The Potential Angiosuppressive Activity of the Ethanolic Extract from the Leaves of Uray (*Amaranthus spinosus* Linn, Family Amaranthaceae) using Chorioallantoic Membrane (CAM) Method

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Introduction

Plant Extracts as Inhibitors

- Plants have provided a source of inspiration for novel drug compounds since ancient times.
- Plant extracts as inhibitors of angiogenesis has been a stepping stone in the treatment of tumor progression. (Verceles & Acebedo, 2013)
- Philippine is well-known for its rich and natural resources and a wide variety of plants that grow abundantly.

Uray *(Amaranthus spinosus)*

- local weed found throughout Philippines.
- for relief of bruises, abscesses, burns, wound, inflammation, menorrhagia, gonorrhoea, eczema and inflammatory swelling

Uray *(Amaranthus spinosus)*

- Very nutritious and contin high quality protein.
- Calcium
- Iron
- VitaminA
- Vitamin K
- Vit B6
- Riboflavin
- Folate
- Dietary minerals

Studies on Uray Leaves

- Potential antitumor activities (Samuel et. al, 2010)
- potent antioxidant activity (Kumar, et. al., 2010)
- have the ability to inhibit cell proliferation
- induce apoptosis in cancers of breast, colorectal, liver and normal cell lines (Dinesh, et. al, 2014).

General Objective

Investigate potential angiosuppressive effect of leaf extracts of different concentrations of *Amaranthus spinosus* using in *vivo* Chorioallantoic Membrane (CAM) assay.

Specific Objectives

1. Determine the phytochemical constituents present in uray leaves.
2. Determine the Total Phenolic and Flavonoid content.
3. Determine the effect of different extract concentration on the vascularity of the Chorioallantoic Membrane
4. Determine which dose exhibited the highest angiosuppressive activity using CAM method.
5. Determine if there was a significant difference between the angiosuppressive activity between Tamoxifen and extract of Uray.

Significance of the Study

- Uray can be found throughout the Philippines, and unknown to many, this plant has many potential medicinal benefits that people can further utilize.
- This study could provide a cheap herbal alternative to combat cancer that can be readily found and accessible.

