ANTI-INFLAMMATORY ACTIVITY OF SPANISH NEEDLE (*Bidens pilosa L.*) ROOTS ETHANOLIC EXTRACT ON DINITROCHLOROBENZENE-INDUCED SPRAGUE-DAWLEY RATS

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ABSTRACT

This research study focuses on the anti-inflammatory activity of the ethanolic root extract of the plant Spanish Needles (Bidens pilosa L.). Inflammation has been induced with Dinitrochlorobenzene on Sprague Dawley Rats. Spanish Needles (Bidens pilosa L.) ethanolic root extract was prepared by maceration. In the anti-inflammatory activity, the ethanolic extract was administered in three different doses (100 mg/kg, 200 mg/kg, and 300 mg/kg). Prednisolone at 2.5 mg/ kg was used the positive control. Allergic contact dermatitis was induced with Dinitrochlorobenzene on the right ear of the rats for 8 days. Right ear thickness was measured before and after induction of ACD. Administration of treatment groups started on the 10th day of the protocol. The right ear thickness was measured with a Vernier caliper. The Spanish Needles (Bidens pilosa L.) root extract was administered orally. As evident on the results, it was found out that there is a significant difference on all the doses of Spanish Needles (Bidens pilosa L.) root extract (100 mg/kg, 200 mg/kg and 300 mg/kg) and the positive control. Furthermore, statistical analysis using Student t-test and One- Way Analysis of Variance (ANOVA) showed that the ethanolic extract of Spanish Needles (Bidens pilosa L.) is comparable with the standard drug, Prednisolone. Based on the data gathered, the researchers concluded that Spanish Needles (Bidens pilosa L). is effective as an anti-inflammatory agent.

Key words: *Bidens pilosa L., dinitrochlorobenzene, ethanolic extract, inflammation, Sprague Dawley rats*

INTRODUCTION

One of the body's major defense mechanisms is Inflammation. It is a pathophysiological process by which the immune system acknowledges and eliminates harmful stimuli and initiates the healing process. In general, there are two types of inflammation: acute and chronic inflammation. Chronic inflammation is being referred to as slow, long-term inflammation lasting for prolonged periods of several months to years. Generally, the extent and effects of chronic inflammation vary with the cause of the injury and the ability of the body to repair and overcome damage from the stimuli (Pahwa et al. 2018).

Nearly 25% of the population of developed countries is afflicted with allergic disorders majorily associated with inflammation, such as eczema, hay fever, asthma, and anaphylaxis. Repetitive exposure to allergens which are intrinsically not harmful substances found commonly in the environment leads to chronic allergic inflammation (Galli et al. 2008).

Estimated statistics is formed from surveillance on occupational studies. Roughly 30% of all occupational diseases are associated with the skin, 90% of this account are an irritant and contact dermatitis. On an annual Incidence report of such diseases imposes to 13 to 34 cases per 100,000 workers. The commonly associated agents causing ACD included latex materials, acrylics, resins, soap, cleaners and protective equipment's. On a separate study was taken from the general population thru patch testing and retrospective studies included metals, preservatives, chemical hair products, fragrances, topical antibiotics, topical corticosteroids, plastic, and glue as the most common allergen groups.

The non-steroidal option such as herbal drug treatments has been employed since the dawn of time. Plant extractives formulated into suitable dosage forms such as topical ointments and creams are some of these herbal treatments. The Philippines has wide biodiversity, majority of these are herbs. Spanish Needles (*Bidens pilosa L.)* is an erect, erect perennial herb which usually grows on tropical regions like the Philippines. The plant has been widely used as a traditional medicine which is usually used to treat a broad range of complaints e.g. to sooth pain, inflammations. Results of studies on suspension and boiling water extract of dried powder from the aerial parts of Spanish Needles (*Bidens pilosa L.) var radiata Scherff* inhibited histamine release and production of IgE, suggesting it may be clinically useful in the prevention of type 1 allergic disease (Horiuchi et al.2008). Motivated by these observations, this study aimed to determine the anti-inflammatory and anti-allergic activities of Spanish Needle (*Bidens pilosa L.)* roots. In this paper, a Dinitrochlorobenzene-induced ACD rat model was utilized to explore the anti-inflammatory and anti-allergic effects of Spanish Needle Roots (*Bidens pilosa L.)*.

Research Questions

1.) What are the preliminary phytochemical constituents present in the local variety of Spanish Needle Roots (*Bidens pilosa L.)*?
2.) Is there a significant difference in the anti-inflammatory activity of Spanish Needle Roots *(Bidens pilosa L.)* ethanolic extract when grouped according to treatment groups?
    a.) 100 mg/kg
    b.) 200 mg/kg
    c.) 300 mg/kg

d.) positive control
e.) negative control

3.) Is there a significant difference in the anti-inflammatory activity of Spanish Needle Roots *(Bidens pilosa L.)* ethanolic extract, when grouped according to the timing of treatment?
a.) before treatment (after completion of inflammation induction days)
b.) after completion of treatment

Hypotheses

Ho_1: There is no significant difference in the anti-inflammatory activity between the positive control, which is the Prednisone at 2.5 mg/ kg, and the experimental controls of Spanish Needles *(Bidens pilosa L.)* ethanolic root extract, with its three doses of 100mg/kg, 200mg/kg, 300mg/kg, respectively.

