



Evaluation of the analgesic property of the semi-purified flavonoids from the fronds of *Diplazium esculentum* ethanolic extract

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Abstract

Diplazium esculentum or “Pako” is an edible fern popularly used in homemade salads in the Philippines and the other form is used as an ornamental plant. According to the findings of Chawla *et al.* (2015) *D. esculentum* is a potential analgesic drug in treating pain and the active constituents inferred to be responsible for the effect were flavonoids and sterols. The present study was done to evaluate the analgesic property of the semi-purified flavonoids of *D. esculentum* ethanolic extract. Phytochemical analysis shown the presence of saponin glycosides, flavonoids, proteins and fixed oils. The evaluation used acetic acid induced writhing method in five groups of swiss albino mice given the dose of (25, 100, 300 and 500 mg/kg) of the plant extract compared to the positive control (Aspirin). The study used One Way Anova and Tukey’s Multiple Comparison test in the analysis of the data. The computed F which is 153.7977528 is greater than the F-critical which is 2.866081402 therefore there is a significant difference between the groups. The comparison used 95% level of confidence with a P value of 0.1209. These data may indicate that the semi-purified flavonoids of *D. esculentum* has showed analgesic effect as the dose increases.

Keywords: analgesic, *Diplazium esculentum*, flavonoids, pteridophytes, aspirin

Introduction

Philippines is a tropical country with great biodiversity reason why many plants were discovered and cultivated until today. In ancient times, Filipinos utilize folklore herbal medicines in treating various illnesses such as pain. The availability of plants is not a problem to consider, the nature itself of the country supports the needs of its people in searching for unconventional medicines. However because of the changing society, people ascertained different technologies and ways on how to obtain drugs in a convenient way. Nowadays, several drugs of chemical source emerged in the market and one of these are the analgesics. Large number of the population prefers chemically obtained drugs rather than the natural or plant sources in the remedy of pain.

Pain is a tormenting unpleasant sensation that can lead from general to localized discomfort. The sensation is characterized as both physical and emotional. Patients can now select their treatment from a wide range of analgesic drugs in the market and some are available over the counter or those which are dispensed without a prescription. Aside from chemical origin, plants are also a basis of analgesic properties in which the researchers are interested to.

“Pako” or *Diplazium esculentum* is an edible fern popularly used in homemade salads in the Philippines and the other form is used as an ornamental plant. It belongs to the family Athyriaceae and widely distributed in Asia and to other parts of France, North America, Hawaii and Polynesia. The plant usually originates on stony bars and near banks of streams. The stipe grows 20-50 centimeters long and green in color (Quisumbing). They are rich in calcium, phosphorus and a good source of iron and vitamin B. This plant has a lot of

beneficial uses and the researchers focused on identifying its medicinal use.

According to the recent findings of Chawla *et al.*, 2015^[7]. *D. esculentum* is a potential analgesic drug in the treatment of pain and further studies should be conducted to develop the property of this plant. It concluded that the active constituents responsible for the protective action were the flavonoids and sterols. Therefore, the researchers of this study decided to continue the determination of the constituent that elicit the desirable effect and flavonoids from the ethanoic extract of *D. esculentum* was subjected for evaluation of its analgesic property.

Background of the Study

Several drugs for pain such as low-back pain, headache, arthritis pain, cancer pain, and other conditions have several limitations including restricted long-term efficacy, tolerance problems and other adverse effects. Therefore, herbal medicine is the answer for a natural way of treating pain and Pteridophytes is an example of a plant with various uses in food, medicine and other necessities in life.

In this study, the researchers used *Diplazium esculentum* also known as “Pako” in the Philippines. The fresh young ferns of this plant is commonly served as a salad which is rich in vitamins and other nutrients while the matured plant is usually used as a decoration in flower arrangements just like the other types of ferns in the same family. Studies regarding this plant are emerging with different findings and conclusions such as its antioxidant, antimicrobial, cytotoxic and analgesic activity. Therefore, the researchers of this study were focused in the evaluation of its property to lessen the different types of pain

in the body without producing such tolerance problems and adverse effects like those drugs commercially available in the market.

