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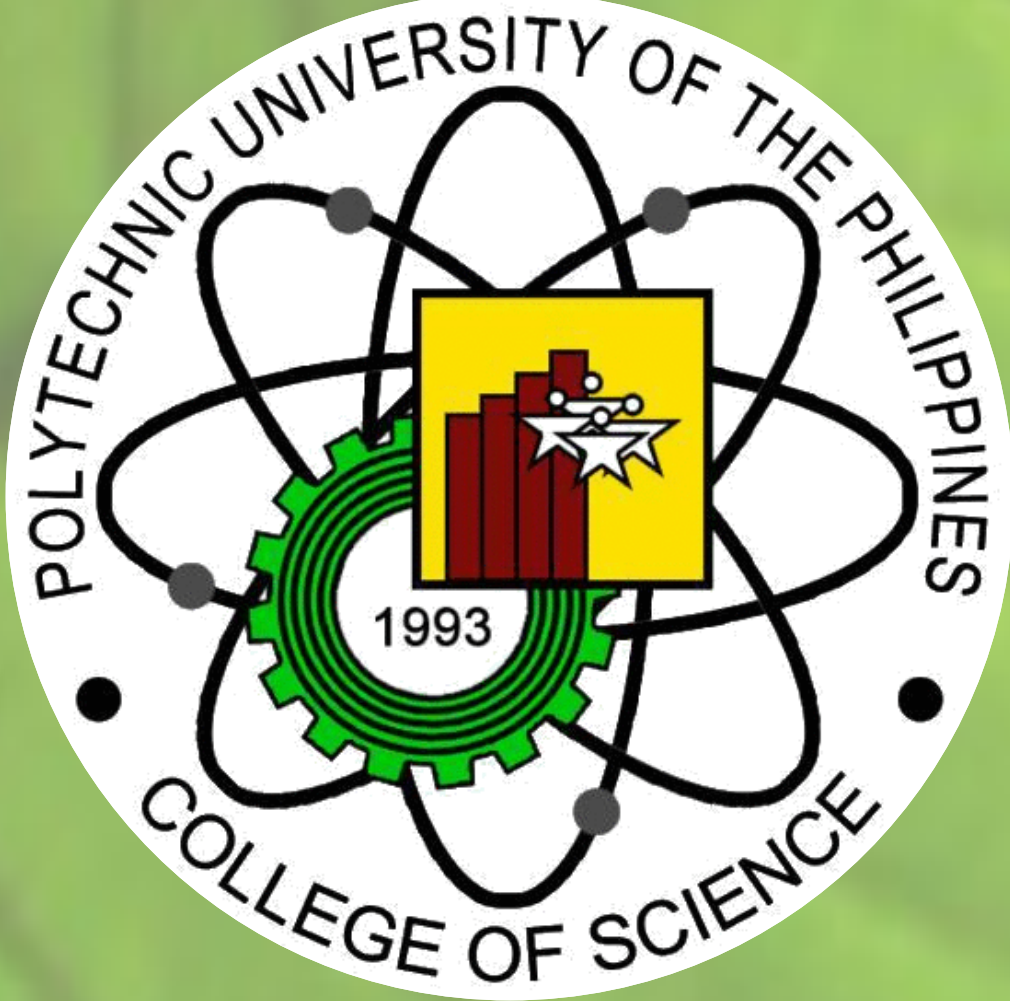
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Angiosuppressive activity of *Dillenia philippinensis* Rolfe ethanolic leaf extract using Chorioallantoic membrane assay in *Anas platyrhynchos* Linn. embryos



