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Bioactivity studies on the extracts and the phlebotonic diosmetin isolated from Premna *odorata* Blanco *(Lamiaceae)*

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ABSTRACT
Extracts and the phlebotonic diosmetin isolated from the leaves of the Philippine ethnomedicinal plant Premna odorata Blanco (Lamiaceae) were assayed against brine shrimp, the PI-4 kinase, and four bacteria. The LC_{50} of the crude ethanolic extract to brine shrimp was 564 µg/mL while that of the decoction was 685 µg/mL. The isolated diosmetin had IC50 to PI-4 kinase at 118 µg/mL. The zone of inhibition of both the decoction and crude ethanolic extract were in the range 0-11 mm against all four test bacteria. The MIC of the decoction and the isolated diosmetin against all four test bacteria ranged from 4-8 µg/mL to 256 – 512 µg/mL.The different extracts and the isolated diosmetin were not significantly active against the test bacteria, nor were they significantly inhibitory to PI-4 kinase. However, the presence of the phlebotonic diosmetin makes P. odorata a medicinal plant indeed.
Keywords: phlebotonic diosmetin, Premna odorata, Bioactivity

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INTRODUCTION

Premna odorata Blanco is a native tree of the Philippines and other Asian countries. It used to be classified under the Verbenaceae family, but reclassified under *Lamiaceae.* It is widely distributed in the secondary forests around the Philippines where its leaves are used as a vaginal wash and as antitubercular. It is one of the seven components of a local herbal preparation called "pito-pito". The leaves are also used as natural insecticide against poultry lice.

Diosmetin was first isolated from *Scrophularia nodosa* in 1925 but has been found in only a few species so it is considered as non-widespread [2]. It has been isolated and purified from the leaves of the *P. odorata.* Its identity was determined by LC/MS/MS and NMR spectroscopic analyses [3].

Aside from being a venous phlebotonic agent [4], several studies report that diosmetin is anti-allergic [5], anti-inflammatory [6], and chemopreventive [7,8,9]. Diosmetin is also reported as a good inhibitor of the enzyme PI-3 kinase [10] whose products are observed in colorectal tumors [11].

In this study, samples were screened for their general bioactivity, antibacterial activity and inhibitory activity on the enzyme PI-4 kinase. The PI-4 kinase acts on phosphotidylinositols (PIs) to synthesize phosphatidylinositol-4-phosphate or PI(4)P which is, in turn, acted on by the enzyme PI(4)P-5 kinase to synthesize phosphatidylinositol-4,5-diphosphate or $PI(4,5)P_2$ (12). The PI-4,5-P_2 has a crucial role in cell migration which is the central process in the development and maintenance of multicellular organisms. The orchestrated cell migration to specific locations is required in tissue formation during the development of the embryo, the healing of wound, and immune responses. Errors during cell migration may lead to mental retardation, vascular diseases, tumor formation and metastasis (13).

MATERIALS AND METHODS

The sample collection, extraction and solvent partitioning of the plant extracts, as well as the structural elucidation of the isolated diosmetin, were covered by an earlier published report [3]. Except for the technical grade 95% EtOH, the various brands of solvents used were of analytical or HPLC grade.

General Bioactivity

Samples were subjected to the Brine Shrimp Lethality Test to evaluate their general bioactivity. Screening was done on 100-µg/mL samples and the % Brine Shrimp Lethality was evaluated after a 24-

hr exposure. To determine the median lethal concentration (LC_{50}), screening was done on three (3) different concentrations: 50-, 100-, and 500-µg/mL. By using the *Excel Software* and by means of regression analysis, the best-fit line equation was statistically acceptable with $R^2 > 95\%$**.** Significant bioactivity was inferred at $LC_{50} < 30$ µg/mL (14).

Enzyme Inhibition Activity

Samples were screened, using the Membrane Capture Method, for inhibitory effect on the enzyme PI-4 kinase. The reference standard or positive control used was the crude ethanolic extract of the *Brucea ammarissima* bark at four (4) varying concentrations, ranging from 3.6 to 9.6 mg/mL; all of which showed 0% phosphorylation or 100% inhibition of the enzyme PI-4 kinase. As for the isolated diosmetin, six (6) concentrations were assayed to determine the median inhibitory concentration (IC_{50}). By using the Excel *Software* and by means of regression analysis, the best-fit line equation was statistically acceptable with $R^2 > 90\%$. Significant inhibitory activity was inferred at $IC_{50} < 30$ µg/mL (**15**).