Theoretical Framework

Diplazium esculentum is one of the most common pteridophytes and distributed widely in Asia. According to Quisumbing, trunk bears many black wiry roots; it is erect, woody and thick. The stalks are green in color and somewhat smooth; it is also 20-50 cm long. The tip is clothed with brown linear scales. Fronds are 2 or 3 pinnate, 50-80 cm long, and about half as wide as long. The pinnate are broad ovate, 2-3 cm long and rather coarsely toothed. The clusters of spore are located on the side of the veins or veinlets.

The recent findings of Chawla *et al.*, stated that the plant was found to be a potent analgesic and flavonoids and sterols were the attributes that may be considered in eliciting the protective action. The intention of the researchers is to evaluate the flavonoids of *D. esculentum* using ethanolic extract and in its semi-purified form. Flavonoids are polyphenols commonly found in fruits, vegetables and plants. This constituent is significantly used as natural antioxidant and has been shown to possess antimutagenic and antimalignant effects. The solvent ethanol has the ability to extract more phytochemical constituents such as phenolics and flavonoids.

In the evaluation of the analgesic property acetic acid induced through the process of writhing method was utilized and aspirin served as the positive control. The method is usually applied in peripherally acting analgesics and aspirin is a non-opioid that has analgesic property that acts by suppressing pain substances in the peripheral tissue. Furthermore, pain perception involves the production of prostaglandin and flavonoids were reported to inhibit prostaglandin synthetase.

Literature Review

This chapter provides information regarding literature and studies conducted about *Diplazium esculentum*. Further, it presents the foreign and local literature and foreign and local studies.

Local Literature

“Fiddlehead fern” also known as “Pako” is from the family Athuriaceae which is abundant in the southern parts of the island of Luzon up to the entire Central Visayas. This study was entitled “Nutritional and Phytochemical Screening, and Total Phenolic and Flavonoid Content of *Diplazium esculentum* (Retz.) Sw. from Philippines” by Jovale Vincent V. Tongco *et al.* Publish last 2014.

The study concluded that methanol is more toxic and the solvent used in the study was ethanol. For its ability to respond to specific bioassays and its ability to extract more constituents like phenolic compounds and flavonoids rather than water.

Foreign Literature

Diplazium esculentum is one of the most common pteridophytes and distributed widely in Bangladesh and Philippines. This study was entitled, “Investigation of In vitro Antioxidant, Antimicrobial and Cytotoxic activity of *Diplazium esculentum* (RETZ). SW.” by Akter *et al* published

last 2014. Accordingly, the dried rhizomes of the plant are mainly used as insecticides and it is also used for coughs and haemoptysis through the method decoction. In the folklore medicine, tribal and ethnic groups utilized the various parts of the plant in the treatment of various ailments. The leaves are used for headache, pain, fever and wounds. The plant is also used in dysentery, glandular swellings, indigestion, diarrhea and various skin infections. Phytochemical constituents are steroids, triterpenoids, phenols, flavones, flavonoids such as myricetin and alphatocopherol.

For the determination of the total flavonoid content, Folin-Ciocalteu reagent was used. The methanol extract revealed the presence of various plant secondary constituents while the chloroform extract yielded no result for carbohydrates. Flavonoids are polyphenols commonly found in fruits, vegetables and plants. Flavonoids contribute significantly as natural antioxidant. Flavonoids have been shown to possess antimutagenic and antimalignant effect along with its role in preventing diseases like cancer and inhibiting low density lipoprotein (LDL) oxidation induced by free radicals. Flavonoids have also shown activity as antibacterial, antiviral, antitumor and anti-inflammatory.

The study concluded that *D. esculentum* leaf extracts possess antimicrobial activity and cytotoxic activity. The antioxidant potential of the plant was exhibited by the reducing capacity of flavonoids and cupric ion.

Local study

A local study of Amoroso *et al* published last January 2014, from Central Mindanao University, entitled “Antimicrobial, Antipyretic, and Anti-Inflammatory Activities of Selected Philippine Medicinal Pteridophytes” provided the researchers the necessary information in conjugation to the phytochemical screening of the plant *Diplazium esculentum*. According to the study, Pteridophytes are described to as herbal medicines used in the treatment of various illnesses yet scarce literature regarding the medicinal potentials of this plant have been conducted. The study used 15 species of Philippine medicinal pteridophytes in the determination of the constituents of each plant specifically alkaloids, flavonoids, saponins, tannins and anthraquinones. The antimicrobial activity of the plants were tested on *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas Aeruginosa*. Egg albumin induced paw edema was the method used in the anti-inflammatory activity and yeast induced pyrexia for antipyretic activity.