Ho_2: There is no significant difference in the anti-inflammatory activity of Spanish Needle Roots *(Bidens pilosa L.)* ethanolic extract when grouped according to the timing of treatment.

Significance of the study

This study determined whether there is an anti-inflammatory activity of Spanish Needle root (*Bidens pilosa L.*) which will, therefore, provide alternative organic management for the inflammatory disorder that is used by a person who suffers from allergic contact dermatitis. This study will be providing a substitute treatment for people who are currently taking anti-inflammatory and anti-allergic drugs who are suffering from undesirable side effects.

Spanish Needles (*Bidens pilosa L.*) which is commonly known as “Burburtak” in Tagalog, is found in several native towns here in Cagayan. It is known as an herbal plant that is used in the treatment of various skin diseases by the native people in the above-mentioned place. Thus, this study will be advantageous to the community by raising the awareness of the proper usage of natural and alternative medications on the treatment of inflammation and allergy since it is abundant in the locality. This study will also promote the usage of a nearby source of treatment especially to the community where medical services are difficult to acquire.

This study will be an advantage in the context of healthcare by providing a natural and accessible preparation for inflammation and allergy. This study could lead future researchers to formulate a new drug of discovery utilizing the herbal plant and will serve as an eye-opener to the future researchers to conduct further studies.

Literature Review

Inflammation

The body’s major defense mechanism against harmful stimuli or allergens is inflammation. It is a pathophysiological process by which the body acknowledges and eliminates harmful stimuli and initiates the healing process. In general, there are two types of inflammation: acute and chronic inflammation. Chronic inflammation is characterized by slow, long-term inflammation lasting for prolonged periods of several months to years. The extent and effects of chronic inflammation vary with the cause of the injury and the ability of the body to repair and overcome the damage from the stimuli. This article reviews chronic inflammation (Pawha et al. 2018). Characterized by delayed hypersensitivity is Allergic contact dermatitis which rises from the exposure of an allergen broken out between sensitive subjects. Such disease is being mediated by T lymphocyte cells together with sensitized T lymphocytes.

In contemporary clinical practices, the therapy given to patients with ACD would entirely be consist of antibiotics, chemical topical and oral drug preparations and steroids which may cause harmful adverse drug reaction on long-term users. Thus inculcation of herbal, traditional, and low-toxicity substitute for ACD sets up new feet of advancement to the health care providing industry (Xiao et al. 2018).

Nearly 25% of the population of developed countries is afflicted with Allergic disorders majorly associated with inflammation, such as eczema, hay fever, asthma, and anaphylaxis. Repetitive exposure to allergens which are intrinsically not harmful substances found commonly in the environment leads to chronic allergic inflammation. The implication renders long-term metamorphoses the structure of the affected organs and notable abnormalities in their normal function. Giving importance to the further understanding of the characteristics and upshot of chronic allergic inflammation, and in particular to identify how mast cells contribute to some of the features of this maladaptive pattern of immunological reactivity (Galli et al. 2008).

Estimated statistics is formed from surveillance on occupational studies. Roughly 30% of all occupational diseases are associated with the skin, 90% of these accounts are an irritant and contact dermatitis. On an annual Incidence report of such diseases imposes to 13 to 34 cases per 100,000 workers. The commonly associated agents causing ACD included latex materials, acrylics, resins, soap, cleaners and protective equipment's. On a separate study was taken from the general population thru patch testing and retrospective studies included metals, preservatives, chemical hair products, fragrances, topical antibiotics, topical corticosteroids, plastic, and glue as the most common allergen groups.

In contrast to the desired study, a Dinitrochlorobenzene-induced ACD rat model was utilized to explore the anti-inflammatory and anti-allergic effects of *Bidens pilosa* extract. A study conducted by Chinese researchers on the anti-inflammatory effect of Fructus Kochiae, Sprague- Dawley rats were used as their animal model on their experiment. Dinitrochlorobenzene (DNCB) was used to induce Allergic Contact Dermatitis (ACD) according to a diffusely employed protocol based on (Xiao et al. 2018). The Dinitrochlorobenzene was dissolved in a mixed solvent (acetone/ olive oil, 4:1, v/v); 7% and 1% Dinitrochlorobenzene solution were prepared, respectively. Right ear thickness and right ear weight after recovery were some of the parameters of this study (Xiao et al. 2018).

The Eczema Area Severity Index (EASI) scoring system uses a defined process to grade the severity of the signs of allergic inflammation (eczema) and the extent affected. Erythema, edema papulation, excoriation, and lichenification are the parameters involved in this system (EASI Guidance, Dec. 2014). A study conducted on the anti-inflammatory effect of Fructus Kochiae on Allergic Contact Dermatitis Rats, the oral administration of the total flavonoids of Fructus Kochiae displayed a significant anti-inflammatory activity in ACD rat, including the inhibition of monocyte infiltration and epidermal keratinization and mediation of cytokines in ear tissue and serum (Xiao et al. 2018).

Prednisone is a synthetic form of a natural substance (corticosteroid hormone) made by the adrenal gland. It is used to treat conditions such as arthritis, blood problems, immune system disorders, skin and eye conditions, breathing problems, cancer, and severe allergies. It decreases the immune system's response to various diseases to reduce symptoms such as pain, swelling, and allergic-type reactions.

