

BUTTERING CANCER: A Novel Phytonutraceutical in Cancer Prevention and Treatment from *Bunchosia Glandulifera* (Peanut Butter Tree)

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ABSTRACT

Cancer is a disease characterized by an abnormal growth of cells that can start in any organs affecting tens of millions of people every year. Conventional therapy of disease offers a success but remained as a challenge due to its negative adverse effects. This study aims to determine the phytonutraceutical, anti-angiogenic, and antioxidant properties of *Bunchosia glandulifera* (Peanut Butter Tree). Ethanolic leaf extract of *B. glandulifera* was subjected to phytochemical screening and tested for anti-angiogenic, cytotoxic, and antioxidant activity. Secondary metabolites screening indicates the presence of alkaloids, flavonoids, saponin, and phenols. Test for angiosuppressive activity revealed a positive anti-angiogenic property at 25%, 50%, and 75% concentration. The probit analysis of the leaf extract was found to be 309.7 μ g/ml. LC50 of the extract was higher than 100 microgram which indicates that the extract has low toxicity towards human cells. Evaluation for antioxidant property of the leaf extract exhibits an IC50 value of 35.39 μ g/ml. The results revealed that *B. glandulifera* exhibit a promising source of phytonutraceutical, anti-angiogenic, as well as antioxidant property which can be a novel cancer prevention and treatment. Isolation of potent bioactive compounds and in vivo testing of *B. glandulifera* must be done to further validate the claim of the study.

Keywords: anti-angiogenic, antioxidant, *Bunchosia glandulifera*, cytotoxicity, phytonutraceutical

INTRODUCTION

Characterized by an abnormal growth of cells that can start in any organs, cancer is considered to be the second leading type of disease in the world today [4]. Currently in United States, 1 out of 5 kids diagnosed with cancer and in other countries, 4 out of 5 kids dies because of cancer [11]. According to World Health Organization (WHO), approximately 9.6 million loss their lives due to the emergence of this disease. The first metastatic cancer treatment was discovered in 1956 when methotrexate was used to treat the rare tumors called choriocarcinoma [8]. This resulted to the discovery of the targeted cancer treatment which uses drugs or other substances to attack a certain cancer cell. This type of treatment can cause adverse effect (such as fatigue, high blood pressure, mouth sore, or even worst it can form a hole in gastrointestinal tract) depending on the type of targeted cancer treatment used [12]. Angiogenesis is the formation of new blood vessels that plays an important role in tumor progression, it works by slowing or stopping tumor growth [1]. Following this, anti-angiogenesis is a method that delays the growth or spread of tumor by preventing the growth on new blood vessels and, it also improves tumor immune microenvironment.

Bunchosia glandulifera belongs to a family of malpighiaceae and is commonly found in tropical countries. It contains bioactive compounds such as carotenoids, phenolics, and flavonoids, which makes the plant a promising source of antioxidants [3]. According to Farhan et al. in 2023, flavonoids have anticancer, antioxidant, antiviral, and anti-inflammatory. The treatment with flavonoids also induces differentiation in malignant cells isolated from several solid tumors. This bioactive compound found in *B. glandulifera* may be further validated by the present study in the presentation of its phytonutraceutical property.

Methods and Experimental Details

Ethanolic Extraction

Seventy-five grams of leaves were soaked in 750 mL of 95% ethanol in room temperature. After 48 hours, the extract was filtered using filter paper. The collected ethanolic crude extract was subjected for rotary evaporation in PHILEXPORT Quality Control Laboratory, General Santos City, Philippines to obtain the pure extract.



Figure 1. Soaking of *B. glandulifera* and its crude extract

Phytochemical Screening

Ethanol was used to extract the bioactive compounds in *Bunchosia glandulifera* following the protocols of Tiwari et al. in 2011.

Test for Alkaloids (Wagner's Test)

Two (2) mL of *B. glandulifera* filtrates were treated with Wagner's reagent and was added along the sides of the test tube. The presence of reddish or brown precipitates indicates the presence of alkaloids.

Test for Flavonoids

One (1) mL of the plant extract was treated with sodium hydroxide solution. The formation of intense yellow color until it becomes colorless on addition of hydrogen chloride (dilute acid) indicates the presence of flavonoids.

