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The anti-angiogenic and antibacterial effect of *Tinomiscium philippinense* Miers. (Menispermaceae) leaf extract

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ABSTRACT

Objective: To determine the toxicity profile, anti-angiogenic and antibacterial activity of the crude and semi-crude leaf extracts of *Tinomiscium philippinense* (*T. philippinense*).
Methods: The leaves of *T. philippinense* were extracted with methanol and partitioned with solvents of different polarities, namely, hexane, dichloromethane and butanol. The extracts were subjected to duck chorioallantoic membrane assay to establish its anti-angiogenic property. Microwell assay was utilized to determine the minimum inhibitory concentration and minimum bactericidal concentration of the different extracts of the plant.
Results: The dichloromethane leaf extract of *T. philippinense* at 1 000 µg/disc showed the highest anti-angiogenic activity with 37.46% inhibition. All the fractions exhibited a bacteriostatic and bactericidal effect on the three bacterial strains with *Pseudomonas aeruginosa*, a Gram negative lactose fermenter exhibiting a higher sensitivity to dichloromethane semi-crude extract among the treatment groups. For the toxicity test, no mortality and no change in behavior were observed in the Sprague-Dawley rats 14 days after the oral administration of the plant extracts. The methanolic leaf extract of *T. philippinense* is non-toxic at a maximum dose of 5 000 mg/kg.
Conclusions: The dichloromethane leaf extract of *T. philippinense* is a potential anti-angiogenic endemic plant species. This plant extract is also a potential antibacterial candidate as determined by microwell assay. The anti-angiogenic and antibacterial activity of the plant may be attributed to the essential oil, steroid, flavonoid, sterol and triterpene content of the plant.

1. Introduction

Angiogenesis is a process that entails the generation of new blood capillaries from current blood vessels. This is a tightly regulated and self-limiting process that occurs in embryogenesis, ovulation and wound healing[1]. The key steps in such a complex series of events include the reactivation of endothelial cells that results to weakening of the basement membrane and other changes in its morphological properties[2,3].

Angiogenesis is an extremely slow process in adults and is activated by special physiological processes such as those mentioned above and retains its quiescent state through a well-coordinated balance between the angiosuppressors and angiopromoters in the body[2]. However, if the normal pattern of this activity has been deregulated, angiogenesis plays an important role in the etiology of tumor progression and metastasis[4].

According to World Health Organization[5], death due to cancer accounts for about 10% of the total deaths caused by non-communicable diseases. Cancer also ranked third among the leading causes of mortality in the Philippines based on the list released by the Department of Health[6]. Approximately 59 000 people in the country were dying of cancer and this causes alarm since the number of affected individuals keeps on increasing every

year[6].

Recognition of angiogenesis as a significant event in tumor progression and metastasis has led to the innovation of new strategies in the field of oncology[7]. This approach has several advantages over the traditional anti-proliferative therapy, including the reduced risk of developing hematopoietic and gastrointestinal toxicity, a general broad spectrum of activity and less probability of developing acquired drug resistance[7,8].

Synthetic angiogenic inhibitors have been produced but only few studies have investigated the natural sources of these compounds[9]. The Philippines has a diverse collection of plants but these resources are not yet well explored in pursuit of novel anti-angiogenic agents[10]. According to Laudico *et al*.[11], more than 80% of Philippine families cannot afford out-of-pocket expenses needed for basic medical care. With the use of herbal plants, several studies have been performed to search for more dynamic and cost-effective treatments[10]. From the data gathered, there is a need to discover potential new drug candidates.

From the study made by Alibek *et al*.[12], it was documented that some antimicrobial agents had antiproliferative effect, such as doxorubicin, neomycin and erythromycin[13-15]. However, the exact mechanism of action on how they elicit such activity is not yet well-established as of this time. In relation to this, another area of the study focuses on the possible antibacterial effect of *Tinomiscium philippinense* (*T. philippinense*).

