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RESEARCH ARTICLE

ANTI-BACTERIAL AND ANTI-INFLAMMATORY PROPERTY EVALUATION OF PARAMERIA LAEVIGATA (LUPIIT) FOR THE FORMULATION OF AN OINTMENT.

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Abstract

This study evaluated the antibacterial and anti-inflammatory property of *Parameria laevigata* in the form of topical ointment. Chloroform and ethanolic solvents were used in the plant stem extraction. The extracts were subjected to phytochemical testing in which both extracts were positive with steroids and phenolic compounds. The antibacterial property was evaluated using paper disc diffusion method. The ethanolic and chloroform extracts with different concentration 2.5mg/ml; 5mg/ml; and 7.5mg/ml were evaluated against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. The study showed that ethanolic crude extract of lupiit with the different concentrations have negative results while the chloroform extract was found to be partially active (10-13mm) on *Staphylococcus aureus* but not active to *E. coli* and *B. subtilis*. The chloroform crude extract was used in preparing the topical ointment. The anti-inflammatory activity was studied using albino rats through wound excision. Different concentrations were made 100 mg/kg BW of rat; 300 mg/kg BW of rat and 500 mg/kg BW of rat. The positive control used in the study was Betamethasone Mupirocin (Foskina-B) while the negative was distilled water. Based from the result of the study, all ointments made with different concentrations can be comparable to positive control. There was no significance difference in time duration among the different concentrations of ointment and the positive control. The best formulation was the 500mg ointment and was made as the final product. Therefore, the study showed that *Parameria laevigata* can be an antibacterial and anti-inflammatory ointment and it can be made available in the market.

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Introduction:-

The world's population has insufficient access on having life-saving medicines. Further, a large percentage of the world's population has a limited or no access in attaining the proper medical treatment needed for their condition according to UN Millennium Project (2005). In addition, majority of the world's population cannot manage to pay for the cost of western style drugs. In many cases, the Third World countries are experiencing and the reason why

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traditional medicines are far distributed on the globe. Such that, herbal medicines are much easier to offer without the rules necessary to register drugs nowadays. And with this, the significance of the medicinal plant's safety and efficacy had been challenged by western academic medicine. Hence, the progress of evidence-based traditional medicines appears to be one of the options to satisfy skeptic western physician according to Tabish (2008).

As defined by World Health Organization (2000), traditional medicine is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. The use of traditional medicines has prolonged globally and gained popularity not only in the use of primary health care of the poor in developing countries but also in countries where conventional medicine is leading in the national health care system.

In the Philippines, Republic Act No. 8423, also known as the Traditional and Alternative Medicine Act (TAMA) of 1997, Article 1, Section 3 promotes and advocates the use of traditional, alternative, preventive and curative health care modalities that have been proven safe, effective, cost effective and consistent with government standards on medical practice. Moreover, Ehrlich (2015) mentioned that traditional medicines are useful resources that is essential to collect, profile and document in order to widen knowledges on traditional medicine for treating diseases. In relation to this, bacterial exacerbations are associated with systemic inflammation according to Murphy (2006). The purpose of the immune system is to prevent or eradicate bacterial infections recognized in the body and it varies to which site has been invaded and the nature of the invader.

In the study conducted by Cuellar, Giner, Recio, Manez, & Rios (2001) on the topical anti-inflammatory activity of extracts of *Cassia angustifolia*, *Rheum palmatum*, *Coptis chinensis*, *Phellodendron amurense* and *Scutellaria baicalensis* plants in East Asia as medicine against different skin disorders found out that all the extracts significantly inhibited the edema induced by 12-O-tetradecanoylphorbol-13-acetate (TPA), in single or multiple application, oxazolone, and arachidonic acid (AA). Another study also demonstrated the antibacterial and anti-inflammatory property of capric acid and lauric acid and found out that it exerts anti-bacterial and anti-inflammatory effect (Huang, 2014).

