asiatica*) LEAVES

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## ABSTRACT

This study aimed to determine the anti-inflammatory activity of the ethanolic leaf extract of Takip Kuhol (*Centella asiatica*) particularly the leaves, to determine the presence of anti-inflammatory activity considering the basis of use in the traditional medicine. The inflammation was induced by injecting 0.1 ml of 1% w/v carrageenan suspension into the sub-plantar region of the left hind paw of the rat. This was done by treating Diclofenac as a positive control and Experimental group which is the ethanolic extract of Takip Kuhol (*Centella asiatica*). Measuring the paw edema of the rats was done using volume displacement method to assess if the inflammation has subsided. Results showed that the 300 mg/kg, 150 mg/kg and 100 mg/kg of the extract exhibited its anti-inflammatory effect during the 3rd day which was assessed basing on the baseline and after carrageenan administration in the hind paw of the rat. Therefore, based on the methods used and the results obtained in this study, the ethanolic leaf extract of Takip Kuhol (*Centella asiatica*) statistically proved that it is effective as an anti-inflammatory with a dose of 300 mg/kg, 150 mg/kg and 100 mg/kg.

**Key words:** *anti-inflammatory activity, carrageenan, inflammation ethanolic, leaf-extracts, volume displacement*

## INTRODUCTION

Inflammation is the reaction of living tissues to injury, infection or irritation (Chippada et al., 2011). Living tissues respond to the injury in a complex way depending on its rigorousness (Jain et al., 2014). Which can occur from lysosomal enzymes released during inflammation produce a variety of disorders which lead to the tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are assumed to be responsible for certain pathological conditions such as heart attacks, septic shocks and rheumatoid arthritis (Chippada et al., 2011). Symptoms of inflammation include redness, swelling, heat, and pain (George et al., 2009). The purpose of inflammation is to limit the effects of harmful bacteria or injury by destroying the organism and by limiting its spread throughout the body (Black & Hawks, 2005). However, inflammation deregulated response that involves active inflammation, tissue destruction and attempts at tissue repair (Jain et al., 2014).

According to the global disease burden of health grove, an inflammatory condition has an overall impact and specific effects on demographic groups in the

Philippines and one of these conditions that has a high prevalence in our country is rheumatoid arthritis. To overcome the challenges of inflammatory disorders, several classes of anti-inflammatory drugs have been used. These include non-steroidal anti-inflammatory drugs (NSAIDs), Immunoselective Anti-inflammatory Derivatives (ImSAIDs), corticosteroids etc. (Jain et al., 2014). Nowadays, Non-steroidal anti-inflammatory drugs (NSAIDs) among the most commonly used drugs worldwide and their analgesic, anti-inflammatory, and anti-pyretic therapeutic properties are thoroughly accepted. They are weak organic acids and are the most commonly used drugs in orthopedics and trauma practice. They provide mild to moderate pain relief (Kheiri et al., 2014). However, their everyday and long-term administration is limited due to their renal side effects and gastrointestinal side effects (Jain et al., 2014), which include the peptic ulcer. NSAIDs, when used for weeks or months can damage the lining of the digestive tract, causing an ulcer. The greatest disadvantage of the present remedy of synthetic drugs like NSAID lied in their toxicity and relapse of symptoms after discontinuation of treatment (Olajide et al., 2004). Therefore, medicinal plants are the common source of therapeutically active chemical substances with lesser side effects (Saha et al., 2013).

Hence, the study was conducted to judge its claimed uses of *Centella asiatica* or often referred as Takip Kuhol, particularly the leaves, to determine the presence of anti-inflammatory activity considering the basis of use in the traditional medicine. This study aimed to provide scientific evidence on the basis of the traditional use of Takip Kuhol (*Centella asiatica*) in treating inflammation.

**Research Questions**

Generally, the aim of this study was to determine the anti-inflammatory activity of the ethanolic extract of the leaves of Takip Kuhol (*Centella asiatica*) in rats. Specifically, it aimed to answer the following questions:

1. What are the phytochemical constituents of Takip kuhol (Centella asiatica)?
2. What is the degree of paw edema/ inflammation as evidenced by the volume of water displacement of the different treatment groups?
    a. Baseline
    b. Post-induction of Carrageenan
    c. 1 day post-treatment
    d. 3 days post treatment
    e. 5 days post treatment
    f. 7 days post-tretament
3. Is there a significant difference in the degree of inflammation of subjects after induction of inflammation and after 1, 3, 5 and 7 days post-treatment?
4. Is there a significant difference in the degree of inflammation of the different treatment groups 1, 3, 5 and 7 days post-treatment?
    a. Negative control
    b. Positive control (25mg/kg Diclofenac Sodium)

c. 300mg/kg *C. asiatica* extract
d. 150mg/kg *C. asiatica* extract
e. 100mg/kg *C. asiatica* extract
f. 75mg/kg *C. asiatica* extract

**Hypotheses**

1. There is no significant difference in the degree of inflammation of subjects after induction of inflammation and after 1, 3, 5 and 7 days post-treatment.
2. There is no significant difference in the degree of inflammation of the different treatment groups 1, 3, 5 and 7 days post-treatment.