Methods and Materials

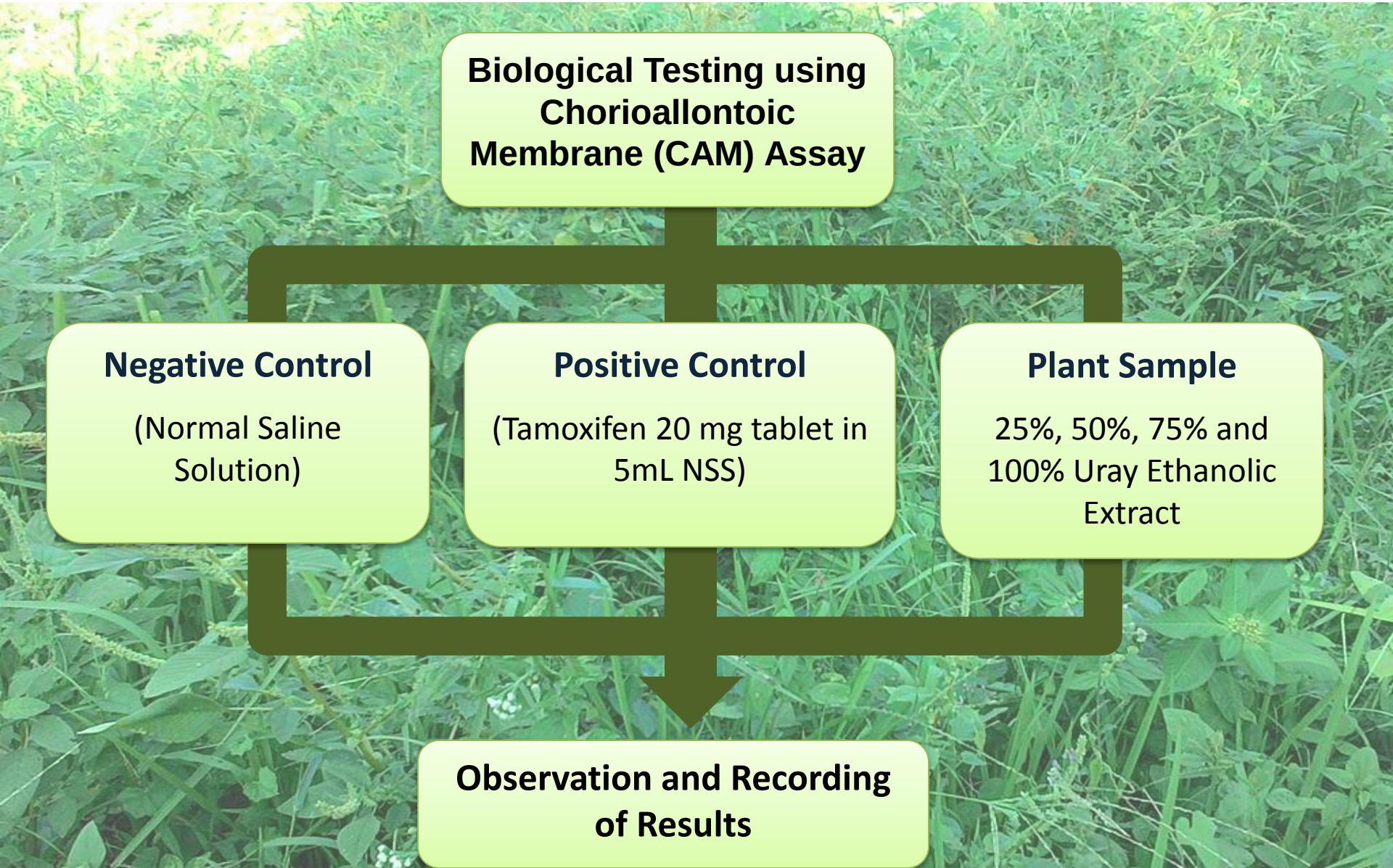
Research Methodology

Collection and Authentication, Preparation of Plant Sample.

Soil analysis (pH, moisture content &Nitrogen (N)content)

Phytochemical Screening, Total Phenolic Content (TPC) , Total Flavonoid Content (TFC)

Research Methodology



Comparison of Test Results

1. **One-Way Analysis of Variance (ANOVA) -** comparison of the differences of vascularization or angiosuppressive activity between groups.

   > **Post hoc analysis (scheffe test**) - performed following the result of the ANOVA that is significant.

   >These tests were performed at the 0.05 level of significance.

2. **The *t-test*** - used to compare the number of branching between each extract concentration and tamoxifen.

*performed at the 0.05 level of significance

Scope and Delimitations

- The study was limited to the evaluation of potential angiosuppressive activity of the crude ethanolic extract of uray leaves
- No other properties are observed.
- No other plant parts were used
- No other extraction solvents aside from ethanol.
- Macroscopic observation.
- Specific active constituents that may be responsible for angiosuppressive activity were not determined.
- No toxicity testing was done and no other test animals were used aside from duck eggs.

Results

Phytochemical Constituents

Sterols	(++)
Triterpenes	(+)
Flavonoids	(+)
Alkaloids	(+)
Saponins	(++)
Glycosides	(+)
Tannins	(-)

Thin Layer Chromatography

SPOT	Rf	Visible	UV 365nm	UV, 254nm
A	0.10	_	Orange	_
B	0.95	_	Blue	_

Total Flavonoid Content

Sample	Absorbance Reading	Computed mg quercetin / g sample	Average mg quercetin / g sample
A.spinosus in EtOH	0.811	3.5296000603	3.75 ± 0.13
	0.906	3.9768019884	
	0.856	3.7414325525	