Antibacterial Activity

Samples were screened for antibacterial activity, using the Agar Diffusion Method, against *Bacillus subtilis* (*B. subtilisi*) (BIOTECH 1754H), *Escherichia coli* (*E. coli*) (BIOTECH 1428H), *Salmonella typhimurium* (*S. typhimurium*) (TA 98-BIOTECH B 1326) and hospital isolate oxacillin-resistant *Staphylococcus aureus* (*S. aureus*). After 24h of incubation, the zone of inhibition, ZI, around each paper disc was measured. Activity was categorized as: inactive (ZI < 10 mm), partially active (10 = ZI < 13 mm), active (13 = ZI < 19 mm), and very active (ZI > 19 mm).

Minimal Inhibitory Concentration (MIC)

For the MIC determination, Serial Tube Dilution Technique was used. The test bacteria were the *B. subtilis* (BIOTECH 1754H), *E. coli* (BIOTECH 1428H), *S. marsescens* (ATCC 13880) and the hospital isolate oxacillin-resistant *S. aureus*. The breakpoints were MIC ≤ 2 µg/mL for susceptibility to the test sample and MIC ≥ 4 µg/mL for resistance.

RESULTS AND DISCUSSION

General Bioactivity

At 100 µg/mL, the percent lethality to brine shrimp of the crude ethnolic extract (CEX) was 26.7%. Among the partitioned fractions, the hexane-soluble fraction (HSF) had the highest bioactivity at 81.2% lethality, which was followed by the dicloromethane-soluble fraction (DSF) at 49.9% lethality. Both the ethyl acetate-soluble fraction (ESF) and the water-soluble fraction (WSF) had the least bioactivity at 25.0% lethality. So, the trend was: HSF > DSF > CEX > ESF = WSF.

The diosmetin, which was isolated from the DSF, had 76.9% lethality. This meant that there was no observable synergistic effects on diosmetin's bioactivity by other principles present in the CEX and in the DSF. This was significant as it could justify the isolation of diosmetin and the use of pure diosmetin as medicinal.

The LC_{50} of the CEX was 564 µg/mL ($R^2 = 0.9989$) while that of the decoction was 685 µg/mL ($R^2 = 0.9994$). Though CEX was more lethal to brine shrimp than the decoction, both were inferred to be not significantly bioactive.

Enzyme Inhibition Activity

The inhibitory activity of the samples on the PI-4 kinase followed the trend: **ESF > CEX > WSF > HSCX = Decoction.** The isolated diosmetin had IC_{50} at 118 µg/mL ($R^2 = 0.9465$), and was therefore inferred as not a significant inhibitor of the PI-4 kinase.

Antibacterial Activity

At 400 µg/mL, the decoction was inactive (ZI = 0 mm) against all four test bacteria (Table 1). The crude ethanolic extract at 400 µg/mL was inactive against *E. coli* (ZI = 0 mm), *S. typhimurium* (ZI = 0 mm) and *S. aureus* (ZI = 9 mm), but partially active against *B. subtilis* (ZI = 11 mm).

Table 1 Antibacterial activity of the decoction and the crude ethanolic extract against four test bacteria

	Test Organism with the Average Zone of Inhibition (mm) [Disc Size: 6 mm diameter]			
Test Sample at 400 µg/mL	***B. subtilis***	***E. coli***	***S. typhimurium***	***S. aureus***
Decoction	0 (inactive)	0 (inactive)	0 (inactive)	0 (inactive)
Crude ethanolic extract	11 mm (partially active	0 (inactive)	0 (inactive)	9 mm (inactive)

Minimal Inhibitory Concentration (MIC)

With the MIC of more than 4 µg/mL (Table 2), it appeared that all test bacteria were resistant *in vitro* to both the decoction of *P. odorata* leaves and the isolated diosmetin, with the oxacillin-resistant *S. aureus* as the least resistant.

Table 2 The minimum inhibitory concentration of the decoction and the isolated diosmetin against four test bacteria

	Minimum Inhibitory Concentration (MIC) Range			
Sample	***B. subtilis***	***E. coli***	***S. aureus***	***S. marsescens***
Decoction	128 – 256 µg/mL (Resistant)	128 – 256 µg/mL (Resistant)	64 – 128 µg/mL (Resistant)	128 – 256 µg/mL (Resistant)
Isolated diosmetin	256 – 512 µg/mL (Resistant)	128 – 256 µg/mL (Resistant)	4 – 8 µg/mL (Resistant)	256 – 512 µg/mL (Resistant)

CONCLUSION AND RECOMMENDATIONS

The different extracts, fractions, and the isolated diosmetin were determined not to be significantly active against the four test bacteria, nor were they significantly inhibitory to PI-4 kinase. The medicinal compounds that might be directly related to the plant's traditional uses could be present in other fractions. However, finding the phlebotonic diosmetin in its leaves makes it reasonable to conclude that *P. odorata* is indeed a medicinal plant. Other Philippine plants, native and introduced, could also be screened for the presence of the health-benefitting diosmetin.