Published studies also reported that *P. laevigata* leaves and bark are found to have embryoprotective and anti-teratogenic role (Cajuday & Herrera, 2011). According to Vital (2011), the ethanolic extracts of leaves of *P. laevigata* A. L. Juss Moldenke can inhibit parasites. Thus, the plant ethanolic extracts can possibly be used to produce alternative forms of antimicrobials. And also, in the study conducted by Tolentino (2014), *P. laevigata* was evaluated in terms of its anti-inflammatory activity using paw edema method and it was found to treat inflammation. Though in the study, it revealed that *P. laevigata* ethanolic extract have no antimicrobial activity to establish its safety when used as a medication whether topical or internal. Henceforth, this study was conducted to determine the antibacterial property of the *P. laevigata* plant crude ethanolic and chloroform extract. Furthermore, the researchers deemed to evaluate the anti-inflammatory activity of the *P. laevigata* stem using the chloroform extract and to formulate an anti-inflammatory topical ointment. Specifically, the study sought to determine the phytochemical components of *P. laevigata* (Lupiit) stem; determine the antibacterial property of the crude ethanolic and chloroform extract of *P. laevigata* (Lupiit) stem; formulate different concentrations of anti-inflammatory ointment from the chloroform extract of *P. laevigata* (Lupiit) stem; and to determine significant difference between the control group and the formulated concentration of the lupiit ointment in terms of time duration.

Research Methodology:-

Research Design

Experimental research design was used in determining anti-bacterial and anti-inflammatory property of *P. laevigata* (Lupiit) ethanolic and chloroform extract. Figure 1 below summarizes the research undertaking.

