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RESEARCH ARTICLE

New peperomin and polyketides from dichloromethane extract of *Peperomia blanda* Jack. (Kunth)

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ABSTRACT

Much of the new research and investigation in pharmacy sciences are concerned with developing therapeutic agents, and identifying and finding new drugs with their chemical structure to treat different human diseases such as infectious diseases from natural products. Therefore, the present findings relate to isolating five new compounds the dichloromethane extract of *Peperomia blanda* (Jacq.) Kunth grown on Socotra Island, Yemen. two new secolignans; which have been proposed as peperomin I & J. These compounds were isolated together with the other two polyketides presented as surinone D and dindygulerione F. The chemical structures were elucidated and confirmed with nuclear magnetic resonance (NMR) and liquid chromatography-mass spectroscopy (LCMS) analysis. These compounds were first isolated and reported from this plant. These new compounds' antimicrobial activity has been evaluated, and minimum inhibitory concentration has been recorded in the range of 125-250 µg/mL. The pharmacotherapeutic spectrum of compounds was predicated using PASS software which showed potential activity.

Keywords: Peperomin; PASS; antibacterial; cytotoxic; poyketides.

INTRODUCTION

Infectious diseases are one of the main causes of mortality, especially in communities with low sanitary condition, commonly present in developing countries, such as Yemen. Yemen is also facing other infectious diseases, including diphtheria, measles, cholera, and COVID-19. The conflict and the collapse of the healthcare system have made it challenging to control these diseases, putting the lives of millions of Yemeni people at risk. The high incidence of antibiotic resistance could be either by drug-induced selection or drug-mediated mutagenesis (de Castro *et al*., 2014; Sílva *et al*., 2017). Therefore, searching for new active compounds to be used in antimicrobial therapy is of great concern. Recently, natural products from micro-organisms, animals, plants, and algae have been proven to be excellent sources of antimicrobial compounds (Antoraz *et al*., 2015; Deshmukh *et al*., 2015; Carter *et al*., 2016; Lacerda *et al*., 2016). Plants-derived compounds are also attractive candidates for bioprospecting programs due to their high chemical diversity, which results in the inhibition of a range of microbial pathways (Sílva *et al*., 2017). Traditional communities have commonly used plants due to their supposed medicinal properties. This widespread knowledge has directed lot of researches aiming to provide scientific evidence of these actions (Bezerra dos Santos *et al*., 2015; Zhang *et al*., 2015; Sɒlva *et al*., 2017; Hashemi Gahruie *et al*., 2020).

Peperomia blanda (Jacq.) Kunth is a fleshy annual herbaceous plant of the Piperaceae family that grows in Asia's tropical and subtropical regions. *Peperomia blanda,* cultivated in Socotra Island (Yemen), has been used as antibacterial, antifungal, and cytotoxic against different cancer cell lines such as HepG2, HL-60, and MCF-7 (Al-Madhagi *et al*., 2018, 2019a). *Peperomia blanda* belongs to the *Peperomia* genus, which is considered the most abundant source of bioactive compounds in the Piperaceae family (Lɳpez *et al*., 2010). This genus reported having many uses, such as inflammation, antibacterial, gastric ulcer, asthma, analgesic, and anticancer (Cheng *et al*., 2003; Coseri, 2009; Mbah *et al*., 2012; Gutierrez *et al*., 2016). Based on these different uses, there is rising interest in identifying new compounds from *Peperomia*. New polyketide compounds have been isolated from petroleum ether extracts from *P. blanda*. They have displayed antibacterial activity (Al-Madhagi *et al*., 2019ba). Therefore, the present study has been concerned about isolating

bioactive compounds from the dichloromethane extract of *P. blanda* (JACK.) Kunth, followed by predication of pharmacological activities of the active isolated compounds by using PASS.

MATERIALS AND METHODS

General reagents

Ethyl acetate, methanol, n-hexane, chloroform (analytical grade), thin-layer chromatography plates (silica gel 60 PF_{254}), silica gel (0.063-0.200 mm) for column chromatography were obtained from Merck (Darmstadt, Germany).

Sample preparation

The whole fresh *Peperomia blanda* (Jacq.) Kunth plant was collected from Diksam-Sikand Socotra *Island* Yemen in March 2013. Plant sample was identified by a botanist Abdul Wali Al-Khulaidi, College of Sciences, Taiz University. Its voucher specimen (No. PPLS/2013/90/1) was deposited in the Faculty of Pharmacy, Sana'a University. The whole fresh plant was oven dried at 30°C for several days. The dried plant samples were ground into powder and stored in airtight container. Then, the dichloromethane (DCM) extracts of *P. blanda* crude have been prepared for the whole plant by the maceration method (Al-Madhagi *et al*., 2018) where the dried samples have been macerated in DCM solvent with a ratio (1:10) three times and filtrated. The collected filtrate were combined and dried, the yield of the dichloromethane is 4.7%. Then the dichloromethane extract has been evaluated as antimicrobial (Al-Madhagi *et al*., 2018). The active DCM extract (11.7 g) was chromatographed over a silica gel, and eluted with $CHCl_3$:EtOAc (1:0–0:1) to afford three fractions (D1-D3). Fraction D2 (10.15 g) was purified by column chromatography with n-hexane-EtOAc (7:3) to yield five pure compounds: peperomin A, peperomin I, and peperomin J surinone and dindygulerione F. The whole isolation process was summarized in Figure 1.

Structural Elucidation

Chemical structures of the isolated compounds were elucidated using FT-IR/FT-FIR (ATR, Perkalin), GCMS (QP2010 Plus, Shimadzu, Japan), 1D-NMR (^1H, ^{13}C, and DEPT-135) and 2D NMR (COSY, NOSY, HMBC) (JEOL) spectroscopies at 500 MHz with deuterated chloroform ($CDCl_3$) as a solvent.

Tested Bacterial and fungal strains

Among the micro-organisms used for antimicrobial tests, five micro-organisms were obtained from American Type Culture Collection (ATCC) and Microbiology Laboratory, Medical Faculty, University Putra Malaysia, two of which were Gram-positive bacteria *Staphylococcus epidermidis* (a clinical isolate) and *Staphylococcus aureus* (ATCC 25923) and three Gram-negative bacterial *Pseudomonas aeruginosa* (ATCC27853), *Escherichia coli* (a clinical isolate) and *Klebsiella pneumoniae* (ATCC 2513). The other two micro-organisms were fungi obtained from the Institute for Medical Research (IMR), Kuala Lumpur, which were *Candida albicans* (C2213) and *Aspergillus niger* (A121).