Test for Saponins (Foam test)

One (1) mL of extract was diluted with 2 mL distilled water. The suspension was shaken in a graduated cylinder for 15 minutes. If the foam persist for ten minutes indicates the present of saponins.

Test for Tannins

One (1) mL of the extract was diluted with 1% gelatin solution containing 10% NaCl. The presence of white precipitates indicates the presence of tannins.

Test for Phenols

One (1) mL of extract was treated with 34 drops of ferric chloride solution. The formation of bluish black color indicates the presence of phenols.

Duck Chorioallantoic Membrane (CAM) Assay

The angiostatic activity using CAM assay conducted in this study followed the protocol of Cruz et al. in 2022. An 8-day old duck egg was used in this study. The eggs were candled to see the embryo's position. Sterile forceps were used to create holes around the airspace of the eggs and the opening was covered using parafilm. The outer part of the eggs was sprayed with 70% ethanol to minimize the inflammation. The eggs were treated with 25%, 50%, 75% concentration of *B. glandulifera* extracts diluted to distilled water in 1mL quantities which are obtained using the $C1V1=C2V2$ formula, anticancer drug (positive control), and distilled water (negative control). The eggs were treated with 0.1mL of the treatments respectively and incubated at 37.5 °C for 48 hours.



Figure 2. CAM of an 8-day old duck embryo

Brine Shrimp Lethality Assay

The cytotoxicity test conducted followed the protocol of Sarah et al. in 2017. The *Artemia salina* species were hatched in a rectangular aquarium that was divided in to 2 unequal compartments by a perforated (2mm) dam and filled with artificial seawater that was prepared through dissolved 25 grams of table salt to 1 liter of distilled water (salinity of natural seawater ~25ppt) [6]. An air pump was attached at the bottom of the aquarium to maintain the proper aeration.

Fifteen grams of brine shrimp eggs were sprinkled at the top water level of the aquarium and mixed with the saltwater. A 60100 watts light bulb was put a few inches away from the aquarium. The set up of eggs and photophilic nauplii was incubated with a temperature of 28°-30°C for a period of 20-24 hours. The hatched nauplii was separated from the empty eggs. It was done by turning off the air switch and lamp. The empty eggs floated while brine shrimps were concentrated in the water column [9].

A 10 nauplii was transferred to each test tube using Pasteur pipettes. The nauplii was exposed to: T_1 : 0, T_2 : 100µg/ml, T_3 : 500µg/ml, and T_4 : 1000µg/ml concentration of *Bunchosia glandulifera* extract. After 24 hours, the number of survivors were counted and calculate the percentage of death using percentage of death formula.

$$\% \text{Death} = \frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii} + \text{Number of live nauplii}} \times 100$$



Figure 3. Brine Shrimp Hatching Set-up for Cytotoxicity Test

Scavenging Assay

The hydrogen peroxide scavenging assay conducted followed the protocol of Ruch et al. in 1989 to assess the ability of the plant extract to scavenge hydrogen peroxide free radicals. Forty (40) mM of hydrogen peroxide is prepared in phosphate buffer solution (pH 7.4, 50mM). *B. glandulifera* extract and standard were prepared in various concentration (200µg/mL, 400µg/mL, 600µg/mL, 800µg/mL, and 1000µg/mL). Ascorbic acid was used as reference or standard. 0.1mL of the samples and standard were added to 0.6mL of hydrogen peroxide solution and incubated in a dark room at 30°C for 10 minutes to complete the reaction. The absorbance was estimated at 230nm in a spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. Hydrogen peroxide in phosphate buffer will be used as control.

$$H_2O_2 \text{ Scavenging Activity \%} = \frac{\text{AbsC} - \text{AbsS}}{\text{AbsC}} \times 100$$

Where AbsC is the absorbance of the control and AbsS is the absorbance of the sample

RESULTS AND DISCUSSION

Phytochemical Screening

The presence of the bioactive compounds like alkaloids, flavonoids, terpenoids, and phenols exhibits anti-angiogenic and anti-metastatic action. The table below shows the bioactive compounds present in *Bunchosia glandulifera* leaf extract.