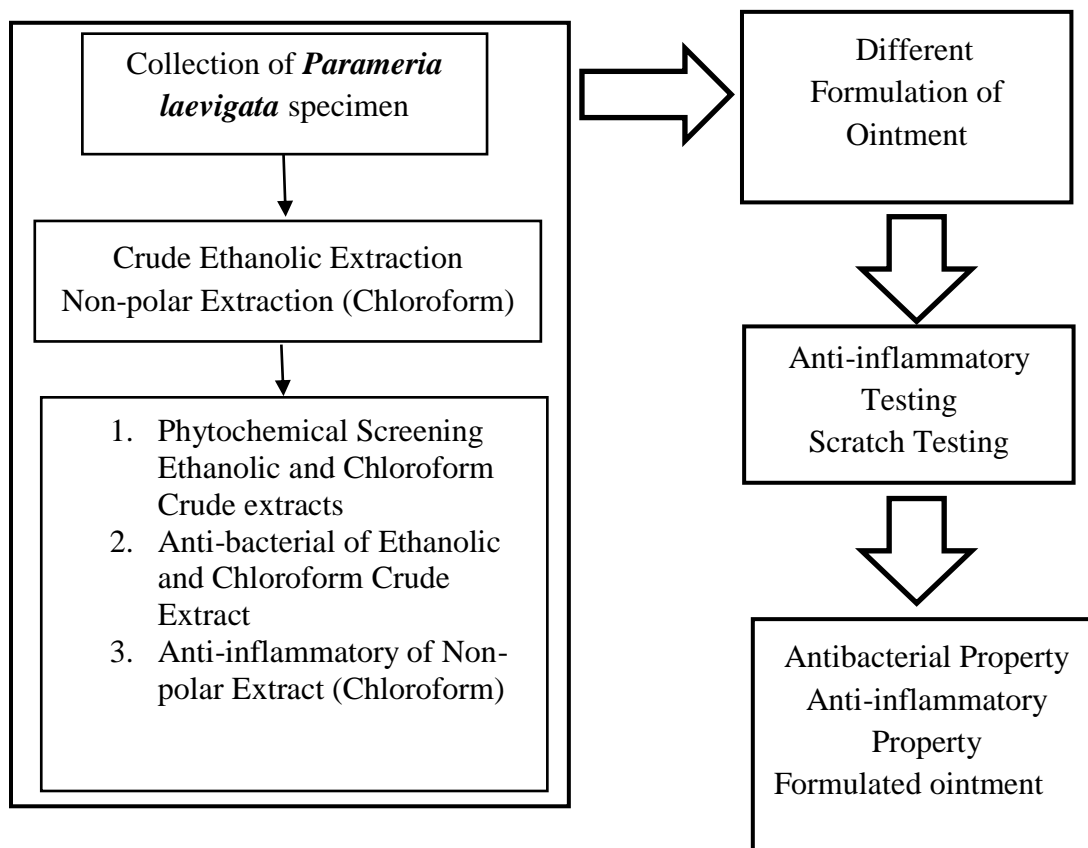


Fig 1:-Schematic diagram of the research process

Collection of Lupiit stem

The plant *P. laevigata* were collected from Mount Singian, Villaverde, Nueva Vizcaya, Philippines. The authenticity of the plant was verified by the local residents of Barangay Bintawan Sur, Villaverde, Nueva Vizcaya, Philippines and examined by licensed Forest Resource Manager. The plant analyses were conducted at the Pharmacy Laboratory and Arts Sciences Laboratory of Saint Mary's University, a private institution in the province of Nueva Vizcaya, Philippines.

Preparation of lupiit stem extract

Four (4) kilograms of *P. laevigata* was freshly powdered using a blender. The powdered lupiit stems were soaked in 80% ethanol and in chloroform for 72 hours. The ethanol and chloroform extracts were placed in steam bath at 40-45 °C until crude extracts was produced. The crude ethanolic and chloroform extracts were weighed and stored in the laboratory refrigerator until phytochemical screening, anti-microbial assay and anti-inflammatory was established.

Laboratory Analyses

Preparation of the Plant Extraction for Phytochemical Screening

The crude extract was filled into the Thin Layer Chromatography plates using a capillary tube and placed into a developing chamber with ethanol, 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 tested.

Table 1:-Spray Reagents and the Compounds Tested Through Phytochemical Analysis.

Spray Reagents	Compounds tested	Indication of Positive Result
Vanillin-Sulfuric Acid	Phenol, Steroids	Triterpenes and Sterols appear mainly as blue violet spots. Essential oils from zones with wide range of color.

	Essential oils	
Alpha-Naphthol-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

Anti-microbial screening

Test for the anti-microbial activity of the extracts on *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* was done using the protocol of Guevara (2005).

Formulation of Ointment

Preparation of ointment

Incorporation method was used in the study. The components were mixed until a uniform preparation is attained. Extemporaneous compounding was used, components were mixed using mortar and pestle, or a spatula was used to rub the ingredients together on an ointment slab (a large glass or porcelain plate or pill tile). Non- absorbable bent parchment paper was used to cover the working surface. The ointment was prepared by thoroughly rubbing and working the components together on the hard surface and the powdered components, previously reduced to fine powder is mixed with a portion of the base until uniform. Geometric dilution was used and continued until all portions of the powder and base were combined thoroughly and uniformly blended.

Wound Excision

Excision wound model was based from the study of Mulisa, Asres, & Engidawork (2015). On wounding day, animals were anesthetized using subcutaneous injection of lidocaine (1 ml/kg) and the back hair of the animals was depilated by shaving. about 1 cm² circular area was marked and the full thickness of the marked area was carefully excised by using sharp sterilized scissors. After 24h of wound creation, the ointment was applied gently once daily, according to the respective grouping as described under grouping and dosing section, to cover the wounded area until complete healing was achieved. Wound contraction, epithelization period was monitored. Wound contraction was measured every 2 days until complete wound closure was achieved.

Application of Ointment and Animal Disposal

A sufficient quantity of ointment was applied on the inflamed area of rats. The Albino rats that were used in the experiment were killed painlessly using chloroform and was buried at the mini forest of Saint Mary's University.

