ANTIPROLIFERATIVE, PHENOLIC CONTENT AND DETERMINATION OF TOTAL ANTIOXIDANT PROPERTY USING 2'2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) RADICAL SCAVENGING ACTIVITY OF *Pittosporum resiniferum*

An Undergraduate Research Study Presented to the Faculty of

HONORATO C. PEREZ, SR. MEMORIAL SCIENCE HIGH SCHOOL

Mabini Extension, Cabanatuan City

In Partial Fulfilment

of the Requirements for the Subject

SCIENCE RESEARCH II

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MARCH 2020

Table of Contents

TITLE PAGE **1**

APPROVAL SHEET **3**

ACKNOWLEDGMENT **4**

TABLE OF APPENDICES **5**

TABLE OF FIGURES **6**

TABLE OF TABLES **7**

ABSTRACT **11**

INTRODUCTION **11**

Statement of the Problem 12

Significance of the Study 12

Scope and Delimitations 12

METHODOLOGY **13**

Methods of Research Used 13

Materials 13

Schematic Diagram 13

Research Design 14

RESULTS AND DISCUSSION **15**

RECOMMENDATIONS **16**

BIBLIOGRAPHY **16**

CURRICULUM VITAE **17**

APPROVAL SHEET

We, the members of the Board of Oral Examiners, have found the thesis of

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entitled

ANTIPROLIFERATIVE, PHENOLIC CONTENT, AND DETERMINATION OF TOTAL ANTIOXIDANT POTENTIAL USING 2'2 DIPHENYL-1-PICRYLHYDRAZYL RADICAL SCAVENGING ACTIVITY OF *Pittosporum resiniferum*

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ACKNOWLEDGMENT

With sincere appreciation, thanks are due to the following people, whose unwavering support and help led to the realization of this academic pursuit:

Dr. Raquel Moana M. Abolencia, the researchers' adviser and Science Research II teacher, for the trust and confidence she has given them; for her motherly advice and concern; and for her brilliant insights and suggestions which facilitated the completion of this research work;

Mrs. Filipina Misalang, the Research Consultant, for the professional and technical assistance extended to the researchers towards the fruition of this study;

Mr. Dennis S. Bunag, Mr. Ismael A. De Lara, Mr. Mark Clester A. Rufino, Mr. Gladys Joy B. Caoile, Ms. Shiela M. Reyes, and Miss Faye Marion C. Sebastian, the members of the Board of Oral Examiners, for their analytical comments, suggestions, and substantial time spent probing and scrutinizing the content of the study;

Dr. Leonora C. De Jesus, the school principal, for her kind approval through the permission she had extended in all the requests appurtenant to the conduct of this study;

Mr. Anthony S. Lagrata, Mrs. Florita B. Lagrata, Mr. Mariano Solano and Mrs. Joelyn I. Solano, the researchers' parents, whose understanding, spiritual, moral, & financial support, and love prodded the researchers to pursue and complete this study;

And finally, to the Giver of life, wisdom, compassion without hesitation, and success, the **Lord God Almighty**, none of these would ever be possible if not because of Him and His everlasting love. This moment is a symbol of His Eternal Goodness.

L.P.B.L.

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2020

TABLE OF APPENDICES

APPENDIX	PAGE
A: Preparation of Petroleum Nut Oil	vii
B: Analyzation of DPPH and Phenolic Content	ix
C: Analyzation of Antiproliferative Property	x

TABLE OF FIGURES

FIGURE	PAGE
1. Phases of the general procedures	13

TABLE OF TABLES

TABLE	PAGE
1. Antiproliferative property	14
2. Phenolic content	15
3. Antioxidant property	15
4. Antiproliferative property of the petroleum nut to the onion cell	15
5. Total phenolic content of the petroleum nut.	15
6. Total antioxidant property of the petroleum nut.	15