The ethanol extraction used 200 g of the fresh plant were weighed separately and 95% ethyl alcohol was used and the plant was completely submerged for 24 hours, filtered and concentrated using a rotary evaporator. The results of the phytochemical screening showed that *D. esculentum* has flavonoids. The anti-inflammatory and antipyretic activities of the plants are coined in the presence of flavonoids which is an important pharmacological constituent. Based on the table of phytochemical analysis of the ethanolic extract, *D. esculentum* is negative in the test for leucoanthocyanins (Bate-Smith and Metcalf). Anthraquinones were undetected in all species of Pteridophytes used in the study. The absence of anthraquinones in the pteridophyte extract may prevent the occurrence of diarrhea when the plant extracts are

administered orally as herbal remedy.

Extract from three species of pteridophytes namely: *Equisetum ramosissimum*, *Pyrrosia piloselloides*, and *Selaginella usterii* did not manifest any antibacterial activity and all extracts showed significant effect in reducing yeast-induced pyrexia when compared with the negative control (NSS).

Foreign Study

The plant *Diplazium esculentum* was recently studied by Chawla *et al.* in the year 2015 from the Department of Pharmacy, Himachal Institute of Pharmacy, India entitled "Analgesic Activity of Medicinally Important Leaf of *Diplazium esculentum*". The prospect of the study entails the use of medicinal plants in modern era in the cure of pain. Fresh plants were gathered and were air dried at temperatures ranging from 25°C to 35°C, reduced, and preserved. The researchers used four solvents (Extract I-distilled water, Extract II-petroleum ether, Extract III-chloroform, and Extract IV-ethanol) in extracting the plant constituents and the phytochemical screening yielded flavones, flavonoids, proteins, steroids, and triterpenoids respectively.

Acetic acid induced the practice of writhing process. It was utilized for the determination of the analgesic activity. Six (6) groups of albino mice of both genders ranging from 20 to 30 grams in weight were grouped into two and each group includes 5 mice. The first group serves as the control and the second group was treated with the standard drug (aspirin). Route of administration was intraperitoneally at a dose of 25mg/kg and the biological animals were placed and observed under a glass jar. The response such as trunk twists, extension of the hind limbs and abdominal contractions were regarded as the writhing responses. It was calculated that the reactions produced by the extract commenced after 10 minutes of administration. Based on the level of significance the results of the acetic acid induced writhing method states that extract IV or the ethanol solvent is greater than the results of extracts I, II, and III.

The study concluded that the aqueous extracts of *Diplazium esculentum* were found to be potent analgesic. Based on the observations it has a marked advantageous effects against centrally, peripherally and inflammatory pain models. Flavonoid and sterols were the attributes that may be considered in eliciting the protective action. Accordingly, the study encourages further studies on the matter to identify the exact active constituent accountable for the analgesic activity of the plant. These reports may serve as a footstep in the research of a potent analgesic drug. (Chawla *et al.*, 2015)^[7].

Research Methodology

The study was conducted to evaluate the analgesic property of *Diplazium esculentum* fronds. Randomized control trials were used in the manipulation of the quantitative and expected results. Experimental method was used by the researchers to be a tool in the evaluation of the analgesic property of *D. esculentum*. The use of experiments in the course of the study was to predict and to answer the stated problems by manipulating the given variables. The method was most applicable in every aspect of the study from the extraction of plant constituents to the isolation and biological tests.

The study used laboratory animals in the evaluation of the analgesic property of *D. esculentum* and the 30 Swiss albino mice were purchased from the Pharmacology and Toxicology Department of University of the Philippines, Manila. The researchers submitted documents for Application of Authorization and Animal Care and Use Statement (IACUC) at Our Lady of Fatima University. The study also passed the OLFU Institutional Ethics Review Committee (IERC).

The fresh plant samples were collected near banks of streams at Gloria 1 Sindalan City of San Fernando, Pampanga on the month of August. For the certification of authenticity, the plant materials were brought to the Botany Division of the National Museum, Manila.