Antimicrobial assay

Fraction and pure compounds were subjected to an antimicrobial assay using a microdilution method where the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Two-fold serial dilution of the DCM fractions ranging from 0.56 to 100 µg/mL was used for the MIC assays (Ndi *et al*., 2007), and for pure compounds, 3.90 to 500 µg/mL were used in 96-well with Mueller Hinton Broth as diluent. Duplicate tubes of each dilution were seeded with test organisms to the concentration of the standard (5 x 105 cfu/ml). The plates were incubated at 37°C for 24 hours for bacteria and 25°C for 48 hours for fungi. The least concentration of the tested samples showing no visible growth was taken as the MIC (Moulari *et al*., 2006). Then,

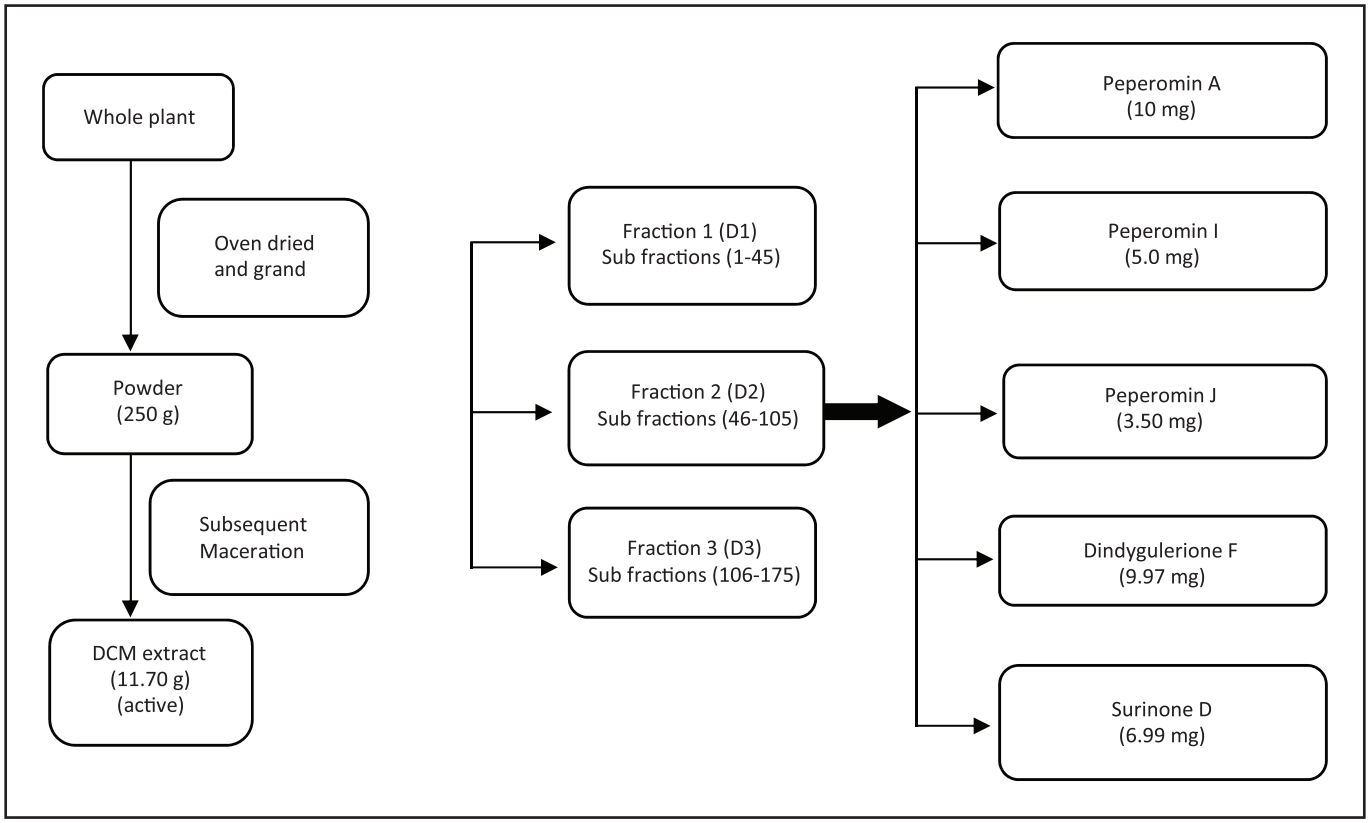


Figure 1. A schematic diagram of bioassay-guided fractionation until isolation of compounds from the crude extract of dichloromethane extracts.

the minimal bactericidal concentration (MBC) and the minimal fungicidal concentration (MFC) were determined by aspirating 0.1 ml of culture medium from each well showing no apparent growth and sub-culturing it on the fresh NA and PDA plate, respectively. The MBC and MFC were read as the least concentration showing no visible growth on NA and PDA sub-culture.

Biological activity predictions using PASS

Activity Spectra Prediction for Substances (PASS) (Geronikaki *et al*., 2004) software has been used to predict the biological activity of compounds using the online predication; www.way2drug.com. Instantly, PASS predicts 4000 types of activities, including pharmacological effects, mechanisms of action, toxic and adverse effects, interactions with metabolic enzymes and transporters, and effects on gene expression with an average accuracy of about 85% based on the chemical structure of the compound. From the structure-activity relationship database (SARBase), the biological activity spectrum of the tested compounds was evaluated, the input files were either MOL or SDF files, and the output results were interpreted as biologically 'active' (Wang *et al*., 2014) or 'inactive' in the form of compound probabilities (Pi). These probabilities were acquired by joining contributions from groups of atoms in the compound that favored or disadvantaged the particular operation described in the software backend structure-activity database. The higher the Pa-Pi values, the greater the probability of the compound showing activity on a scale of 0-1.

RESULTS

Physical, chemical and spectroscopic data of the compounds

The compound purity is detected with different tests such as the NMR, IR and GCMS/LCMS.

i. Peperomin I

Yellowish green oil, UV ($CHCl_3$) λ_{max} nm: 289, 291. IR(ATR) u_{max} cm^{-1}: 2958, 2870 (C-H), 1667 (C=C), 1462 (OC=O), 1389, 1363, 1311, 1220, 1190 (CH). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 6.47 (d, 4H, *J*= 2.30 Hz, H-2',2",6',6"), 4.30 (dd, 1H, *J*= 9.15 & 8.05 Hz, H-4b), 3.87 (m, 1H, H-4a), 3.84 (s, 6H, 2xOCH_3), 3.80 (s, 6H, 2xOCH_3), 3.79 (s, 6H, 2xOCH_3), 3.50 (d, 1H, *J*= 11.45 Hz, H-5), 2.91 (m, 1H, H-3), 2.33 (m, 1H, H-2), 0.93 (m, 2H, H-7), 0.87 (t, 3H, *J*= 6.90 Hz, H-8). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 174.8 (C-1), 153.6 (C-5',5''), 153.5 (C-3',3''), 137.6 (C-1',4'), 137.1 (C-1'',4''), 104.8 (C-6',6''), 104.7 (C-2''), 104.6 (C-2'), 70.4 (C-4), 61.0 (5''-OCH_3), 60.9 (5'-OCH_3), 56.7 (C-5), 56.3 (4xOCH_3), 47.4 (C-2), 40.6 (C-3), 29.7 (C-6), 15.6 (C-7). HRMS (ESI-TOF) m/z calcd. for $C_{25}H_{32}O_8$[M+1]$^+$: 460.2097 (100.0%), 461.2131 (27.0%), 462.2164 (3.5%), 462.2140 (1.6%), found: 460.5131 (75), 461.2017 (24), 462.1220 (3.5).