Appendix A. Preparation of Petroleum Nut Oil

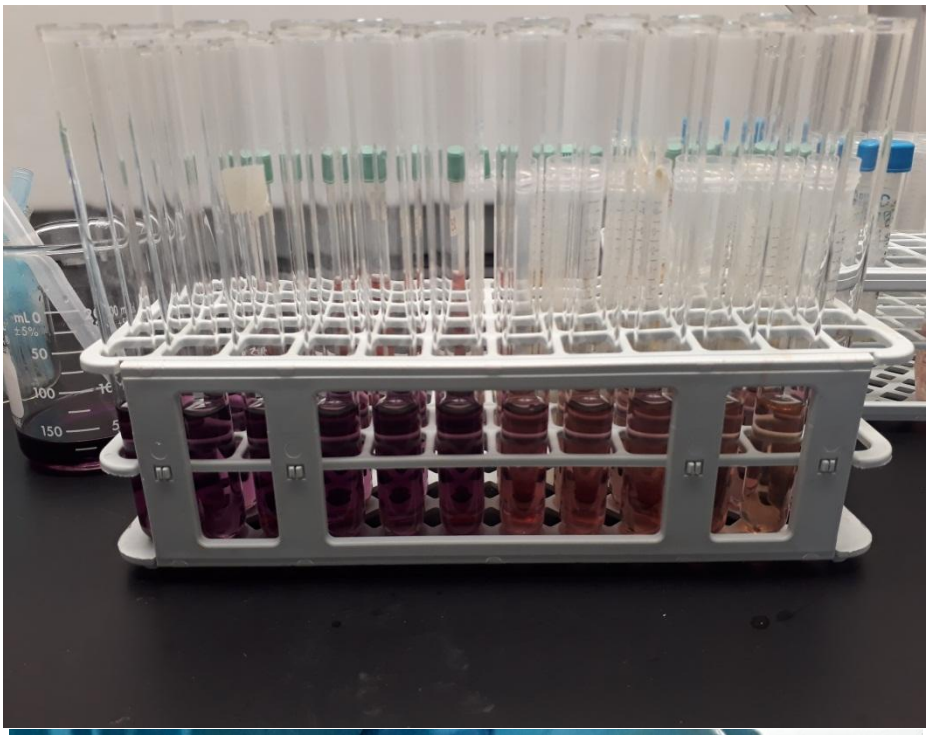


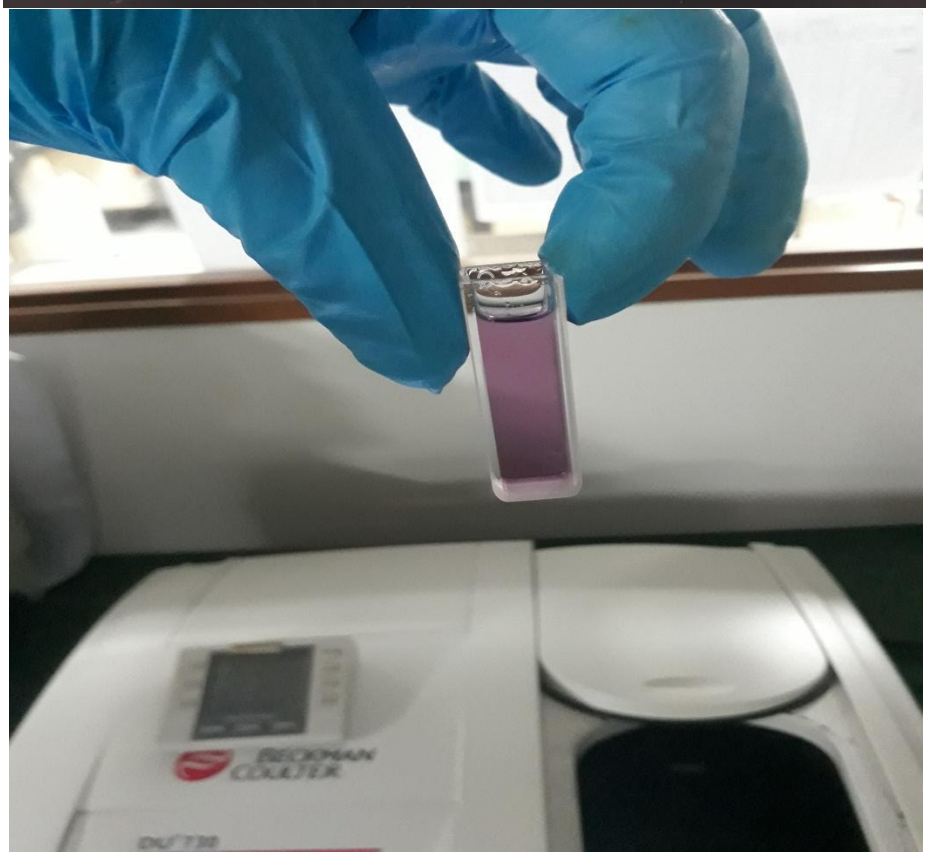




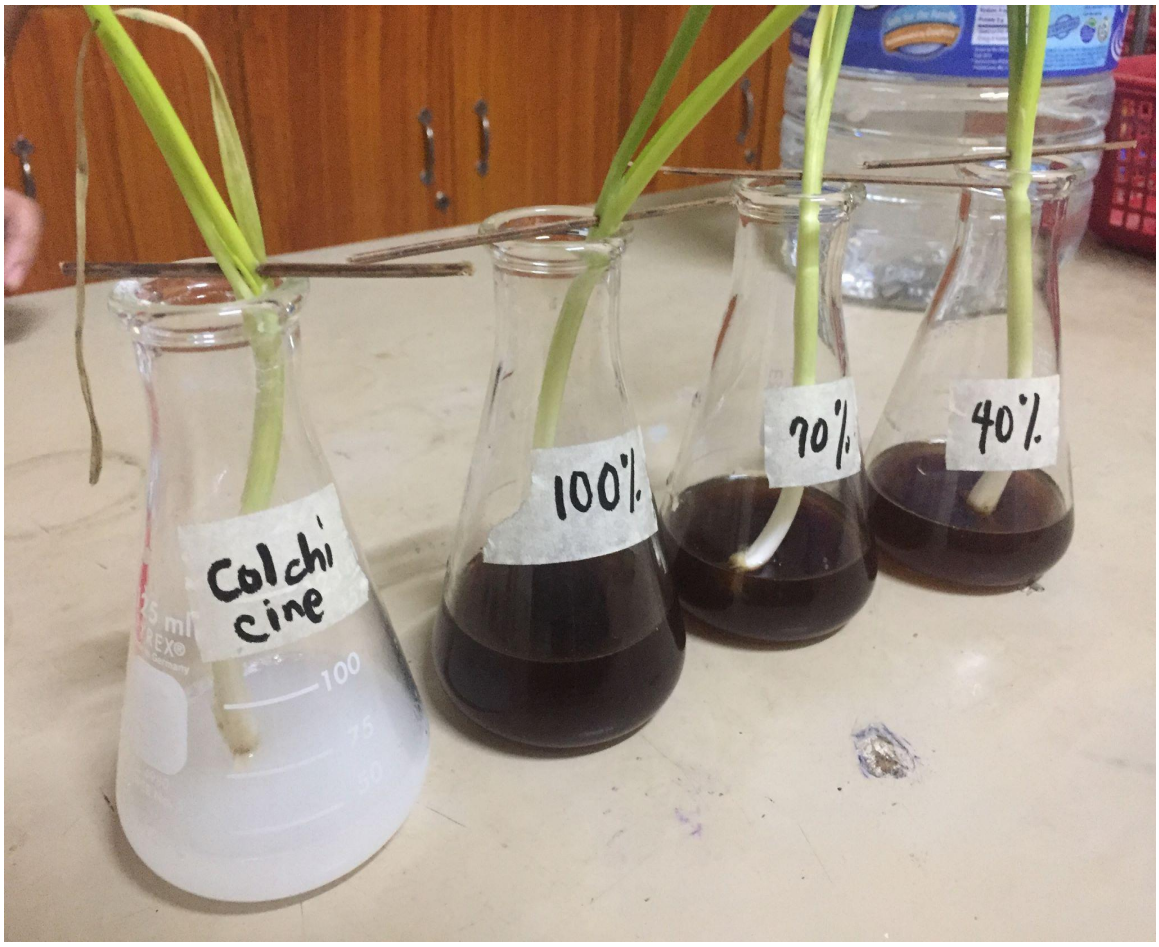


Appendix B. Analyzation of DPPH and Phenolic Content





Appendix C. Analyzation of Antiproliferative Property



ANTIPROLIFERATIVE, PHENOLIC CONTENT, AND DETERMINATION OF TOTAL ANTIOXIDANT PROPERTY USING 2'2, DIPHENYL-1-PICRYLHYDRAZYL (DPPH) RADICAL SCAVENGING ACTIVITY OF *Pittosporum resiniferum*

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ABSTRACT

Petroleum nut, also known as "Pittosporum resiniferum", is a plant that is tested for medicinal purposes that is not scientifically proven. It is also used in producing biofuels. But people have not looked deep into it and discover that it may have other purposes.