**Significance of the Study**

This study provides a significance to lessen the risk of the side effects of the synthetic anti-inflammatory agents that are available to the market. To help the public to provide a safe and effective folkloric medicinal plant that is affordable and more available in the community. To discover and develop new therapeutic or dietary supplement drugs made out of Takip kuhol (*Centella asiatica*) leaves.

**Literature Review**

Takip Kuhol (*Centella asiatica Linn*.) belonging to the family of umbeliferae is a very useful plant (Xavier et al., 2014). It is commonly found in parts of India, Asia and the Middle East (Somchit et al. 2004). C. asiatica is commonly known as “Gotu kola, Asiatic pennywort, Indian pennywort, Indian water navelwort, wild violet, and tiger herb” in English (Mala & Tulka. 2014). It is known as ‘Pegaga' in Malaysia, ‘Luei Gong Gen' in China, ‘Vallarai' in India and ‘Daun Kaki Kuda'in Indonesia, In the Philippines it is known as ‘Takip Kuhol' in Tagalog. The leaves, which are edible, are in yellowish-green color, thin, alternate with long petioles, orbicular, or oblong-elliptic shapes with seven veins.

*Centella asiatica* is one of the chief herbs for treating skin problems, to heal wounds, for revitalizing the nerves and brain cells; hence it is primarily known as “brain food” in India. The use of *Centella asiatica* in food and beverages has increased over the years basically due to its health benefits such as antioxidant, as an anti-inflammatory, wound healing, and has a memory enhancing property and many others.

It has been recently introduced in modern medicine as an alternative remedial solution and has been included in clinical studies testing memory in relation to reaction time and as a topical remedy for skin ulcers and wound (Hamidpour et al. 2015). In addition, it has been shown to promote fibroblast proliferation and collagen synthesis (Abdulla et al., 2010). The bioactive pentacyclic triterpenoid compound of *C. asiatica, asiaticoside* and *madecassoside*, have been

widely studied and reported to have various biological properties such as wound healing and ulcer healing. The present study demonstrated that hydrophobic solvent extraction of *Centella asiatica* yielded better results in terms of phenolic, asiaticosside and madecassoside contents and that this is associated with better anti-inflammatory potential (Nurlaily et al., 2012). The CA extracts (CAE) have been used traditionally for wound healing and the research has been increasingly supportive of these claims. A preclinical study reported that various formulations (ointment, cream, and gel) of an aqueous CAE applied to open wounds in rats (3 times daily for 24 days) resulted in increased cellular proliferation and collagen synthesis at the wound site, as shown by an increase in collagen content and tensile strength. The authors found that the CAE-treated wounds epithelialized faster and the rate of wound contraction was higher when compared to the untreated control wounds. Healing was more prominent with the gel product. It is believed to have an effect on keratinization, which aids in thickening skin in areas of infection. Also, it was shown to inhibit the inflammatory process which may provoke hypertrophy in scars and improves the capillary permeability (Gohil et al., 2010). *C. asiatica* extract has been shown to contain anti-inflammatory activity and it is speculated that the gastroprotective effect exerted by *C. asiatica* extract could be attributed to its anti-inflammatory activity.

This anti-inflammatory activity could also be a key factor in the prevention of gastric ulcer (Abdulla et al., 2010). The juice of *Centellaa asiatica* (fresh whole plant) was found to possess significant antiulcer activity against ethanol-, aspirin-, cold restraint stress-, pylorus ligated- induced gastric ulcers in rats (Sairam et. al., 2000).

Takip Kuhol plant has been used for medicinal purposes since prehistoric times. Traditional healers from the South Sea Islands, Indonesia, Madagascar, and from near the Malay Peninsula have used fresh extracts of *Centella asiatica* topically as a treatment of wounds and internally for therapeutic applications (Kartnig, 1988). People from India and Africa have also used preparations of the plant for the treatment of leprosy (Kartnig 1988; Madaus 1938) and useful application of *Centella asiatica* in treating of leprosy was reported for the first time in 1852 (Madaus, 1938). Sine (1887) reported more wide-spread lineal use of *Centella asiatica* as a therapeutic agent for the treatment of leprous lesions has been documented.