Infection is one of the major causes of death and is an important cause of morbidity and mortality in cancer patients particularly those who are receiving intensive chemotherapy due to a decrease in their white blood count[16,17]. At present, there are only 10 medicinal plants approved and recognized by the Philippine Department of Health in spite of the many efforts of different Filipino scientists. Among the 10 plants in the list, only two are antibacterials, namely, guava (*Psidium guajava*) and akapulko (*Cassia alata*). In developing countries like the Philippines, people from remote communities use folk medicine in treating infectious diseases and because of the increase of new and re-emerging diseases, as well as the antimicrobial drug resistance, there is a continuous need to discover new antimicrobial compounds[18,19].

At present, 80% of the world's population depends mainly on plants and plant extracts for medicinal purposes. Plants play an important role in providing sources for new drug formulation intended to fight many diseases that affect humans since they contain components having therapeutic value[16,20]. Numerous pharmacological properties have been attributed to natural herbs[21]. The plants *Tinospora rumphii*, *Tinospora cordifolia* and *Tinospora glabra* had shown potential results as anti-angiogenic agents while *Tinospora cordifolia*, *Tinospora capillipes* and *Tinospora tuberculata* showed biological characteristics as potential antibacterial candidates[10,22-28]. All of these plants belong to the family Menispermaceae.

A plant belonging to the same family, Menispermaceae, is *T. philippinense*, an endemic plant that is locally known as "bayatíng" (Pampanga), "lagtáng" (Laguna), and "timbang-tímbang"

(Tayabas). No study has been made to explore the anti-angiogenic and antibacterial properties of this plant though relatives of this plant under the same family were proven to possess the anticipated pharmacological activities.

This study aims to evaluate the angiosuppressive and antibacterial property of *T. philippinense* which may be a potential source of phytochemical active compounds with the use of the duck chorioallantoic membrane (CAM) and microwell assay, respectively.

2. Materials and methods

2.1. Chemicals

Technical grade reagents bought from RTC laboratories were used for extraction. Analytical grade reagents were used for the phytochemical screening and biological assays of the air-dried, ground leaves of *T. philippinense*. Retinoic acid was bought from Sigma-Aldrich and used for the positive control group while absolute ethanol was used for the negative group in the CAM assay. Gentamicin purchased from Sigma-Aldrich was used as a reference antibiotic for the determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

2.2. Plant material

Fresh leaves of *T. philippinense* were collected from the forest area of San Benito, Buhi, Camarines Sur, north of Manapao Lake at an altitude of 120 m above sea level. The plant sample was identified and authenticated by Mr. Noe Gapas, museum researcher II of the Botany Division, at the National Museum located at P. Burgos St., Manila, Philippines with accesion No. 57604. The leaves were air-dried under shade for three weeks until they become brittle. The leaves were pulverized with the use of a Wiley mill grinder and weighed.

2.3. Test animals

A total of six female, non-pregnant, nulliparous Sprague-Dawley rats, aged 8–12 weeks, weighing 100–160 g were selected for the toxicity test. The test animals were procured from the Food and Drug Administration at Alabang, Muntinlupa City, Philippines and were kept at animal house facility, Research Center of Natural and Applied Sciences, University of Santo Tomas. The animals were subjected to a 7-day acclimatization. An approval from the Institutional Animal Care and Use Committee was obtained as a requirement for the animal testing.

2.4. Egg preparation

Fertile duck eggs were obtained from a supplier from Kalawaan, Pasig, Philippines. A total of fourteen groups composed of five zero-day old eggs were used in the assay. The eggs were incubated

with the use of the Incubox automatic turning model with a 1-hour rolling interval at (38 ± 1) °C in humidified incubator until embryonic development Day (EDD) 8. Clean water was placed beneath the egg trays which had a wire mesh bottom to prevent excessive humidity that might allow surface organisms to penetrate the shell[29].

2.5. Bacterial strains

The following bacterial strains were obtained from University of Santo Tomas Collection of Microbial Strains: *Staphylococcus aureus* (ATCC 25923) (*S. aureus*), *Escherichia coli* (ATCC 25922) (*E. coli*) and *Pseudomonas aeruginosa* (ATCC 27853) (*P. aeruginosa*). The bacterial cultures were well kept on a nutrient agar (NA) slant at 4 °C and then sub-cultured on nutrient broth for 24 h before the antibacterial assay.