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ABSTRACT

Angiogenesis involves the formation of new blood vessels. However, imbalance in angiogenesis may lead in tumor growth. Angiosuppression is a new path as alternative treatment in inhibiting the development of tumor cells. Bioactive compounds present in plants have potential angiosuppressive agents. This study focuses on exploring the angiosuppressive activity of *Dillenia philippinensis* collected from Infanta, Quezon. Ethanolic leaf extract of *D. philippinensis* was lyophilized then partitioned to different concentrations of 25%, 50%, 75% and 100% v/v. The 90% v/v ethanol and retinoic acid were used as negative and positive control, respectively. Duck eggs were disinfected and incubated for 10 days at 37°C. On the 10th day, a window in the egg shell was made to expose the chorioallantoic membrane (CAM). The extract was absorbed on the filter paper disc and placed directly onto the CAM. Then, the treated eggs were incubated for two days. At day 12, the CAM area covered by the filter paper disc was examined for the number of blood vessel branching points. The results revealed that angiogenesis was induced in the negative control while inhibition on the growth of blood vessels were observed on the treatments with *D. philippinensis* extract and positive control. However, there was no significant difference ($\alpha_{0.05} < 0.89$) among the concentrations used in the study. Moreover, 25% and 50% concentration have comparable effects on blood vessel formation. Highest inhibition of blood vessels was observed at 100% (22 ± 3.57) followed by 75% (60 ± 4.23).

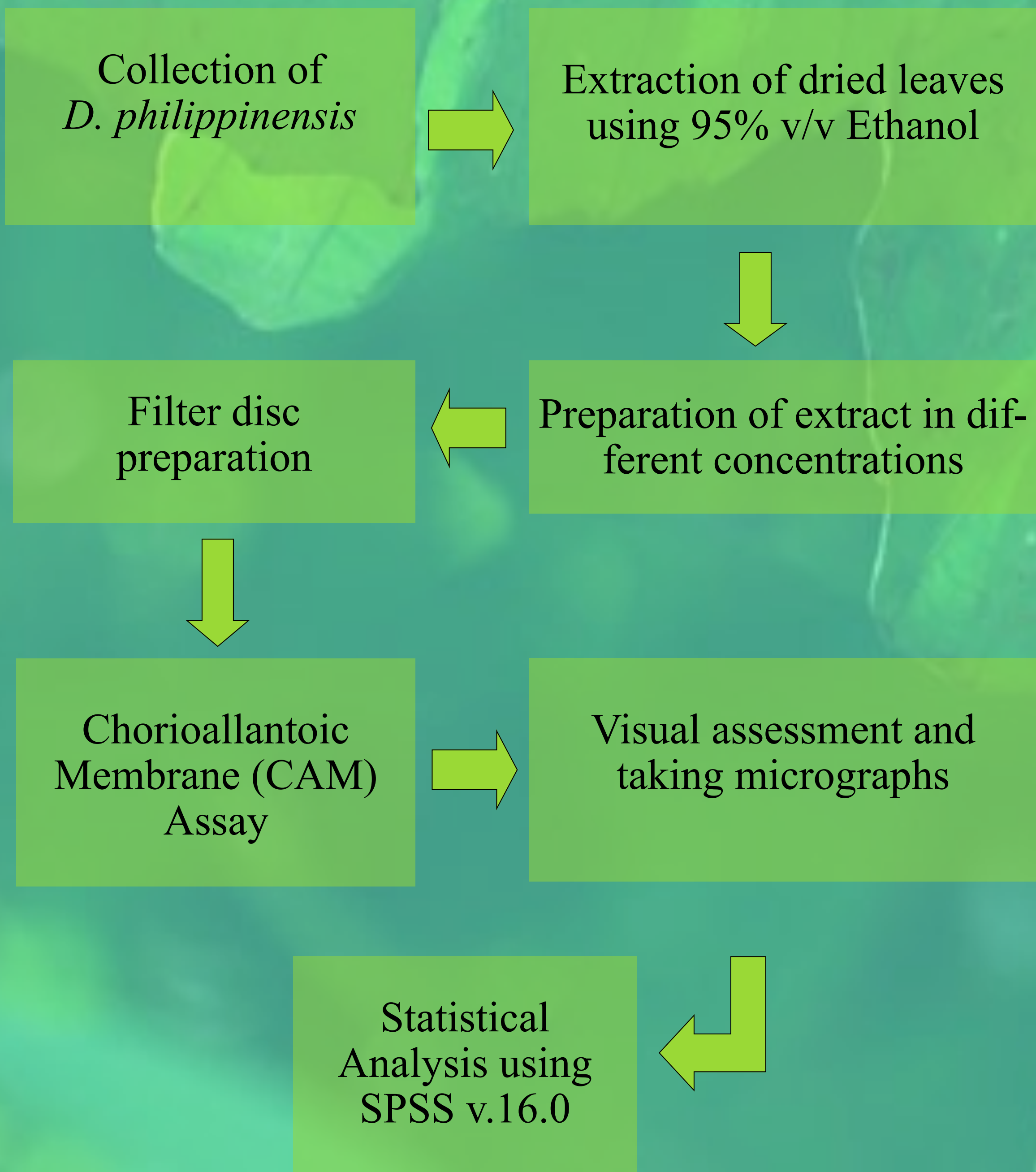
Keywords: Angiogenesis, Angiosuppressive, *Dillenia philippinensis*, CAM assay, and *Anas platyrhynchos*