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REFERENCES

1. Campanero MA, Escolar M, Perez G, Garcia-Quetglas E, Sadaba B, Azanza JR. (2010).Simutaneous determination of diosmin and diosmetin in human plasma by ion trap liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry: Application to a clinical pharmacokinetic study. *J Pharma Biomed Anal* [Online] **51**(4)875-81. *http://www.sciencedirect.com/science/article* (accessed Sept. 17, 2011).
2. Valant-Vetschera KM, Wollenweber E. Flavones and flavonols. In: Andersen M, Markham KR, editors (2006). *Flavonoids: Chemistry, biochemistry and applications.* London:Taylor and Francis Group; p. 628-44.
3. Pinzon LC, Uy MM, Sze KH, Wang M, Chu IK (2011). Isolation and characterization of antimicrobial, anti-inflammatory and chemopreventive flavones from *Premna odorata* Blanco. *J Med Plants Re.* [Online] **5(**13)2729-35. *http://www.academicjournals.org/journal/JMPR/abstract/47151C816799*. (accessed Jan. 17, 2012).
4. Martinez MJ, Bonfill X, Moreno RM, Vargas E, Capella D (2005). Phlebotonics for venous insufficiency. *Cochrane Database Syst Rev* [Online] Jul 20 (3):CD003229. http://pubmed.gov (accessed Jan. 26, 2016).
5. Cheong H, Hoo SY, Oak MH, Cheon SH, Yoo GS, Kim KM (1998). Studies of structure-activity relationship of flavonoids for the anti-allergic actions. *Arch Pharmacal Res*. [Online] **21**:4. *http://www.springer.com. (*accessed Sept. 11, 2010).
6. Lopez-Posadas R, Ballester IF, Abadia-Mollina C, Suarez MD, Zarzuelo A, Martinez-Augustin O, de Medina FS (2008). Effect of flavonoids on rat splenocytes, a structure-activity relationship study*. Biochem Pharmacol.* [Online] **4**(76)495-506. http://www.ncbi.nlm.nih.gov/pubmed/18590707. (accessed Jan. 17, 2010)
7. Androutsopoulos VP, Wilsher N, Arroo RR, Potter G (2009). Bioactivation of the phytoestrogen diosmetin by CYP1 Cytochromes P450. *Cancer Lett.* [Online] **274**:54-60.*http://www.sciencedirect.com/science /article/pii/S0304383508007052. (*accessed Sept. 17, 2010).

8. Androutsopoulos VP, Mahale S, Arroo RR, Potter G (2009). Anticancer effects of the flavonoid diosmetin on cell cycle progression and proliferation of MDA-MB 468 breast cancer cells due to CYPI activation. *Onco Rep.* [Online] ;**20**:1525-28. *http://www.uniprot.org/citations/19424633*. (accessed Sept.17, 2010).

9. Androutsopoulos VP, Spandidos DA (2013). The flavonoids diosmetin and luteolin exert synergistic cytostatic effect in human hepatoma HEPG2 cells via CYPP1a-catalyzed metabolism, activation of JNK and ERK an P53/P21 up-regulation. *J Nutr. Biochem.* [Online] **24**: 2, 496-504. *http://www.ncbi.nih.gov/pubmed/22749133* (accessed Sept. 17, 2014)

10. Agullo G, Gamet-Payrastre L, Manenti S, Viala C, Remesy C, Chap H, Payhrastre (1997). Relationship between flavonoid structures and inhibition of phosphatidylinositol 3-kinase: A tyrosine comparison with kinase and protein kinase C inhibition. *Biochem Pharmacol.* [Online] **53:**1649-57. *http://www.ncbi.nlm.nih.gov/ pubmed/9264317* (accessed Feb. 4, 2010)

11. Phillips WA, St. Clair F, Munday AD, Thomas RJS, Mitchell CA (1998). Phosphatidylinositol 3-kinase activity in colorectal tumors*. Cancer Res. [Online]* **83**:41-47. *http://www.ncbi.nlm.nih.gov/pubmed/9655291* (accessed Feb. 4, 2010)

12. Knight ZA, Feldman ME, Balla A, Balla T, Shokat KM (2007). A membrane capture assay for lipid kinase activity. *Nature Protocols*. **2**(10)2459-66.

13. Ling K, Schill NJ, Wagoner MP, SunY, Anderson RA (2006). Movin' on up: The role of PtdIns(4,5)P_2 in cell migration. *Trends in Cell Biology*. **16**(6)16.

14. Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV (2005). Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *Intl J Applied Sc Eng*. **3**(2)125-34.

15. Vlahos CJ, Matter WF, Hui KY, Brown RF (1994). A specific inhibitor of phosphatidylinositol-3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (ly294002). *J Biol Chem*. **269**(7)5241-48.

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