2.6. Plant extraction and fractionation

A total of 1.17 kg of the ground leaves was macerated with 95% methanol for 24 h and the percolate was collected thereafter. This procedure was repeated twice. The collected percolate was concentrated *in vacuo* at 40–45 °C by using a Buchi heating bath B-490 rotary evaporator until a crude extract with a thick syrupy mass was obtained.

Solvent fractionation was done by dissolving 105 g of the methanolic leaf extract of *T. philippinense* (MLETP) in 500 mL of distilled water and adding 200 mL hexane to the mixture. Two layers were formed corresponding to the hexane and aqueous layers and were then partitioned with the use of a separatory funnel. This procedure was repeated until the color of the hexane layer became faint. Anhydrous sodium sulfate was added to the pooled hexane layer to remove the residual water; then the mixture was filtered. The filtrate was concentrated *in vacuo* at 45 °C to obtain the hexane semi-crude leaf extract of *T. philippinense* (HLETP). To the aqueous layer, 200 mL of dichloromethane was added. Two layers were formed and separated. This procedure was repeated until the color of the dichloromethane layer lightened. Anhydrous sodium sulfate was added to the collected dichloromethane layer and after several hours, was filtered and concentrated at 45 °C to yield the dichloromethane semi-crude leaf extract of *T. philippinense* (DLETP). To the aqueous layer, 200 mL of *n*-butanol was added forming two layers. The layers were separated and the *n*-butanol layer after several collections was concentrated under reduced pressure at 45 °C to obtain the butanol semi-crude leaf extract of *T. philippinense* (BLETP) and the aqueous layer was discarded. Plant extracts were tightly stoppered at temperature between 0 and 5 °C.

2.7. Phytochemical screening

Phytochemical screening was done by using thin layer chromatography sprayed with color reaction reagents. The sample was applied by using a capillary tube on a strip of pre-coated silica plates (Merck EMD TLC). After the sheet was air-dried, it was developed in a chamber that was a beaker containing the most suitable solvent covered with a watch glass. For the MLETP, HLETP and DLETP, a solvent system of hexane and ethyl acetate (4:1) was used. On the other hand, the BLETP was developed with dichloromethane, methanol and glacial acetic acid (9.5:0.5:0.1). The developed chromatogram was air-dried and visualized by spraying with a suitable reagent for the desired constituents.

2.8. Toxicity test

The MLETP was assessed for toxicity by using the acute oral toxicity test according to the Organization for Economic Cooperation and Development Guideline 423[29]. One dose level of 2 000 mg/kg and one dose level of 5 000 mg/kg body weight were performed. Initially, the animals were selected randomly and equally divided into two groups designated as Group A and Group B. The rats were marked for proper identification and were subjected to a 7-day acclimatization. Prior to dosing, the rats were fasted overnight (12 h) by withholding food but not water. The fasted weight was recorded and the suitable dose for each rat was computed. The MLETP to be administered was diluted with 10% Tween 80. One dose level of 2 000 mg/kg body weight was given to Group A via oral gavage. General toxicity signs and behavioral changes were observed daily for a total of 14 days and were noted. With no mortality in a 5-day observation period, Group B was given a one dose level of 5 000 mg/kg body weight. Same observation done on Group A was executed for Group B. After the testing period, the rats were sacrificed through cervical dislocation and were subjected to gross necropsy. Liver and kidney samples were collected for histopathological evaluation by Dr. Cynthia Ochona, a licensed veterinarian pathologist, at the Department of Science and Technology, Bicutan, Taguig.

2.9. CAM assay

Prior to the 8th day of incubation, the crude and semi-crude leaf extracts of *T. philippinense* were diluted with ethanol to prepare a stock solution of 30 000 μg/mL. Ethanol was used to dissolve the crude and semi-crude extracts without adverse effects on the CAM since it would evaporate during drying of the discs[30]. Through serial dilution of the stock solution with ethanol (1:9), concentrations of 3 000 μg/mL and 300 μg/mL were prepared. An aliquot of 33.33 μL was pipetted from each prepared solution to prepare 1 000 μg, 100 μg and 10 μg for each disc (6 mm filter disc).