INTRODUCTION

Angiogenesis involves a series of coordinated events including the formation of new blood vessels. However, imbalance between the stimulating and inhibiting factors may lead to the development of tumor cells (Herrera, 2010). Up to date, phytochemical compounds in plants has not been fully utilized in search of angiosuppressive agent (Tantiado and Tan, 2012). *Dillenia philippinensis*, is a Philippine endemic species, which exhibits potential angiosuppressive effect because of the compounds, triterpenes betulinic acid and 3-oxoolean-12-en-30-oic acid that shows pharmacological activity (Ragasa *et al.*, 2009).

The aim of the study is to determine the angiosuppressive activity of *D. philippinensis* ethanolic leaf extract using chorioallantoic membrane assay in *Anas platyrhynchos* embryos by observing percent vascularity in duck chorioallantoic membranes.

METHODOLOGY



RESULTS AND DISCUSSION

Results revealed that angiogenesis was induced in the negative control while inhibition on the growth of blood vessels were observed on the treatments with *D. philippinensis* extract and positive control. Figure 1 shows the effects of control and different concentrations of *D. philippinensis* to the vascularity of duck embryos.

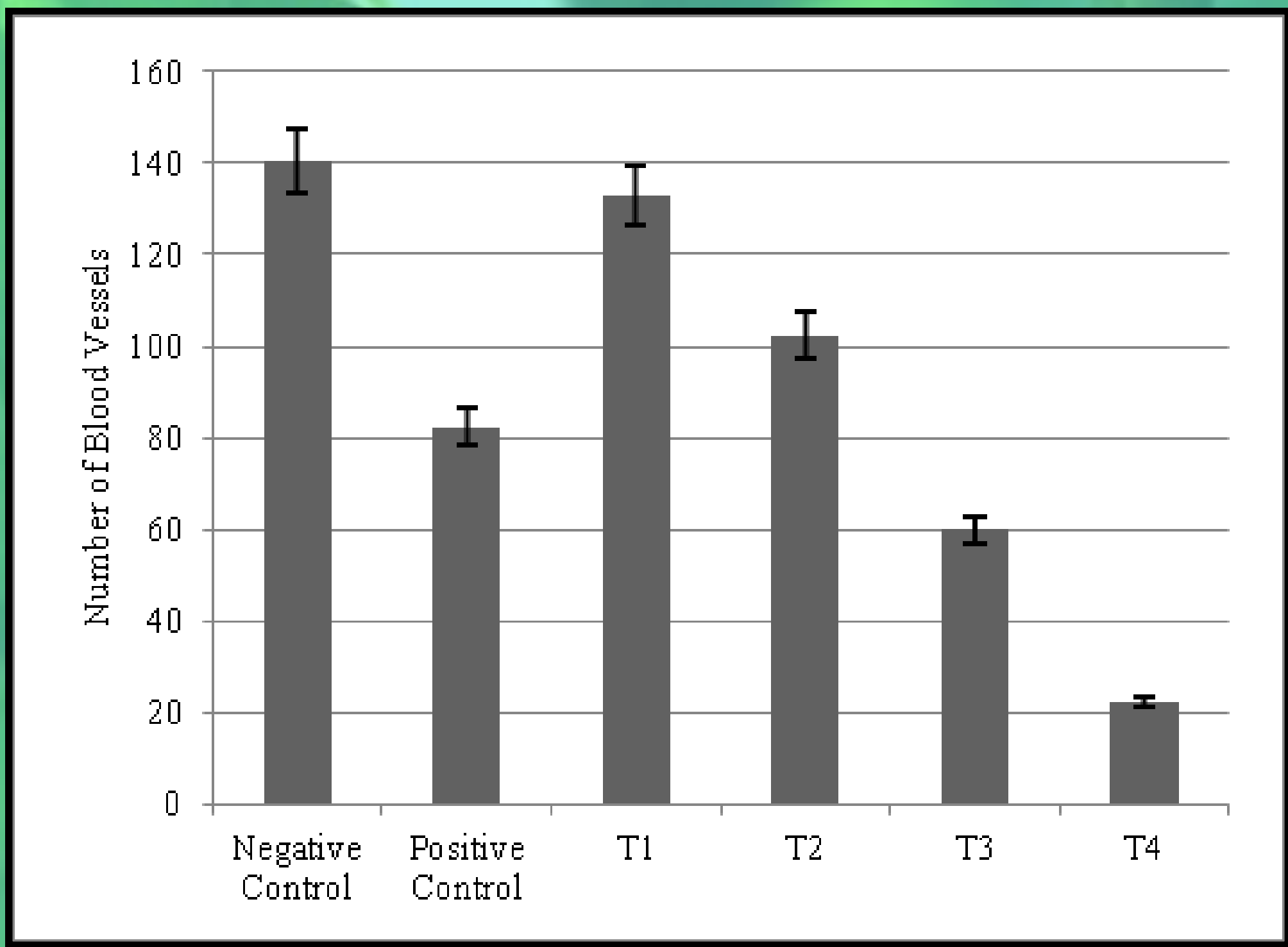


Figure 1. Effect of the controls (negative, 90% ethanol and positive, retinoic acid) and *D. philippinensis* leaf ethanolic extract (T1=25%; T2=50%; T3=75% and T4=100%) in the vascularity of CAM of duck embryo (mean \pm standard error).

