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Secondary metabolite composition & Anti-inflammatory potential of Pansit-pansitan (*Peperonia pelludica* Linn): An indigenous vegetable

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Abstract

The research was conducted to determine if Pansit- pansitan (*Peperomia pellucida* Linn) aqueous leaf extract have in – vitro anti-inflammatory potentials. Fresh leaves collected were air dried and subjected to aqueous extraction and prepared in different doses (200, 400, 600, 800 and 1000ug/mL). Inhibitions of HRBC lysis and protein denaturation were used to evaluate in-vitro anti-inflammatory activity of the extract. Based on the findings of the study, Pansit- pansitan (*Peperomia pellucida* Linn) shows that with increasing dosage, membrane stabilization and protein protective property also increases. The anti-inflammatory effect of the aqueous extract on the plant can be attributed to the presence of its secondary metabolites which is known to have anti-inflammatory property only that these metabolites are not sufficient enough when used in low dosage.

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Introduction

Herbal medicine, although dates back from thousands of years, still accounts to improve health because of its therapeutic and rehabilitative properties. Locals rely on herbal plants because of its medicinal value and is easily grown in the community (Tolentino *et al.,* 2019). This is why World Health Organization, Regional Office for South-East Asia (2010) published traditional herbal medicines for Primary health care to promote the proper use and preparation of herbal medicine.

Pansit-pansitan (*Peperomia pelludica* Linn*)* is a fleshy herb, widely grown and found abundantly in the Philippines. It is also found in other tropical countries including Central and North America. This plant was used locally for the ailment of headache, fever, gout, conjunctivitis, and rheumatic pains. (Mosango, 2008)

The therapeutic potential of plants are due to the presence of wide range of phytochemicals or secondary metabolites. According to studies, pansit-pansitan contains tannins, saponins, alkaloids, phenolics, flavonoids and terpenoids (Raghavendra & Prashith, 2018). Some literature have shown that pansit-pansitan has anti-microbial, cytotoxic, antioxidant, anti-diabetic, anti-hypertensive, fracture healing (Florence *et al.,* 2017), anti-angiogenic, anti-inflammatory & analgesic bioactivities (Alves, Setzer & da Silva, 2019; Raghavendra & Prashith, 2018). In addition, Pansit-pansitan is also an important sources of minerals and nutritional compounds and can be a functional food (Ooi, Iqbal & Ismail, 2012).

In this study, pansit-pansitan is investigated for its in-vitro anti-inflammatory properties by inhibiting lysis on Human red blood cell and inhibiting denaturation of proteins.

Materials and methods

Collection and preparation of Plant extracts

Fresh leaves of Pansit-pansitan were collected from mountainous area of Rizal, Cagayan. The leaves were subjected to air drying in a clean and dry area for 3 weeks at Room temperature turning the leaves regularly to ensure that they dry evenly.

The dried leaves of Pansit-pansitan (10g) were soaked with 90mL aqueous solvent. It was heated at 50°C for 2 hours with continuous stirring. The extract was then stored at 4°C for further use. Different dosages (200, 400, 600, 800 and 1000ug/mL) were produced to serve as treatments for the study.

Phytochemical screening

Standard protocols were conducted for the qualitative tests of secondary metabolites. The plant extract were tested for the presence of alkaloids, carotenoids, anthraquinones, flavonoids, saponins, steroids, tannins, terpernoids and xanthoproteins.

Human Red Blood Cell (HRBC) Membrane Stabilization Assay

The assay was adopted from Chowdhury *et al.* (2014). HRBC suspension was made by mixing collected blood from healthy volunteers who have not taken NSAIDs for two weeks prior the experiment with Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, 0.42% sodium chloride and 100mL distilled water) and centrifuged at 3,000rpm. A 10% (v/v) HRBC suspension was prepared with isosaline after it has been washed.

To each prepared dosages of extract (200, 400, 600, 800 and 1000ug/mL) and Aspirin (reference drug) were added equal amount of HRBC suspension. The solution was incubated at 37°C for 30 minutes, followed by centrifugation at 3,000rpm for 20 minutes. The supernatant was measured using spectrophotometer at 560 nm for haemoglobin content.

To calculate for the percent inhibition of lysis, the formula are as follows:

$$\% \text{ inhibition of lysis} = \frac{100 - \text{Absorbance of test}}{\text{absorbance of control}} \times 100$$

Here, the control served as the Alsever's solution containing blood without the reference drug or the different concentrations of extract.

Inhibition of Protein Denaturation Method

Inhibition of protein denaturation was adopted from Osman *et al.* (2016).