Traditional and Alternative Medicine Act (TAMA) of 1997

The use of herbal medicines has been employed and practiced ever since man has started to develop consciousness in their environment. Ethnic groups and various cultures utilize their available resources in order to prevent and manage diseases. Republic Act 8243 (RA 8243), also known as the Traditional and Alternative Medicine Act of 1997, is an act creating the Philippine Institute of Traditional and Alternative Health Care (PITAHC), accelerating the development of traditional and alternative health care in the Philippines. The act focuses on developing different traditional health-related management in the country.

Drugs for the prevention, treatment, mitigation, and cure of diseases, lessening signs and symptoms, and maintaining a healthy lifestyle with lower price are needed to be explored and developed. Alternative medications employed for such purposes undergo proper methods of compounding. This law strongly

encourages indigenous people living in the Philippines to share their traditional medicines and for people to study more about the pharmacological and toxicological implications of these alternative medicines. Health care professionals should become aware of these medications and promote it to their community. By this, our countrymen would encounter more alternative medicines coming from a lesser price than that of the existing drugs. The cheaper the medicines get, the higher the chance of patient medication compliance (Nolledo, 2015).

Ethnopharmacology of Spanish Needles (*Bidens pilosa L.*)

There are 230 to 240 identified species of Bidens. Among these species, Spanish Needles (*Bidens pilosa)*. Spanish Needles (*Bidens pilosa)* is a representative perennial herb which is globally distributed across temperate and tropical regions like the Philippines. The plant has been traditionally consumed as food and used as a medicine without obvious adverse effects. Spanish Needles (*Bidens pilosa)* is an easy-to-grow herb. It is considered to be a rich source of food and medicine for humans and animals. There is increasing global interest in the use of Spanish Needles (*Bidens pilosa)* as shown by the many studies conducted on the plant in recent years. The traditional use of Spanish Needles (*Bidens pilosa)* has been recorded continentally namely: America, Africa, Asia, and Oceania. Carolus Linnaeus was the first to collect and name the plant "*Bidens pilosa"* in 1753.

Taxonomically, it belongs to the plant family of Asteraceae and genus of Bidens. Bidens genus is estimated to include 230 to 240 species globally. Several varieties of Spanish Needles (*Bidens pilosa)* includes var. radiata, var. minor, var. pilosa, and var. bisetosa. Alongside examination of morphological traits, authentication of Spanish Needles (*Bidens pilosa)* can be aided by chemotaxonomy and molecular characterization. Spanish Needles (*Bidens pilosa)* is an erect, perennial herb. The plant is either glabrous or hairy, with green opposite leaves that are serrated, lobed or dissected. It has white or yellow flowers, and long narrow ribbed black achenes (seeds). It grows to an average height of 60 cm and a maximum of 150 cm in favorable environments.

Bidens species and their varieties bear vernacular names based on their characteristics. Bidens species are known by such names: Spanish needles, Beggar's ticks, Devil's needles, Cobbler's pegs, Broomstick, Pitchforks, and Farmers' friends. These names are associated because of their sticky achenes, or seeds. The plant is also known as xian feng cao ("all bountiful grass") in Chinese because of their prosperous growth. All parts of Spanish Needles (*Bidens pilosa)* plant, the whole plant, the aerial parts (leaves, flowers, seeds, and stems), and the roots, fresh or dried, are used as ingredients in folk medicines. It is frequently prepared as a dry powder also by decoction, maceration or tincture.

Generally, this plant is applied as dry powder or tincture when used externally, and as a powder, maceration, or decoction when used as an internal remedy (Bartolome, Villaseñor, et al 2013). Different research studies were conducted to prove the anti-inflammatory and anti-allergic activity of various plants in the world like the inhibitory effects of *Calophyllum inophyllum* extract on atopic dermatitis induced by DNCB in mice. It was found out that *C. inophyllum* extracts efficiently inhibit inflammatory lesion of DNCB induced mouse skin and show superior redundant of itching behavior and inflammation-related signal molecules of disease with low toxicity (Um, Jo et al, 2016).

Improvement of the Anti-inflammatory and anti-allergic activity of Spanish Needles (*Bidens pilosa)*. var. radiata SCHERFF treated with the enzyme (Cellulosine) was also conducted. Results from the study suggest that Spanish Needles (*Bidens pilosa)* var. radiata SCHERFF has anti-inflammatory and anti-allergic activity and that enzymatic digestion enhances its anti-allergic activity by the inhibition of histamine release from the mast cells due mainly to an increase in caffeic acid and flavonoids (Horiuchi, Seyama, et al, 2008).