The result of this paper is determined by experimental method through conducting an antiproliferative effect against Allium cepa and DPPH property of the extract.

In conducting the study, the researchers used ½ kg of Pittosporum resiniferum, 15 pcs. of Allium cepa, 10 ml water, 50 ml 85% Ethanol, 250 ml Coconut oil, 25x80 mm thimble, Vortex, Centrifuge Tube, Filter paper, 85% aqueous methanol for the antiproliferative effect against the Allium cepa while 2.5 ml Folin-Ciocalteu reagent and 2 ml 7.5% sodium carbonate for the DPPH property of the Pittosporum resiniferum. Phenolic compounds have received increasing interest in the human health due to their effects against several diseases like cancers attributed to their antioxidant activity. The antioxidant activity of the petroleum nut was evaluated using the procedure reported by Brand-Williams et al. (1995) with some modifications.

The study was with positive conclusions in comparison with the positive control, Colchicine, an anti-gout medicine. All the extract concentrations ranging from 40%-100% stopped the onion's cell production dipped into the extract concentrations, also into the Colchicine mixture and was observed for 2-3 weeks to see if there is any sign of root growth. If the onion had grown its roots, then it has no antiproliferative property. Therefore, the researchers conclude that Pittosporum resiniferum has an antiproliferative effect and can be used as an anticancer medicine.

INTRODUCTION

The tree called petroleum nut, *Pittosporum resiniferum* grows in the northern part of the Philippines. It is rich in heptane, myrcene, dihydropyrrole and e-pinene which are gasoline substances. It has a carbon rating of 54 much higher than *Jatropha curcas* which has only 41.

Humans basically know that plants are used for ornamental and medicinal purposes. But people have looked deep into it and some plants may have other purposes. *Pittosporum resiniferum* is a tree that grows in the Philippines

and Malaysia, particularly in the wilderness surrounding the Mayon Volcano and in the Cordillera of the Philippines and Mount Kinabalu of Sabah, Malaysia. The petroleum nut has derived its name from the resemblance of the fruit's odor to petroleum based- fuels.

The fruits of the tree burn brightly when ignited and can be used for illumination as torches or candles. Its fruit is also highly suitable for use in producing biofuel. This use was encouraged by the Philippines Department of Agrarian Reform and the Philippine Coconut Authority.

Petroleum nut tree is an aromatic tree to 30m tall, but probably smaller in its forest habitats (perhaps even epiphytic); fruiting when only 6-12 m tall. Leaves aromatic, coriaceous, entire (possibly evergreen), thickest above the middle, innately nerved, with a short acumen at the tip. Flowers are fragrant, white, clustered on the stems. The Pine Tree Cordillera Ecological Center here said that the petroleum nut tree has shown promises of becoming a viable source of fuel that could be used for cooking and to also run vehicles.

In the Philippine Cordilleras, petroleum nut is found among other trees like oak and other mossy forest species. It can also grow well with pine trees. Herbalists in the Philippines use he petroleum nut as a universal medicine. An infusion of the fruit is used as a remedy for intestinal and stomach pains. An oleoresin obtained from the fruit is used externally as a cure for leprosy and other skin diseases also to bring relief from muscular pains.

A decoction of the nuts is used in the treatment of colds. The crushed nuts, combined with coconut oil, are used to bring relief from myalgia. A decoction of the leaves is taken in the treatment of coughs. The sap is used to treat ringworm, but firm evidence is still lacking. (Bruce M.E., 1977).