ii. Peperomin J

Brown oil, UV ($CHCl_3$) λ_{max} nm: 289. IR (ATR) u_{max} cm^{-1} ($CHCl_3$): 2962 (C-H), 1564 (O-C=O) 1260, 1215 (C-O). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 6.66 (d, 1H, J= 1.75 Hz, H-6'), 6.61 (d, 2H, *J*= 1.70 Hz, H-2''), 6.59 (d, 1H, *J*= 1.15 Hz, H-2'), 5.93 (m, 1H, H-6''), 5.89 (s, 2H, OCH_2O), 4.30 (m, 2H, H-6), 4.02 (m, 1H, H-4), 3. 88 (s, 6H, 2xOCH_3), 3.56 (d, 1H, *J*= 11.45 Hz, H-5), 2.76 (m, 1H, H-3), 2.48 (m, 2H, H-2), 2.32 (m, 2H , H-8), 1.62 (m, 2H, H-9), 0.85 (t, 3H, J=6.30 Hz, H-10).^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 179.4 (C-7), 175.9 (C-1), 149.4 (C-3'), 149 (C-3''), 143.6 (C-5'') , 143.0 (C-5'), 136.9 (C-4',4''), 136.0 (C-1',1''), 108.9 (C-6'), 108.0 (C-6"), 101. 6 (C-2'), 101. 6 (C-2"), 101.6 (OCH_2O), 70.4 (C-6), 70.0 (C-4), 57.0 (2xOCH_3), 56. 2 (C-5), 47.2 (C-3), 40.0 (C-2), 32.0 (C-8), 24.6 (C-9), 15.9 (C-10). HRMS (ESI-TOF) m/z calcd for $C_{27}H_{30}O_{11}$ [M+1]$^+$: 530.1788(100%), 531.1822(29%), 532.1855(4%), found: 530.1982(75%), 531.1916(25%).

iii. Surinone D

Yellowish oil, UV ($CHCl_3$) λ_{max} nm: 288. IR (ATR) u_{max} cm^{-1} ($CHCl_3$): 2975(C-H), 1401(C-O). ^{1}HNMR (500 MHz, $CDCl_3$) δ ppm: 6.79 (dd, 1H, J=8.05, 9.75 Hz, H-16'), 6.69 (m, 1H, H-9'), 6.65 (d, 1H, J=9.15 Hz, H-12'), 6.57 (d, 1H, J=9.20 Hz, H-13'), 6.06 (dd, 1H, J= 7.40, 16.00 Hz, H-10'), 5.88 (d, 1H, J=10.3 Hz, OCH_2O), 4.01 (m, 1H, H-4), 3.04 (m, 1H, H-2'b) ,2.93 (m, 1H, H 2'a), 2.76 (m, 2H, H-6) ,2.46 (m, 2H, H-8') ,2.38 (m, 1H, H-5b), 1.79 (m, 1H, H-5a), 1.58 (m, 8H, H-4'-7').^{13}CNMR((500 MHz, $CDCl_3$) δ ppm: 214.4 (C-1'), 205.9(C- 1), 197.9 (C-3), 149.1 (C-15'), 147.1 (C 14'), 130.3 (C-9'), 130.2 (C-16'), 128.9 (C-10'), 122.2 (C-13'), 121.0 (C-12'), 108.0 (C-2), 100.7 (OCH_2O), 71.4 (C-4), 40.1(C-6), 35.7 (C-2'), 31.1 (C-8'), 29.1 - 29.7 (C6'-7'), 27.1 (C-5'), 24.7 (C 3',4'), 22.2 (C-5). GCMS m/z [M+1]$^+$ 401 (10), 355 (60), 281(30), 221(30), 147(30), 73(100). HRMS (ESI-TOF) m/z [M+1]+ 401.0500, (calcd for $C_{23}H_{28}O_6$: 400.4624). HRMS (ESI-TOF) m/z calcd for $C_{23}H_{26}O_6$ [M+1]$^+$: 398.1729(100%), 399.1763(25%), 400.1796(3%), found: 398.1839(100%), 399.1903(25%).

iv. Dindygulerione F

Brownish oil, UV ($CHCl_3$) λ_{max} nm: 291. IR (ATR) u_{max} cm^{-1} ($CHCl_3$): 3411, 2928 (NH_2), 1659 (C-O), 1261 (C=C), 1027 (C O).^{1}HNMR (500 MHz, $CDCl_3$) δ ppm: 7.25 (m, 5H, H 12' 16'), 7.15 (br s, 1H, NH), 5.33 (m, 4H, H7'-8'), 4.01 (dd, 1H, J=5.75, 10.85 Hz, H4), 3.08 (m, 1H, H-6eq), 2.77 (m, 2H, H 10'), 2.58 (q, 4H, J= 7.45 Hz, H-6', 9'), 2.33 (m, 1H, H-2') 1.82 (m, 1H, H-5ax), 1.62 (m, 6H, H3'-5').^{13}CNMR(500 MHz, $CDCl_3$) δ ppm: 206.2 (C 1), 197.9 (C-3), 178.3 (C-1'), 110.3 (C-2), 142.9 (C-11'), 128.2-128.4 (C 12'-16'), 125.5 (C-7',8'), 71.6 (C-4), 40.2 (C-6), 36.0 (C-2'), 31.3 (C-6'), 29.1 (C-4', 5'), 24.7 (C-3'), 24. 5 (C-5). GCMS m/z [M+1]$^+$: 356(40), 265(10), 167 (100), 139 (60), 91(90), 55(60). HRMS (ESI-TOF) m/z calcd for $C_{22}H_{27}NO_3$[M+1]$^+$: 353.1991(100%), 354.2024(23%), 355.2058(3%), found: 353.2001 (100%), 354.2003(25%).

MIC determination for fractions

The active DCM extract (Al-Madhagi *et al*., 2018) as an antibacterial against Gram-negative (*P. aeruginosa, K. pneumonia, and E. coli*) and Gram-positive bacteria (*S. epidermis* and *S. aureus)* has been selected for further isolation of pure active compounds from this extract. The collected fractions from DCM extracts were tested for their antibacterial properties in this study. Most of the fractions exhibited significant activity against five tested bacteria in the experiments; two Gram-positive bacteria (*S. aureus* and *S. epidermidis*), three Gram-negative bacteria which were, *E. coli*, *P. aeruginosa*, *K. pneumoniae,* with MIC values ranging from 50 – 100 µg/mL and MBC values of 50-100 µg/mL as presented in Table 3. This study had found out that the DCM fractions recorded a low MIC value of 12.5– 25 µg/mL and an MBC value of 25 - 50 µg/mL against *P. aeruginosa*. The antibacterial activity of the different fractions was higher than the recorded activity ampicillin but lower than gentamicin.

Moreover, compounds isolated from petroleum ether extract of *P. blanda* (cultivated in Socotra island) were dindygluerine A, dindygluerine E, peperomin A and flavokawain A that possessed antibacterial activity (Al-Madhagi *et al*., 2019a). Therefore, the presence of a large number of compounds and rare compounds such as secolignans named peperomins, polyketides of 2-acyl-cyclohexane-1,3-dione type, and the chromenes make this genus a source of unique compounds. The isolation of compounds from active *P. blanda* DCM crude extracts to narrow down the metabolites number and increase our probabilities of gaining active compounds and concerning to GCMS data (Al-Madhagi *et al*., 2018) that lead to the isolation of five two new secolignans, called *Peperomin I* and *Peperomin J*. In addition to Peperomin A and two new polyketides of 2-acyl-cyclohexane-1,3-dione type known as surinone D and

Table 1. Summary of ^{1}H and ^{13}C NMR spectroscopic data of secolignan compounds isolated from DCM extract