A reaction vessel was prepared consisting of 200uL of hen's egg albumin, 1400uL of phosphate-buffered saline (pH 6.4) and 1mL of the different concentrations of extract and/or diclofenac sodium (reference drug). A double-distilled water was used as negative control instead of extracts. The reaction mixture was incubated at 37°C for 15 minutes and heated at 70 °C for 5 minutes. The mixture was cooled and the absorbance was measured spectrophotometrically at 660nm by using the vehicle as a blank.

The percentage inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition of denaturation} = \frac{\text{Absorbance of test}}{\text{absorbance of control}} \times 100$$

Statistical Analyses of Results

All data obtained were expressed as mean ± standard deviation of five replicates. The results were analysed for statistical significance using One-way Analysis of variance (ANOVA) at $p < 0.05$ level of significance.

Results and discussion

Secondary metabolites are mostly by-products of primary metabolism which might be essential for the plants survival and reproduction (Böttger *et al.,* 2018). Studies have shown that secondary metabolites derived from plants serves as source for discovery and development of drugs like inflammatory agents. In this study, phytochemical screening on Pansit-pansitan showed the presence of secondary metabolites Tannins, Saponins and Alkaloids (Table 1). Many recent studies have documented that Tannins (Hossain *et al.,* 2014; Alinejhad *et al.,* 2016) Saponins (Jang *et al.,* 2013; Hassan *et al.*, 2011; Choi *et al.,* 2005) and Alkaloids (Souto, 2011; Khan, 2017; Barbosa *et al.,* 2006) have anti-inflammatory effects (Mohammed & Osman *et al.,* 2014). This findings is consistent with the literature review conducted by Raghavendra & Prashith (2018) which shows the presence of tannins, saponins and alkaloids in Pansit-pansitan.

Table 1. Phytochemical analysis of *P. pelludica*.

Secondary Metabolite	Result
Tannins	+
Saponins	+
Alkaloids	+

Table 2. Effect of extracts on membrane stabilization.

Dosage	% inhibition
200 µg/mL	-21.6 ± 20.7
400 µg/mL	31.5 ± 22.5
600 µg/mL	47.3 ± 16.6
800 µg/mL	86.9 ± 26.8
1000 µg/mL	118.5 ± 27.3
Reference Drug	128.5 ± 34.8

Inflammation is the process where the body responds to cell injury brought about by foreign material, trauma, toxins or chemicals, and/or microorganisms. (Pahwa *et al.,* 2020; Ansar & Ghosh, 2016). Inflammatory response causes the release of mediators and biomarkers (Stone, Basit & Burns, 2019). These mediators include inflammatory cells (neutrophils, lymphocytes & macrophage) and chemical mediators like cytokines, enzymes, plasma proteins and many more that ultimately results in healing and tissue restoration. (Ansar & Ghosh, 2016; Abdulkhaleq *et al.,* 2018).

Acute Inflammation, winding up for not more than 48 hours, is initiated by cellular mediators (mostly neutrophil) along with other coagulation factors (Solomkin & Simons, 1983). An inflammation that persists because of failure to address the causative agent of inflammation due to defects of cellular mediators, being exposed to an irritant for a long period of time and/or recurrent exposure to acute inflammation and autoimmune disorder is categorized as chronic inflammation (Pahwa, Goyal & Barsal, 2020). Chronic inflammation also releases cellular mediators like monocytes/macrophages, lymphocytes (Stone, Basit & Burns, 2019) and plasma cells (King *et al.,* 2007; Kradin, 2017). Some diseases arises from chronic inflammation including Rheumatoid Arthritis, diabetes, cardiovascular disease, cancer (Brenner *et al.,* 2014) and neurodegenerative disorders (Pahwa, Goyal & Barsal, 2020).

In this study, in vitro anti-inflammatory analysis was conducted using inhibition of lysis of HRBC and inhibition of protein denaturation. Both methods has been widely used to evaluate in vitro inflammatory activity because it is an easy and reliable method used for natural products (Kumari & Yasmin, 2015; Sangeetha & Vidhya, 2016).

Protein denaturation happens when there is disruption of its structure causing alteration in the biological, chemical and physical property of the protein (Eckersall, 2008). Denaturation of proteins has been linked to cause disorders like Rheumatoid Arthritis, diabetes and cancer. Therefore the prevention of protein denaturation may help prevent inflammatory conditions. The present study shows the effectiveness of Pansit-pansitan extract in inhibiting protein denaturation where a maximum of 248.2 ± 53.7% inhibition was observed at 1000 µg/mL (Table 3).

Table 3. Effect of extracts on protein denaturation.