In the Philippines, the sap of leaves is used as curative for sclerotic wounds. The decoction of leaves used as a diuretic and considered useful for gonorrhea also useful in the treatment of chronic and obstinate eczema. The sap of the leaves is used on the wound in skin sores, scratches, and superficial burns. Different subspecies are also present in the country like *Centella boninensis, Centella glohidiata, Centella hirtella, Centella tussilaginifolia, Hydrootyle asiatia, Hydrootyle biflora, Hydrootyle ficarifolia, Hydrocotyle lurida* and many more.

Republic Act of 8423 also known as the Traditional and Alternative Medicine Act of 1997 focuses on developing different traditional health-related management in the country. Drugs for prevention, cure, lessening sign and symptoms, diagnosing and maintaining a healthy lifestyle with lower prize are needed to be explored and developed. The alternative medications undergo method of proper compounding (Nolledo, 2015).

This law encourages the indigenous people to share their traditional medicines and for people to study more about the safety effectiveness of these alternative medicines. The healthcare professional should be aware of these alternative medications and promote it to their patients. By this, countrymen would encounter these alternative medicines coming from that cost much lesser than the existing drugs. The cheaper medicines get, the more patient will comply with medication (Nolledo, 2015).

**Research Paradigm**

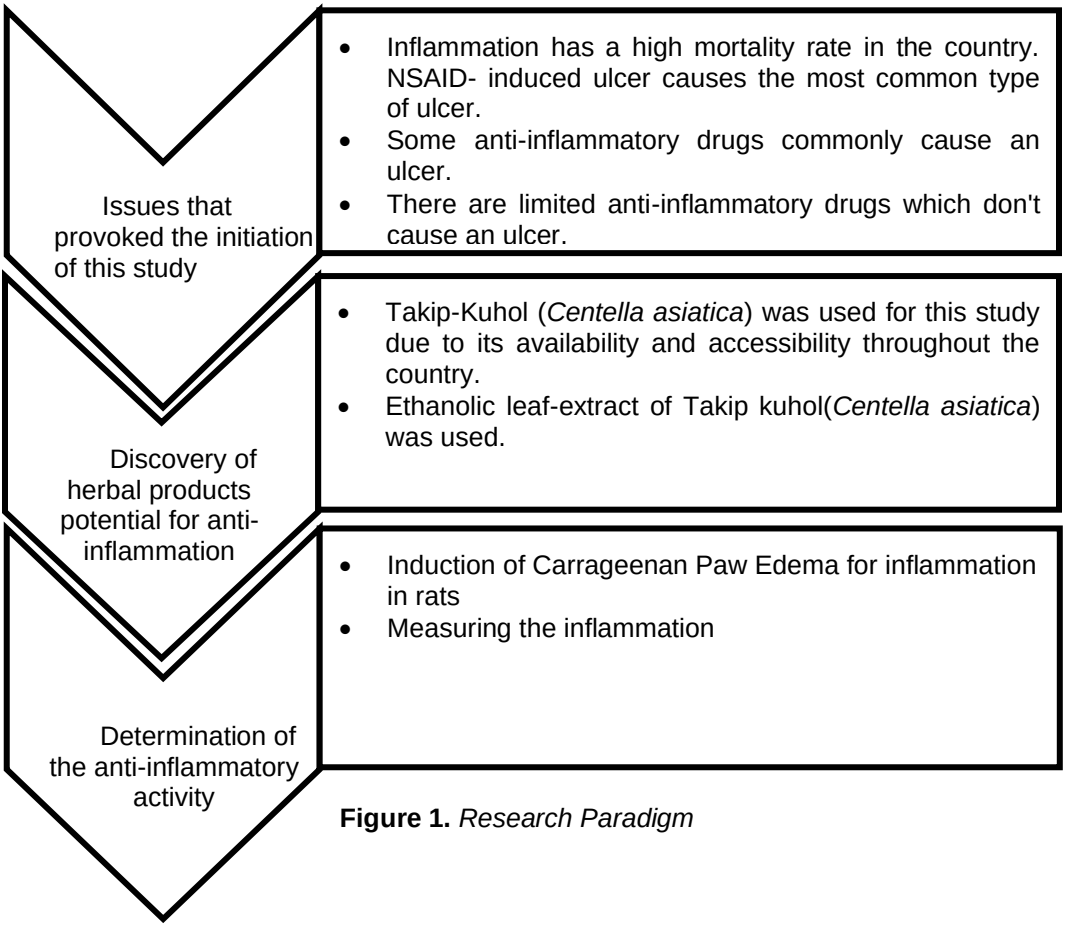


**Figure 1.** *Research Paradigm*

This paradigm shows the reasons why this study was conducted and the way to prove the anti-inflammatory activity of the extract.