The 100 grams of the plant materials were air dried for seven days. The dried fronds were reduced in particle size, weighed and placed in a wide mouth glass container. The pulverized plant samples were extracted using the solvent 80% ethyl alcohol and macerated for 72 hours with frequent agitation.

The extracted fronds of *Diplazium esculentum* were screened for the presence or the absence of flavonoids, alkaloids, glycosides, proteins, carbohydrates, saponins, tannins, and phenolic compounds.

To obtain the semi-purified flavonoids, the collected plant samples were air dried for 7 days and the 100 grams of the plant was submerged and macerated with 80% ethyl alcohol for 48 hours. Using a Rotary Evaporator, the crude extract was concentrated until a viscous solution was obtained. This was evaporated to dryness in a water bath at 60 degrees Celsius. The residue was treated with almost 10 mL of 2M HCl and equal amounts of ethyl acetate. The treated extract was evaporated to incipient dryness and the residue left was the flavonoids. The presence of the constituent was confirmed using alkaline reagent test.

The semi-purified flavonoids were subjected to organoleptic evaluation, solubility, confirmatory tests and acute oral toxicity tests.

Acute oral toxicity test was performed following the Organization for Economic Cooperation and Development (OECD) guideline # 423. The test animals that were used in the study were nulliparous, non-pregnant, and healthy female albino swiss mice.

The test animals were kept and placed in an experimental room with temperatures ranging from 22°C (+3°C). Animals were given unlimited supply of water and will be grouped. For the preparation, the test animals were randomly selected, given identification and were placed in the cage 5 days prior to dosing. The samples were administered in a constant volume according to the range of doses per concentration. Prior to dosing, the mice were fasted for 3-4 hours. Administration of the test substance was of single dosing and was administered via oral gavage using a suitable intubation cannula. After the administration of the test substance the mouse was withheld from food for 1-2 hours. The doses that were administered were 5, 50, 300 and 2000 mg/kg body weight and were observed for 30 minutes, 24 hours until 14 days.

For the determination of the analgesic property of *D. esculentum*, six groups of swiss albino mice weighing 20 grams of the female gender containing 5 in each group was used in the study. Four groups were for the plant extract with

different dose (25, 100, 300 and 1000mg/kg), one group for the negative control, and one group for the standard drug which was aspirin (positive control). The method used was acetic acid induced writhing method. All the extracts and the standard drug were dissolved in distilled water. The negative control (NSS), were administered via oral gavage 1ml/100g of normal saline. At a dose of 25 mg/kg, the standard drug (aspirin) was administered orally as well as the 25, 100, 300, and 1000 mg/kg of the semi-purified flavonoids. After 15 minutes, 3% of acetic acid solution (1ml/100g) was administered intraperitoneally. The animals were individually observed in a glass jar. The trunk twist response, extension of limbs and abdominal contractions were recorded for 10 minutes.

Results and Discussions

In this chapter, data is presented, analyzed and interpreted. Presentation and analysis were accomplished through tables and narratives. Data were interpreted in light of information gathered during the review of relevant theories, literature and studies.

Table 1: Results of the Phytochemical Screening of the *D. esculentum* Ethanolic Extract

Phytoconstituents	Test	Remarks
Saponin Glycosides	Froth Test	-
	Liebermann-burchard's Test	++
Cardenolides and Bufanolides	Keller Kelliani Test	-
Flavonoids	Leucoanthocyanins (bate-smith and metcalf's test)	-
	Willstatter cyanidin test	++
Anthraquinone	Borntrager's Test	-
	Modified Borntrager's Test	-
Alkaloids	Wagner's Test	-
	Mayer's Test	-
Total Phenolic Compounds	Gelatin's Test	-
	Ferric chloride Test	-
Carbohydrates	Fehling's Test A and B	-
	Moore's Test	-
Proteins	Xanthoproteic Test	++
	Millon's Test	-
Fixed Oils and Volatile Oils	Stain Test	Fixed Oil

The table 1 presents the result of the phytochemical screening of the ethanolic extract of *D. esculentum*. Based on the test performed, the group obtained positive result on saponin glycosides, flavonoids, proteins and fixed oils. This means that the constituents are present on the sample.

According to Tongco *et al.*, ethanol is desired in the analysis of the phytochemical constituents due to its ability to produce more constituents than water alone.