Position	Peperomin I (20)		Peperomin J (21)	
	^{1}H (δ)[a]	^{13}C (Singh *et al*.)	^{1}H (δ)[a]	^{13}C (Singh *et al*.)
1		174.7 (q)[c]		175.9(q)[c]
2	2.33, m, 1H	47.4 (t)	2.48, 2H, m	47.0 (s)
3	2.91, m, 1H	40.3 (t)	2.76, 1H, m	40.0 (t)
4a,4b	3.87, m, 1H4.30, dd, 1H, J_{dd}= 9.15, 8.05 Hz	70.4 (s)	4.02, 1H, m	70.0 (t)
5	3.50, d, 1H, *J*=11.45 Hz	56.7 (t)	3.56, d, 1H, *J*=11.45	56.2 (t)
$1^{/}$		137.6 (q)		134.3 (q)
$2^{/}$	6.47, d, 1H, *J*=2.30 Hz	104.6 (t)	6.59, d, 1H, *J*= 1.15 Hz	101.6 (t)
$3^{/}$		153.5 (q)		149.4 (q)
$4^{/}$		137.6 (q)		136.9 (q)
$5^{/}$		153.6 (q)		143.0 (q)
$6^{/}$	6.47, d, 1H, *J*=2.30 Hz	104.8 (t)	6.66, d, 1H, *J*=1.75 Hz	108.9 (t)
$1^{//}$		137.1 (q)		136.0 (q)
$2^{//}$	6.47, d, 1H, *J*=2.30 Hz	104.7 (t)	6.61, d, 1H, *J*=1.70 Hz	101.6 (t)
$3^{//}$		153.5 (q)		149.0 (q)
$4^{//}$		137.6 (q)		136.9 (q)
$5^{//}$		153.6 (q)		143.6 (
$6^{//}$	6.47, d, 1H, *J*=2.30 Hz	104.8 (t)	5.93, IH, m	108.0 (t)
OCH_2O	3.84, s, 3H	56.3 (p)	5.90, s, 2H	100.7 (s)
	3.84, s, 3H	56.3 (p)	5.90, s, 2H	100.7 (s)
	3.87, s, 3H	56.3 (p)	5.90, s, 2H	101.6 (s)
	3.87, s, 3H	56.3 (p)	5.90, s, 2H	101.6 (s)
$3^{/}$-OCH_3	3.79, s, 3H	60.9 (p)	3.84, s, 3H	57.0 (p)
$3^{//}$-OCH_3	3.79, s, 3H	61.0 (p)	3.84, s, 3H	57.0 (p)
6	0.86, t, 3H, *J*= 6.90 Hz	15.6 (p)	4.2,4.0^{over,} m 2H,	70.4 (s)
7				179.4.9 (q)[c]
8			2.32, m, 2H	32.0 (s)
9			1.62, m, 2H	24.6 (s)
10			0.85, t, 3H, *J*= 7.45 Hz	15.9 (p)

dindygluerine F. The results of the isolated compounds showed more potent activity against Gram-positive bacteria than Gram-negative bacteria with MIC ranging between 125-250 µg/mL and MBC value of 500 µg/mL. The varied activity of the compounds against tested bacteria that could be involved in wounds and infection seems to be consistent with traditional uses of *P. blanda*. Another important finding was that peperomin A and peperomin I had been displayed antifungal activity against *A. niger*.

Prediction of biological activity using PASS (Prediction of Biological Activity for Substances)

Virtual screening of the isolated compound was done by PASS in order to have an idea about the possible biological activity of compounds as well as their possible mechanisms of action, metabolism-related actions, transporter terms, and toxic/adverse effects (Lagunin *et al*., 2000). The activity with a probability of activity, Pa, greater than 0.3, was included. Peperomin I gave a higher prediction value with Pa > 0.707 as chemoprotective in comparison with other secolignan; peperomin I and A. In addition, they showed prediction activity as antineoplastic ranged 0.5 < Pa < 0.7. Besides, surinone D exhibits better prediction activity as an antineoplastic and apoptosis agonist with Pa > 0.707. Dindygulerione F gave a prediction value of Pa 0.697 as an antineoplastic and apoptosis agonist. Moreover, the prediction activity of the tested compound as antimicrobial showed low prediction value of Pa < 0.5 for the secolignan derivatives (peperomin A, peperomin I, peperomin J). At

Table 2. Summary of ^{1}H and ^{13}C NMR spectroscopic data of N-containing polyketides compounds isolated from DCM extract of *P. blanda*

Position	Dinhygulerinone F (26)		Surinone D (25)	
	^{1}H (δ)[a]	^{13}C (Singh *et al*.)	^{1}H (δ)[a]	^{13}C (Singh *et al*.)
1		206.2 (q)		205.9 (q)
2		110.3 (q)		108.0 (q)
3		197.9 (q)		197.9 (q)
4	4.01, dd, 1H, J_{dd}= 5.75, 10.85 Hz	71.6 (t)	4.01, m, 1H	71.4 (t)
5	1.82, 1H, m, H-5_{ax}	24.5 (s)	1.79, m, 1H	22.2 (s)
	2.36,1H, m, H-5_{eq}		2.38, m, 1H	
6	2.33, m, 2H	36.0 (s)	2.76, m, 2H	40.1 (s)
1/		178.3 (q)		214.4 (q)
2/	2.90, m,1H	40.2 (s)	2.93, m, 1H	35.7 (s)
	3.08, m,1H		3.04, m, 1H	24.7 (s)
3/	1.62, m, 8H (3'-5')	24.7 (s)	2.16, m, 2H	24.7 (s)
4/		29.1 (s)	1.58, m, 8H	27.1 (s)
5'		29.1 (s)		29.7 (s)
6'	2.58, q, 2H, *J*=7.45 Hz	31.3 (s)		29.7 (s)
7'	5.33, m,2H	125.5 (s)		31.1 (s)
8'	5.33, m,2H	125.5 (t)	2.46, m,2H	130.3 (t)
9'	2.58, q, 2H, *J*=7.45Hz	33.8 (s)	6.69, m, 1H	128.9 (t)
10/	2.77, m, 2H	31.5 (s)	6.06, dd, 1H, J_{dd}= 7.40, 16.00 Hz	130.3 (q)
11/		142.9 (q)		121.0 (t)
12/	7.25, m,1H	128.2 (t)	6.65, d, 1H, *J*= 9.15	122.2 (t)
13/		128.4 (t)	6.57, d, 1H, *J*= 9.20	147.1 (q)
14/-15/		128.2 (t)		149.1 (q)
16/		128.4 (t)	6.79, dd, 1H, J_{dd}= 8.05, 9.75 Hz	130.26 (t)
18/				
4-OH	3.93, s,1H		4.04, s	
1/-NH	7.15, brs, 1H			
OCH2O			5.88, d, 2H, *J*= 10.3	100.7 (s)

the same time, surinone D gave a varied prediction value ranging 0.5 < Pa < 0.7. Besides, PASS prediction results of the tested compounds as an antioxidant were low Pa < 0.5, as shown in Table 4.

The mechanisms of action prediction indicate the binding of biologically active ligands with biological entities at the macromolecular level. Secolignans compounds the expected mechanism as antimitotic with prediction value (Pa = 0.504 – 0.425) of the peperomins. In addition, PASS predicts some new mechanisms, including myc inhibitor (regulator gene) with (Pa = 0.557) for peperomin I, hepatoprotective (Pa = 0.570), neurotrophic factor enhancer (Pa = 0.506), and peripheral vasodilator (Pa = 0.516) activities for peperomin J. Meanwhile, dindygulerione F showed a high prediction value as Myc inhibitor (Pa = 0.593). Moreover, PASS predicts another mechanism such as transcription factor NF kappa B inhibitor with a prediction value (Pa = 0.510, 0.531 and 0.499) for surinone D and dindygulerione F, respectively, as illustrated in Table 4. The toxic/adverse effect reflect the chemical compounds' specific toxicities or adverse reactions. Sensitization was the highly predicted toxic/adverse effects for peperomin I, peperomin J and surinone D with Pa = 0.378, 0.633 and Pa = 0.634.