Table 1 Phytochemical Analysis of *Bunchosia glandulifera* leaf extract

Bioactive Compounds	Leaf Extract
Alkaloids	+
Flavonoids	++
Saponins	++
Phenols	++
Tannins	-

(+): present; (-): absent

The secondary metabolites screening done in the ethanol leaf extract of *B. glandulifera* indicated the presence of alkaloids, flavonoids, and saponin.

CAM Assay

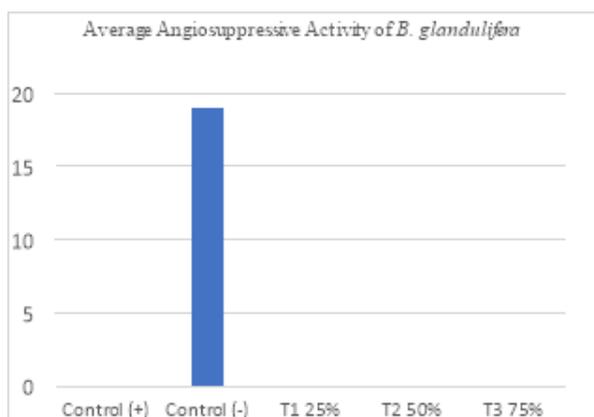


Figure 4. Result of Angiosuppressive Activity of leaves

Figure 4 shows the average angiosuppressive ability of *Methotrexate* (positive control) and *B. glandulifera* extract. The number of blood vessels in the duck embryos after 48 hours of administration of the positive control exhibits degeneration of the blood vessels. It is found that concentrations >25% exhibits toxicity toward the duck eggs leading to death. The concentrations have comparable result with the positive control.

Table 2 Descriptives of Angiosuppressive Activity of *Bunchosia glandulifera* Leaves

Treatments	N	Mean	Std. Deviation
Negative	3	19.3333	3.21455
Positive	3	.0000	.00000
25%	3	.0000	.00000
50%	3	.0000	.00000
75%	3	.0000	.00000
Total	15	3.8667	8.09644

Table 2 shows the descriptive of treatments. The negative control has the mean of 19.3333 with the standard deviation of 3.21455. Positive control and the concentrations have .0000 on both. Therefore, *B. glandulifera* exhibits angiosuppressive property.

Table 3 Analysis of Variance Angiosuppressive Reaction of Leaves

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	897.067	4	224.267	108.516	.000
Within Groups	20.667	10	2.067		
Total	917.733	14			

^a. 0.05 Level of Significance

Table 3 presents the results of Analysis of Variance shows that p=0.000 which is lower than the standard 0.05 confidence level. There is a significant difference between the comparison of number of blood vessel branch points in the treatments. This implies that the *B. glandulifera* leaves extract has anti-angiogenic property based on the table above.

Table 4 Table for Multiple Comparison for the Angiosuppressive Activity

(J) Treatment	(I) Treatment	Mean Difference (I-J)	p-value
Negative	positive	19.33333*	.000
	25%	19.33333*	.000
	50%	19.33333*	.000
	75%	19.33333*	.000
Positive	negative	-19.33333*	.000
	25%	.00000	1.000

	50%	.00000	1.000
	75%	.00000	1.000
25%	negative	-19.33333*	.000
	positive	.00000	1.000
	50%	.00000	1.000
	75%	.00000	1.000
50%	negative	-19.33333*	.000
	positive	.00000	1.000
	25%	.00000	1.000
	75%	.00000	1.000
75%	negative	-19.33333*	.000
	positive	.00000	1.000
	25%	.00000	1.000
	50%	.00000	1.000

Table 4 shows the multiple comparisons among the treatments. The p-value found between the negative control and the other treatments is 0.000, which indicates that there is a significant difference between and are comparable for its significant value is lower than the standard significant level at 0.05. On the other hand, the positive control and 25%, 50%, and 75% concentrations of *B. glandulifera* leaf extract have comparable results in terms of its angiosuppressive activity with a p-value of 1.000. Thus, there is no significant difference and are comparable. The results revealed that concentrations >25% of the leaf extract is at par with the result of the positive control in causing degeneration of the blood vessels in the duck embryo leading to death.