Anti-inflammatory Assay

Albino rats weighing 110-120g were used in the study and were acclimatized at the CNS greenhouse with free access to food and water ad libitum. The animals were divided into five groups, each group containing three animals.

Treatments

Group 1: negative control: white petrolatum and white wax; Group 2: positive control: Bethametasone + Mupirocin; Group 3: 100 mg/kg body weight of the rat; Group 4 : 300 mg/kg body weight of the rat; and Group 5: 500 mg/kg body weight of the rat

Scratch Test

The protocol for the sensitivity test to local and topical applications were done using the protocol of Guevara (2005). The researchers only conducted the scratch test. Using the scratch test, the absence, presence and severity of edema was evaluated based on the parameters listed on Table 2.

Table 2:-Interpretation of Edema Formation.

Indication	Interpretation
0	No edema
1	Very slight edema
2	Slight edema
3	Moderate edema
4	Severe edema

For the scratch test in sensitivity test, albino rats were used. The skin lateral to the spinal groove was shaved and was cleaned. The left side of groove in the animal was utilized as the negative (-) control site and the right side as the test drug site. The negative control refers to the vehicle of the drug. The site was cleaned with 70% alcohol and is immediately followed by the application of the test drug. Both sites are covered with sterilized gauze (1x1 cm in size). Surgical tapes were used to keep the gauze in place, and left undistributed for about 24 to 27 hours during which in time all the test animals are rendered immobile, in scratch test, the application of the test drug, the skin was abraded, lateral to the spinal groove of the test animal, by slightly scratching the skin five (5) to seven (7) times with scalpel.

Treatment of Data

Values were expressed as mean \pm standard error of the mean. Statistical significance was calculated using analysis of variance (ANOVA). The values were considered statistically significant when the p-value is less than 0.05 (<0.05).

Results and Discussions:-

Computation of Yield Value of the Crude Ethanolic and Chloroform Extract.

Table 3:-Computed Yield Value.

Ethanolic Extraction	Chloroform Extraction
Formula: (collected mass/initial mass) X 100% = %	
80% 3250 ml of Ethanol (420 g/2000g) X 100= 21%	3, 750 ml of Chloroform (380g/2000g) X 100= 19%

Based in Table 3, 21% of crude can be extracted from 2kg of *P. laevigata* that were soaked in 3250 ml of ethanol and 19% of crude extract can be extracted from 2 kg powdered stem that were soaked in 3,750 ml of chloroform. This shows that in the ethanolic extraction, there is more % yield compared with the chloroform extraction.

Determination of the Phytochemical Components of *P. laevigata* (Lupit) Stem.

Phytochemical screening of the crude solvents extract afforded several metabolites summarized in Table 4. Ethanolic and Chloroform stem extract of *P. laevigata* contains flavanoids, triterpenes, sterols, essential oils, fatty acid, steroids, antraquinones, coumarines, and anthrones. The bioactive compounds provide semi-qualitative information on the active constituents of the extract.

Table 4:-Results of Phytochemical Screening of Ethanolic and Chloroform Extract.

Metabolites	Rf values (Ethanol)	Rf values (Chloroform)
Alkaloids	0.28,0.68	0.36, 0.7
Anthraquinones	0.3, 0.7	0.56
Fatty acids	0.66	0.32
Flavonoids	0.64	0.42, 0.72
Phenols	0.64	0.42, 0.72
Steroids	0.7	0.72
Tannins	0.64	0.42, 0.72
Essentials oils	0.63	0.66

Essential oils were found in the Lupit ethanolic and chloroform extracts which provide scent of the plant. Essential oils are used in antiseptics and liniments. Specifically, it is used in aromatherapy as form of substitute medicine