Meanwhile, dindygulerione F showed a lacrimal secretion stimulant with Pa = 0.506 (Table 4). As in PASS, the spectrum of biological activity provides a theoretical estimate of the general biological potential of the compound under analysis, and the probability of prediction of the compounds as chemoprotective and antineoplastic was high for compounds, which could offer hope of promising activity for those compounds. The PASS prediction results were compared to the results obtained as antiproliferative, antimicrobial and antioxidant for the compounds to validate the PASS finding.

Table 3. Minimum inhibitory concentration (µg/mL) of isolated compounds against selected microbes

	P. aeruginosa		*K. pneumoniae*		*E. coli*		*S. epidermis*		*S .aureus*		*A. niger*		*C. albican*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
	µg/mL													
Peperomia A	125	500	125	NA	NA	500	250	500	125	500	62.5	500	250	500
Peperomia I	125	500	125	500	125	500	250	500	125	500	31.25	500	125	500
Peperomia J	125	500	125	500	125	NA	NA	125	250	500	NA	NA	NA	NA
Surinone D	125	500	125	500	125	500	125	250	250	500	NA	NA	NA	NA
Dindygulerione F	125	500	125	500	125	NA	NA	NA	NA	NA	NA	NA	NA	NA
D1	25	NA	50	NA	50	NA	50	NA	100	NA	ND	ND	ND	ND
D2	12.5	NA	25	NA	25	100	25	NA	25	100	ND	ND	ND	ND
D3	25	NA	25	NA	50	100	50	NA	25	25	ND	ND	ND	ND
Ampicillin	250	500	NA	NA	125	250	125	250	125	250	NA	NA	NA	NA
Gentamicin	15.6	31.25	15.6	31.25	15.6	31.25	7.8	31.25	15.6	31.25	NA	NA	NA	NA
Ketocanazol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	250	500	62.5	125

ND; not determined, Dichloromethane fractions 1-3 (D1-D3). *Pseudomonas aeruginosa (P. aeruginosa); Klebsiella pneumoniae (K. pneumoniae); Escherichia coli (E. coli); Staphylococcus epidermis (S. epidermis); Staphylococcus aureus (S. aureus); aspergillus niger (A. niger); Candida albican (C albican).*

Table 4. Pharmacological, mechanism of action, and adverse effect prediction by PASS on selected isolated compounds

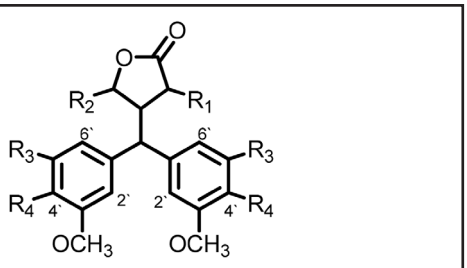
Tested compounds	Pharmacological Effects			Mechanism of action			Toxic and Adverse Effects		
		Pa	Pi		Pa	Pi		Pa	Pi
Peperomin I	Cardiovascular analeptic	0.717	0.05	Myc inhibitor	0.557	0.021	Ulceration	0.378	0.049
	Chemo protective	0.707	0.003	Antimitotic	0.442	0.012	Sensitization	0.322	0.041
	Antineoplastic	0.566	0.068	Transcription factor inhibitor	0.443	0.038	Teratogen	0.310	0.066
	Antimitotic	0.442	0.012	Vasodilator, peripheral	0.446	0.043	Embryo toxic	0.282	0.071
	Antifungal	0.459	0.031	Transcription factor NF kappa B inhibitor	0.434	0.034	Carcinogenic, group 1	0.266	0.057
	Antibacterial	0.364	0.042	Sodium/bile acid cotransporter inhibitor	0.346	0.041	Eye irritation, high	0.217	0.039
	Antioxidant	0.177	0.049	Lipoxygenase inhibitor	0.356	0.051	Cytotoxic	0.219	0.051
Peperomin J	Anti metastatic	0.591	0.004	Hepato protectant	0.570	0.021	Sensitization	0.633	0.009
	Antineoplastic	0.623	0.053	Neurotrophic factor enhancer	0.506	0.004	Carcinogenic, mouse, male	0.317	0.035
	Apoptosis agonist	0.580	0.021	Antimitotic	0.504	0.009	Carcinogenic, mouse	0.318	0.038
	Hepato protectant	0.570	0.012	Vasodilator, peripheral	0.516	0.026	Skin irritative effect	0.295	0.023
	Antimitotic	0.504	0.009	Anesthetic general	0.464	0.023	Teratogen	0.309	0.066
	Antibacterial	0.413	0.031	Vasodilator	0.464	0.0231	Cardio depressant	0.296	0.056
	Antifungal	0.383	0.044	Psychostimulant	0.407	0.032	Embryo toxic	0.302	0.067
	Antioxidant	0.200	0.038	Vasodilator, coronary	0.393	0.056	Cytotoxic	0.252	0.040
Surinone D	Vasodilator, peripheral	0.721	0.005	Vasodilator, peripheral	0.721	0.005	Sensitization	0.634	0.009
	Antineoplastic	0.730	0.027	Hepatoprotectant	0.664	0.007	Embryotoxic	0.497	0.036
	Apoptosis agonist	0.703	0.011	Vasodilator	0.658	0.008	Teratogen	0.426	0.045
	Spasmolytic	0.675	0.008	Vasodilator, coronary	0.543	0.015	Cardiodepressant	0.399	0.026
	Hepatoprotectant	0.664	0.007	Transcription factor NF kappa B inhibitor	0.510	0.024	Cytotoxic	0.380	0.025
	Antiprotozoal (Leishmania)	0.651	0.004	Immunosuppressant	0.510	0.045	Genotoxic	0.361	0.010
	Antibacterial	0.552	0.014	Antipruritic	0.474	0.032	Abortion inducer	0.337	0.008
	Antifungal	0.517	0.024	Lipoxygenase inhibitor	0.465	0.023	Carcinogeni, mouse, female	0.342	0.030
	Antioxidant	0.287	0.018	GABA aminotransferase inhibitor	0.444	0.005	Carcinogeni, mouse	0.344	0.032
Dindyguleri-one F	Antineoplastic	0.697	0.011	Myc inhibitor	0.593	0.016	Lacrimal secretion stimulant	0.506	0.015
	Apoptosis agonist	0.697	0.011	Transcription factor NF kappa B inhibitor	0.531	0.021	Cytotoxic	0.441	0.020
	Antiallergic	0.590	0.014	Transcription factor inhibitor	0.525	0.024	Teratogen	0.427	0.045
	Antifungal	0.531	0.022	Immunosuppressant	0.533	0.040	Embryotoxic	0.415	0.045
	Immunosuppressant	0.533	0.040	Angiogenesis stimulant	0.482	0.007	Abortion inducer	0.297	0.012
	Antibacterial	0.412	0.032	Vasodilator, peripheral	0.423	0.051	Carcinogeni, group 1	0.296	0.041
	Antioxidant	0.236	0.029	Interleukin agonist	20.40	0.034	Carcinogenic, mouse, female	0.293	0.039

DISCUSSION

Chemical constituents from *Peperomia blanda*

i. Secolignans compounds

Peperomin is a specific type of secondary metabolites called secolignan that are reported in *Peperomia* species. They have been previously isolated from *P. blanda* (Felippe *et al*., 2008, 2012), *P. dindygulensis* (Wu *et al*., 2005; Li *et al*., 2007; Wang *et al*., 2014), *P. glabella var. nervulosa* (Soares *et al*., 2006), *P. heyneana* (Zhang *et al*., 2007), *P. japonica*, *P. pellucida* (Xu *et al*., 2006), and *P. sui*. (Cheng *et al*., 2003; Cheng & Chen, 2008) In this study, three peperomins were isolated from D2-fraction namely peperomin A (Al-Madhagi *et al*., 2018), peperomin I and peperomin J (Figure 2).



