Daud MNH et al. Adv Appl Chem Biochem 2018(1): 01-08.
https://doi.org/10.33513/ACBC/1801-01

OCIMUM

Advances in Applied Chemistry and Biochemistry

Research Article

In Vitro 5-Lipoxygenase Inhibiting Activity of Selected Malaysian Plants and Isolation of Constituents

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Received Date: 10 August, 2018
Accepted Date: 19 September, 2018
Published Date: 04 October, 2018

Citation

Daud MNH, Jabit ML, Khamis S, Ali DAI, Shaari K (2018) *In Vitro* 5-Lipoxygenase Inhibiting Activity of Selected Malaysian Plants and Isolation of Constituents. Adv Appl Chem Biochem 2018(1): 01-08.

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Abstract

5-Lipoxygenase (5-LO) is the key enzyme in biosynthesis of inflammatory mediators known as leukotrienes which are responsible for asthma, allergic inflammation and innate immunity. This paper reports the evaluation of 30 species of Malaysian plant extracts potential in inhibiting 5-LO activity. Five plant extracts exhibited >80% inhibition against 5-LO activity which include *Phyllanthus watsonii, Euphorbia hirta, Anacardium occidentale, Acalypha wilkensiana* and *Piper betle*. Nine plant extracts exhibited moderate value of inhibition (79-40%), while sixteen others exhibited <40% inhibition. From the screening work via inhibition of 5-LO activity, *P. watsonii* showed the highest inhibition with value of 97.3%, followed by *E. hirta* with 90.9% inhibition. Both plant extracts were further fractioned using organic solvent which include Dichloromethane (DCM), Ethyl Acetate (EA) and Butanol (BuOH) for evaluation of their inhibiting effects on 5-LO activity. For *P. watsonii*, DCM fraction exhibited the highest value with 75% inhibition, while for *E. hirta*, EA fraction exhibited the highest value of 72.5% inhibition. Both active fractions were further subjected to isolation and purification work in order to identify the major compounds. Subsequently, four major compounds managed to be isolated and purified which further identified as quercetin-3-*O*-rhamnoside (E1), myricetin-3-*O*-rhamnoside (E2), 26-nor-D: A-friedoolean-14-en-3*β*-ol (PW1) and glochidonol (PW2) using spectroscopic technique and their data comparison with literature.

Keywords

5-Lipoxygenase; Inflammatory; Inhibition; Leukotrienes; Major Compounds; Plant Extracts

Introduction

Over 374,000 plant species of which approximately 308,312 are vascular plants, with 295,383 flowering plants exist on this planet [1]. Malaysia is one of countries in the world with a rich biodiversity and claim to possess medicinal value. More than 1000 species of medicinal plant have recorded in Peninsular of Malaysia [2], and were reported to possess some important biological activities such as antioxidants, anti-cancer, anti-inflammatory and anti-microbial [3].

Inflammation is a complex process towards the formation of diseases such as arthritis, asthma and bronchitis [4]. It involves many effector mechanisms that produce a multiplicity of vascular and cellular reactions. Inflammation is accompanied with pain, vasodilation, increased microvascular permeability,

chemotaxis, or cellular activation, mediated by the local production and release of several specific mediators such as leukotrienes. Leukotrienes are potent mediators of bronchitic asthma and allergic reactions [5].

Lipoxygenases (LO) are precursor enzymes that play an important role in Arachidonic Acid (AA) metabolism by catalyzing the oxygenation of the polyenoic fatty acids to the corresponding lipid hydroperoxides [6]. They belong to a family of soluble cytosolic enzymes found in the lung, platelets, mast cells and white blood cells. They are generally classified on the basis of their oxygenation sites on AA, i.e., 5-, 2- and 15-lipoxygenases. 5-Lipoxygenase (5-LO) is the key enzyme in leukotriene biosynthesis [7]. Thus, inhibition of the 5-LO pathway of AA metabolism may have a therapeutic potential in a variety of inflammatory diseases, in particular bronchitic asthma and allergies. Hence for the first phase of this study, this paper reports the evaluation of 30 selected Malaysian medicinal plants ability in inhibiting 5-LO activity. The selection of plant and part used were based on their traditional testimony claims as anti-inflammatory therapeutics. The major constituents from the most active plant crude extract and fraction were isolated using chromatographic methods. Finally, the structure elucidation of the isolated constituents were carried-out using spectroscopic methods.