(Ramanath, Nagaveni, & Burte (2012). In aromatherapy, essential oils are believed to improve the mood and mental function. They can be used for skin issues, respiratory illnesses and as antiseptics. This is because phenols and phenolic compounds have been widely used in disinfection and remain the standard with which added bactericides are compared. Phenolic compounds are able to inhibit either the production or the action of pro-inflammatory mediators, resulting in anti-inflammatory capacity (Perez, 2015). Phenolic compounds are primarily recognized for their activity as anti-microbial agents in clinical dentistry, and their anti-inflammatory activity is thought to be due to their action as non-specific counter-irritants. Fatty acid may also be responsible for antibacterial and anti-inflammatory effect. One study showed the anti-bacterial and anti-inflammatory property of capric acid and lauric acid and was found to exert anti-bacterial and anti-inflammatory effect (Huang, 2014). In connection with this, *P. laevigata* is positive for phenolic compounds like the flavonoids; hence, it can be said to have potential in suppressing inflammation.

Apparently, in the study, steroids have been detected through phytochemical screening implying that the plant has potential in suppressing inflammation. It is responsible in suppressing the body's immune system, decreasing inflammation as well as blocking the chemical called histamine (causes allergic reaction). The compound is also known as corticosteroids which are used to treat diseases (Tidy, 2015). Corticosteroids are also known as glucocorticosteroids, glucocorticoids or just steroids which are among the most widely used drugs in the world and are effective in many inflammatory and immune diseases (Barnes, 2006). Topical steroids (corticosteroids) are extremely effective anti-inflammatory agents. They suppress various parts of the inflammatory reaction. Topical steroids are available as ointments, creams, gels, lotions, solutions, aerosols and in other forms that can be applied locally on the skin. Moreover, different topical steroids have different potency ranging from mild to very potent topical steroids.

Determination of the Antibacterial Property of the Crude Ethanolic and Chloroform Extract of *P. laevigata* (Lupiit) Stem

Antibacterial Assay: Paper disc diffusion method

The antibacterial assay was done as presented in Guevara (2005) and Benson (2002). The ethanolic and chloroform extracts were tested for their activities against the following microorganism; *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. Bacterial cell suspension was prepared from cultures, and swabbed on petri plates pre-filled with Nutrient Agar (NA). Control disks (Streptomycin for positive control; distilled water for negative control). The moistened filter paper discs containing different concentrations (A-2.5mg/ml, B-5mg/ml and 7.5mg/ml) of the crude extract and the positive control drug are laid on the solidified agar using sterile wooden sticks and immediately the petri dishes were covered. The petri dishes were incubated within 24 hours at 37 degree Celsius. After 24 hours of incubation, the petri dishes were observed for any zone of inhibition. The diameters of the zone of inhibition were measured using Vernier caliper then the measurements were recorded. The measured diameters of the zones were expressed in millimeter (mm). Assays were in 3 replicates. Table 5 summarizes the observed zones of inhibition.

Table 5:-Determination of Antibacterial Property.

Extract (Chloroform)	Staphylococcus aureus	E. coli	Bacillus subtilis
7.5 mg/ml	Partially active	inactive	inactive
5.0mg/ml	Inactive	inactive	inactive
2.5 mg/ml	Inactive	inactive	inactive
Positive (+)	Very active	Very active	Very active
Negative (-)	Inactive	inactive	inactive
Extract (Ethanol)	Staphylococcus aureus	E. coli	Bacillus subtilis
7.5 mg/ml	Inactive	inactive	inactive
5.0 mg/ml	Inactive	inactive	inactive
2.5 mg/ml	Inactive	inactive	inactive
Positive (+)	Very active	Very active	Very active
Negative (-)	Inactive	inactive	inactive

The zones of inhibition were measured and means were computed. These measured zones of inhibition among the Lupiit ethanolic and chloroform extracts and with the standard (Streptomycin) were observed and compared with the positive control. And based from Table 5, all of the concentrations showed no antibacterial property against

Bacillus subtilis and *E. coli*. Only the chloroform crude extracts exhibit antibacterial property on *Staphylococcus aureus* at 7.5mg/ml of the Lupiit extract.

Formulation of the Anti-inflammatory Ointment from the Different Concentrations of *P. laevigata* (Lupiit) Stem.