The CAM assay was performed based on the method of West *et al.*[30], with minor modifications on the choice of control agents. Retinoic acid was used as positive control agent and ethanol as vehicle control.

At the 8th day of incubation, the surface of the egg was swabbed with 70% alcohol. A hole was made at the pointed end of the egg and with a use of a 5-mL syringe with a 21G needle, at least 2 mL of albumen was removed. A small window was made at the side

of the egg. The square shell was removed with the use of a forceps exposing the CAM. A filter disc embedded with test solution was administered on top of the CAM. The hole was completely sealed with a "magic" tape and the egg was returned to the incubator for 48 h. After 48 h, the egg was opened and the general condition of the embryo and the CAM was visually assessed. The embryo and the CAM were photographed with the use of a macro lens with 7×–20× magnificiation. A picture of each quadrant at the site of application was analyzed by means of a software called AngioQuant, an automated image analysis tool, for quantification of angiogenesis. The same procedure was applied for the rest of the eggs. After all results had been obtained, percent inhibition of each test group was determined by using the following formula[31]:

$$\% \text{ Inhibition} = \frac{\text{The number of branch points (treated) - the number of branch points (control)}}{\text{The number of branch points (control)}} \times 100$$

2.10. MIC and MBC

The MIC and MBC of the different extracts were determined by using microwell assay. Conventional serial dilution method was used to prepare 5, 2.5, 1.25 and 0.625 mg/mL of the plant extract. The extracts were diluted to a concentration of 10 mg/mL, placed in microwells, then serially diluted (1:2) into 8 wells to a final volume of 50 µL for each test organism. Before the assay, bacterial suspension was compared with 0.5 McFarland standard to have a concentration of 1.5×10^8 CFU/mL. The suspension was adjusted by adding culture broth to attain the same turbidity. A total of 40 µL of broth was then added to each well followed by 10 µL of bacterial suspension and was incubated at 37 °C for 24 h. The test tubes were analyzed for the presence or absence of growth. All wells with no growth were then sub-cultured by streaking 0.01 mL of the contents of the tube into NA plates. The plates were inverted and incubated for 24 h at 37 °C. After the incubating period, the plates were observed for growth of colonies. The lowest concentration of extract with no growth on the NA plates after 24 h was reported as the MBC, the concentration or dilution of the plant that killed 99.9% of the test organism. The MIC was the lowest concentration of the assayed antimicrobial that inhibits the growth of the bacterium being tested and is usually evaluated by purely visual examination of the wells. All setups were done in triplicate for each extract. Control setups were also tested. These included setups with dimethylsulfoxide only as the growth control and as sterility control.

2.11. Statistical analysis

SPSS version 21 for Windows was used to analyze the results. One-way ANOVA was performed and the results were considered significant at $P \leqslant 0.05$. Further analysis was done through using the *post hoc* analysis at 95% Duncan's multiple range test. Results were expressed as mean ± SD.

3. Results

3.1. Plant extraction

The dried leaves of *T. philippinense* weighing 1.17 kg yielded 130 g of MLETP obtaining a percentage yield of 11.11%. In addition, 105 g of MLETP yielded 1.05% of HLETP, 2.35% of DLETP and 0.85% of BLETP.

3.2. Phytochemical screening

The phytochemical compositions of methanolic leaf extract and semi-crude extracts of *T. philippinense* were detected by using various spray reagents as shown in Table 1.

Table 1

Phytochemical composition of *T. philippinense* leaf extract.

Spray reagents	Chemical content	MLETP	HLETP	DLETP	BLETP
Dragendorff's reagent	Alkaloids	-	-	-	-
Borntrager reagent	Anthrones	+	+++	++	+
Potassium ferricyanide-ferric chloride	Flavonoids	+	++	+++	++
Van-Urk-Salkowski reagent	Indole	-	-	-	-
Vanillin-sulfuric acid	Sterols	++	+	+++	-
	Triterpenes	++	+	+++	-
	Essential oils	++	+	+++	-
α-Naphthol-sulfuric acid	Sugar	-	-	-	-
Antimony chloride	Flavonoids	++	++	+++	+

+: Presence of color; ++: More intense color of spots; +++: Most intense color of spots; -: Absence of spot.