Materials and Methods

General instrumentation

UV spectra for isolated compounds were recorded on a Varian UV-Vis CARY 100. UV Spectra Max Plus 38 was used for reading absorbance values in the 5-LO assays. IR spectra (in KBr disc) for isolated compounds were recorded on a Perkin Elmer 1650 FT-IR spectrometer. All ^{1}H and ^{13}C NMR were recorded on a Varian Unity Inova (500 MHz ^{1}H and 125 MHz ^{13}C) equipped with Pulsed Field Gradients (PFG), using and Indirect Detection Probe. Deuterated solvents were used and chemical shifts ($\delta_H$and δ_C) were given in ppm.

Plant material

Plants for preliminary biological screening were collected throughout of Peninsular Malaysia. The plants were air dried for 3 days in order to reduce moisture.

Preparation of crude extract for 5-LO screening

Sample (250g) was soaked with 100% Methanol (MeOH) for 4 days at room temperature after which the extract was decanted. The plant material was replenished with fresh MeOH and the same extraction procedure repeated two more times. The extracts collected from each soaking were pooled and rotary evaporated to dryness using Buchi Rotavapor R210 at 45°C.

5-Lipoxygenase (5-LO) inhibition assay

Plant extracts were assayed preliminarily by the 5-LO assay according to method developed by [8]. The 5-LO enzyme from soybean and linoleic acid (substrate) were obtained from Sigma (5-LO - CAS Number: 9029-60-1; linoleic acid - CAS Number: 60-33-3). The substrate solution (2.9ml) was transferred into a quartz cuvette. Then, 50µl of the sample was added. For the blank run and positive control, this was replaced by 50µl borate buffer (pH 9.0) and 50µl of phenidone (Sigma CAS Number: 92-43-3) solution, respectively. The enzyme reaction was initiated by the addition of 50µl of the enzyme solution. The absorbance at 234nm of the reaction mixture was followed on a UV spectrophotometer over a six-minute interval, readings taken every 30 seconds. The absorbance values were plotted against time and the slope of the linear segment of the reaction curve was calculated. The % inhibition of 5-lipoxygenase activity was then calculated using the following equation:

$$\% \text{ Inhibition} = (1 - A_t / A_c) \times 100\%$$

where A_t: slope of test sample
A_c: slope of blank/ standard

Isolation of major compounds from active crude extracts

From the screening work, *E. hirta* and *P. watsonii* extracts exhibited the highest inhibition against 5-LO. Hence, both extracts were subjected to isolation of major compounds.

E. hirta (1.2kg) was soaked with 100% Methanol (MeOH) for 4 days at room temperature after which the extract was decanted. The plant material was replenished with fresh MeOH and the same extraction procedure repeated two more times. The extracts collected from each soaking were pooled and rotary evaporated to dryness, yielding 57.60g crude extract. The crude methanolic extract of *E. hirta* was then resuspended in distilled water and solvent partitioned into Dichloromethane (CH_2Cl_2), Ethyl Acetate (EA) and Butanol (BuOH) to give 2.8g, 12.0g and 22.6g of the respective solvent fractions. All the fractions were subjected to a second phase of biological screening using the same 5-LO assay used in the preliminary screening. From the results of the assay, further work was carried out on the ethyl acetate fraction, which was found to be the most active fraction. The bioactive ethyl acetate fraction (11.0g) was first subjected to Vacuum Liquid Chromatography (VLC). Vacuum

elution started with 100% Chloroform ($CHCl_3$) followed by mixtures of $CHCl_3$: MeOH of increasing polarity, yielding a total of 8 fractions. Based on similar TLC patterns, fractions 2 to 3 (1.73g) and 4 to 8 (6.40g) were recombined and the two set of combined fractions separately subjected to open column reverse phase chromatography using MeOH: H_2O as mobile phase, starting from a more polar to a less polar gradient. The combined fractions, respectively afforded 200 mg of E1 and 30 mg of E2 both in form of yellow amorphous.