Research Paradigm

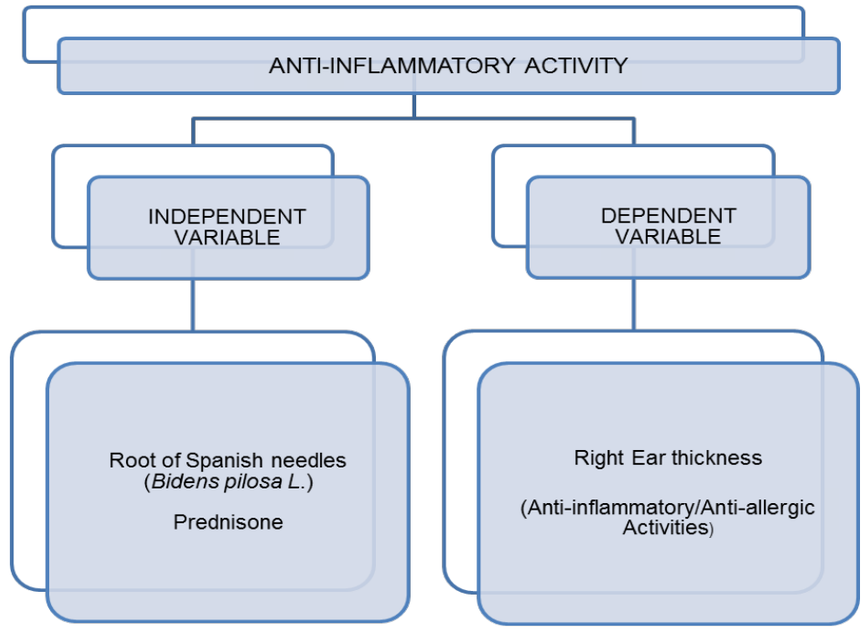

Figure 1. *Research Paradigm*

The figure above was patterned from the research study of Andaya, Herrera, Pajarillo, Wanya. The figure above shows how the independent variable which is the roots of Spanish needles (*Bidens pilosa L.*) as the experimental control and the positive control which is Prednisone can affect the Degree of Allergic Inflammation in experimental animals and on how the dependent variable responds to the different controls.

METHODS

This section consists mainly of various equipment, apparatuses, procedures, and protocols in the aforementioned research study. Found here also are the subjects of the study, plant sample technique, phytochemical analysis, together with the extraction method, and the biological assays.

Research Design

Experimental method was applied in this research endeavor, which included plant authentication and extraction, animal acclimatization, disease induction protocol, and biological assay. It was conducted in the Pharmacy and Chemistry laboratories of the University of Saint Louis, Tuguegarao City, Cagayan and in the Philippine Institute of Traditional and Alternative Health Care (PITAHC), Carig Sur, Tuguegarao City, Cagayan.

The study aimed to determine the anti-inflammatory and anti-allergic activities of the ethanolic extract of Spanish Needle roots (*Bidens pilosa L.)* that were administered orally on Sprague-Dawley rats at a dose of 100 mg/ kg, 200 mg/kg and 300 mg/kg of the plant root extract.

The infusion of Spanish Needle roots (Bidens pilosa L.) did not produce acute oral toxicity at a limit dose of 2000 milligram/ kilogram, did neither produce toxicity after the application of repeated doses of 1000 milligram/ kilogram during 28 days, being produced some beneficial changes on hematological and biochemical variables in the sentinel group. So much the gel as the cream prepared from the infusion of Spanish Needle roots (Bidens pilosa L.) did not produce dermal irritation (Cárdenas, M. B. et al. 2006). Accordingly, Prednisone (2.5 milligrams/ kilogram) was used as the standard drug.

Right ear thickness was measured using a Vernier caliper accordingly and right ear weight on the last day of the experimentation. Right ear thickness was measured to determine if there is a significance on the anti-inflammatory activity of Spanish needle roots *(Bidens pilosa L.)* when grouped according to treatment groups and according to the timing of treatment.

Subjects of the Study

The roots of the Spanish Needle plant was used for this research. The plant material was collected from Tallang, Baggao, Cagayan. Male Sprague Dawley rats (*Rattus norvegicus domesticus)* on the other hand, was bought from a registered and duly licensed breeder in Malvar, Santiago City, Isabela. The rats were housed in the Philippine Institute of Traditional and Alternative Health Care (PITAHC) at Carig Sur, Tuguegarao City, Cagayan.

Sample Technique

The identification and authentication of the plant sample were conducted at the Department of Agriculture Regional Center, Carig Sur, Tuguegarao City, Cagayan.