## METHODS

### Research Design

Experimental methods were used in the thesis entitled 'Anti-inflammatory activity of the ethanolic extract of Takip Kuhol (*Centella asiatica*) leaves in rats'. This study was done by treating rats with carrageenan-induced paw edema with the ethanolic leaf extract of Takip Kuhol (*Centella asiatica*) and the Diclofenac as the positive control. Values for edema was expressed in milliliter and compared to control using one way-ANOVA. Data were statistically analyzed using +S.E.M and $p < 0.05$ was considered being the criterion for statistical significance (Abdullah et al., 2010). The anti-inflammatory activity of the extract was evaluated in 6 groups of 7 rats in each group.

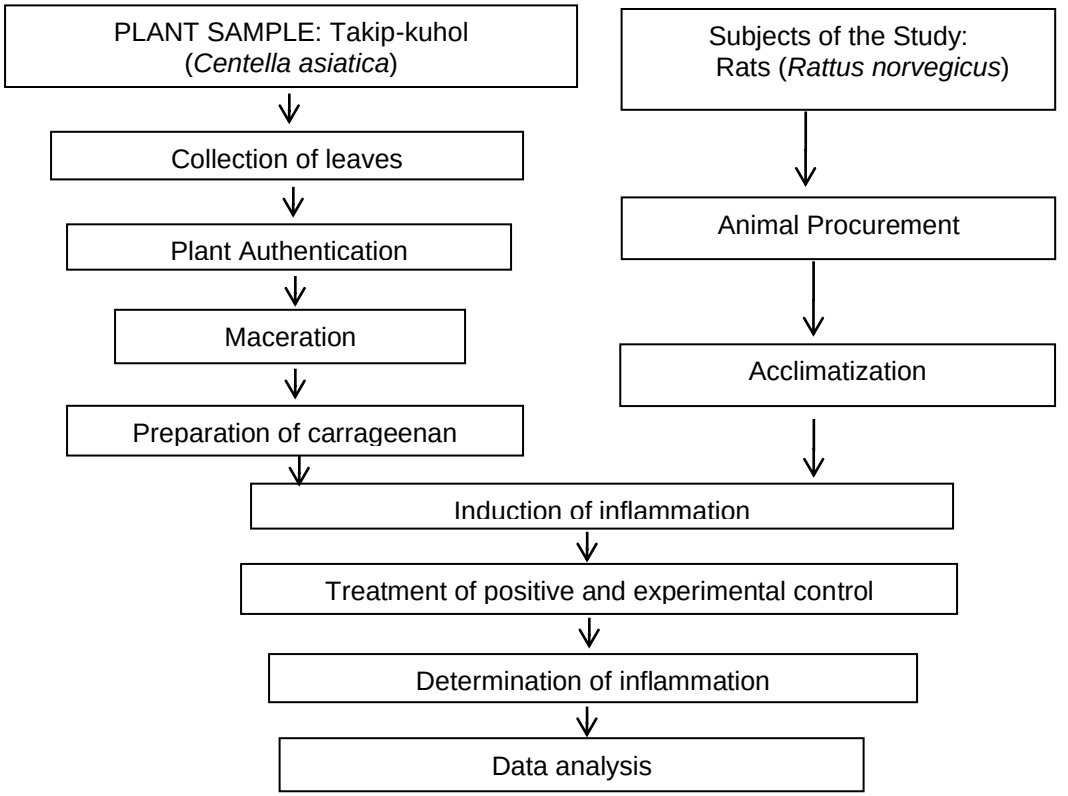


**Figure 2.** *Methodological flow chart*

Figure 2 shows the general methods that the researchers used in the study, from the collection of the leaves of Takip Kuhol (*Centella asiatica*) to the ethanolic extraction of these leaves and the testing of their anti-inflammatory activity.

**Locale of the Study**

The Takip Kuhol (*Centella asiatica*) leaves that were used by the researchers were collected from Tabuk City, Kalinga. Mature leaves were collected in the morning characterized with darker green in color. The leaves were washed and freed from extraneous dirt using distilled water.

**Sampling Technique**

The verification and authentication of the Takip kuhol were done at the Bureau of Plant Industry Malate, Manila after which the sample was prepared and was used for the determination of its anti-inflammatory activity.

**Procedures for Data Gathering**

**1. Collection and Preparation of Plant Sample**

Centella asiatica leaves were collected freshly from Tabuk City, Kalinga. The leaves were washed with distilled water to remove any extraneous dirt. After which the leaves were then transferred to University of Saint Louis Tuguegarao, Pharmacy Laboratory for drying under shade, and made into coarse powder by grinding and extraction.