The results of this study support the efficacy of these valuable oils and extracts as an antiproliferative agent for cancer cells, and it suggests that they may be provided as a potential huge source to current chemotherapeutic researchers. Antiproliferative tends to suppress cell growth, especially the growth of malignant cells into surrounding tissue.

Thus, the researchers aimed to find out if the plant could also be used as an anti-cancer medicine and as an antioxidant.

Statement of the Problem

This study aims to develop an antiproliferative and an antioxidant, specifically, this study sought to answer the following:

1. Does the following *P. resiniferum* Extract concentrations have the ability stop the onion's cell production and have and antiproliferative ability;
   a. 40%;
   b. 70%;
   c. 100%?
2. Does the *P. resiniferum* have an antioxidant scavenging property?
3. Would the *P. resiniferum* have enough phenolic content to be an antibacterial medicine?

Significance of the Study

This research study aims to help the:

To the individual: This study would help the individuals to further acknowledge the effects and medicinal purposes of the plant *Pittosporum resiniferum*

To the society: To give knowledge about the plant *Pittosporum resiniferum.*

To the researchers: This research aids them to explore and improve their abilities on finding out what would be more efficient for the society.

To the future researchers: This research may be used as a baseline for researchers with the same idea in medicinal purposes of plants.

Scope and Delimitations

This study entitled "Antiproliferative, Phenolic Content and determination of Antioxidant Property Using 2'2, Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity of *Pittosporum resiniferum*" tested the effect of the chosen plant to the onion's cell production.

Several tests are conducted to achieve reliable results and conclusions when using *Pittosporum resiniferum* as an antiproliferative and an antioxidant.

The researchers used ½ kg *Pittosporum resiniferum*, 15 pcs. *Allium Cepa,* 10 ml water, 50 ml 85% Ethanol, 250 ml Coconut oil, 25x80 mm thimble, Vortex, Centrifuge Tube, Filter paper, 85% aqueous methanol for the antiproliferative effect against the Allium cepa while 2.5 ml Folin-Ciocalteu reagent and 2 ml 7.5% sodium carbonate for the experiment.

For the Determination of Total Antioxidant Property using 2,2' Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity, first is the extraction of Phytochemicals, about 1g of petroleum nut was weighed into a centrifuge tube and extracted at room temperature using 10 ml of 85% aqueous methanol. The mixture was shaken for 12 h and centrifuged at 3000 rpm for 10 minutes. The extract was transferred into a centrifuge tube and stored at 4℃ until analysis.

For the Total Phenolic Content (TPC) using the method of Singleton et al., (1999) with some modifications, petroleum nut extract (0.5 ml) was mixed with 2.5 ml freshly prepared Folin-

Ciocalteu reagent (1:10 dilution). After 15 minutes of incubation, 2 ml of 75% sodium carbonate was added to the mixture. It was then allowed to stand for 1 h for the optimum color formation.

The absorbance of the resulting blue color was measured at 760 nm against a blank. Gallic acid (GA) was used as a standard and TPC was expressed as mg GA equivalent/g sample.

As for the determination of antiproliferative property of the petroleum nut, the plant was extracted into 3 types o extract: oil, ethanol, and water extract and used colchicine as a positive control. The onion was placed on the extracts to see if there are growths and was left for 2-3 weeks

The researchers started the experiment as early as possible to have more trials done. The researchers started the experiment on May 2019-August 2019.

METHODOLOGY

CRD (Completely Randomized Design) is the research design used in this research study, the *Pittosporum resiniferum* can be used as an alternative for anti-cancer medicine.

Methods of Research Used

Experimental research is a method of research used in this study.

Materials

- ½ kg *Pittosporum resiniferum*
- 15 pcs. *Allium cepa*
- 50 ml water
- 50 ml 85% Ethanol
- 250 ml Coconut oil
- 25x80 mm thimble
- Vortex
- Centrifuge Tube
- Filter paper
- 85% aqueous methanol
- 2.5 ml Folin-Ciocalteu reagent
- 2 ml 7.5 % sodium carbonate