Table 6:-Anti-inflammatory Reading every 2 Days' Time Intervals.

Ointment Concentrations (mg/kg BW of rat)	Phase 1 (inflammatory phase)		Phase 2 (Epithelialization)	Phase 3 (maturation phase)				Phase 4 (healing phase)	
	Day2	Day4	Day6	Day8	Day10	Day12	Day14	Day16	Day18
100	1.367	1.267	1.433	1.333	1.233	1.100	1.033	1.567	0.333
Ointment Concentrations (mg/kg BW of rat)	Phase 1 (inflammatory phase)		Phase 2 (Epithelialization)	Phase 3 (maturation phase)				Phase 4 (healing phase)	
Ointment Concentrations (mg/kg BW of rat)	Day2	Day4	Day6	Day8	Day10	Day12	Day14	Day16	Day18
300	1.367	1.167	1.133	1.267	1.267	1.167	0.900	0.633	0.267
500	1.267	1.267	1.100	1.333	1.267	0.967	0.600	0.367	0.167
+ Control	1.200	1.200	1.200	0.900	1.100	0.700	0.400	0.100	0.000
- Control	1.300	1.100	1.100	1.100	1.100	1.100	0.733	1.100	1.100

Table 6 shows the anti-inflammatory effect of the chloroform extract administered in every after 2-day intervals. Inflammation has four phases. The first phase called inflammatory phase of inflammation occurred at time 0-1. At time 2, phase 2 occurred, in which there was observed epithelialization. The sign and symptoms observed in rats were pain, palpable tenderness, swelling and muscle range in motion. There was a violent reaction of rats so there was tendency of scratching their back. Third phase which is maturation phase occurred at time 3 to time 7. In this phase there was formation of collagen characterized by less warmth, swelling palpable tenderness decreases, pain felt with tissue resistance. Phase 4 occurred at time 8 in which there was removal of epithelialization and the surrounding skin pigmentation.

Initially, around 1cm² was inflicted to the rats as to determine the anti-inflammatory property of the Lupiit chloroform extract. And based from table 3, the topical administration of chloroform extract in the form of ointment markedly raised the size of the incurred wound 2 days after wound excision (Time 0). However, across time, it can be gleaned from the table that the sizes of inflammation decreases indicating that the 100mg/kg BW of rat, 300mg/kg BW and 500mg/kg BW of rat concentrations are relatively effective of reducing the skin inflammation.

ANOVA Test Results of the Significant Difference between the Control Group and the Formulated Concentration of the Lupiit Ointment in Terms of Time Duration.

Table 7:-Significant Difference between the Control Group and the Formulated Concentrations of the Lupiit Ointment in Terms of Time Duration.

ANOVA						
Day		Sum of Squares	Df	Mean Square	F	Sig.
Day 2	Between Groups	0.060	3	0.020	0.471	0.711
	Within Groups	0.340	8	0.043		
	Total	0.400	11			

Day 4	Between Groups	0.023	3	0.008	0.176	0.909
	Within Groups	0.340	8	0.043		
	Total	0.363	11			
Day 6	Between Groups	0.203	3	0.068	4.784	0.034
	Within Groups	0.113	8	0.014		
	Total	0.317	11			
Day 8	Between Groups	0.389	3	0.130	1.922	0.205
	Within Groups	0.540	8	0.068		
	Total	0.929	11			
Day 10	Between Groups	0.057	3	0.019	0.204	0.891
	Within Groups	0.740	8	0.093		
	Total	0.797	11			
Day 12	Between Groups	0.383	3	0.128	2.473	0.136
	Within Groups	0.413	8	0.052		
	Total	0.797	11			
Day 14	Between Groups	0.740	3	0.247	3.364	0.076
	Within Groups	0.587	8	0.073		
	Total	1.327	11			
Day 16	Between Groups	0.517	3	0.172	3.626	0.064
	Within Groups	0.380	8	0.048		
	Total	0.897	11			
Day 18	Between Groups	0.189	3	0.063	1.484	0.291
	Within Groups	0.340	8	0.043		
	Total	0.529	11			