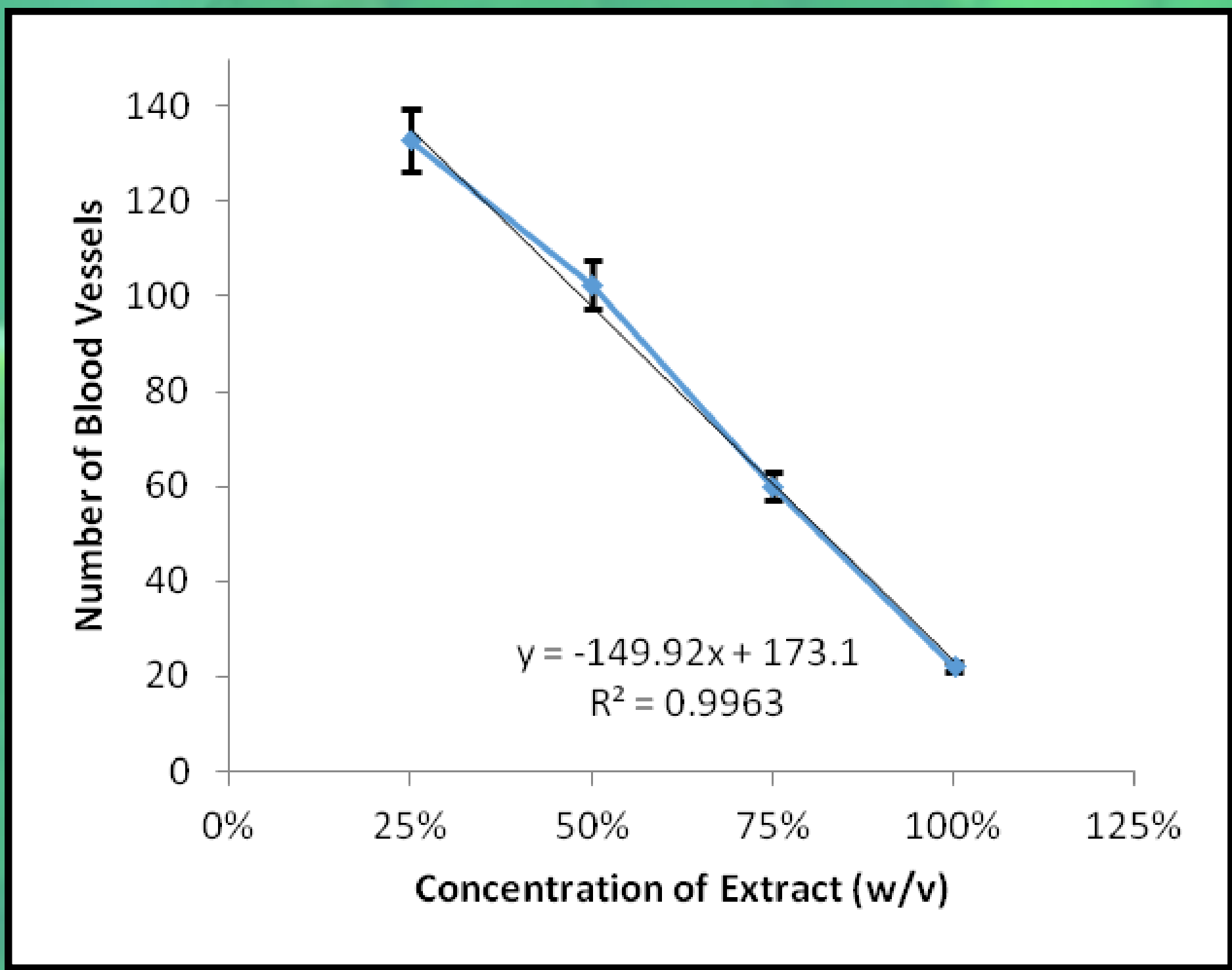


Figure 2. Relationship between the concentration of extract and the number of blood vessels. Number of blood vessels were presented as mean \pm standard error.