Schematic Diagram

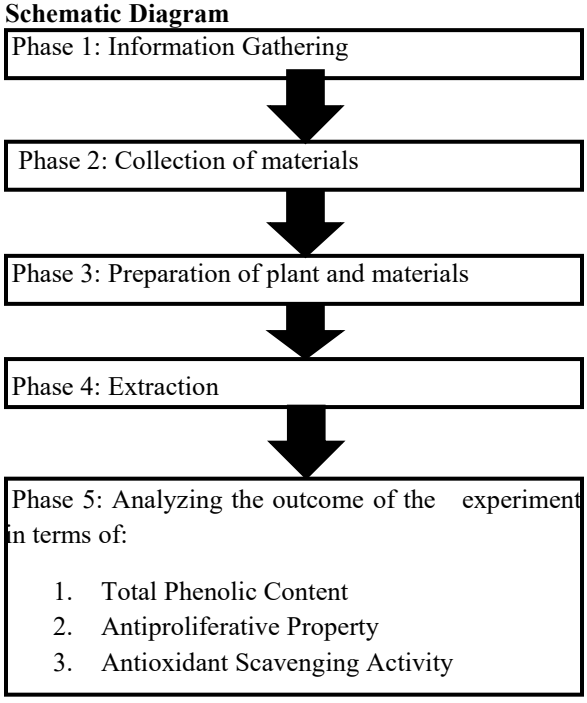


Figure 1. The diagram shows the phases of the general procedures.

For the first phase, the researchers gathered the needed information about the needed materials, such as the quantity needed and the place where to find each material.

For the second phase, the collection of the needed materials is conducted; collecting each material helped the researchers identify the missing materials needed for the study.

For the third phase, the researchers prepared all the materials and learned the use and the benefits of each material to the study.

For the fourth phase, the researchers made the three types of extracts (water, ethanol, oil). For the Ethanolic extraction, every 1 gram (± 0.001) of crushed, air-dried plant material is added to 10 ml of 85% ethanol; vortex to mix and let stand for 12 hours with occasional shaking.

After extraction, the solution must be centrifuged at 3000 rpm for 15 minutes and transfer the supernatant to a centrifuge tube, cover and store at 4◦C until further analysis.

As for the water extraction, every 1 gram (±0.001) of crushed, air-dried plant material is added to 10 mL of water; boil (100◦C) for 15 minutes, filter (using a Whatman no.1 filter paper), and store in a centrifuge tube at 4◦C until further analysis.

For the oil, the researchers boiled the whole petroleum nut fruit cut in half in a 250ml coconut oil for not less than 6hrs or until the color changes from red to red orange thoroughly over low heat.

Finally, the researchers tested the three types of crude extracts into the onion roots which was observed for two to three weeks, to see if there is any effect to the production of onion cells. The antiproliferative assay was conducted at Araullo University.

As for the Determination of Total Antioxidant Activity using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) Radical-Scavenging Activity, first is the Extraction of Phytochemicals, about 1 g of petroleum nut was weighed into a centrifuge tube and extracted at room temperature using 10 mL of 85% aqueous methanol.

The mixture was shaken for 12h and centrifuged at 3000 rpm for 10 min. The extract was transferred into a centrifuge tube and stored at 4^0C until analysis.

The petroleum nut extract was evaluated following a procedure reported by Brand-Williams et al. (1995) with some modifications. Mixtures containing 0.5 mL extract/standard and 5 mL freshly prepared 0.1 mm DPPH was incubated for 60 min. The absorbance was read at 517 nm against a blank and Trolox standards.

The second phase is to test the Total Phenolic Content (TPC) using the method of Singleton et al., (1999) with some modifications. Petroleum nut extract (0.5 mL) was mixed with 2.5 mL freshly prepared Folin-Ciocalteu reagent (1:10 dilution). After 15 min of incubation, 2 mL of 7.5% sodium carbonate was added to the mixture. It was then allowed to stand for 1 h for the optimum color formation. The absorbance of the resulting blue color was measured at 760 nm against a blank. Gallic acid (GA) was used as a standard and TPC was expressed as mg GA equivalent/g sample.

Meanwhile, the Determination of Total Antioxidant Activity using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) Radical-Scavenging Activity content of the *P. resiniferum* was conducted at the Philippine Rice Research Institute-Rice Chemistry and Food Science Division, Science City of Muñoz, Nueva Ecija.