Based from Table 7, across time observation periods revealed no significant difference wherein computed p values were greater than 0.05 excluding Day 4 wherein computed p values was less than 0.05, $p=0.034$. This indicates that the different concentrations do not vary in terms of healing effect with respect to the wound incurred to the rats. The conducted study agrees with the results acquired by Tolentino (2015) in her study on anti-inflammatory property of the ethanolic extract of *P. laevigata* using paw edema method (oral gavage). The results of the study revealed that there is no significant difference in the different concentrations when compared to the control group wherein p value was greater than 0.05. Similarly, as stated by Nordqvist (2015), the inflammation can cause unlikable result since it can become self-perpetuating because more inflammation is created in response to the prevailing inflammation. From the initial incurred wound of 1cm^2 , 2 days after excision, the wounds have increased in sizes. The sizes of inflammation become wider across the concentrations. On the other hand, Wassung (n.d.) stated that the inflammatory response is a natural defense mechanism that is triggered whenever body tissues are damaged in any way. Most of the body defense elements are located in the blood and inflammation is the means by which body defense cells and defense chemicals leave the blood and enter the tissue around the injured or infected site. Moreover, inflammation occurs in response to physical trauma, intense heat and irritating chemicals, as well as to infection by viruses and bacteria. In the conducted study, the topical ointment method was used to determine the anti-inflammatory property of the chloroform extract of *P. laevigata*. It resulted to no significant difference across concentrations in terms of time excluding day 6. To determine the particular group with significant difference, further test was performed through Bonferroni multiple comparison. Using multiple comparisons, there is no significant difference between the concentrations of ointment across time intervals. This implies that the concentrations have relative effect on the wound of the experimental rats. The concentrations 100mg/kg, 300mg/kg, 500mg/kg, and the positive control group concentration have p values greater than 0.05. This indicates that the different concentrations of chloroform extract of *P. laevigata* (Lupit) are relatively comparable with the positive control Bethametasone + Mupirocin. Further, the data suggest that the different concentrations can reduce size of the inflammation and treat the inflammation of the experimental rats. Based on the result conducted in scratch testing, it did not show any edema; hence, the experimental animal has no sensitivity with the 500mg/kg concentration.

Conclusions and Recommendations:-

Based on the results, there was no significance difference among the concentrations and positive control in terms of time duration. The researchers also concluded that *P. laevigata* ointment is effective as anti-inflammatory agent and

it was partially active against *Staphylococcus aureus*. The best formulation of the ointment was made from 500mg concentration. Hence, the researchers recommended that addition of perfume and colorants can be mixed with the ointment to enhance the appearance. The researchers also recommend that the next researchers may focus on the isolation of the compound responsible in the anti-inflammatory effect.

