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Anti-Inflammatory Property Evaluation of *Parameria laevigata* (Lupiit) Plant

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***Abstract*—** *Parameria laevigata is a perennial woody vine and has been used for many generations for its extensive natural medicinal properties. The study evaluated the anti-inflammatory properties of P. laevigata using qualitative and experimental research method to provide a scientific basis for its utilization in folkloric medicine as treatment for sprains. Also, the study determined the effectiveness of P. laevigata as treatment for sprains in folkloric medicine from the testimonies of patients, determined its phytochemical components, as well as the anti-inflammatory response of Mus musculus when treated with P. laevigata extract using the carrageenan-induced rat paw edema assay. Informal interviews revealed that the plant is used as treatment for their ailments, two methods of preparation were revealed: one by decoction through boiling with drinking water, and the other by extraction by soaking it with liquor, both of which were claimed by the respondents to be effective. In the phytochemical screening of the ethanolic extract of its root, essential oils, higher alcohols, steroids, anthraquinones, coumarins, tannins, flavonoids, and phenols were detected which would possibly explain its anti-inflammation benefits. In the inflammation experiment, the carrageenan-induced mice paw edema assay was conducted revealing that from the in-vivo test, 100mg/kg (extract/body weight) was comparable to the known anti-inflammatory drug, Diclofenac. The stem crude extract produced a variety of secondary metabolites and exhibited a considerable anti-inflammatory property.*

***Keywords*—** *Alternative medicine, Ethnobotany, folk medicine, Lupiit, non-timber forest product.*

I. INTRODUCTION

The plant domain provides an enormous source of biologically active compounds with medicinal properties. Medicinal plants primarily serve as source of traditional medicine which were considered in the last decades as main focus for rigid pharmacological studies due to their therapeutic worth and being sources of novel compounds in the drug development [1]. Plants produce a wide variety of secondary metabolites that have evolved as defensive mechanism against herbivores, pathogens and other microbes [2]. Medicinal plants are commonly used in treating and preventing specific ailments and diseases that are generally considered to be harmful to humans. These plants are either wild plant species that grow spontaneously in self maintaining populations in natural or semi-natural ecosystems and could exist independently of direct human actions or domesticated plants species which may have arisen through human actions such as selection, breeding or management [3]. This use of herbal drugs that has been in practice among locals for centuries is now defined by the World Health Organization as traditional medicine [4], which only indicates that the indigenous practices of utilizing herbs as treatment for ailments is now recognized even by the premier health organization in the world. Yet, many of the indigenous utilizations of plant materials in health care still have to be evaluated for their efficacy, especially that nowadays, the importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, among others. Recent estimates suggest that several thousands of plants have been known with medicinal applications in various cultures [5]. These attributes of the plants are obviously accounted to the phytochemicals they contain including *Parameria laevigata*. Published studies had reported that *P. laevigata* leaves and bark are found to have embryoprotective and anti-teratogenic role [6]. Some other unverified webpages claim that *P. laevigata* can be used to treat infertility and arthritis. In lieu of the above, this study sought to investigate the biological activity of a locally know plant, the *P. laevigata*, particularly its anti-inflammatory property.

II. MATERIALS AND METHODS

A. Research Design

Survey method through structured interview was conducted personally to determine how patients perceive the effectiveness of *P. laevigata* as treatment to their sprains. Experimental research design was used in determining the anti-inflammatory activities of *P. laevigata* crude extract.

B. Structured Interview

Patients in some local communities of Nueva Vizcaya and Isabela who received *P. laevigata* as treatment for their inflamed body parts were conveniently sampled for interview regarding their perceptions as to the effectiveness of this so-called herbal treatment.

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C. Plant Materials Collection and Authentication

The plant *P. laevigata* were collected from the Mount Singian of Villaverde, Nueva Vizcaya, Philippines. The authenticity of the plant was verified by a licensed Forester.

D. Extraction

The air-dried stem of *P. laevigata* were successively extracted with 90% ethanol within 72 hours soaking. The solvent was evaporated by electric water bath (50^{o}C) to yield a dry condensed residue.

E. Phytochemical Analysis of the Plant Samples

The crude extract was filled into the Thin Layer Chromatography plates using a capillary tube and placed into a developing chamber with methanol, toluene-acetic acid (8:1:1). Chromatograms were sprayed with the different reagents in order to have an idea on the bioactive compounds of the extract responsible for its anti-inflammatory property. Table 1 summarizes the spray reagents used and the corresponding compounds that they test with the expected outcomes.

TABLE 1. Spray Reagents and Corresponding Compounds.