3.3. Acute oral toxicity test

The animals showed no mortality and no change in behavior for 14 days after the administration of the crude methanolic extract of *T. philippinense*. Histopathological examination showed that there was no structural damage to the tested organs. Liver histology evaluation revealed normal hepatocytes. No necrosis, inflammatory reactions, fibrosis or local fatty degeneration was noted. Moreover, the kidney histology evaluation showed that the glomeruli, Bowman's capsules and Bowman's space appeared normal. Results obtained from the acute oral toxicity test showed that MLETP was practically non-toxic at a maximum dose of 5 000 mg/kg body weight.

3.4. CAM assay

There was a noticeable decrease in blood vessel density as compared with the negative control group (Figure 1). Furthermore, One-way ANOVA was used to compare the means of groups. Results showed that the different treatment groups exhibited varying response based on their mean blood vessel count (Figure 2). All treatments were not statistically significant except butanol at 10 µg/disc extract, which was statistically significant at $P = 0.05$. This meant that all extracts had anti-angiogenic properties except for butanol.
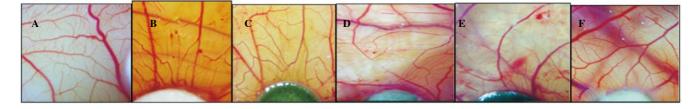
Figure 1. Vascularization of the duck eggs incubated until EDD 10 at (38 ± 1) °C of a humidified incubator.
The negative control, absolute ethanol A: has normal blood vessels while the positive control, retinoic acid at 30 μg/disc; B: MLETP; C: HLETP; D: DLETP; E: at 1000 μg/disc showed a reduction in the blood vessel density while BLETP at 1000 μg/disc had no apparent change; Pictures taken with a 20× macrolens; Mean ± SD blood vessels were determined by using AngioQuant.

The data from the statistical analysis indicated that all the extracts except butanol at 10 μg/disc exhibited a response that was comparable with retinoic acid. Thus, the MLETP, HLETP, DLETP in all the given concentration and BLETP at 100 and 1000 μg/disc semi-crude extract of *T. philippinense* showed anti-angiogenic effect. Butanol semi-crude extract at 10 μg/disc concentration produced a response that was similar to ethanol, meaning that this particular fraction and concentration had an angiopromotive effect.

From Figure 3, a dose-dependent pattern was observed for the percent inhibition of the three extracts particularly, the MLETP, HLETP and DLETP. Percent inhibition (Figure 3) revealed that DLETP at 1000 μg/disc produced the highest activity with a 37.46% inhibition which was higher than the positive control, retinoic acid at 30 μg/disc, with a 30.50% inhibition. From the data gathered, retinoic acid provided a more effective response than DLETP since the latter produced an activity that was only 10.50% but at a concentration that was approximately 33 times greater than retinoic acid. However, DLETP seemed to be a promising source of anti-angiogenic compounds yielding a better response than the purified drug in its semi-crude form. Among the fractions at 1000 μg/disc concentration, BLETP showed the least activity with 2.76% inhibition.

Another important observation was that BLETP at 10 μg/disc concentration showed an angiopromotive effect with a 3.54% inhibition. However, BLETP demonstrated an anti-angiogenic property as the dose increased.

3.5. Antibacterial assay

3.5.1. MIC

The potency of the extracts was assessed quantitatively by determining the MIC and MBC. MIC of the extracts was determined against the three bacterial strains. Based on the results in Table 2, all the extracts inhibited the growth of *E. coli* and *S. aureus* with MIC of 1.250 mg/mL. The dichloromethane semi-crude extract showed the highest bacteriostatic effect against *P. aeruginosa* at MIC 1.250 mg/mL in relation to the remaining fractions that exhibited their effects at MIC 2.500 mg/mL. Although the dichloromethane semi-crude extract inhibited the growth of the bacterial strains, its bacteriostatic capacity was not comparable with the antibiotic standard (gentamicin), which demonstrated a high bacteriostatic effect at a much lower concentration. Dimethylsulfoxide, exhibited no antibacterial activity against the microorganisms tested.