The extraction of *P. watsonii* (1.0kg) followed the same procedure described previously for *E. hirta*. The crude methanolic extract of *P. watsonii* was obtained in a yield of 42.7g. Similarly, the crude extract was resuspended in distilled water and solvent partitioned into Dichloromethane (CH_2Cl_2), Ethyl Acetate (EA) and Butanol (BuOH) yielding 10.0g, 21.6g and 9.8 g of the respective fractions.

The dichloromethane fraction contained substantial amounts of green pigments and thus was subjected to gel column chromatography. The stationary phase used was Sephadex LH-20, was purposely to remove these chlorophyllic constituents. Fractions devoid of the green pigments were recombined and subjected to open column chromatography over silica gel, eluted with varying gradients of hexane: $CHCl_3$ solvent system, starting from a less polar to a more polar gradient. Twenty fractions were collected and from this yielded 50 mg of PW1 in the form of white crystalline powder. Based on similar TLC profile, fractions 14 to 17 were recombined and rechromatographed over silica gel, eluted with varying gradients of $CHCl_3$: EA solvent system, starting from a less polar to a more polar gradient. From this yielded 6.0 mg of PW2 in the form of white crystalline powder.

Statistical analysis

All experiments were run in triplicates. Statistical analyses were conducted with the Statistical Analysis System (SAS) 9.1.3 software package. Analyses of variance were performed by ANOVA procedures. Significant differences ($P < 0.05$) were determined by least square means comparison.

Results and Discussion

5-Lipoxygenase (5-LO) inhibition assay

A total of 30 plant extracts have been preliminary screened for anti-inflammatory activity using the 5-LO inhibition assay. The list of species assayed and their results are shown in table 1.

Species	Local name	Part	Inhibition
Acalypha wilkensiana	Ekor kucing	Stem	88.0 ± 0.2
Aleurites moluccana	Buah keras	Stem	25.0 ± 0.1
Anacardium occidental	Gajus	Stem	90.3 ± 0.1
Anisophyllea corneri	Buah bongkok	Whole	35.0 ± 0.1
Artabotrys sp.	Akar belah	Whole	22.0 ± 0.1
Calatropis giganteae	Lembiaga	Whole	13.5 ± 0.1
Carica papaya	Betik	Leaves	57.5 ± 0.1
Coscinium fenestratum	Sekunyit	Whole	13.2 ± 0.1
Cratoxylum maingayi	Geronggang	Whole	38.5 ± 0.2
Didisandra sp.	Meroyan kobus	Whole	16.8 ± 0.1
Euphorbia hirta	Aratanah	Whole	90.9 ± 0.1
Gonocaryum gracile	Pokok cabang tiga	Whole	16.0 ± 0.2
Leea indica	Memali	Whole	18.7 ± 0.1
Lophatherum gracile	Rumput banyak anak	Whole	58.5 ± 0.1
Michelia champaca	Cempaka	Bark	54.9 ± 0.1
Ocimum basilicum	Selasih	Whole	61.9 ± 0.2
Peperomia pellucida	Sirih tanah	Whole	63.4 ± 0.1
Pereskia citrifolia	Lidah jin	Whole	44.0 ± 0.2
Phyllanthus watsonii	Dukung	Leaves	97.3 ± 0.1
Piper betle	Sirih	Leaves	85.5 ± 0.1
Piper porphyllum	Sirih sarang punai	Whole	12.9 ± 0.2
Piper sp.	Kaduk hutan	Whole	18.8 ± 0.1
Piper sp.	Sireh gajah	Leaves	5.0 ± 0.2
Pothos peninsularis	Akar seruntum	Whole	24.3 ± 0.2
Rhapidophora karthalsii	Tapak buaya	Whole	51.0 ± 0.1
Smilax sp.	Akar janggut baung	Whole	14.0 ± 0.2
Sonneratia caseolaris	Berembang	Whole	35.6 ± 0.1
Thottea grandiflora	Hempedu beruang	Whole	41.6 ± 0.1
Tristaniopsis whiteana	Palawan	Whole	62.0 ± 0.1
Vitex negundo	Lemuni hitam	Stem	29.5 ± 0.2

Table 1: Inhibition of selected crude extracts against 5-LO activity.