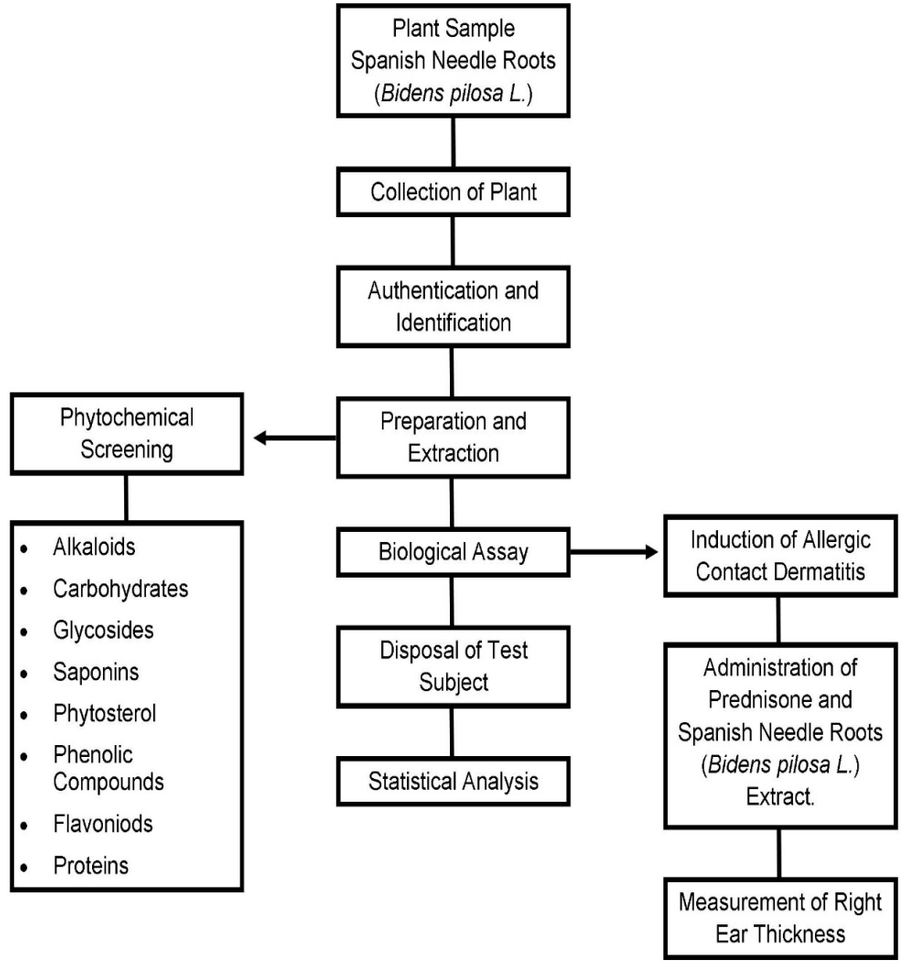

Figure 2. *Methodological Flowchart*

Procedures of Data Gathering

1. Collection and Preparation of Plant Control

1.1. Plant Authentication

Prior to the conduct of the experiment, authentication of the plant was accomplished at Department of Agriculture, Regional Office, Carig Sur, Tuguegarao City. Department of Agriculture authenticated the plant sample as Spanish Needles (*Bidens pilosa var. pilosa.).*

1.2. Plant Collection

Roots of the Spanish Needle plant were used for this research. The fresh plant material was collected from Tallang, Baggao, Cagayan. The roots were washed thoroughly with tap water to remove soil particles and debris. After washing, the roots were sun-dried for 2- 3 days.

1.3. Powdered Plant Roots

The sun-dried plant root materials were again dried in a drying oven at 150 deg. Celsius for 2 hours. After drying, the roots were finally reduced to a powder form using a blender. The powder was passed through a powder sieve to remove debris.

1.4. Preparation of Bidens pilosa root extract for the phytochemical analysis

1.4.1 The preparation of the plant root extract for phytochemical analysis was based on the textbook of (Guevarra et al) on Phytochemical Analysis of Plants.

1.4.2 Ethyl alcohol, 80% was prepared by diluting 80 mL of ethyl alcohol with distilled water to 100mL.

1.4.3 Ground dried plant material was weighed at around 100 g in an Erlenmeyer flask and was treated with sufficient 80% ethyl alcohol to completely submerge the plant material. The volume of alcohol used was noted (Guevarra et al).

1.4.4 The plant suspension was macerated for 48 hours.

1.4.5 After maceration, the plant material was washed with fresh portions of alcohol in a Buchner funnel. The plant material residue was discarded.

1.4.6 The collected filtrate from the Buchner filtration process was filtered again using a filter paper.

1.4.7 Then it was subjected to the rotatory evaporator for 1 hour to completely remove the ethanolic component and concentrate.

1.4.8 After an hour, a sample extract was subjected to a flame test to confirm traces of ethanol. If positive, it will be subjected again to rotatory evaporation for 10 minutes. If the flame test is negative, the final extract will then be weighed using an analytical balance.

1.4.9 The weight and the final concentration of the plant extract were noted.

1.4.10 It was stored and labeled in a clean Erlenmeyer flask.

2. Preliminary Phytochemical Screening of Constituents

Phytochemical Screening of the plant constituents was conducted at the University of Saint Louis Baguio by the Natural Sciences Research Unit.

3. Biological Assay

3.1 Preparation of Spanish Needle (*Bidens pilosa*) roots extract for administration

3.1.1 900 ml of Ethyl alcohol, 80% was prepared by diluting 757 mL of ethyl alcohol, 95 % with 143 ml of distilled water.

3.1.2 Ground dried plant material was weighed at around 100 g in an Erlenmeyer flask and was treated with 900 ml of ethyl alcohol, 80% to completely submerge the plant material.

3.1.3 The plant suspension was macerated for 48 hours.

3.1.4 After maceration, the plant material was filtered using a laboratory filter paper and glass funnel. The plant material residue was discarded.

3.1.5 The collected filtrate from the filtration process was filtered again. The volume of the collected filtrate was 650 ml.

3.1.6 The filtrate was refluxed until the volume was reduced to half.

3.1.7 The extract was subjected to flame test accordingly until no traces of ethanol.

3.1.8 The final concentration of the plant extract was 100 g/ 100 ml of *Bidens pilosa* extract.

3.2 Preparation of the Test Subjects

Ethics on laboratory animal used was secured prior to experimentation. Application for the conduct of scientific research was submitted to the Philippine Institute of Traditional and Alternative Healthcare (PITAHC). The permit to conduct was granted by the Bureau of Animal Industry (BAI). Prior to experimentation, this study had passed

through the Animal Ethics Board of the University of Saint Louis. Sprague-Dawley rats were purchased from United Doctors Animal Clinic, Malvar, Santiago City, Isabela. Each group of animals had access to the same food and water ad libitum. All animal anti-inflammatory experiments on conscious animals were performed with the NIH guide for the care and use of laboratory animals and Ethical Issue of the International Association for the Study of Pain (Mandred, Z. et al 1983).