**2. Preparation of Plant Extract**

To 20 g of each dried plant powder form, 500 ml of 80% ethanol was added and the contents of the flask were mixed thoroughly by gentle shaking. Flasks were kept for two to three days with frequent shaking. After the completion of maceration process, the extract was obtained and the solvent was evaporated to get the crude extract using simple distillation.

**3. Induction of Paw Edema**

The paw edema was induced by injecting 0.1 ml of 1% w/v carrageenan suspension into the sub-plantar region of the left hind paw of rat (Gupta et al., 2014). The method that was used for measuring the edema is volume displacement.

## 4. Testing of the Product

### 4.1 Anti-inflammatory Test

Each animal from each group containing 7 animals was treated with the following doses of the sample. The negative control group was orally administered with plain water. The positive control group received oral dose of 25mg/kg of Diclofenac. Experimental groups were orally administered with 75mg/kg, 100mg/kg, 150mg/kg and 300mg/kg of ethanol extract of *C. asiatica* leaf.

**Data Analysis**

All values were reported as mean + S.E.M. The statistical significance of differences between groups was assessed using one-way ANOVA. A probability value of $p < 0.05$ was considered being the criterion for statistical significance (Abdulla et al., 2010).

**Ethical Consideration**

The researchers requested permission from PITAHC regarding the housing of the animal models, in which proper cages and disposal of animal models were taken into consideration to avoid contamination and spread of diseases. And from the Department of Agriculture- Bureau of Animal Industry for the conduction of specimens and to legally request a permit for utilization of specific materials needed in the study. The researchers requested the authentication of the plant and animal subjects which were utilized in the study. The Research also underwent University Research and Ethics Board (UREB) review of protocols.

**RESULTS**

This chapter discusses the finding of the study from the experiments conducted to the plant sample. All data from the previous chapter were presented here together with the phytochemical screening and the anti-inflammatory testing result.

**Table 1.** *Phytochemical Screening of the ethanolic extract of Takip kuhol leaves*

| CONSTITUENT | RESULT |
|---|---|
| Alkaloids | (+) |
| Carbohydrates | (-) |
| Glycosides | (-) |
| Saponins | (+) |
| Phytosterol | (+) |
| Phenolic | (+) |
| Flavonoids | (+) |

| | |
|---|---|
| Proteins | (+) |

Phytochemical screening of the ethanolic leaf extract of Takip Kuhol (*Centella asiatic*a) is positive for the presence of alkaloids, saponins, phytosterol, phenolic, flavonoids, and proteins.

**Table 2.** *Degree of Paw Edema/ Inflammation (Volume of Water Displacement) of the Different Treatment Groups Pre and Post-Treatment*

| **Treatment Groups** | **Before Carrageenan Administration** | **After Carrageenan Administration** | **1st Day** | **3rd Day** | **5th Day** | **7th Day** |
|---|---|---|---|---|---|---|
| Negative control (plain water) | 0.5714 | 0.8857 | 0.9000 | 0.9143 | 0.8571 | 0.7286 |
| Positive (Diclofenac 25mg/kg) | 0.4143 | 0.8289 | 0.6571 | 0.5286 | 0.4286 | 0.3429 |
| Experimental group 1 (300mg/kg) | 0.4429 | 0.7429 | 0.6000 | 0.4571 | 0.4143 | 0.3571 |
| Experimental group 2 (150mg/kg) | 0.4000 | 0.8714 | 0.7857 | 0.6429 | 0.5286 | 0.4143 |
| Experimental group 3 (100mg/kg) | 0.4714 | 0.8286 | 0.8000 | 0.7000 | 0.5857 | 0.4714 |
| Experimental group 4 (75mg/kg) | 0.6429 | 0.9857 | 0.8857 | 0.7714 | 0.6857 | 0.6143 |

Table 2 reveals that the positive control and experimental groups which are the 300mg/kg, 150mg/kg and 100mg/kg have the same mean number of anti-inflammatory effect to the tested rats during the 1st, 3rd, 5th and 7th day. Almost all of the rats paw edema returned to the baseline during the 7th day. The experimental group 300mg/kg is somehow close to positive control.

Meanwhile, inflammation level of negative control and experimental group 75mg/kg have evidently slow onset of anti-inflammatory action on the paw edema of the rats.