Peperomin A (19) $R_1 = CH_3$	R_2 = H	R_3, R_4 = OCH2O
Peperomin I (20) $R_1 = CH_2CH_3$	R_2 = H	$R_3 = R_4 = OCH_3$
Peperomin J (21) R_1 = H	$R_2 = CH_2COCH_2CH_2CH_3$	R_3, $R_4 = OCH_2O$

Figure 2. Chemical structure of Secolignans.

ii. Peperomin I

Peperomin I was isolated from DCM extract as a yellow oil. Peperomin I is a new secolignan and is considered as peperomin C derivative, with following characteristics; molecular ion peak of $[M+H]^+$ 460.5131 corresponding to $C_{25}H_{30}O_8$. The fragment at m/z 347 for bis (3,4,5-tri methoxyphenyl) methane and the IR absorption was observed at 1667 (C=C stretching), 1462, 1220 (Ar-CO bending) 1190 (C-O bending) cm^{-1}. The ^{1}HNMR spectrum displays presences of thirty protons that gave resonances of four meta-coupled aromatic hydrogens at δH 6.47 ppm (d, H-2',2'', *J* = 2.30 Hz), 6.47 ppm (d, H-6',6'', *J* = 2.30 Hz), six methoxy groups at δH 3.84 ppm (s, H-3',3''), δH 3.87 ppm (s, H-4',4'') and δH 3.79 ppm (H-5',5'') attached to C-3'/C-3'', C-4'/C-4'' and C-5'/C-5'', respectively. Besides, a triplet at δH 0.86 ppm (t, H-7, *J* = 6.90 Hz) was assigned to the methyl group, multiplets at δH 3.87 ppm (H-4a) and 4.30 ppm (H-4b) due to the methylene group of the butyrolactone moiety, and resonances observed at δH 2.33 ppm (m, H-2), 2.91 ppm (m, H-3), and δH 3.50 ppm (d, H-5, *J* = 11.45 Hz) are due to three methine groups. The ^{13}C-NMR data confirmed the presence of the butyrolactone system; it shows the presence of carbonyl signal at δC 174.7 ppm, signals at δC 47.4, 40.3 and 70.4 ppm relate to C-2, C-3 and C-4 and the ring, and the signal at δC 29.7 and 15.6 ppm, corresponds to ethyl group C-6 and C-7, respectively which is bonded to C-2, as shown in Table 1. Also, the signal appeared at δC 56.7 ppm is assigned to a C-5 (methine) where the three rings of the structure are bonded. The six methoxyl groups attached to the aromatic rings display signals at δC 56.3, 60.9 and 61.0 ppm. A δ-butyrolactone ring was also confirmed by the HMBC between H-2, H-4, and H-6 and the lactone carbonyl carbon δC 179.9 ppm (C-1) and the union of the three rings in the C-5 was confirmed by the presence of correlation of hydrogen at δH 3.50 ppm (H-5) with the carbons at δC 40.3 ppm (C3) and δC 70.4 ppm (C-4), δC 104.6 ppm (C-6', C-6") and δC 104.8 ppm (C-2' / 2") of aromatic rings. DEPT assigned the presence of CH_3 at δC 15 ppm and CH_2 at δ 29 ppm. Moreover, signals absorbed at δC 70.0 ppm (CH) confirm the attachment of carbon to oxygen and relate to C-4 and C-6. Moreover, analysis of the proton COSY spectrum shows the existence of connectivity of H-2, H-6, H-3, and H-4 and the HMBC then confirmed the correlation of H-5 with C-2, C-6, C-4, C2'-2'' to prove the presence of δ-butyrolactone ring and the bis (3,4,5-trimethoxyphenyl) methane. The spatial interactions detected in the experiments of NOESY 1D between the hydrogens at δH 2.3 ppm (H-2) and 3.3 ppm (H-3), indicate the cis configuration between them, which is further confirmed by the observed interactions between hydrogen in δH 3.6 (H-5) and the methyl with hydrogen (H-6), thus determining its relative configuration and the two possible configurations (2S,3R) or (2R,3S). In addition, the comparison of these data to those available in the literature (Felippe *et al*., 2011), made it possible to identify substance as (2R, 3S)-2-ethyl-3- [bis (3', 4', 5'-trimethoxyphenyl) methyl] butyrolactone.

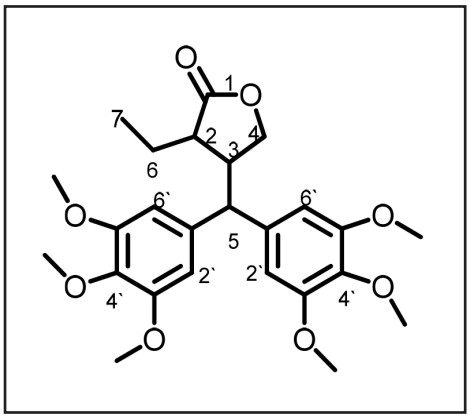


Figure 3. Chemical structure of peperomin I.

iii. Peperomin J

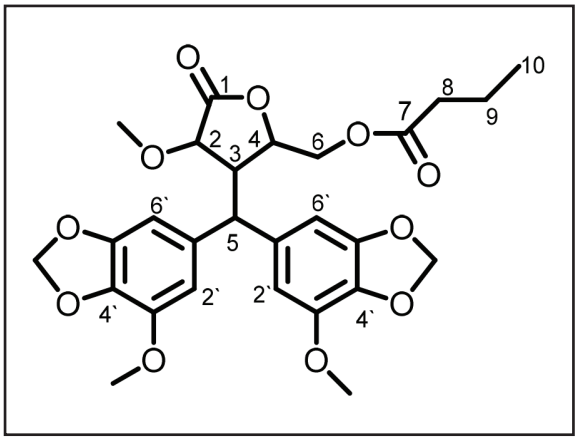


Figure 4. Chemical structure of peperomin J (21).

Peperomin J is the third secolignan isolated from *P. blanda* and it is a new compound with molecular ion peak $[M+H]^+$ = 499.0913 that was corresponding to $C_{27}H_{30}O_{11}$ with a calculated mass equal to 530.1982. Mass fragmentation at m/z 315 and m/z 263 which represents the fragments [bis (3,4-methylenedioxy-5-methoxyphenyl) methane] and [(3,4-methylenedioxy-5-methoxyphenyl), δ-butyrolactone) methyl group, respectively. The IR spectrum recorded absorption peaks at 1260-1215 (C-O-C st-as), 1018, 1013 (C-O-C st- sy) cm^{-1}. Meanwhile, the ^{1}HNMR relieved the presence of twenty-eight protons that displayed as four meta-coupled aromatic hydrogens at δ_H 5.93 ppm (H-6'', m), 6.59 ppm (d, H-2', *J* = 1.15 Hz), 6.61 ppm (d, H-2'', *J* = 1.70 Hz) and 6.66 ppm (d, H-6', *J* = 1.75 Hz). A singlet at δH 5.90 ppm was