Brine Shrimp Lethality Assay

Table 5 Percentage Mortality and LC₅₀ of *Bunchosia glandulifera* Leaf Extract

Concentration (µg/mL)	log10 (concentration)	No. of Mortality			Total	Mortality %	Probit	LC50
		R1	R2	R3				
1000	3	8	7	6	21	70	5.32	309.7
500	2.699	5	6	4	15	50	4.68	
100	2	4	4	3	11	36	4.19	
0	0	0	0	0	0	0	0	

Table 5 shows the percentage mortality and LC₅₀ of *Bunchosia glandulifera* leaf extract. The extract was found active at 100µg/ml against the brine shrimps. Results show that the computed LC₅₀ is 309.7µg/mL which is greater than 100µg/mL. This indicates that the *Bunchosia glandulifera* extract is considered as nontoxic toward human cells. This suggests that the extract will not have adverse effect upon pharmacological applications.

Hydrogen Peroxide Scavenging Assay

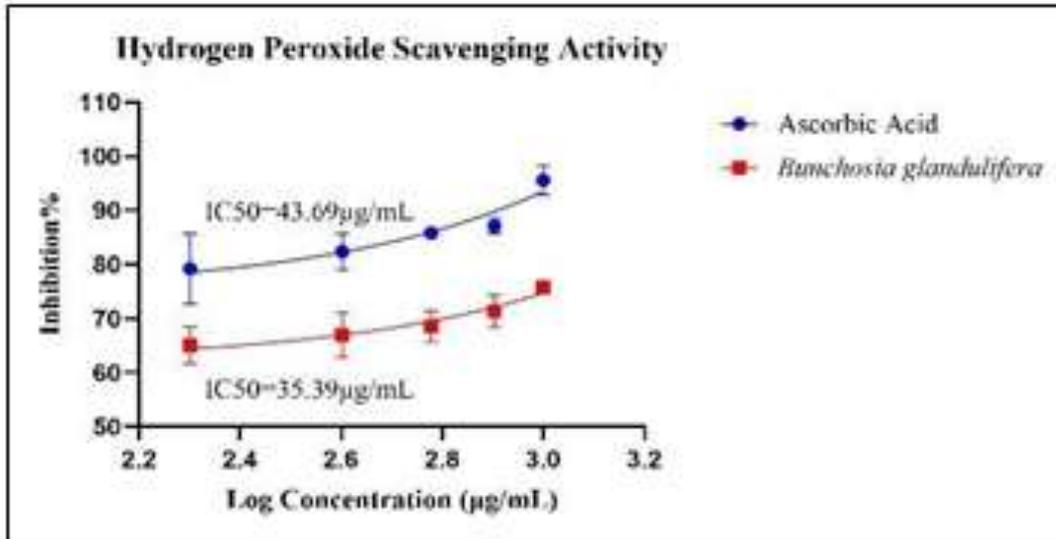


Figure 5. Hydrogen Peroxide Scavenging Assay

Figure 5 shows the ability of *B. glandulifera* extract and ascorbic acid to scavenge hydrogen peroxide free radicals. The reduced absorbance of the concentrations is an indication of scavenging activity. *B. glandulifera* extract revealed an IC₅₀ value of 35.39µg/mL, and the ascorbic acid, as a reference or control, has an IC₅₀ value of 43.69µg/mL. The ability of the leaf extract to scavenge free radicals indicates an antioxidant property. The phenolic compounds may act as free radical because of their ability to donate hydrogen and scavenging activity [2].

CONCLUSION

The results of the study supported the phytonutraceutical, anti-angiogenic, and antioxidant properties of the ethanolic leaf extract of *Bunchosia glandulifera*. The bioactive compounds (Alkaloids, Flavonoids, Saponin, and Phenols) indicate a promising source of phytonutraceuticals as it can be applied for further pharmacological use. Cytotoxicity analysis revealed that *B. glandulifera* will not have an adverse effect towards human cells. The anti-angiogenic and antioxidative properties of the extract showed that it can be a novel cancer prevention and treatment. A promising lead for pharmacological application in battling and ending cancer. On the other hand, isolation of potent bioactive compounds should be done to further validate the claim of the study.

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