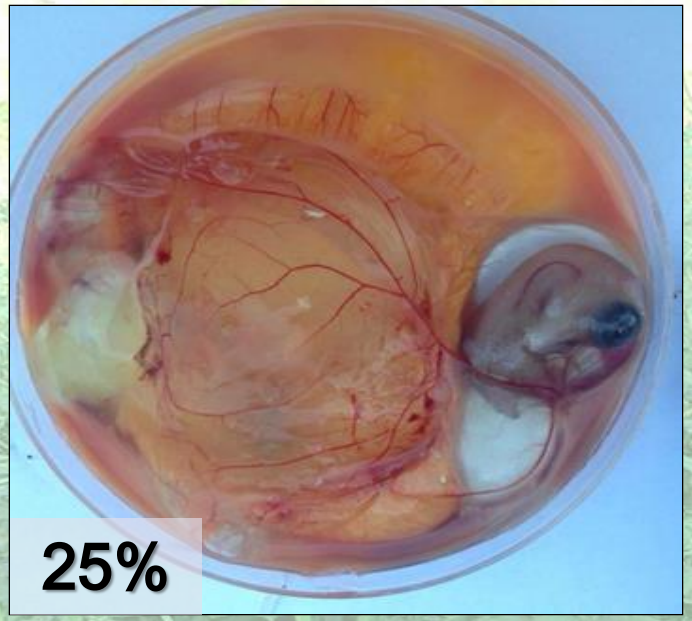
Total Phenolic Content

Sample	Absorbance Reading	Computed mg gallic acid/ g leaf exract	Average mg gallic acid/ g leaf exract
A.spinosus in EtOH	0.853	5.0801894124	5.04 ± 0.04
	0.852	5.0732502110	
	0.836	4.9622229871	

Controls

NSS (Negative Control)