3.3 Acclimatization of Animals

A total of 25 Sprague-Dawley male rats with a weight range of 70-150 grams were purchased from the United Doctors Animal Clinic, Malvar, Santiago City, Isabela used in this were kept in 5 cages, 5 rats per cage under standard condition (temperature of 25±2 deg. C, 12 hour light and 12 hour dark cycle). All animals were fed with commercially formulated rat feed and water ad libitum. The animals were acclimatized for a period of 2 weeks.

3.3.1 Rat ACD Model Construction and Treatment

3.3.1.1 In this paper, a Dinitrochlorobenzene -induced Allergic Contact Dermatitis (ACD) rat model was utilized to explore the anti-inflammatory and anti-allergic effects of Spanish Needle roots (*Bidens pilosa L.*) extract. Sprague- Dawley male rats were used as the animal model, weighing 70- 150 grams.

3.3.1.2 Dinitrochlorobenzene was used to induce Allergic Contact Dermatitis (ACD) according to a diffusely employed protocol employed in the study of (Xiao et al. 2018). Dintrochlorobenzene (Sigma Aldrich) was purchased from Chemline Scientific Philippines. The Dinitrochlorobenzene was dissolved in mixed solvent (acetone/ olive oil, 4:1, v/v); 7% and 1% Dinitrochlorobenzene solution was prepared, respectively.

3.3.1.3 After shaving the abdomen of the rats with a sterile hair shaver (Shick), sodium sulfide was used to completely remove the hair in the area of 2 cm2.

3.3.1.4 Firstly, 50 mcl of 7% Dinitrochlorobenzene solution was applied to the rat's abdomen to sensitize it on the first day.

3.3.1.5 On the second day, 50 mcl of 1% Dinitrochlorobenzene solution was coated on the right ear of the rats to reinforce sensitization.

3.3.1.6 20 mcl of 1% Dinitrochlorobenzene solution was evenly coated on the right ear of the rats on the fifth day and repetitive operation was carried out on the subsequent three days.

3.3.1.7 The drug administration started on the fifth day with a frequency of twice a day. There are five groups in our experiment: Dinitrochlorobenzene group (Negative control) was treated with Dinitrochlorobenzene; Dinitrochlorobenzene + 100 mg/kg BPE; Dinitrochlorobenzene + 200 mg/kg; Dinitrochlorobenzene + 300 mg/kg; and Dinitrochlorobenzene + Prednisone Acetate (2.5 mg/kg) as the positive control group. A brief diagram adopted from the study of (Xiao et al. 2018) of the ACD model construction treatment protocol is shown in **Figure 3.**

- **Group 1:** Negative control (Distilled water)
- **Group 2**: 100 milligram per kilogram of Spanish Needle Roots (*Bidens pilosa L.)* Extract + Dinitrochlorobenzene
- **Group 3:** 200 milligram per kilogram of Spanish Needle Roots (*Bidens pilosa L.)* Extract + Dinitrochlorobenzene **Group 4:** 300 milligram per kilogram of Spanish Needle Roots (*Bidens pilosa L.)* Extract + Dinitrochlorobenzene
- **Group 5:** Positive control (Prednisone, 2.5 milligrams per kilogram) + Dinitrochlorobenzene

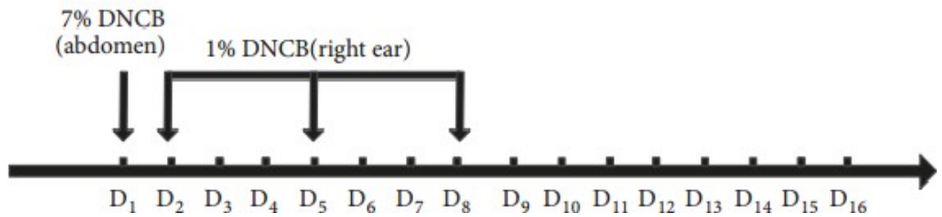

Figure 3. *A brief diagram of the ACD model construction and treatment groups*

3.1 Determination of the anti-inflammatory/anti-allergic activity of Spanish Needles (Bidens pilosa) extract

Sprague- Dawley rats were used as the animal model for inducing Allergic Contact Dermatitis (ACD). After the induction of ACD and confirmation of the visual parameters for skin sensitization, drug treatment started on the 10th day. The Eczema Area Severity Index (EASI) scoring system was used to grade the severity of the signs of allergic inflammation (eczema) and the extent affected. The intensity of redness, Thickness/ swelling, Scratching and Lichenification are the parameters involved that were evaluated. Right ear thickness was measured using a Vernier caliper and the right ear weight (end of experimentation) was evaluated to determine the significance of the control groups.

Statistical Analysis

This study aimed to determine the anti-inflammatory effects of Spanish Needle roots (*Bidens pilosa L.*) extract on Sprague-Dawley rats using student t-test and
one- way analysis of variance (ANOVA).

Disposal of Dead Animals

The researchers followed the IACUC Disposal Rodent Carcasses Policy using a high dose of anesthetics (Revised June 2013). After taking a right ear specimen for weighing, the rats were suffocated and killed with absorbent cotton dipped in chloroform. The carcasses were placed in a black plastic bag. The plastic bags were finally placed at the designated disposal area at the PITAHC.