assigned to the two methylenedioxy groups attached to C-4'/C-5' and C-4''/C-5'', respectively. In addition, two methoxy singlets at δH 3.84 ppm (3H) attached to the C-3' and C-3'' carbons. This set of signals characterized two 3,4-methylenedioxy-5-methoxyphenyl rings with magnetically non-equivalent methylenedioxy protons as a result of the aromatic ring. A chain of a triplet at δH 0.85 ppm (t, H-10, *J* = 7.45), multiplets at δ_H 1.62 ppm (m H-9), and δH 2.32 ppm (H-8, m), were then confirmed by HMBC, assigning the correlation of H-8 to C-9, C-10, and carbonyl ester C-7. Besides, multiplets at δ_H 4.02 ppm and 4.2 ppm were assigned for hydrogen attached to neighboring ester group at C-4 and C-7, respectively. DEPT spectrum confirms the presence of C-6 (CH) and C-7 (CH2) at δc 70.0 and 70.4, respectively. Moreover, ^{1}H NMR spectrum displays resonances at δ_H 2.84 (m, H-2), 2.76 (m, H-3), and 3.56 (d, H-5, *J*=11.45 Hz) are due to the three methine groups of δ-butyrolactone ring that has been corroborated by ^{13}C-NMR data that shows the presence of carbonyl signal at δ_C 179.6, signals at δ_C 47.1, 40.2 and 70.0 ppm correspond to carbons C-2, C-3 and C-4 of the ring, and signal at δ_C 4.2 is related to the group methyl C-6 bonded to the C-4, as shown in Table 1. Also, the signal appeared at δC 56.2 assigned to a C-5 (methine) where the three rings are bonded, the methoxyl groups attached at the 3' and 3'' positions of the aromatic rings display signals at δC 57.0 ppm and the two methylenedioxyl groups showed signals in δC 100.7 and 101.6 ppm. The HMBC spectrum confirmed the correlation between H-2, H-4, and H-6 and the lactone carbonyl carbon 179.9 (C-1) of the δ-butyrolactone ring. In addition, it confirms the union of the three rings at C-5 by the presence of correlation of hydrogen at δH 3.56 ppm (H-5) with the carbons at δC 40.0 ppm (C-3) and δC 70.0 ppm (C-4) of the ring, δC 101.1 ppm (C-2', C-2'') and δC 108.0 ppm (C-6',6") of the aromatic rings. The spatial interactions identified in the experiments of NOESY 1D between the hydrogens at δH 2.48 ppm (H-2) and 2.76 ppm (H-3) indicate the cis configuration between them, confirmed by the observed interactions between hydrogen in 3.56 ppm (H-5) and the methyl with hydrogen in 4.02 ppm, thus determining its configuration relative of (2R,3S). Therefore, it suggests that substance 21 as (2R,3S)- 4- methyl butanoate -3-[bis (4',5'-methylenedioxy-3'-methoxyphenyl) methyl] butyrolactone.

iv. Surinone D

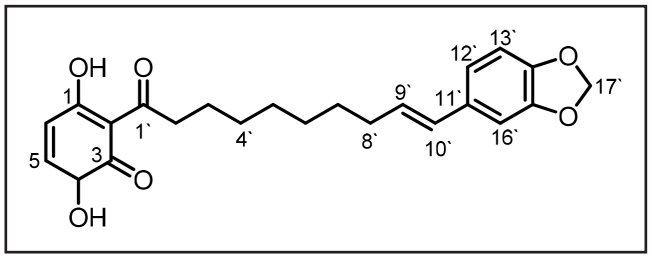


Figure 5. Chemical structure of surinone D.

Surinone D is a new 2-acyclocyclohexane-1,3-diones obtained from *P. blanda* as a yellow oil which was isolated from DCM extract with a molecular ion mass m/z of 398.1763 and molecular formula $C_{23}H_{28}O_6$. The UV absorption band was recorded at 288 nm indicating the presence of the cyclic polyketone group (triketone). The IR absorption recorded peaks at 3411, 1214, and 1046 cm-1, which is an indication of the presence of different groups named hydroxyl, conjugated carbonyl, benzyl ring and methylenedioxy (Cheng *et al*., 2003). ^{1}H NMR spectrum displays resonance of three aromatic hydrogens appear in δH 6.65 ppm (d, H-12', *J* = 9.15 Hz), 6.57 ppm (d, H-13', *J* = 9.20 Hz), and 6.79 ppm (dd, H-16', *J* = 8.05 and 9.75 Hz). The signal observed at δH 5.88 ppm (s, 2H), confirms the methylenedioxy group as substituent of the aromatic ring at the 18'/19' positions. Analysis of the ^{13}C spectrum confirmed the presence of the aromatic ring with signals at δC 130.3, 121.0, 122.2, 147.1, 149.1 and 130.2 ppm that are assigned to C-11' till C-16', respectively. In addition, olefinic hydrogens gave resonance at δH 6.69 ppm (H-9', m) and 6.06 ppm (dd, H-10', *J*= 7.40, 16.00 Hz) that was compared to corresponding peaks reported for 2-acyclocyclohexane-1,3diones from P. alata (Ferreira *et al*., 2014). In addition, cyclohexane-1,3-dione ring was validated by the presence of oxymethine at δH 4.01 (m, H-4), two DE shielded methylene at δH 1.79, 2.38 ppm (H-5a, m) and (H-5b, m) and at δH 2.76 (m, H-6) and ^{13}C NMR spectrum showed the presence of enol and two keto carbonyls at δC 197.9, 205.9 and 214.4 ppm. The ^{1}H-^{1}H COSY spectrum were assigned their respective correlations. Moreover, the methylenedioxyl substituent group gives resonance at δC 100.7 ppm. On that note, it is important to mention that these results were validated in the findings based on a literature review for the cyclohexane-1,3-dione derivative (Mudd, 1981; Ferreira *et al*., 2014).

The correlations observed in the HMBC indicated the correlation between the carbon in 35.6 (C-15') and the aromatic hydrogens in 6.65 (C-12') and 6.79 (C-16'), which allows to conclude the allylic chain binding to C-16 'of the aromatic ring, and also the correlation of the hydrogens of the methylenedioxyl group in 5,88 with the carbons at 147.1 (C-14') and 149.1 (C-15') of the same rings. The stereochemistry at position 4 could be determined after measuring the optical activity of the substance having a value of [α]D= +97.5° with a concentration of 2.05 mg / mL, in $CHCl_3$ and also by the high coupling constant (*J* = 13.0Hz) between H-5 (-axial) and H-4, suggesting that the hydroxyl OH-4 is in the -equatorial position. These data were compared to data available in the literature (Seeram *et al*., 2000; Cheng *et al*., 2003; Ferreira *et al*., 2014) led to the identification of substance as 2-(10-(benzo[d][1,3]dioxol-5-yl) decanoyl)-3,6-dihydroxycyclo hexan-1-ne.

v. Dindygulerione F

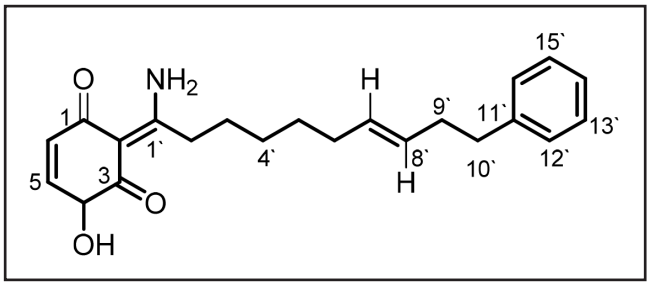


Figure 6. Chemical structure of dindygulerione F.