**Table 3.1.** *Test of Significant Difference of the Degree of Inflammation of Subjects under the Negative Control Treatment after Induction of Inflammation and Post-treatment*

| Pairs | t-value | p-value | Decision |
|---|---|---|---|
| Post-Carrageenan Administration- 1 day Post Treatment | -.354 | .736 | Accept Ho |
| Post-Carrageenan Administration- 3 days Post Treatment | -.444 | .673 | Accept Ho |
| Post-Carrageenan Administration- 5 days Post-Treatment | .603 | .569 | Accept Ho |
| Post-Carrageenan Administration-1 day Post Treatment | 2.976 | .025 | Reject Ho |

The table shows that the degree of paw edema or inflammation of the test subjects did not change significantly after 1, 3 and 5 days of administering sterile water. However, there was a significant decrease in the degree of inflammation after 7 days of administering sterile water.

**Table 3.2.** *Test of Significant Difference of the Degree of Inflammation of Subjects under the Positive Control Treatment after Induction of Inflammation and Post-treatment*

| Pairs | t-value | p-value | Decision |
|---|---|---|---|
| Post-Carrageenan Administration- 1 day Post Treatment | 4.076 | .007 | Reject Ho |
| Post-Carrageenan Administration- 3 days Post Treatment | 5.196 | .002 | Reject Ho |
| Post-Carrageenan Administration- 5 days Post-Treatment | 8.198 | .000 | Reject Ho |
| Post-Carrageenan Administration-1 day Post Treatment | 7.249 | .000 | Reject Ho |

It can be gleaned on the table above that the degree of paw edema or inflammation of the test subjects significantly decreased after 1, 3, 5 and 7 days of administering Diclofenac Sodium.

**Table 3.3.** *Test of Significant Difference of the Degree of Inflammation of Subjects under the Experimental Group 1 after Induction of Inflammation and Post-treatment*

| Pairs | t-value | p-value | Decision |
|---|---|---|---|
| Post-Carrageenan Administration- 1 day Post Treatment | 7.071 | .000 | Reject Ho |
| Post-Carrageenan Administration- 3 days Post Treatment | 10.954 | .000 | Reject Ho |
| Post-Carrageenan Administration- 5 days Post-Treatment | 11.500 | .000 | Reject Ho |
| Post-Carrageenan Administration-1 day Post Treatment | 27.000 | .000 | Reject Ho |

It can be gleaned on the table above that the degree of paw edema or inflammation of the test subjects significantly decreased after 1, 3, 5 and 7 days of administering 300mg/kg dose of extracted *C. asiatica*.

**Table 3.4.** *Test of Significant Difference of the Degree of Inflammation of Subjects under the Experimental Group 2 after Induction of Inflammation and Post-treatment*

| Pairs | t-value | p-value | Decision |
|---|---|---|---|
| Post-Carrageenan Administration- 1 day Post Treatment | 3.286 | .017 | Reject Ho |
| Post-Carrageenan Administration- 3 days Post Treatment | 8.000 | .000 | Reject Ho |
| Post-Carrageenan Administration- 5 days Post-Treatment | 9.295 | .000 | Reject Ho |
| Post-Carrageenan Administration-1 day Post Treatment | 6.358 | .001 | Reject Ho |

It can be gleaned on the table above that the degree of paw edema or inflammation of the test subjects significantly decreased after 1, 3, 5 and 7 days of administering 150mg/kg dose of extracted *C. asiatica*.

**Table 3.5.** *Test of Significant Difference of the Degree of Inflammation of Subjects under the Experimental Group 3 after Induction of Inflammation and Post-treatment*

| Pairs | t-value | p-value | Decision |
|---|---|---|---|
| Post-Carrageenan Administration- 1 day Post Treatment | 1.000 | .356 | Accept Ho |
| Post-Carrageenan Administration- 3 days Post Treatment | 4.500 | .004 | Reject Ho |
| Post-Carrageenan Administration- 5 days Post-Treatment | 8.167 | .000 | Reject Ho |
| Post-Carrageenan Administration-1 day Post Treatment | 17.678 | .000 | Reject Ho |

It can be gleaned on the table above that the degree of paw edema or inflammation of the test did not change significantly after one day of treatment with 100mg/kg dosage of *C. asiatica* extract. However, significant decrease in paw edema/ inflammation was observed after 3, 5 and 7 days of administering such dose.

**Table 3.6.** *Test of Significant Difference of the Degree of Inflammation of Subjects under the Experimental Group 3 after Induction of Inflammation and Post-treatment*

| Pairs | t-value | p-value | Decision |
|---|---|---|---|
| Post-Carrageenan Administration- 1 day Post Treatment | 2.646 | .038 | Reject Ho |
| Post-Carrageenan Administration- 3 days Post Treatment | 3.873 | .008 | Reject Ho |
| Post-Carrageenan Administration- 5 days Post-Treatment | 5.196 | .002 | Reject Ho |
| Post-Carrageenan Administration-1 day Post Treatment | 5.766 | .001 | Reject Ho |

It can be gleaned on the table above that the degree of paw edema or inflammation of the test subjects significantly decreased after 1, 3, 5 and 7 days of administering 75mg/kg dose of extracted *C. asiatica*.