From the primary screening, five plant crude extracts demonstrated more than 80% inhibition against 5-LO activity, and these are *Phyllanthus watsonii, Euphorbia hirta, Anacardium occidentale, Acalypha wilkensiana* and *Piper betle. Phyllanthus watsonii* showed the highest inhibition against 5-LO activity with value of 97.3%, followed by *Euphorbia hirta* and *Anacardium occidental* with value of 90.9% and 90.3 % inhibition, respectively. From these, as reported in many studies [9-11], only *E. hirta* and *A. occidental* have been known to possess an anti-inflammatory potential. Based on previous report, anti-inflammatory activities are due to certain

phytochemicals such as flavonoids [12], phenolic acids [13] and terpenes [14]. The presence of functional group such as hydroxyl (OH) or carboxylic in their structure are believed to contribute to the anti-inflammatory ability [13].

Nine species of crude extracts shows moderate inhibition (40-79%) against 5-LO activity which include *Peperomia pellucida* (63.4%), *Tristaniopsis whiteana* (62.0%), *Ocimum basilicum* (61.9%), *Lophatherum gracile* (58.5%), *Carica papaya* (57.5%), *Michelia champaca* (54.9%), *Rhapidophora karthalsii* (51.0%), *Pereskia citrifolia* (44.0%) and *Thottea grandiflora* (41.6%). Previously, some of the species such as *P. pellucida* [15], *O. basilicum* [16], *L. gracile* [17], *C. papaya* [18] and *M. champaca* [19] have been known to possess anti-inflammatory effects.

Sixteen species crude extracts exhibited inhibition less than 40% inhibition against 5-LO activity. Although some of the species including *S. caseolaris* [20], *V. negundo* [21], *A. moluccana* [22] and *L. indica* [23] have been reported to possess an anti-inflammatory effect, the extracts was evaluated via different assays such as Prostaglandin (PG) synthesis inhibition.

Based on their strong activity against 5-LO and the more substantial quantity of crude extract available for study as compared to the other active extracts, *P. watsonii* and *E. hirta* was selected for further phytochemical analysis.

The crude extract of *E. hirta* and *P. watsonii* were further solvent fractionated using Dichloromethane (DCM), Ethyl Acetate (EA) and Butanol (BuOH). The fractions were further evaluated for 5-LO inhibition and the results are shown in table 2. It could be observed that the fractions exhibited much lower activity from the crude extract, indicating a loss of some activity as a result of the fractionation. The fractionation could have separated synergistically acting compound which could have contributed to the total activity observed in the crude extract.

Fraction	% Inhibition	
	E. hirta	*P. watsonii*
Dichloromethane (DCM)	61.8 ± 0.1^{c}	75.0 ± 0.1^{a}
Ethyl Acetate (EA)	72.5 ± 0.2^{a}	60.5 ± 0.1^{b}
Butanol (BuOH)	64.0 ± 0.1^{b}	58.3 ± 0.1^{c}

Table 2: Percentage of 5-LO inhibition by solvent fractions of *E. hirta* and *P. watsonii.*

For *E. hirta*, ethyl acetate fraction exhibited the strongest inhibition against 5-LO activity with percent inhibition value of 72.5%, followed by butanol (64%) and dichloromethane (61.8%). This indicate the polar fractions (EA and BuOH) possess higher ability in inhibiting 5-LO activity compared to non-polar (DCM fraction). Usually in polar fraction, chemical constituents such as flavonoids either individual aglycone or with glycosides could be found [24]. As for *P. watsonii*, dichloromethane fraction showed the strongest inhibition against 5-LO activity with percent inhibition value of 75%, followed by ethyl acetate (60.5%) and butanol (58.3%). The results indicate that the moderate polar fraction possess higher potential in inhibiting 5-LO activity compared to polar fraction. In moderate polar fraction, usually chemical constituents such as terpenes, aglycone flavonoids and phenolic acids could be found [25].