A new N-containing polyketides isolated from *P. blanda* in the form of brown oil with a molecular ion mass of $[M+H]^+$ m/z 353.2001 and corresponding to the molecular formula of $C_{22}H_{27}NO_3$. The UV absorption band was recorded at 291 nm could be assigned to a cyclic polyketone group. The spectrum of IR was recorded for absorption at 3411, 2928, 1659, 1261, 1450 and 1027 cm-1 indicating the presence of hydroxyl, amino, conjugated carbonyl, conjugated chelated carbonyl and vinyl groups. The ^{1}H NMR spectrum displayed presence of twenty-eight protons that recorded peaks at the lowest field and was of well-defined doublet at δH 4.01 ppm (dd, H-4, *J*=5.75, 10.85 Hz) due to an oxymethine proton and peak for unchelated hydroxyl group (br s, 1H) and two deshielded methylene pairs at δH 1.82 ppm (m, H-5a), 2.36 ppm (m, H-5b), and 2.33 ppm (m, H-6). The oxymethine and two of the deshielded methylene groups formed one sequence, H-4, H-5 and H-6 that has been detected by ^{1}H–^{1}H COSY spectrum. The J values in the multiplets for this fragment signified that this sequence was part of a six-member ring with the oxymethine H-4 (*J* = 10.85) being the φ-axial and the hydroxyl group as the φ-equatorial, this ring was similar to proctoricone B (Seeram *et al*., 2000) and surinone C (Cheng *et al*., 2003) that had been previously isolated from *P. suri* and *P. proctorii*.

The carbonyl which appeared at δc 206.2 ppm (C-1) was linked to the methylene at C-6 and the olefinic quaternary carbon at δc 110.3 (C-2). Similarly, the carbonyl carbon at δc 197.9 ppm (C-3) could also be connected to C-4. The connection of O=C1-C2=1' C-NH_2 was detected. The HMBC cross-peaks between the C-1' at δc 178.3 ppm and the protons at δH 2.90 ppm (m, H-2a) and δH 3.08 ppm (m, H-2b) showed their linkage. The downfield chemical shift of the remaining olefinic quaternary carbon δC 178.3 (C-1') suggested that it was substituted at the β-position of the α-substitution at β-unsaturated ketone while in connection to a hetero-atom. Since there was only one hetero-atom (N) left and there were still two active H atoms in the ^{1}HNMR spectrum δH 7.12 ppm (1H, br.s, 1-NH)], as compared to N-containing polyketide derivatives isolated from *P. dindygulensis* (Wang *et al*., 2013). The olefinic signals at δC 125.5 ppm in ^{13}C NMR and δ 5.33 (2H, m) in the ^{1}H NMR spectrum denoted the presence of a double bond and were comparable with the ^{13}C NMR shifts of the vinyl carbons reported in the literature review (Mudd, 1981). The ^{1}H-^{1}H COSY spectrum displayed the attachment of 5', 8' to olefinic C6'-7' with mono substituted aromatic ring at δH 7.21-7.3 ppm (Table 2). The trans-geometry of the double bond at C-7' could be elucidated from the carbon shift of the allylic to the double bond DCM extracts of *P. blanda.*

(C-6', 9'), which both appeared at δC 31.3 and 33.8 ppm which differ significantly from the carbon shifts of the positions allylic to cis double bond at δC 25.7 ppm (Wang *et al*., 2012). The stereochemistry at position 4 could be confirmed after determining the optical activity of substance having a value of [α]CH_3Cl = +44.4° with a concentration of 2.5 mg / mL, in $CHCl_3$ and also by the high coupling constant (*J* = 13.0Hz) between H-5 (-axial) and H-4, suggesting that the hydroxyl OH-4 is in the -equatorial position. These data were compared to data available in the literature (Seeram *et al*., 2000; Cheng *et al*., 2003; Ferreira *et al*., 2014) led to the identification of substance 26 as (E)-2-(1-amino-10-phenyldec-7-en-1-ylidene)-4-hydroxycyclohexane-1,3-dione.

MIC determination for fractions

The antibacterial activity of the DCM crude extracts was weakened may be due to the combined effect of the compounds and fractionation of the extracts could lead to separate some of the inactive constituents or the active compounds infractions were more concentrated. This antimicrobial activity further supports the traditional uses of *P. blanda* for wounds and infection treatment. Moreover, the antimicrobial activity of *P. blanda* crude extracts was stronger than the antibacterial activity of *P. vulcanica, P. fernandopoioana* recorded against *S. aureus* (ATCC 33862) (Mbah *et al*., 2012) and are consistent with data obtained from *P. pellucida* against *P. aeruginosa* (Khan & Omoloso, 2002). The results of the isolated (peperomin A, peperomin I, peperomin J, surinone D, dindygluerine F) showed more potent activity against Gram-positive bacteria than Gram-negative bacteria with MIC ranged between 125-250 µg/mL and MBC value of 500 µg/mL. The varied activity of the compounds against tested bacteria that could be involved in wounds and infection seems to be consistent with traditional uses of *P. blanda*. Another important finding was that peperomin A and peperomin I displayed antifungal activity against *A. niger*. This activity matched those observed in earlier studies for compounds isolated from Peperomia species such as the polyketides derivatives derived from *P. trineura* and recorded a rather potent antifungal activity against *C. cladosporioide*s, and *C. sphaerospermum* (Ferreira *et al*., 2014) and *P. alata* extract gave two fungicidal polyketides against *C. cladosporioides* and *C. sphaerospermum*, respectively (Gutierrez *et al*., 2016). In addition, *P. glabella* var. nervulosa extract yielded four polyketides and four secolignans with antifungal activity.

Prediction of biological activity using PASS (Prediction of Biological Activity for Substances)

The highest prediction value (Pa > 0.7) as chemoprotective, antineoplastic or apoptosis were recorded from dindygluerione F (polyketides) and followed by peperomin J, peperomin I and *peperomin A*. Prediction probability of the compounds as antimicrobial showed the less experimental chance of activity Finding with prediction value ranged 0.5 < Pa < 0.7. Based on the antimicrobial finding, the antimicrobial activity of compounds was the different and are comparable to ampicillin but weaker than gentamicin. The antifungal activity of compounds was showed that peperomin I was the highest activity. The antimicrobial activity of compounds seems to be constituents of PASS prediction. Thus, the correct prediction of compounds would provide new insights into the other biological activities encountered in the PASS prediction to be a starting point for further study. Moreover, the results of PASS prediction support the concept that Peperomia species have many uses and have correlated their activity with the presence of polyketides and secolignans where our compounds were from these groups. the clinical significant biological of the DCM extract or the isolated compounds have proved the importance of those compounds as anti bacterial, anticancer and antioxidant. The antineoplastic or apoptosis wasrecorded from dindygluerione F (polyketides) and followed by peperomin J, peperomin I and peperomin A by the computational study and has been supported by the cytotoxic activities of the compounds. Therefore, the need for further studies on those compounds is needed to find new promising compounds.

CONCLUSIONS

The DCM extract of *peperomia blanda* has been proven its effectiveness as anticancer, antimicrobial and antioxidant activities. Those promising activities are raised from the presence of the unique and new peperomin and polyketides. The activity of the new compounds has proved strong activities and as new drugs in the future as anticancer. Moreover, the Computational study for the compounds provides important insights into the rapid screening, hit identification, and hit-to-lead development.

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