**Table 4.** *Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 1, 3, 5 and 7 days Post-treatment*

| Pairs | F-value | p-value | Decision |
|---|---|---|---|
| 1 day Post-treatment | 2.400 | .056 | Reject Ho |
| 3 days Post-treatment | 5.791 | .001 | Reject Ho |
| 5 days Post-treatment | 5.866 | .000 | Reject Ho |
| 7 days Post-treatment | 5.817 | .000 | Reject Ho |

The table above shows that there is a significant difference in the degree of paw edema/ inflammation among the different treatment groups (negative control, positive control, experimental groups 1, 2 and 3) after administration of the respective treatments for 1, 3, 5, and 7 days.

**Table 5.1.** *Post-Hoc Analysis of the Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 1 day Post-treatment*

| | Mean | Negative Control | Positive control | Exp. Group 1 | Exp. Group 2 | Exp. Group 3 | Exp. Group 4 |
|---|---|---|---|---|---|---|---|
| **Negative control** | 0.9000 | | | | | | |
| **Positive Control** | 0.6571 | .034* | | | | | |
| **Exp. Group 1** | 0.6000 | .010* | .608 | | | | |
| **Exp. Group 2** | 0.7857 | .307 | .252 | .101 | | | |
| **Exp. Group 3** | 0.8000 | .371 | .204 | .078 | .898 | | |
| **Exp. Group 4** | 0.8857 | .898 | .046 | .014* | .371 | .442 | |

**The mean difference is significant at the 0.05 level*

The table above shows that after 1 day of treatment, the rats treated with 300mg/kg of *C. asiatica* extract manifested significantly the same effect as those treated with the positive control (Diclofenac Sodium).

**Table 5.2.** *Post-Hoc Analysis of the Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 3 days Post-treatment*

| | Mean | Negative Control | Positive control | Exp. Group 1 | Exp. Group 2 | Exp. Group 3 | Exp. Group 4 |
|---|---|---|---|---|---|---|---|
| **Negative control** | 0.9143 | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Positive Control** | 0.5286 | .000* | | | | | |
| **Exp. Group 1** | 0.4571 | .000* | .467 | | | | |
| **Exp. Group 2** | 0.6429 | .008* | .248 | .064 | | | |
| **Exp. Group 3** | 0.7000 | .034* | .086 | .017* | .560 | | |
| **Exp. Group 4** | 0.7714 | .150 | .017* | .003* | .194 | .467 | |

**The mean difference is significant at the 0.05 level*

The table above shows that after 3 days of treatment, the rats treated with 300mg/kg, 150mg/kg and 100mg/kg of *C. asiatica* extract manifested significantly the same effect as those treated with the positive control (Diclofenac Sodium). Moreover, there is no significant difference in the anti-inflammatory effect between 300mg/kg and 150mg/kg doses of *C. asiatica*. However, the 300mg/kg dosage manifested significantly better anti-inflammatory effect than the 100mg/kg dose.

**Table 5.3.** *Post-Hoc Analysis of the Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 5 days Post-treatment*

| | **Mean** | **Negative Control** | **Positive control** | **Exp. Group 1** | **Exp. Group 2** | **Exp. Group 3** | **Exp. Group 4** |
|---|---|---|---|---|---|---|---|
| **Negative control** | 0.8571 | | | | | | |
| **Positive Control** | 0.4286 | .000* | | | | | |
| **Exp. Group 1** | 0.4143 | .000* | .885 | | | | |
| **Exp. Group 2** | 0.5286 | .002* | .315 | .251 | | | |
| **Exp. Group 3** | 0.5857 | .009* | .118 | .089 | .564 | | |
| **Exp. Group 4** | 0.6857 | .089 | .013* | .009* | .118 | .315 | |

**The mean difference is significant at the 0.05 level*

The table above shows that after 5 days of treatment, the rats treated with 300mg/kg, 150mg/kg and 100mg/kg of *C. asiatica* extract manifested significantly the same effect as those treated with the positive control (Diclofenac Sodium). Moreover, there is no significant difference in the anti-inflammatory effect among 300mg/kg, 150mg/kg and 100mg/kg doses of *C. asiatica*.