Structure elucidation of isolated compounds

Compound E1 and E2

Phytochemical work-up of the bioactive EA fraction yielded 200mg of E1and 30mg of E2 as yellow amorphous solids.

The ^{1}H NMR spectrum of E1 was typical of a flavonoid glycoside, with resonances for aromatic protons of ring A and B of the flavonoid unit (δ_H 6.2 to 7.4) and resonances for a sugar unit (δ_H 3.7to 5.3). A methyl doublet at δ 0.96indicated that the sugar unit is rhamnose. The anomeric proton of the sugar could be observed at δ 5.37as doublet (J = 1.5Hz). Based on the small coupling constant (J < 4Hz) the anomeric proton was deduced to be in the α orientation. The ^{1}H NMR spectra of E1 also showed the presence of a pair of meta-coupled protons at δ 6.23 (1H, d, J = 2 Hz) and δ_H 6.39 (1H, d, J = 2 Hz), and an ABX spin system at δ_H 6.93 (1H, d, J = 8.5 Hz) δ 7.34 and (2H, m) for the aromatic resonances. The ^{13}C NMR for E1 exhibited resonances for 21 carbons comprising of a C=O (δ_c 178.5). Based on ^{1}H NMR and ^{13}C NMR data and comparison with available literature [26] as shown in table 3, E1 is assigned as quercetin-3-*O*-rhamnoside.

The ^{1}H NMR spectrum of E2 was very similar to that of E1, typical of another flavanone rhamnoside. The sugar protons were visible at δ 0.96 for, the methyl group at δ_H 5.33 for the anomeric proton and at δ_H 3.53, 3.81 and 4.24 for the rest of the sugar proton. The two aromatic protons on ring A of flavanone aglycone was observed to be similar as E1, appearing as a meta coupled doublet at around the same chemical shift region at δ 6.22 (d, J = 2 Hz) and δ 6.38 (d, J = 2 Hz). However, the ABX spin system for ring B was not present. Instead the spectrum of E2 exhibited 2H as a singlet at δ_H 6.97 for two equivalent aromatic protons indicating that ring B is substituted at C-3', 4' and 5'. This gave the possible assignment of E2 as myricetin 3-*O*-rhamnoside. The ^{13}C NMR exhibited 21 carbons comprising of a carbonyl at δ_c 178.5, a CH_3 (for rhamnosyl moiety) at δ_c 16.5, an anomeric carbon at δ_c 108.4, four sugar

Position	Compound E1		Carvalho et al.,		Compound E2		Carvalho et al.,	
	δC	δH	δC	δH	δC	δH	δC	δH
1	-	-		-	-	-	-	-
2	158.2		158.5	-	158.3	-	158.4	-
3	135.1		136.2	-	135.1		136.2	-
4	178.5		179.6	-	178.5	-	179.6	-
5	162.1		163.2	-	162	-	163.1	-
6	98.6	6.23	99.8	6.2	98.7	6.22	99.8	6.19
7	164.8		165.8	-	164.9	-	165.7	-
8	93.5	6.39	94.7	6.36	93.6	6.38	94.7	6.35
9	157.4		159.3	-	157.4	-	159.4	-
10	104.7		105.9	-	105	-	105.8	-
1’	121.8		122.9	-	121	-	121.9	-
2’	115.8	7.34	116.4	7.34	108.4	6.97	109.6	6.95
3’	145.3		146.4	-	145.7	-	146.7	-
4’	148.7		149.8	-	137	-	137.8	-
5’	115.2	6.93	116.9	6.92	146	-	146.7	-
6’	121.7	7.34	123	7.31	108.4	6.97	109.6	6.95
1”	102.4	5.37	103.5	5.36	102.5	5.33	103.5	5.31
2”	72.1	3.37	72	3.37	72.2	3.38	72	3.79
3”	70.9	3.77	72.1	3.77	70.9	3.81	72.1	3.59
4”	70.9	3.44	73.2	3.44	70.8	3.53	73.3	3.34
5”	70.7	4.24	71.9	4.24	70.7	4.24	71.8	4.23
6”	16.5	0.96	17.7	0.96	16.5	0.96	17.6	0.96