Spray Reagents	Compounds tested	Indication of Positive Result
Vanillin-Sulfuric Acid	Phenol Steroids Essential oils	Triterpenes and Sterols appear mainly as blue violet spots. Essential oils from zones with wide range of color.
Alpha-Napthol-Sulfuric acid	Sugars	Blue spots
KOH-MeOH (Methanolic Potassium Hydroxide)	Anthraquinones Coumanins Antrones	Give orange coloration. React to form blue colored (UV 365nm). Give yellow (UV 365nm)zones.
Potassium Ferricyanide Ferric chloride	Tannins Flavonoids Phenols	Blue spots
Dragendorff's Reagent	Alkaloids	Brown-orange visible spots immediately or spraying color are not stable.
Antimony (III) chloride	Flavonoids Steroids	Intense yellow orange upon spraying for glycoside flavonoids; Flourescent colors under UV 365 nm

F. Experimental Animals

Male mice (20–30 g) were used for the study and were acclimatized at the Center for Natural Sciences greenhouse with free access to food and water ad libitum. The animals were divided into four groups, each group containing five animals: Group 1 served as negative control, which received no treatment (only NSS); Group II animals were treated with ethanol extract at 50mg/kg BW (dissolved in NSS) of *P. laevigata*, Group III were treated with ethanol extract at 100mg/kg BW (dissolved in NSS) of *P. laevigata* and; Group IV served as the positive control which were treated with standard drug diclofenac at 10mg/kg BW (dissolved in NSS).

G. In-Vivo Screening Method for Anti-Inflammation

The Carrrageenan-induced mouse paw edema assay was used in this study. This assay was based on the ability of the anti-inflammatory agent (*P. laevigata*) to inhibit the edema produced in the hind paw of the mouse by the injection of an inflammation agent (carrageenan).

The needed amount of the *P. laevigata* extract to be injected on the test animal was calculated relative to the individual body weight per mouse.

H. Preparation of 1% Carrageenan Suspension

Carrageenan suspension was prepared by mixing 100 mg of carrageenan sodium salt (mister gulaman powder) into a 10 mL NaCl (0.9%). The mixture was stirred properly and let it stand for one hour before administration to the experimental animals

I. Anti-inflammatory Assessment

Twenty mice were fasted for 16 hours prior to the test. The mice were weighed in order to calculate the needed amount of the drug materials (*P. laevigata* extract and diclofenac) with respect to their body weight.

The drug materials were administered orally through gastric gavage following the volume of 1 mL/ 100 g BW. One mL tap water was orally given to each mouse after administration of the drug materials. The left hind paws of the mice were measured using a digital caliper one hour after administration of drug materials. The measurements were carefully observed and tabulated.

One hour after administration of the drug materials, 20 µl of freshly prepared 1% carrageenan suspension in normal saline was administered to the mice by single sub-plantar injection. Swelling or edema formation was measured by the increase in paw thickness which was recorded periodically for six days. The increases in paw thickness and percent inhibition were calculated as follows [7].

$$\text{Increase in paw thickness in control/treatment} \qquad \frac{P_c}{P_T} = P_t - P_0$$

$$\text{Percent inhibition} = \left(\frac{P_c - P_T}{P_c}\right) \cdot 100$$

Where Pt is paw thickness at time t, Po is initial paw thickness, Pc is the increase in paw thickness of the control group and PT is the increase in paw thickness of the treatment groups.

J. Animal Disposal

Mice that were used in the experiment were killed painlessly using chloroform and were buried at the mini-forest of Saint Mary's University.

III. Results and Discussion

A. Use of P. laevigata as Folkloric Medication

The result of interview with 40 respondents who used *lupiit* as traditional medication for treating their inflammation and the respondents' perception of inflammation that they treat with the plant is presented in tables 2 and 3. There are two general methods of using *lupiit* as medication. One method is by preparing a decoction of the *lupiit* through boiling mixing it with the liquor (Gin) that they drink.

The table below reveals the perception of the respondents that the inflammation that they treat with *lupiit* is due to fracture in the bones. There is, however, a fraction of the

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respondents (17.5%) that perceive inflammation as the result of body pain or infection, and it is interesting to note that they considered *lupiit* as medication [8].

TABLE 2. Respondents' Perception of Inflammation that they Treat with P. laevigata.

Themes	Summary of Responses	Freq	%
1. Bone injury	A crack in the bone or damaged caused by accident or hit by hard things resulting to inflammation	10	25%
	A crack in the bone is said to be a fracture and the body's reaction on the healing process which characterized by the swelling of the area of the injured part	5	12.5%
	Fracture occurs when the bone is broken or cracked. The injured part swells, reddish and in pain.	10	25%
	Fracture is a break or crack in a bone or in ossified cartilage which involves rupturing of the skin, after exposing the bone while inflamma- tion is the swelling in an area of the as a reaction to injury or infection	3	7.5%
	Fracture happens when there is a break or crack in the bones due to accident or injury	5	12.5%
2. Body pain and infection	Inflammation is caused by an injury or infection in an area of the body	7	17.5%

Table 3 shows the number of days when the respondents noticed of improvements in their fracture or inflammation. There were 25% of the respondents who noticed that after a day or two, there was already an improvement in their inflammation/fracture, 62.5% of them noticed that the swollen part of their son/daughter's body slowly healed two to four days after they started drinking wine with *lupiit* or boiled water with *lupiit*, and 12.5% claimed that after one to two weeks, there was a great improvement in their fracture/inflammation because according to them, a year after they met an accident only they have known about the anti-inflammatory property of *lupiit*.