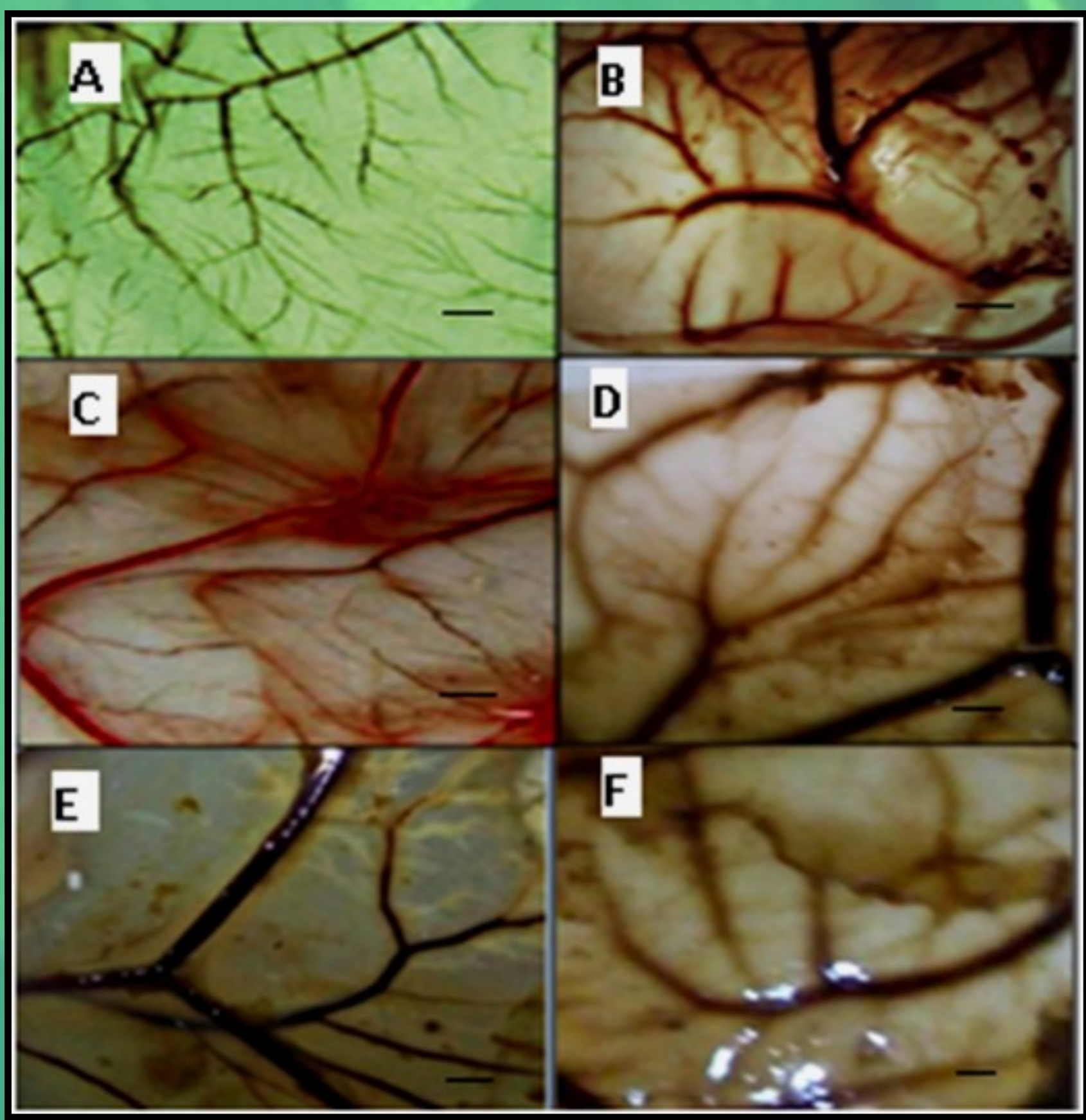


Figure 3. Angiosuppressive response of the duck Chorioallantoic Membrane (CAM) after administration of controls (A) Negative Control, 90% Ethanol; (B) Positive Control, Retinoic Acid and different concentrations (C) 25%, (D) 50%, (E) 75% and (F) 100% of *D. philippinensis* leaf ethanolic extract. (scale bar is present in each image)

There is no significant difference ($\alpha_{0.05} < 0.089$) in the angiosuppressive effect of *D. philippinensis* extract and retinoic acid on the vascularization of duck embryos. Highest inhibition of angiogenesis was observed in 100% v/v (22 ± 3.56) followed by 75% v/v concentration (60 ± 4.23). These two concentrations showed higher angiosuppressive effect than the positive control. In addition, the angiosuppressive effect of the *D. philippinensis* extract is highly dose-dependent as shown in Figure 2, which indicates 0.996 correlation between the concentration of *D. philippinensis* extract and number of blood vessels in CAM.

D. philippinensis constitutes phytochemical compounds such as triterpenoids betulinic acid and 3-oxoolean-12-en-30-oic acid that has a potential angiosuppressive property (Ragasa *et al.*, 2009). Betulinic acid is a pentacyclic triterpene that is derived from the widely distributed natural anti-cancer compound botulin. According to Dehelean *et al.* (2011), betulinic acid does possess an anti-angiogenic activity but it would still depend on the amount of dose. Moreover, betulinic acid inhibits *in vitro* enzymatic activity of aminopeptidase, which is known to play an important role in angiogenesis (Kwon *et al.*, 2002).

CONCLUSION

The study showed the ethanolic leaf extract of *Dillenia philippinensis* exhibit angiosuppressive activity in chorioallantoic membrane of *Anas platyrhynchos*. This study suggests that *D. philippinensis* is a potential source of bioactive compounds that can be utilized as anti-cancer or anti-tumor drug. In addition, results of this study support that Philippine native flora has the potential to be good source for potential active drug.

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