Table 3: Comparison of ^{1}H and ^{13}C chemical shifts data for E1 and E2 with Carvalho [26].

carbons at δ_c 70.7, 70.9(2), 72.2, aromatic CHs at δ_c 102.5, 5 oxygenated Aromatic Quaternary Carbons (ArCO) at δ_c 145.7, 157.4, 158.3, 162.0, 164.9, three aromatic carbons at δ_c 108.4, 93.6, 98.7 and the two quaternary Carbons (=CO) for ring C at δ_c 178.5 and 135.1. Through comparison of compound E2 data (^{1}H NMR and ^{13}C NMR) with available literature [26] in table 3, E2 is assigned as myricetin 3-*O*-rhamnoside.

Quercetin-3-*O*-rhamnoside (E1): UV (MeOH) λ_{max} 257 and 355 nm, IR: 3402 cm^{-1} (Hydroxyl, OH), 2374 (C-H stretching), 1607 (carbonyl, C=O), ^{1}H NMR (500 MHz, CD_3OD): δ_H 6.23 (1H, d, J = 2 Hz, H-6), 6.39 (1H, d, J = 2 Hz, H-8), 6.93 (1H, d, J = 8.5 Hz, H-5’), 7.34 (2H, m), 5.37 (H-1’’), 3.37 (H-2’’), 3.77 (H-3’’), 3.44 (H-4’’), 4.24 (H-5’’), 0.96 (H-6’’), ^{13}C NMR (125 MHz, CD_3OD): δ_C 158.2 (C-2), 135.1 (C-3), 178.5 (C-4), 162.1 (C-5), 98.6 (C-6), 164.8 (C-7), 93.5 (C-8), 157.4 (C-9), 104.7 (C-10), 121.8 (C-1’), 115.8 (C-2’), 145.3 (C-3’), 148.7 (C-4’), 115.2 (C-5’), 121.7 (C-6’), 102.4 (C-1’’), 72.1 (C-2’’), 70.9 (C-3’’), 70.9 (C-4’’), 70.7 (C-5’’), 16.5 (C-6’’).

Myricetin 3-*O*-rhamnoside (E2): UV (MeOH) λ_{max} 263 and 357 nm, IR : 3393 cm^{-1} (hydroxyl, OH), 2367 (C-H stretching) and 1607 (carbonyl, C=O), ^{1}H NMR (500 MHz, CD_3OD): δ_H 6.22 (d, J=2 Hz, H-6), 6.38 (d, J=2 Hz, H-8), 6.97 (2H, H-2’/6’), 5.33 (H-1”), 3.38 (H-2”), 3.81 (H-3”), 3.53 (H-4”), 4.24 (H-5”), 0.96 (H-6”). ^{13}C NMR (125 MHz, CD_3OD) δ_C 158.3 (C-2), 135.1 (C-3), 178.5 (C-4), 162.0 (C-5), 98.7 (C-6), 164.9 (C-7), 93.6 (C-8), 157.4 (C-9), 105.0 (C-10), 121.0 (C-1’), 108.4 (C-2’), 145.7 (C-3’), 137.0 (C-4’), 146.0 (C-5’), 108.4 (C-6’), 102.5 (C-1”), 72.2 (C-2”), 70.9 (C-3”), 70.8 (C-4”), 70.7 (C-5”), 16.5 (C-6”).

Compound PW1 and PW2

The extract of *P. watsonii* yielded 50.0 mg of PW1 6.0 mg of PW2. Both compounds were obtained as white crystalline needles.