**Table 5.4.** *Post-Hoc Analysis of the Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 7 days Post-treatment*

| | Mean | Negative Control | Positive control | Exp. Group 1 | Exp. Group 2 | Exp. Group 3 | Exp. Group 4 |
|---|---|---|---|---|---|---|---|
| **Negative control** | 0.7286 | | | | | | |
| **Positive Control** | 0.3429 | .000* | | | | | |
| **Exp. Group 1** | 0.3571 | .000* | .875 | | | | |
| **Exp. Group 2** | 0.4143 | .001* | .432 | .529 | | | |
| **Exp. Group 3** | 0.4714 | .007* | .162 | .212 | .529 | | |
| **Exp. Group 4** | 0.6143 | .212 | .005* | .007* | .033* | .121 | |

**The mean difference is significant at the 0.05 level*

The table above shows that after 7 days of treatment, the rats treated with 300mg/kg, 150mg/kg and 100mg/kg of *C. asiatica* extract manifested significantly the same effect as those treated with the positive control (Diclofenac Sodium). Moreover, there is no significant difference in the anti-inflammatory effect among 300mg/kg, 150mg/kg and 100mg/kg doses of *C. asiatica*.

## DISCUSSION

The research study was intended to determine the anti-inflammatory activity of Takip Kuhol (*Centella asiatica*). To attain the objective of the study, phytochemical screening and anti-inflammatory testing were done by the researchers.

Ethanolic extraction of Takip Kuhol (*Centella asiatica*) leaves yielded about 40ml from the 40g of the dried plant sample; thus, having a percentage yield of 100% w/v. Phytochemical screening of the ethanolic extract of Takip Kuhol (*Centella asiatica*) revealed that alkaloids, saponins, phytosterol, phenolic compound, flavonoids, and proteins are present. In many research findings, the presence of flavonoids showed evidently the effectiveness of anti-inflammatory activity (Chippada et al., 2011). Inflammation can be induced by administering carrageenan to the hind, paw of the rats for two hours which resulted in edema. Carrageenan induced inflammation is a well-established method to detect orally active anti-inflammatory agents which shows biphasic response. The first phase is mediated through the release of histamine, serotonin, and kinin, whereas the second phase is through the release of prostaglandin (Sudipta Saha., et al.). Crude extract of Centella asiatica showed anti-inflammatory activity in rats by

prostaglandin E2-induced paw edema. Bioactive terpene acids such as Asiatic acid and madecassic acid may be present in the crude extract that may account for the anti-inflammatory activities (Seevaratnam et al., 2012). Asiaticoside promotes fibroblasts proliferation and extracellular matrix synthesis in wound healing (Mala & Tulka, 2014), collagen synthesis, as well as vasodilation activities. These effects are associated with the reduced activation of macrophages and the production of IL-1beta. It is well known that macrophages play an important role in both acquired and nonspecific immune responses. Activation of these leads to various series of responses including the production of pro-inflammatory cytokines which exert their inflammatory effects by activating a diverse spectrum of signaling cascades in the cell that leads to induction of inflammatory genes such as COX-2 (Park et al., 2017).

Diclofenac was used as a reference drug for comparing the anti-inflammatory activity of the ethanolic extract of Takip Kuhol (*Centella asiatica*). Based on the statistical analysis conducted, each showed that there is a significant difference during the 3rd, 5th and 7th day on the anti-inflammatory activity of the ethanolic extract of Takip Kuhol (*Centella asiatica*). It showed that Takip Kuhol (*Centella asiatica*) is comparable with Diclofenac. As evident on the results based on the four different dosages of the sample, it was found that the doses showed no significant difference in the anti-inflammatory activity. And based on the statistical analysis conducted, ethanolic extract of Takip kuhol (*Centella asiatica*) with a dose of 75 mg/kg and the reference drug, Diclofenac showed a significant difference.

## CONCLUSION

In the anti-inflammatory activity, treatment with ethanolic extract Takip Kuhol (*Centella asiatica*) plant significantly decreased the inflammation based on the results and findings of anti-inflammatory activity. The extracts of Takip Kuhol (*Centella asiatica*) with a dose of 300mg/kg, 150mg/kg and 100mg/kg showed that they have comparable anti-inflammatory activity in rats when compared with that of the positive control, the Diclofenac.

## RECOMMENDATION

Based on the findings of the research on the anti-inflammatory activity of the ethanolic extract of Takip Kuhol (*Centella asiatica*) leaves, here are the researchers' recommendations for the enhancement of this study.

1. Adjust the dose of ethanolic extract upon administration to test subject.
2. The community and concerned agency should promote continuous plantation, cultivation, and growth of Takip Kuhol (*Centella asiatica*).
3. Perform additional studies to determine other uses of Takip Kuhol (*Centella asiatica*).

4. Conduct a preclinical study to test the plant's efficacy when it is formulated to a dosage form.

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