Tamoxifen (Positive Control)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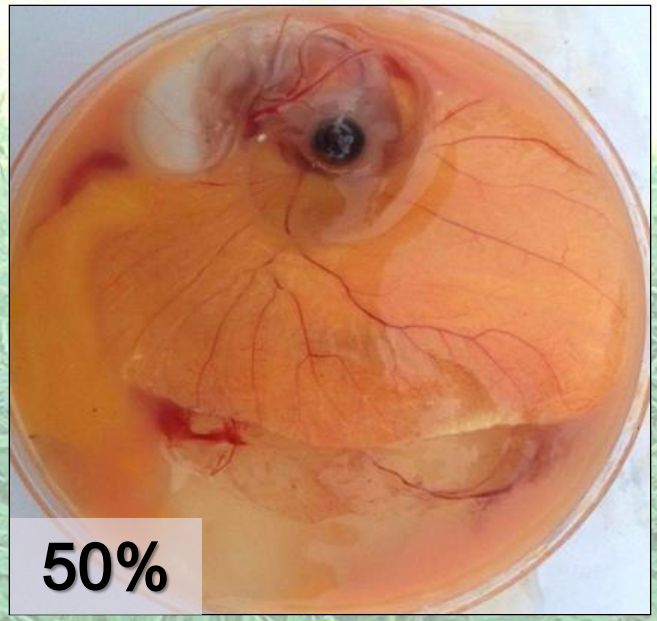
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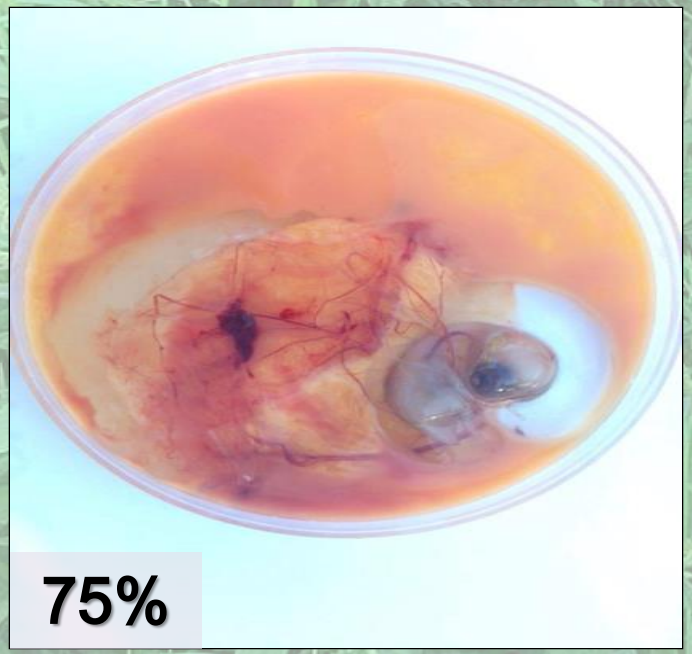
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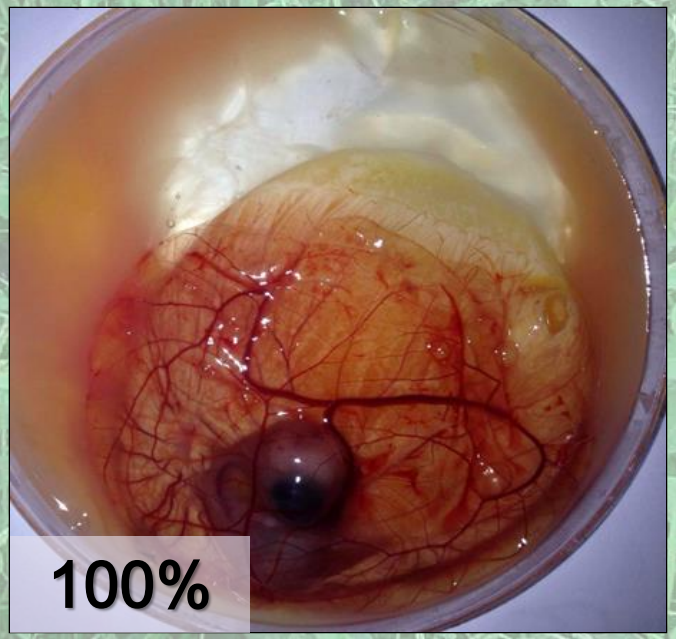
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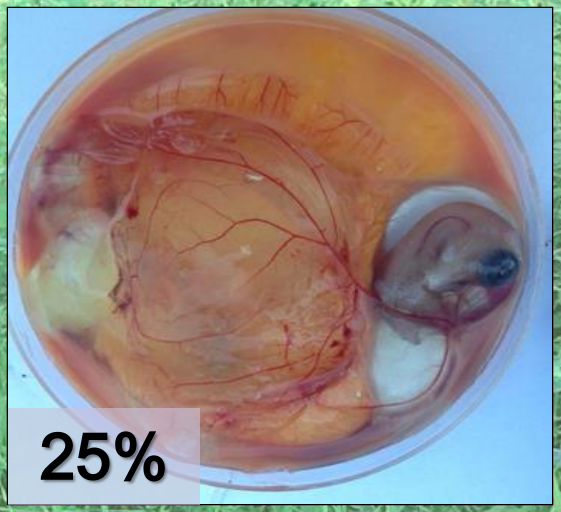
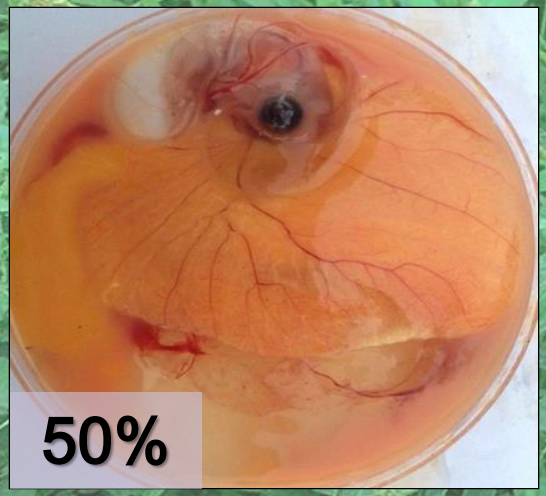
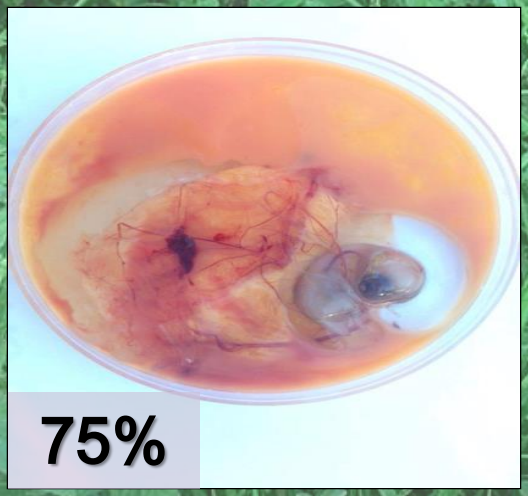
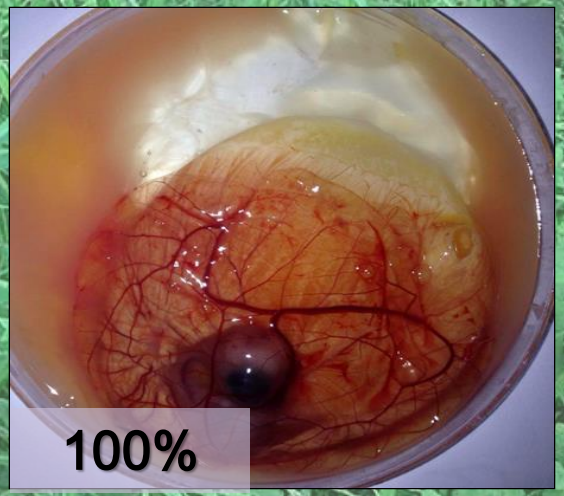
Controls vs. Extracts