Research Design

The research obtains via following the research design to have maximum number of results.

Table 1. The table shows the results obtained via following the research design

T / R	**T1** WATER	**T2** ETHANOLIC	**T3** OIL	**T4** +CONTROL
R1				
R2				
R3				

Legend

R=Extract Concentration
T1=Water Extract
T2=Ethanolic Extract
T3=Extracted Oil
T4=Colchicine

Table 2. The table shows the results obtained via following the research design.

Sample	T1	Average
Petroleum Nut		

Legend

T1=Phenolic Content

Table 3. The table shows the results obtained via following the research design.

T1	Average

Legend

T1=DPPH Radical Scavenging Activity

RESULTS AND DISCUSSION

Table 4. The table shows the result of the antiproliferative property of the petroleum nut to the onion cell.

T / R	Water Extract	Ethanolic Extract	Oil Extract	+ Control
40%				
70%				
100%				

Legend

(-) Negative= No root growth was observed
(+) Positive= There was root growth observed

Antiproliferative property is used to prevent or retard the spread of cells, especially malignant cells, into surrounding tissues.

The antiproliferative property of the petroleum nut that was measured based on all the extract concentrations ranging from 40%-100% stopped the onion's cell production, the onion was dipped into the extract concentrations, also into the colchicine mixture and was observed for 2 -3 weeks to see if there is any sign of root growth. If the onion had grown its roots, then it has no antiproliferative property.

The present study confirmed the findings about the antiproliferative property of the petroleum nut; it was compared to colchicine, which also stopped the onion's cell production.

Table 5. The table shows the results of the total phenolic content of the petroleum nut.

Sample	Total Phenolic Content (mg GAE/g)	Average
Petroleum Nut	38.1	37.9
	38.5	
	37.3	

Phenolic compounds have received increasing interest in the human health due to their benefit effects against several diseases like cancers attributed to their antioxidant activity. The antioxidant activity of the petroleum nut was evaluated using the procedure reported by Brand-Williams et al. (1995) with some modifications; it was calculated using this formula:

$$Antioxidant\ Activity\ \left(\frac{mg}{gTEq}\right)$$

$$\frac{[Abs\ (extract) - Abs\ (blank)] - y\ intercept}{slope} x\ DF$$

The TPC was evaluated using the method of Singleton et al., (1999) with some modifications.

Table 6. The table shows the Total antioxidant property of the petroleum nut.

DPPH Radical Scavenging Activity (mg TE/g)	Average
79.8	81.7
82.9	
82.4	

The total antioxidant property ranged from 79.8 to 82.4 mg TE/g. Having the average of 81.7 The total phenolic content was evaluated using the method of Singleton et al., (1999) with some modifications.

Natural polyphenols are naturally occurring compounds found largely in the fruits, vegetables and are the most antioxidants in human diets, and their radical scavenging activities are related to substitution of hydroxyl groups in the aromatic rings of phenolic.

Dark chocolates are the best type of antioxidants, it has 70 percent of antioxidant content.

The researchers therefore conclude that the following *P. resiniferum* extractions have the ability to stop the onion's cell production and have an antiproliferative ability 40%, 70%; and 100%. With 100% having the most effect on the onion's cell production. The *P. resiniferum* has enough antioxidant property to be an antioxidant. It was tested using Singleton et al., (1999) with some modifications. The plant has enough phenolic content to be an antibacterial medicine and is possible to be an anti-cancer.

RECOMMENDATIONS

To further acknowledge and develop the study, the following recommendations are proposed:

1. To test the extract concentrations on an actual cancer cell.

2. To test if the plant could also be used for other medications other than being an antiproliferative and an antioxidant.

3. To determine the maximum achievable enhancement.

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BIBLIOGRAPHY

Newcastle University, UK Tel: 099477120/2120 Mini Review Open Access A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation July 06, 2015https://www.omicsonline.org/open-access/a-
Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antimicrobial Properties James Redfern, Malcolm Kinninmonth, Dariel Burdass, and Joanna Verran.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4004744/
Reviewed:December 13th 2016 Published:March 8th 2017

https://www.intechopen.com/books/phenolic-compounds-biological-activity/health-benefits-of-phenolic-compounds-against-cancer

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