Table 2: Interpretation of the FTIR Spectrum of the Semi-purified Flavonoids of *D. esculentum* Ethanolic Extract

Wave Numbers (Cm ⁻¹)	Types Of Vibrations	Functional Group
3263.92	OH Stretch	Alcohol-Phenol
2944.96	C-H Stretch	Alkyl
1637.16	C=O Stretch	Amide
1052.58	C-O Stretch	Alkoxy

The identification of the functional groups of *Diplazium esculentum* was conducted using the Fourier Transform Infrared Spectrophotometry. The interpretation of results in table 2 was coined from the infrared absorbance spectrum of the chemical bonds. The wavelength of light absorbed that was seen in the annotated spectrum is hereby recorded. The functional groups of *D. esculentum* were O-H (Alcohol Phenol), C-H (Alkyl), C=O (Amide), Nitro compounds and C-O (Alkoxy) groups. The analysis of results was according to Bruker alpha FTIR Spectrometer.

Table 3: Results of the Organoleptic Evaluation of The Semi-purified Flavonoids of *D. esculentum* Ethanolic Extract

Test	Result
Appearance	Viscous
Color	Yellow
Odor	Pungent

The table 3 represents the result of the organoleptic test of the Semi-purified Flavonoids of *Diplazium esculentum* ethanolic extract.

The semi-purified flavonoids appeared to be viscous, yellow in color with pungent odor. The alkaline reagent test for the confirmation of flavonoids produced the formation of intense yellow color, which disappeared upon addition of diluted acid.

Table 4: Results of the Solubility Test of The Semi-purified Flavonoids of *D. esculentum* Ethanolic Extract

Solvent	Result
Water	Soluble
Petroleum Ether	Insoluble
Methanol	Soluble
Acetone	Soluble
Chloroform	Insoluble

The table 4 represents the result of the solubility test of the Semi-purified Flavonoids of Ethanolic Extract from the *Diplazium esculentum* Fronds. Using the five solvents for the solubility test, the semi-purified flavonoids were soluble in water, acetone and methanol yet insoluble in chloroform and petroleum ether. (Tiwari *et al.*, 2011)

The table 5 shows the results of the 14 days of observation for the potential toxicity of the semi-purified flavonoids according to general. There were no signs of toxicity and mortality among the test animals throughout the entire observations for the acute toxicity studies. All the mice exhibited normal behavior, usual movement patterns and were all in healthy condition after the administration of the doses (5, 50, 300 and 2000 mg/kg). The examination lasted for 14 days and the largest dose produced no signs of mortality. Therefore, the ethanolic extract of the semi-purified flavonoids of *Diplazium esculentum* is safe to be used as a medicinal plant with no toxicity. The median lethal dose (LD50) of this plant was estimated to be above 2000 mg/kg body weight. (Junejo *et al.*, 2015)

Table 5: General behavioral observations for acute toxicity studies of the semi-purified flavonoids of *D. esculentum* ethanolic extract

Observation	Time	Trial no. 1	Trial no. 2	Trial no. 3	Trial no. 4	Trial no. 5
Tremor	30 minutes	Normal	Normal	Normal	Normal	Normal
	24 hours	Normal	Normal	Normal	Normal	Normal
	14 days	Normal	Normal	Normal	Normal	Normal
Eyes and Skin	30 minutes	Normal	Normal	Normal	Normal	Normal
	24 hours	Normal	Normal	Normal	Normal	Normal
	14 days	Normal	Normal	Normal	Normal	Normal
Behavioral Patterns	30 minutes	Normal	Normal	Normal	Normal	Normal
	24 hours	Normal	Normal	Normal	Normal	Normal
	14 days	Normal	Normal	Normal	Normal	Normal
Lethargy and Sleep	30 minutes	Normal	Normal	Normal	Normal	Normal
	24 hours	Normal	Normal	Normal	Normal	Normal
	14 days	Normal	Normal	Normal	Normal	Normal
Allergic Reaction	30 minutes	Normal	Normal	Normal	Normal	Normal
	24 hours	Normal	Normal	Normal	Normal	Normal
	14 days	Normal	Normal	Normal	Normal	Normal
Mortality and Coma	30 minutes	Normal	Normal	Normal	Normal	Normal
	24 hours	Normal	Normal	Normal	Normal	Normal
	14 days	Normal	Normal	Normal	Normal	Normal