References:-

1. Barnes, J. (2006). How corticosteroids control inflammation: Quintiles Prize Lecture 2005. Retrieved January 20, 2017 from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1751559/>.
2. Cajuday, L. & Hererra, A. (2011). An embryoprotective role of *Parameria laevigata* in vitamin A teratogenesis in *Mus musculus*. *The Asian International Journal of Life Sciences*, 20(1).
3. Cuellar, MJ., Giner, RM., Recio, MC., Manez, S. and Rios, JL. (2001). Topical anti-inflammatory activity of some Asian medicinal plants used in dermatological disorders. *Fitoterapia* 72(3), 221-9.
4. Ehrlich, S., (2015). Herbal medicine. Internet article. Retrieved from <http://umm.edu/health/medical/altmed/treatment/herbal-medicine>
5. Guevara, B. (2005). A guidebook to plant screening: phytochemical and biological (Revised Edition). University of Santo Tomas PUBLISHING HOUSE Espana, Manila p.135-175.
6. Huang, W. (2014). Anti-bacterial and anti-inflammatory properties of capric acid against *Propionibacterium acnes*: a comparative study with lauric acid. Retrieved <https://www.ncbi.nlm.nih.gov/pubmed/24284257>.
7. Mulisa, E., Asres, K., and Engidawork, E. (2015). Evaluation of wound healing and anti-inflammatory activity of the rhizomes of *Rumex abyssinicus* J. (Polygonaceae) in mice. *Journal of the International Society for Complementary Medicine Research*, 15, 341.
8. Murphy, T. (2006). The role of bacteria in airway inflammation in exacerbations of chronic obstructive pulmonary disease. National Center for Biotechnology Information online Abstract. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/16645482>.
9. Nordqvist, C., (2015). Inflammation: Causes, Symptoms and Treatment. Internet article. Retrieved from <http://www.medicalnewstoday.com/articles/248423.php>.
10. Perez, D. (2015). Phenolic compounds: Natural alternative in inflammation treatment. A Review. Internet article. Retrieved from <https://www.cogentoa.com/article/10.1080/23311932.2015.1131412>.
11. Ramanath, B., Nagaveni, B., & Burte, N. P. (2012). Analgesic and Antipyretic Effects of the Ethanolic Fruit Extract of the *Momordica cymbalaria* Hook. *Fenzl. International Journal of Toxicological and Pharmacological Research*, 4(3), 45-48.
12. Tabish, SA. (2008). Complementary and alternative healthcare: Is it evidence-based? *International Journal of Health Sciences*, 2(1), 5-9.
13. Tidy, C. (2015). Oral Steroids. Internet article. Retrieved January 20, 2017 from <http://patient.info/health/oral-steroids>.
14. Tolentino, N. (2015). Verification of anti-inflammatory property of *Parameria laevigata* (lupiit) using in-vivo screening method: basis for the preparation of daily learning logs in teaching concepts of anti-inflammation. Unpublished thesis, Saint Mary's University, Bayombong, Nueva Vizcaya, Philippines 3700.
15. UN Millennium Project. (2005). Chapter 1: The problem – UN Millennium Project. Internet article. Retrieved from www.unmillenniumproject.org/documents/TF5-medicines-Chapter1.pdf.
16. van Valkenburg, J. (2001). *Parameria laevigata* (A.L. Juss.) Moldenke, Record from Proseabase. van Valkenburg, J.L.C.H. and Bunyapraphatsara, N. (Editors). PROSEA (Plant Resources of South-East Asia) Foundation, Bogor, Indonesia. Retrieved from <http://www.proseanet.org>.
17. Vital, P. (2002). Antimicrobial activity of *Parameria Laevigata* (A.L. Juss) mondenke and *Voacangan globosa* (Blanco) Merr. Extracts. The Science and Technology Information Network of the Philippines (SCINET-PHIL). Retrieved October 6, 2016 from [http://scinet.dost.gov.ph/union/ShowSearchResult.php?s=2&f=&p=&x=&page=&sid=1&id=Antimicrobial+activity+of+%3Cem%3EParameria+Laevigata%3C%2Fem%3E+\(a.l.+Juss.\)+moldenke+and+%3Cem%3EVoacangan+globosa%3C%2Fem%3E+\(Blanco\)+Merr.+extracts&Mtype=ANALYTICS](http://scinet.dost.gov.ph/union/ShowSearchResult.php?s=2&f=&p=&x=&page=&sid=1&id=Antimicrobial+activity+of+%3Cem%3EParameria+Laevigata%3C%2Fem%3E+(a.l.+Juss.)+moldenke+and+%3Cem%3EVoacangan+globosa%3C%2Fem%3E+(Blanco)+Merr.+extracts&Mtype=ANALYTICS).
18. Wassung, K. (nd). Inflammation and the health process. Internet article. Retrieved from www.viafamilychiropractic.com.
19. World Health Organization. (2000). General guidelines for methodologies on research and evaluation of traditional medicine. Internet article. Retrieved from <http://apps.who.int/iris/bitstream>.