Dosage	% inhibition
200 µg/mL	32.4 ± 20.1
400 µg/mL	29.2 ± 22.4
600 µg/mL	174.1 ± 41.3
800 µg/mL	185.5 ± 55.5
1000 µg/mL	248.2 ± 53.7
Reference Drug	313.5 ± 60.2

Membrane stabilization refers to the process where anti-inflammatory drugs mediates inflammation by stabilizing proteins present in the membrane of red blood cell (Kardile *et al.,* 2016). Non-Steroidal Anti-inflammatory Drugs like Aspirin and Diclofenac protects lysosomal membrane to prevent or limit inflammatory response (Kumari, 2011). Red blood cell membrane is said to be analogous to lysosomal membrane that influence inflammatory process (Jainul & Chowdhurry *et al.,* 2013; Anosike, 2012). During inflammation, lysosomal enzymes are released causing damage on surrounding tissues triggering inflammation. (Patil *et al.,* 2016). In this study, Pansit-pansitan extract was effective in inhibiting lysis of HRBC wherein a maximum of 118.5 ± 27.3% inhibition was observed at 1000µg/mL. However, the non-steroidal anti-inflammatory drug, aspirin, exhibited a greater inhibition capacity of 128.5 ± 34.8%.

The results of both in-vitro anti-inflammatory tests exhibited a concentration-dependent inhibition (Chatterjee *et al.,* 2012; Chandra *et al.,* 2012; Bhattacharya *et al.,* 2012; Rahman *et al.,* 2015; Kumari *et al.*, 2015). The results also showed that there is a significant difference when the different concentration of *P. pelludica* extracts were compared to the reference drugs, Aspirin ($p<0.000$) and Diclofenac ($p<0.000$) for the inhibition of HRBC lysis (table 4) and denaturation of protein (table 6), respectively. Table 5 and 7 shows the comparison of Mean Differences % inhibition of HRBC lysis and protein denaturation, respectively. It is observed that at 1000 ug/mL, the extract showed no significant difference with the reference drug, Aspirin for the inhibition of HRBC lysis. This indicates that Pansit-pansitan extract has comparable anti-inflammatory effect with that of Aspirin.

Table 4. Analysis of Variance on the mean % inhibition of HRBC lysis of *P. pelludica* extract.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	81798.163	5	16359.633	25.181	.000
Within Groups	15592.580	24	649.691		
Total	97390.743	29			

Table 5. Comparison of Mean Differences % inhibition of HRBC lysis of *P. pelludica* extract.

Concentration (ug/mL)	Mean	Mean Differences					
		200 ug/mL	400 ug/mL	600 ug/mL	800 ug/mL	1000 ug/mL	Positive control
200	-21.6	0.00	-53.10*	-68.92*	-108.54*	-140.54*	-150.12*
400	31.5	53.10*	0.00	-15.82*	-55.44*	-87.44*	-97.02*
600	47.3	68.92*	15.82	0.00	-39.62*	-71.62*	-81.20*
800	86.9	108.54*	55.44*	39.62*	0.00	-32.00	-41.58*
1000	118.9	140.54*	87.44*	71.62*	32.00	0.00	-9.58
Positive Control	128.5	15.12*	97.02*	81.20*	41.58*	9.58	0.00

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

Table 6. Analysis of Variance on the mean % inhibition of protein denaturation of *P. pelludica* extract.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	327464.323	5	65492.865	32.011	.000
Within Groups	49103.412	24	2045.975		
Total	376567.735	29			

In conclusion, the results indicate that *P. pelludica* extract exhibited anti-inflammatory potential that is concentration dependent. It is also noted that *P. pelludica* extract is comparable to that of Aspirin. The anti-inflammatory effect of the aqueous extract on the plant can be attributed to the presence of its

secondary metabolites which is known to have anti-inflammatory property only that these metabolites are not sufficient enough when used in low dosage.

Table 7. Comparison of Mean Differences % inhibition of protein denaturation of *P. pelludica* extract.

Concentration (ug/mL)	Mean	Mean Differences					
		200 ug/mL	400 ug/mL	600 ug/mL	800 ug/mL	1000 ug/mL	Positive control
200	32.4	0.00	3.1	-141.6*	-153.1*	-215.8*	-281.1*
400	29.2	-3.1	0.00	-144.8*	-16.3*	-219.0*	-284.2*
600	174.1	141.6*	144.8*	0.00	-11.4	-74.1*	-139.4*
800	185.5	153.1*	156.3*	11.4	0.00	-62.6*	-127.9*
1000	248.2	215.8*	219.0*	74.1*	62.6*	0.00	-65.28*
Positive Control	313.5	281.1*	284.2*	139.4*	127.9*	65.2*	0.00

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

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