The ^{1}H NMR data of PW1 exhibited six methyl peaks, one of which appearing as a doublet, in the region δ 0.88 to 1.04, was typical of a pentacyclic triterpene. This was supported by the ^{13}C NMR data in table 4, which exhibited a total of 29 carbon

https://doi.org/10.33513/ACBC/1801-01

resonances. The ^{13}C NMR spectrum also showed the presence of a carbinol carbon (C-OH) at δ_c 72.9 and two carbons at δ_c 115.7, which usually represent sp^2 methine carbon, 143.3, for a quaternary sp^2 carbon. The values δ 3.78 (^1H, broad) and 5.17 (^1H, doublet J = 6 Hz) could represent the protonated carbons. Based on ^{13}C NMR data comparison with available literature [27] as shown in table 4, PW1 was determined as 26-nor-D: A-friedoolean-14-en-3*β*-ol.

Position	Compound PW1	Matsunaga et al.,	Compound PW2	Puapairoj et al.,
	δC	δC	δC	δC
1	16.7	16.5	79.9	79.6
2	35.3	35.3	45.4	45.1
3	72.9	72.7	216.1	215.9
4	49.2	49	47.4	47.1
5	38	37.8	51.6	51.3
6	40.1	39.9	19.9	19.6
7	21.1	20.9	33.2	32.8
8	47	46.8	43.2	42.9
9	40.5	40.4	50.9	50.6
10	59.2	59	43.2	42.9
11	37	36.8	23.3	22.9
12	35.6	35.1	25.4	25.1
13	39.2	39	38.2	37.9
14	143.3	143	41.4	41.1
15	115.7	115.5	27.7	27.4
16	31.1	30.9	35.8	35.5
17	34	33.8	43.2	42.8
18	46.4	46.2	48.5	48.2
19	39.8	39.6	48.2	47.9
20	31.2	31	150.9	150.7
21	34.7	34.4	30	29.7
22	38.1	37.9	40.2	39.9
23	11.9	11.6	28.2	27.9
24	16.2	16	20.1	19.6
25	15	14.8	12.1	11.8
26	24.3	24.1	16.2	15.9
27	32	31.8	14.7	14
28	34	33.8	18.3	18
29	24.7	24.5	109.7	109.5
30	-	-	19.5	19.2

Table 4: Comparison of ^{13}C chemical shifts for PW1 and PW2 with literatures.

The ^1H NMR data for PW2 was characteristic of a triterpene, exhibiting value for seven methyl groups. All the methyl signal were observable in the region of δ 0.82 to 1.23, except for a deshielded methyl singlet which appeared at δ 1.70. A pair of singlet at δ 4.70 (singlet) and 4.59 (singlet) was typical of a pair exomethylene protons H-29a and H-29b of a lup-20(29) ene-type triterpene, as seen in lupeol and related lupanes. The ^1H NMR also exhibited a broad ^1H singlet at δ 3.92 indicating the presence of an oxygenated or hydroxylated CH (oxymethine) in the molecule. The ^{13}C NMR data (Table 4) exhibited signals for thirty carbons. Altogether, it exhibited signals for the seven methyls, ten methylenes (including the exomethylene), six methines (including the oxymethine), and seven quaternary carbons. A carbonyl signal was observed at δ_c 216.1 for a ketone and two highly deshielded carbons at δ_c 150.9 (quaternary sp^2 C) and 109.7 (sp^2 CH_2) could be

assigned to the exomethylene group of the lup-20(29)ene skeleton, C-20 and C-29 respectively. The other deshielded carbon at δ_c 79.9 belonged to the oxymethine carbon. Based on ^{13}C NMR data comparison with available literature [28] as shown in table 4, PW2 was determined as glochidonol.

Conclusion

The evaluation of 30 species of plant extracts inhibition against 5-LO activity revealed that 5 species exhibited high value with >80%, 9 species with moderate value (40-79%) and 16 species exhibited <40%. The *P. watsonii* exhibited the highest inhibition with value of 97.3%, followed by *E. hirta* with value of 90.9%. Both extracts were further fractionated using organic which include DCM, EA and BuOH. For *P. watsonii*, DCM fraction exhibited the highest value with 75%, while for *E. hirta* EA fraction exhibited the highest of 72.5%. Subsequent isolation and purification of both fractions yielded four major compounds identified as quercetin-3-*O*-rhamnoside (E1), myricetin 3-*O*-rhamnoside (E2), 26-nor-D: A-friedoolean-14-en-3β-ol (PW1) and glochidonol (PW2).

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