Table 6: Number of Writhes of Groups A, B, C, D, E and F

GROUP #	Dose Administered	Number of Writhes					Mean	Percentage inhibition
		Trial no. 1	Trial no. 2	Trial no. 3	Trial no. 4	Trial no. 5		
A (Negative Control)	1 mL/100g	38	35	33	36	35	35.5	Xx
B (Aspirin)	25 mg/kg	15	12	14	16	13	14	60.45%
C (Experimental Control)	25 mg/kg	32	30	29	28	31	30	15.25%
D (Experimental Control)	100 mg/kg	22	24	23	22	21	22.4	36.72%
E (Experimental Control)	300 mg/kg	16	16	17	16	17	16.4	53.67%
F (Experimental Control)	1000 mg/kg	14	10	11	12	11	11.6	67.23%

The table 6 represents the raw data of the number of writhes recorded from the 5 groups of test animals. The data shows the decrease in writhing responses as the dose increases.

The analgesic property was computed using percent inhibition and this was implied by the decrease in writhing responses. In accordance to this, Group F which was the experimental control produced the highest mean of 67.23% and this was considered

to be the dose wherein the plant exhibited its analgesic effect. Based on the raw data of the number of writhings, it was showed that as the dose increases, the number of writhing response decreases. This confirms the conclusion of (Chawla *et al*, 2015) [7] in the study of the medicinal property of *D. esculentum* which is the main objective of this study.

Table 7: One Way Anova Interpretation of Results

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1095.04	4	273.76	153.7977528	1.02338E-14	2.866081402
Within Groups	35.6	20	1.78			
Total	1130.64	24				

The table 7 represents the result of the One Way Anova and the interpretation stated that the null hypothesis is rejected and the alternative hypothesis was accepted.

The study used one way anova and Tukey’s multiple comparison test in the analysis of the data. The statistical treatment between the analgesic property of the positive control (Aspirin 25 mg/kg) and the experimental control (*D. esculentum* semi-purified flavonoids 25, 100, 300, and 1000mg/kg) showed that the computed F which is 153.7977528 is greater than the F-critical which is 2.866081402 in anova, therefore there is a significant

difference between the groups.

The Tukey’s Multiple Comparison Test used 95% level of confidence in the comparison of the individual groups. Group E and Group F versus the positive control resulted in adjusted P value of 0.1209 indicating that among the 5 groups with a P value of <0.0001, Group E and Group F has significant analgesic property.

This data may indicate that the semi-purified flavonoids of *D. esculentum* has showed analgesic effect as the dose increases. (Chawla *et al*, 2015) [7].

Table 8: Tukey's Multiple Comparison Test Interpretation of Results

Tukey's multiple comparisons test	Mean Diff.	Summary	Adjusted P Value
A vs. B	21.4	****	<0.0001
A vs. C	5.4	****	<0.0001
A vs. D	13	****	<0.0001
A vs. E	19	****	<0.0001
A vs. F	23.8	****	<0.0001
B vs. C	-16	****	<0.0001
B vs. D	-8.4	****	<0.0001
B vs. E	-2.4	Ns	0.1209
B vs. F	2.4	Ns	0.1209
C vs. D	7.6	****	<0.0001
C vs. E	13.6	****	<0.0001
C vs. F	18.4	****	<0.0001
D vs. E	6	****	<0.0001
D vs. F	10.8	****	<0.0001
E vs. F	4.8	***	0.0002

The table 8 represents the results of Tukey's Multiple Comparison Test using 95% level of confidence.