Biological Test Results

Test Sample	Average Number of Branching	Interpretation
NSS (Negative Control)	26.4	**A**
25% Extract	4.9	**A**
50% Extract	8.3	**A**
75% Extract	2.1	**B**
100% Extract	3.1	**B**

Biological Test Results

Test Sample	Average Number of Branching	Interpretation
Tamoxifen (Positive Control)	10.5	**B**
25% Extract	4.9	**B**
50% Extract	8.3	**A**
75% Extract	2.1	**B**
100% Extract	3.1	**B**

Discussion

•**Phytochemical Constituents**

✓Sterols
✓Saponins
✓Triterpenes
✓Phenols
✓Flavonoids
✓Alkaloids
✓Glycosides

•Presence of phenolic compounds and flavonoid was confirmed.

•**Flavonoids**- found to have various effects on biochemical pathways such as anti-inflammation, inhibition of cell proliferation, anti-oxidation, detoxification of mutagenic metabolites, apoptosis and inhibition of angiogenesis (Kadioglu, 2013 as cited by Salas &Totaan 2015)

• can serve as chemo-preventive agent

Discussion

- **50% uray extract -** significantly higher number of branching compared to higher extract concentrations.
  - average number of branching is relatively the same compared to the standard drug.
- **Extract concentrations of 25%, 75% and 100% -** significantly lower average number of branching as the standard drug.

- **75% uray extract -** lowest number of branching and therefore indicates that it has the highest angiosuppressive activity.

- **25% extract** was assumed to be the safest dose determined to inhibit neovascularization.

- The angiosuppressive effects of these extracts are **better** than the standard drug, tamoxifen.

Conclusion

- Present study revealed that ethanolic extract from the leaves of Uray (Amaranthus spinosus) exhibited **promising angiosuppressive activity.**

- Phenolic compounds and flavonoid contents – may be responsible for its ability to inhibit neovascularization.

- Uray leaves may become a possible source of therapeutic agent for preventing or treating cancer and other diseases with abnormal angiogenesis.

- Extensive studies are required to further to confirm these promising findings.

Recommendations

- Conduct toxicity test and establish sublethal dose.
- Subsequent tests may be performed to verify angiosuppressive activity of the plant (i.e. rat aortic ring assay, corneal angiogenesis assay etc.
- Disseminate the information that may be useful for other studies, particularly in the treatment of cancer.

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