Ethical Considerations

All animal anti-inflammatory experiments on conscious animals were performed with the NIH guide for the care and use of laboratory animals and Ethical Issue of the International Association for the Study of Pain. The ethical permit was secured from the Department of Agriculture. Application for the conduct of scientific research was submitted to the Philippine Institute of Traditional and Alternative Healthcare (PITAHC). The permit to conduct was granted by the Bureau of Animal Industry (BAI). The ethical permit was also secured from the Animal Ethics Board of the University of Saint Louis. Sprague- Dawley rats were purchased from United Doctors Animal Clinic, Santiago City.

RESULTS

All the end results of every experiment performed on the duration of this research endeavor are stated in this section.

Table 1. *Preliminary Phytochemical Constituents*

Constituent	Result	Constituent	Result
Carbohydrates	+	Physosterol	+
Reducing Sugars	+	Phenolics	+
Anthraquinone Glycosides	-	Tannins	+
Unsaturated Lactones	+	Flavonoids	+
Diterpenes	+	Proteins	+
Triterpenes	+		

Table 1 shows the result of the Phytochemical Screening that *B. pilosa* is positive for the following constituents: Alkaloids, Carbohydrates, Unsaturated lactones, Saponins, Phytosterols, Phenolic Compounds, Flavonoids while negative of Anthraquinone Glycosides and Proteins.

Table 2. *Degree of Inflammation of the Different Subjects after Administration of the Different Treatments*

	1 day Post Treatment	2 day Post Treatment	3 day Post Treatment	4 day Post Treatment	5 day Post Treatment
Negative control (Distilled water)	1.26	1.26	1.22	1.14	1.08
Treatment 1 100 mg/kg Spanish Needle Root Extract	1.28	1.08	.96	.92	.90
Treatment 2 200 mg/kg Spanish Needle Root Extract	1.40	1.18	1.04	.94	.90
Treatment 3 300mg/kg Spanish Needle Root Extract	1.18	1.04	.92	.88	.88
Positive control (Prednisone,	1.22	.98	.88	.86	.86

2.5mg/mL/kg)					

The table above shows that there is a decrease in the degree of inflammation of the test subjects which received the positive control and the different doses of Spanish needle root extract. This therefore signifies that the Spanish Needle extracts have anti-inflammatory effect.

Table 3. *Test of Significant Difference of the Degree of Inflammation of Subjects administered with different doses of Spanish Needle root extract Pre and Post-treatment*

Pairs	t-value	p-value	Decision
Post-Carrageenan Administration- 1 day Post Treatment	2.704	0.000	Reject Ho
Post-Carrageenan Administration- 2 days Post Treatment	4.407	0.000	Reject Ho
Post-Carrageenan Administration- 3 days Post-Treatment	5.700	0.000	Reject Ho
Post-Carrageenan Administration-4 days Post Treatment	6.452	0.000	Reject Ho
Post-Carrageenan Administration-5 days Post Treatment	6.812	0.000	Reject Ho

The table above shows that all concentrations/doses of the Spanish Needle root extract significantly decreased the degree of inflammation of the test subjects.

Table 4.1 *Test of Significant Difference in the Anti-inflammatory Activity of the Different Treatment Groups 1, 2, 3, 4 and 5 days Post-treatment*

	F-value	p-value	Decision
Day 1 post treatment	.605	.664	Accept Ho
Day 2 post treatment	1.573	.220	Accept Ho
Day 3 post treatment	7.175	.001	Reject Ho
Day 4 post treatment	10.433	.000	Reject Ho
Day 5 post treatment	10.944	.000	Reject Ho

The table above presents that there is a significant difference in the degree of inflammation of the test subjects after 3, 4 and 5 days of treatment. This further implies that the significant inflammatory activity exhibited by the positive control and the experimental treatments is manifested only after at least 3 days of treatment.

Table 4.2. *Post-Hoc Analysis of the Test of Significant Difference in the Anti-inflammatory Activity of the Different Treatment 3 days Post-treatment*

Treatment Group	Mean	Negative Control	Treatment 1	Treatment 2	Treatment 3	Positive Control
Negative Control	1.22	1				
Treatment 1	.96	.002*	1			
Treatment 2	1.04	.020	.273	1		
Treatment 3	.92	.000*	.579	.106	1	
Positive Control	.88	.000*	.273	.036	.579	1

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

The table above shows that 100mg/kg and 300mg/kg dose of the Spanish Needle root extract exhibited significantly the same effect as the positive control in decreasing the degree of inflammation of the test subjects after 3 days of treatment.

Table 4.3. *Post-Hoc Analysis of the Test of Significant Difference in the Anti-inflammatory Activity of the Different Treatment 4 days Post-treatment*

Treatment Group	Mean	Negative Control	Treatment 1	Treatment 2	Treatment 3	Positive Control
Negative Control	1.14	1				
Treatment 1	.92	.000*	1			
Treatment 2	.94	.001*	.687	1		
Treatment 3	.88	.000*	.425	.235	1	
Positive Control	.86	.000*	.235	.118	.687	1

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

The table above shows that all doses of the Spanish Needle root extract exhibited significantly the same effect as the positive control in decreasing the degree of inflammation of the test subjects after 4 days of treatment.