TABLE 3. Number of Days of Noticeable Improvements

Theme	Summary of Responses	Freq	%
1. A day or two	After one/two day(s) of drinking lupiit, I noticed an improvement of my inflammation/fracture	10	25
2. Two to four days	Two or three days when I had noticed that the swollen part of my son/daughter body slowly healed	25	62.5
3. One week to two weeks	I have noticed a great improvement of my inflammation after a week or two	5	12.5

B. Phytochemical Screening of the P. laevigata Crude Extract

Table 4 shows that the crude extract of *P. laevigata* had a variety of secondary metabolites namely: phenols, steroids, essential oils, triterpenes, fatty acids, anthraquinones, coumarines, tannins, flavanoids, and phenols. In a review article published in the Journal in Phytopharmacology [9], the secondary metabolites were detertmined as sources of anti-inflammatory substances: terpenoids and essential oils, sterols, saponins, alkaloids, coumarins, flavonoids, gallotannins, thenolic compounds, Condensed tannins. Same classes of compounds were also detected in the root of *P. laevigata*. Secondary metabolites from roots of plants were also shown to produce anti-inflammatory activities [10]. This indicates that the compounds detected from the ethanolic extract of the roots of *P. laevigata* have potentials for anti-inflammatory activities.

TABLE 4. Different Chemical Constituents of the Crude Extract of the P. laevigata.

Reagents	Compound Tested	Positive/Negative Lupiit Extract
Preliminary Test	Organic compounds	
Vanillin-Sulfuric acid	Phenols, Steroids, Essential oils	+
	Triterpenes	+
	Fatty Acids	+
Alpha-napthol-Sulfuric acid	Sugars	-
KOH-MeOH (Methanolic potassium hydroxide)	Anthraquinones,	+
	Coumarins,	+
	Anthrones	-
Potassium ferricyanide-Ferric chloride	Tannins, Flavanoids, Phenols	+
Dragendorff's Reagent	Alkaloids	-
Antimony (III) chloride	Flavanoids, Steroids	+
Magnesium acetate	Anthraquinones	+
Secondary metabolites: *Essential oils, higher alcohols, steroids, Anthraquinones, Coumarins, tannins flavonoids and phenols*		

C. Anti-inflammatory Assay

The white mice, *Mus musculus*, was used for the in-vivo anti-inflammatory assay. Figure 1 summarizes the result of the in vivo anti-inflammatory effect of *P. laevigata* extract on the laboratory mice.

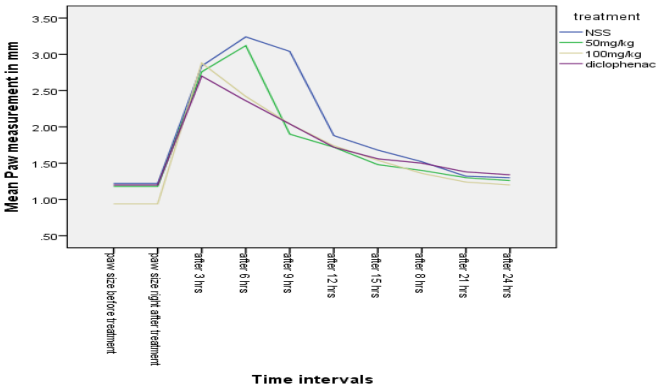

Fig. 1. Summary of the In-Vivo anti-inflammatory Assay.

After induction of carageenan on the paw of the animals, there was a sudden increase in their paw measurement after three hours due to inflammation. The inflammation persisted up to 9 hours for the negative control (NSS-treated) group before it subsided drastically after 12 hours and back to normal after 24 hours. In the group treated with 50mg/kg of extract, the inflammation persisted up to six hours but it subsided drastically after 9 hours.

The highlight of the result as presented in the graph is the observable similarity in the lines for positive control (diclofenac-treated) group and the group treated with 100mg/kg of extract, in which the inflammation of the paw began to subside after three hours. The anti-inflammatory effect of 100mg/kg of P. Laevigata extract is immediate and comparable to diclofenac. This result confirms the

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effectiveness of lupiit in treating inflammation as believed and practiced in folkloric medicine.

IV. Conclusion

P. laevigata is widely claimed among locals of Nueva Vizcaya and Isabela as effective treatment for inflammation. Scientific investigations revealed that the stem crude extract possesses variety of secondary metabolites such as essential oils, higher alcohols, steroids, anthraquinones, coumarines, tannins, flavonoids and phenols, and this extract when assayed in-vivo is comparable to diclofenac in anti-inflammatory activity. The experiences of the locals with *P. laevigata* as effective treatment for inflammation are comparable to the results of anti-inflammatory activities with diclofenac. Based from the salient findings, it is highly recommended that the specific compound responsible for the anti-inflammatory activity of *P. laevigata* should be identified and isolated to allow for preparation of better treatment protocol for inflammation. Other tests for biologic activities should be conducted on this particular plant species to establish its safety when used as medication.

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