Table 2
MIC of the different extracts against different bacterial strains. mg/mL.

Bacterial strains	MIC of *T. philippinense*				
	MLETP	HLETP	DLETP	BLETP	Gentamicin
E. coli	1.250	1.250	1.250	1.500	0.016
P. aeruginosa	2.500	2.500	1.250	2.500	0.004
S. aureus	1.250	1.250	1.250	1.250	0.008

3.5.2. MBC

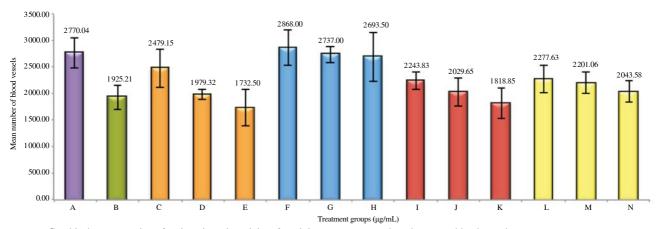MBC determination in Table 3 showed that the extracts could kill

Figure 2. Graphical representation of anti-angiogenic activity of *T. philippinense* extracts based on mean blood vessel count.
A: Ethyl alcohol; B: Retinoic 30; C: DLETP 10; D: DLETP 100; E: DLETP 1000; F: BLETP 10; G: BLETP 100; H: BLETP 1000; I: HLETP 10; J: HLETP 100; K: HLETP 1000; L: MLETP 10; M: MLETP 100; N: MLETP 1000.
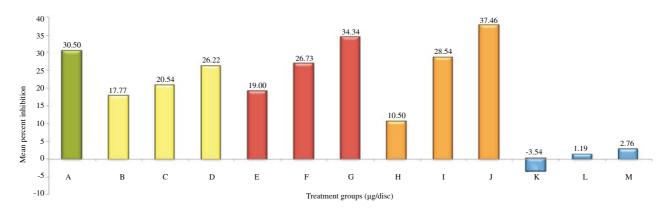
Figure 3. Percent inhibition of the control groups, crude and semi-crude extracts of *T. philippinense* on angiogensis.
A: Retinoic acid 30; B: MLETP 10; C: MLETP 100; D: MLETP 1 000; E: HELETP 10; F: HELETP 100; G: HELETP 1 000; H: DLETP 10; I: DLETP 100; J: DLETP 1 000; K: BLETP 10; L: BLETP 100; M: BLETP 1 000.

E. coli and *S. aureus* at a concentration of 2.500 mg/mL. Again, *P. aeruginosa* showed more sensitivity to the dichloromethane extract with MBC 2.500 mg/mL while the all remaining extracts had an MBC of 5.000 mg/mL. Bactericidal effect of DLETP was lower as compared to the positive control.

Table 3
MBC of the different extracts of *T. philippinense* against different bacterial strains. mg/mL.

Bacterial strains	MBC of *T. philippinense*				
	MLETP	HLETP	DLETP	BLETP	Gentamicin
E. coli	2.500	2.500	5.000	5.000	0.016
P. aeruginosa	2.500	2.500	2.500	5.000	0.004
S. aureus	2.500	2.500	5.000	5.000	0.008

4. Discussion

4.1. Chorioallantoic membrane assay

The plant *T. philippinense* also possesses an anti-angiogenic activity that coincides with the study of its related plants species under Menispermaceae as reflected from previous studies[10,22]. The anti-angiogenic effect of *T. philippinense* may be attributed to phytochemical constituents that are prominent in DLETP such as polyphenols, sterols, triterpenes, flavonoids, steroids and essential oils.

Polyphenols are among the common studies of bioactive plant compounds. The polyphenols exhibit anti-angiogenic effect and metastasis through the regulation of multiple signalling pathways. It was stated that polyphenols may influence some steps in cancer angiogenesis. Red wine polyphenolic compounds and green tea polyphenols act by inhibiting various important events in the angiogenic process such as proliferation and migration of endothelial cells and vascular smooth muscle cells and the expression of vascular endothelial growth factor and matrix metalloproteinases which are two major pro-angiogenic factors[32]. Quercitin, a dietary-derived flavonoids, inhibits the growth of tumor *in vitro* and *in vivo* and also, inhibits the activity of tyrosine kinase. The anti-angiogenic effect of quercitin was confirmed using human

umbilical vein endothelial cells. The chicken CAM assay revealed a reduction in the antiangiogenic effect *in ovo*[33]. Triterpene such as lupeol exhibited anti-cancer effect when tested under *in vitro* and *in vivo* conditions. It was reported that they inhibit the growth of several tumor types by modulating key molecular pathways in the proliferation, survival and apoptosis[34].