Conclusion

The conclusions were drawn based on the observation and findings of the study made on the *Diplazium esculentum* fronds. The semi-purified flavonoids of ethanolic extract of *D. esculentum* yielded positive result in the Wilstatter "Cyanidin" Test. It also possess the characteristic features of the flavonoids proven by the result from the physical, chemical, and instrumental test for assay conducted. The semi-purified flavonoids of the ethanolic extract of *D. esculentum* showed no signs of toxicity and mortality in acute toxicity studies. There is also a reduction in the number of writhings of the experimental control (Group E 300 mg/kg and F 1000 mg/kg) which may indicate that the semi-purified flavonoids of *D. esculentum* has analgesic property as stated on the recent findings of Chawla *et al*, (2015)^[7]. The statistical treatment showed that there is a significant difference between the positive and experimental control and showed that the computed F value which is 153.7977528 is greater than the F-critical which is 2.866081402 in anova, therefore there is a significant difference between the groups. This data may indicate that the semi-purified flavonoids of *D. esculentum* have showed analgesic effect as the dose increases. It resulted in adjusted P value of 0.1209 indicating that among the 5 groups with a P value of <0.0001, Group E and Group F has significant analgesic property.

In accordance to the study of Chawla *et al.* entitled "Analgesic activity of medicinally important leaf of *Diplazium esculentum*" the results have showed that the protective action was attributed between flavonoids and sterols. Therefore the researchers of this study concluded that flavonoids is responsible for the Analgesic property.

Recommendations

The Researchers would like to recommend the following:

1. Further evaluation of the other uses of the semi-purified flavonoids of *Diplazium esculentum* fronds.
2. Further evaluation of other constituents like sterols present in *Diplazium esculentum* that potentially elicits Analgesic property.

3. Further evaluation of other plant parts of *Diplazium esculentum* as source of flavonoids other than its fronds.
4. To conduct further studies on the semi-purified flavonoids of *D. esculentum* such as formulating it into a dosage form.
5. To conduct other methods of determination of the analgesic property of *D. esculentum*.

References

1. Jovale Vincent V Tongco, Ronald Arlet P Villaber, Remil M Aguda, Ramon A Razal. Nutritional and phytochemical screening, and total phenolic and flavonoid content of *Diplazium esculentum* Retz. Sw. from Philippines. 2014; 6(8):238-242
2. Julfikar Ali J, Anirban G, Prodyut M, Lalit N, Kamaruz Z, Khumanthem DS *et al.* *In-vivo* Toxicity Evaluation and Phytochemical, Physicochemical Analysis of *Diplazium esculentum* Retz. Sw. leaves a traditionally used North-Eastern Indian Vegetable. *Adv. Biores.* 2015; 6(5):175-181. DOI:10.15515/abr.0976 4585.6.5.175181
3. Muhammad Razi Ullah Khan, Saeed Ur Rashid Nazir, Musaddique Hussain. Formulation Design, Optimization and Anti- Inflammatory Evaluation of Ibuprofen Cream. 2014; 4(5):762-763.
4. Nitin Kharat, Shylaja H, Viswanatha GL, Lakshman K. Anti- Inflammatory and Analgesic Activity of Topical Preparation of Root Extracts of *Ichnocarpus frutescens*. 2010; 1(3):1103.
5. Prashant Tiwari, BimleshKumar, Mandeep Kaur, Gurpreet. Phytochemical screening and Extraction: A Review. 2011; 1(1):98-106.
6. Ashokkumar R, Ramaswamy M. Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants. 2014; 3(1):395-406.
7. Sonia Chawla, Saurabh Chawla, Veerma Ram, Alok Semwal, Ramandeep Singh. Analgesic activity of medicinally important leaf of *Diplazium esculentum* Dehradun-248161, U.K. India, 2015; 9(25):628-63.
8. Hajare SW, Suresh Chandra, Tandan SK, Sarma K, Jlal. Analgesic and Antipyretic Activities of *Dalbergia Sisoo* Leaves. *Indian Journal of Pharmacology*, 2000, 1-2.
9. Victor B Amoroso, Dorothy A Antesa, Dave P Buenavista, Fulgent P Coritico. Antimicrobial, Antipyretic, and Anti-Inflammatory Activities of Selected Philippine Medicinal Pteridophytes, 2014; 5:18-40.
10. Julfikar Ali Junejo, Anirban Ghoshal, Prodyut Mondal, Lalit Nainwal, Kamaruz Zaman, Khumanthem Deepak Singh, Tapash Chakraborty. *In-vivo* Toxicity Evaluation and Phytochemical, Physicochemical Analysis of *Diplazium esculentum* Retz. Sw. leaves a traditionally used North-Eastern Indian Vegetable.
11. Sujith S. Nair, Molly Mathew, Sreena K. Formulation and Evaluation of Herbal Cream, 2012.