Table 4.4. *Post-Hoc Analysis of the Test of Significant Difference in the Anti-inflammatory Activity of the Different Treatment 5 days Post-treatment*

Treatment Group	Mean	Negative Control	Treatment 1	Treatment 2	Treatment 3	Positive Control
Negative Control	1.08	1				
Treatment 1	.90	.000*	1			
Treatment 2	.90	.000*	1.000	1		
Treatment 3	.88	.000*	.604	.604	1	
Positive Control	.86	.000*	.304	.604	.604	1

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

The table above shows that all doses of the Spanish Needle root extract exhibited significantly the same effect as the positive control in decreasing the degree of inflammation of the test subjects after 5 days of treatment.

DISCUSSION

In this study, phytochemical screening of constituents results show that Spanish Needle roots (*Bidens pilosa L.*) extract is positive for the following constituents: Alkaloids, Carbohydrates, Unsaturated lactones, Saponins, Phytosterols, Phenolic Compounds, Flavonoids while negative of Anthraquinone Glycosides and Proteins. According to (Bartolome, Villaseñor, et al 2013) on their study on the "Botanical Properties, Traditional Uses, Phytochemistry, and Pharmacology of *Bidens pilosa L.*", *B. pilosa* is an extraordinary source of phytochemicals, particularly flavonoids and polyenes. Plant flavonoids are reported to possess anticancer, anti-inflammatory, antioxidant, and other bioactivities. This study also suggests that *B. pilosa* and its phenolics have anti-inflammatory functions. Phenolics and polyenes are major anti-inflammatory phytochemicals present in *B. pilosa* (Bartolome, Villaseñor, et al 2013).

In contrast to the desired study, a Dinitrochlorobenzene-induced ACD rat model was utilized to explore the anti-inflammatory and anti-allergic effects of *Bidens pilosa* extract. A study conducted by Chinese researchers on the anti-inflammatory effect of *Fructus Kochiae*, Sprague- Dawley rats were used as their animal model on their experiment. Dinitrochlorobenzene (DNCB) was used to induce Allergic Contact Dermatitis (ACD) according to a diffusely employed protocol based on (Xiao et al. 2018). The Dinitrochlorobenzene was dissolved in a mixed solvent (acetone/ olive oil, 4:1, v/v); 7% and 1% Dinitrochlorobenzene solution were prepared, respectively. Acetone was used to smear the drug on the skin while olive oil was used to preserve the drug. Right ear thickness and right ear weight after recovery were some of the parameters of this study (Xiao et al. 2018).

Prednisone is a synthetic form of a natural substance (corticosteroid hormone) made by the adrenal gland. It is used to treat conditions such as arthritis, blood problems, immune system disorders, skin and eye conditions, breathing problems, cancer, and severe allergies. It decreases the immune system's response to various diseases to reduce symptoms such as pain, swelling, and allergic-type reactions.

This study was intended to determine whether there is an anti-inflammatory activity of Spanish Needle roots *(Bidens pilosa L.)* which will, therefore, provide an alternative for anti-inflammatory and anti-allergic preparations that may be used by the people who suffer from inflammatory conditions. For the determination of its anti-inflammatory activity, an animal model was used and Sprague-Dawley rats weighing 70-150 grams were selected. The three doses of the extract possessed anti-inflammatory activity in Dinitrochlorobenzene- induced allergic contact dermatitis on Sprague-Dawley rats. Dinitrochlorobenzene was applied to the right ear of the rats topically. Rats right ear were induced with Dinitrochlorobenzene for 7 days. Administration of the treatment groups was made on the 10^{th} day. Measurement of the Right ear thickness was monitored throughout the experimentation. Ear thickness was measured using a Vernier caliper.

With this method, the rat's right ears were treated with the three oral doses of Spanish Needle Roots *(Bidens pilosa L.)* extract, right ear thickness was measured and compared to the oral dose of prednisone, a reference drug for anti-inflammatory/ allergic activity. The results indicate that the three doses of earthworm extract possess a comparable anti-inflammatory activity with that of the prednisone whereas to the negative control, which is the plain distilled water, there was a significant difference between them.

DNCB- induced allergic contact dermatitis is a model of acute inflammation and the agents responsible for reducing the right ear thickness are useful as anti-inflammatory agents. The three doses of Spanish Needle Roots *(Bidens pilosa L.)* showed significant reduction of right ear thickness comparable to that of the prednisone.

Improvement of the Anti-inflammatory and anti-allergic activity of Spanish Needles (*Bidens pilosa)*. var. radiata SCHERFF treated with the enzyme

(Cellulosine) was also conducted. Results from the study suggest that Spanish Needles (*Bidens pilosa)* var. radiata SCHERFF has anti-inflammatory and anti-allergic activity, and that enzymatic digestion enhances its anti-allergic activity by the inhibition of histamine release from the mast cells due mainly to an increase in caffeic acid and flavonoids (Horiuchi, Seyama, et al, 2008).

CONCLUSION

Based on the results and findings of the anti-inflammatory activity, it was found out that the extract of Spanish Needle Roots possessed such activity. The researchers concluded that Spanish Needle Roots *(Bidens pilosa L.)* extract is comparable with the positive control prednisone and such has exhibited a comparable effect on reducing right ear thickness in rats.

RECOMMENDATIONS

Based on the aforementioned findings and conclusions drawn, the following recommendations and suggestions are deemed significant:

- The researchers may perform semi-purification regarding extract of Spanish Needles *(Bidens pilosa L.)*
- The researchers may conduct studies regarding the inhibitory properties of the plant on histamine release using ELISA.
- The researchers should formulate a dosage form for ease of administration (e.g. topical dosage forms).

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