Corticosteroid such as hydrocortisone, cortisone and dexamethasone are one of the well-studied angiostatic compounds used alone or in combination with heparin in preventing edemagenesis or angiogenesis. According to the findings from the study of Nauck *et al.*[35], corticosteroids can reduce edema or prevent new blood vessel formation due to, or at least partly, by hindering the expression of vascular endothelial growth factor. The natural or synthetic ligands of member of the steroid thyroid hormone nuclear receptor superfamily such as retinoic acid receptors, peroxisome proliferator-activated receptor gamma and vitamin D receptor also target specific molecular mediators of the angiogenic response that includes metalloproteinases and angiogenic growth factors[7]. Squalamine, an anti-angiogenic sterol that was isolated from shark liver, possesses an anti-prostate cancer activity[36].

4.2. Antibacterial assay

Results may be due to the high concentration of the following phytochemical components in the dichloromethane semi-crude extract.

Phenolics, such as flavonoids, are widely distributed in the plant kingdom. For a long time, flavonoids have become the mean physiologically active constituents to treat human diseases because they possess antimicrobial and cytotoxic activities[37]. Many phytochemical preparations with high flavonoid content were reported to exhibit antibacterial effect. Examples of flavonoids are apigenin, galangin, pinocembrin, ponceritin, genkwanin and sophoraflavanone G and its derivatives including naringin and naringenin, epigallocatechin gallate, luteolin and luteolin 7-glucoside. The antibacterial mechanisms of action of various flavonoids include the inhibition of nucleic acid synthesis,

inhibition of cytoplasmic membrane function and inhibition of energy metabolism[38].

Essential oils have varying degrees of antimicrobial activity. Examples are eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon[39]. This property may be due to the impairment of a variety of enzyme systems including those involved in energy production and structural component synthesis[40].

Based on the phytochemical constituents, it was reviewed by Agrawal that flavonoids possess good anti-angiogenic and antibacterial activities[41]. In addition, flavonoids and phenolics which have been detected in the present study have been well-cited in the literature as the phytochemical constituents have anti-oxidant activity[42]. Oxidative stress usually occurs when there is an imbalance between the free radical and antioxidant ratio which may be caused by the overproduction of the reactive oxygen species or by inhibiting the pathway that induces the activation of antioxidants. Overproduction of reactive oxygen species can alter the structures of biomolecules such as the proteins, lipids, lipoproteins and nucleic acid[43]. The antioxidant properties are frequently proposed to play an important role in disease prevention caused by oxidative stress, such as cancer coronary arteriosclerosis, and the ageing process[44]. Another possible mechanism how flavonoids can inhibit angiogenesis could be the inhibition of protein kinases, enyzmes that play an important role in signal transduction[45]. Reactive oxygen species also rapidly increase during infection, serving to facilitate pathogen clearance as well as contributing to signaling cascades related to inflammation, cell proliferation, and immune responses[46].

Based on the results obtained, *T. philippinense* is a potential anti-angiogenic endemic plant species with the use of a duck's chorioallantoic membrane. This plant extract is also a potential antibacterial candidate as determined by Microwell assay. Acute oral toxicity test based on OECD 423 shows that the MLETP is non-toxic at a maximum dose of 5 000 mg/kg. The anti-angiogenic and antibacterial activity of the plant may be attributed to the essential oil, steroid, flavonoid, sterol, and triterpene content of the plant based on the thin layer chromatographic phytochemical screening with various spray reagents.

Therefore, further investigation is needed to isolate and identify the biologically active constituent pertaining to the anti-angiogenic and antibacterial effect of *T. philippinense*.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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