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UPLC MS/MS Profile and Antioxidant Activities from Nonpolar Fraction of Patiwala (*Lantana camara*) Leaves Extract

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Abstract: One of the plants used in Indonesian traditional medicine, namely, Patiwala (*Lantana camara*), is traditionally used to treat some diseases, including itching, wounds, ulcers, swelling, eczema, tetanus, malaria, tumors, rheumatism, and headaches. This study aimed to characterize the compound nonpolar fraction of Patiwala leaf capable of scavenging free radicals. The characterization of compound was carried out using the Ultra-Performance Liquid Chromatography–tandem Mass Spectrometry (UPLC-MS/MS) with positive ion method, while the antioxidant testing was carried out using the radical DPPH (2,2-diphenyl-1-picrylhidrazyl) and FRAP (ferric reducing antioxidant power) methods. The results showed that the nonpolar fraction of the methanol extract of *L. camara* leaves was very strong toward DPPH radicals (IC_{50} 34.65 ± 1.26 µg/mL and 40.23 ± 0.18 µg/mL), and FRAP radical (IC_{50} 4.93 ± 0.22 µg/mL and 12.79 ± 0.09 µg/mL). Nineteen compounds identified by UPLC-MS/MS method were Resveratrol dimer, iso-humolones, oleuropein glucoside, quercetin-3-O-glycoside, myricetin, oleuropein, 12-deoxy-16-hydroxy-phorbol, aloeresin A, humulones, ursolic acid, viniferin, Epicatechin, oleanolic acid, 5-hydroxy-3′,4′,7-trimerthoxy-flavanone, Apigenin-6,8-di-C-β-D-glucoside, procyanidin A2, caffeoyl-O-hexoside, tansihnone IIA, and phillyrin. The methanolic extract of *L. camara* leaves can be developed as a source of antioxidants from natural ingredients.

Keywords: *Lantana camara* leaf; antioxidant; UPLC MS/MS; total phenolic; total flavonoid

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1. Introduction

According to WHO, more than 80% of the world's population relies on traditional medicine for primary health care. The use of herbal medicines in Asian societies has a long history of human interaction with the environment. Plants in traditional medicine are used to treat chronic and infectious diseases [1]. The abundance of plant materials that exist in nature causes the interest of researchers to seek new medicinal sources from plants to increase. The plant contains chemical components that can provide certain physiological effects on the human body, such as alkaloids, flavonoids, tannins, and other phenolic groups [2].

One of the plants used in traditional medicine is Patiwala (*Lantana camara*). Patiwala has been used traditionally to treat itching, wounds, ulcers, swelling, eczema, tetanus, malaria, tumors, rheumatism, and headaches [3]. In addition, this plant is also used to treat hypertension, asthma, ulcers, and cancer, carminative, diaphoretic, anti-inflammatory, and antiseptic for wounds [4].

Several studies have shown that extracts of Patiwala exhibit the ability to inhibit radicals. The extract of the younger leaves of Patiwala had stronger inhibitory power against radicals than the older leaves [5]. In addition, the flower and root parts of Patiwala plant have a very strong ability to inhibit free radicals [6,7]. This plant is reported to contain some secondary metabolites, among others are phenolics, flavonoids, triterpenoids, steroids, and saponins [8,9]. The presence of phenolic compounds in the Patiwala plant allows it to have

antioxidant activity. The antioxidant activity in plants is generally related to the total phenolic and flavonoid content [10]. The amount of phenolic compounds influence the strength of antioxidant activity in the sample. Several studies on the antioxidant activity of phenolic compounds suggest that their structure contributes to their activity. The structural activity of phenolic groups depends on the number and location of hydroxyl groups that play a role in scavenging free radicals [11]. Their antioxidant activity lays in their ability to donate a hydrogen or electron and their ability to delocalize the unpaired electron within the aromatic structure. They can also protect biological molecules against oxidation [12]. Several compounds have been isolated and identified in the leaves of *L. camara*, namely: lantandene B, 5-hydroxy6,4′-dimethoxy flavon-7-O-glucopyranose [13], acacetin-7-O-β-D-rutinoside, tricin, hispidulin, 3,5,7,8-tetra hydroxyl-6,3′-dimethoxy flavones, pectolinarigenin, ursolic acid, lantanilic acid, icterogenin, betulonic acid, and betulinic acid [14]. Therefore, the purpose of this study was to characterize the compounds contained in the extract and the nonpolar fraction of Patiwala (*Lantana camara*) leaves using UPLC-MS, which have antioxidant activity.

2. Materials and Methods

2.1. Plant Materials and Reagents

The leaves of fresh Patiwala (Figure 1) were obtained from community gardens in Wakatobi Regency, Southeast Sulawesi Province, Indonesia, which grew wild, at coordinates 5.3802 S and 123.5895 E. The leaves of Patiwala were authenticated in the Laboratory of Biology Departement, Faculty of Teacher Training and Education, Halu Oleo University. The obtained Patiwala leaves were washed thoroughly with running water, stoned, and dried in an oven at 50 °C. 2,2-Diphenyl-1-picryhidrazyl (DPPH), methanol, n-hexane, ethyl acetate quercetin, gallic acid, trichloro acetate acid (TCA), $FeCl_3$, and $K_3Fe(CN)_6$ were purchased from Sigma-Aldrich (Darmstadt, Germany).

Figure 1. Photo of Patiwala herb.

2.2. Extraction

Dried Patiwala leaves were powdered using a 240 W electric blender (SHARP) to obtain *Lantana camara* leaf powder. Then, the extraction was carried out using the maceration method in which 1000 g of leaf powder was soaked in 10 L methanol. Maceration was carried out at room temperature for 3 × 24 h, filtered every 1 × 24 h using Whatman filter paper. Furthermore, the filtrate obtained was concentrated using a rotary evaporator at a temperature of 40 °C to obtain a thick extract of 119 g.

2.3. Fractionation Using Vacuum Liquid Chromatography (VLC)

The methanolic extract of *L. camara* leaf was exploited further in an attempt to isolate the active components exhibiting the antioxidant activity. Different fractions were obtained using vacuum liquid chromatography in the isolation procedure. A sintered glass Buckner funnel attached to a vacuum line was packed with TLC grade silica gel. The silica gel was compressed under a vacuum to achieve a uniform layer for better separation. The greenish colored viscous methanol extract is dissolved in a suitable volatile solvent (methanol) and

added to the same amount (60 g) of silica gel to make a smooth paste. The solvent is evaporated to leave a dried extract which is adsorbed onto the silica gel. The dried extract was then ground to obtain a uniform powder. This powder is transferred to the column again under vacuum to ensure a uniform coating.

Hexane and ethyl acetate were used as mobile phases with increased polarity ratios from hexane to ethyl acetate. The increased polarity ratios were different as shown in Table 1. Each fraction was collected in a crucible. Fractions were monitored by thin layer chromatography. Fractions having a similar TLC profile were pooled together. The combined fraction is concentrated in a vacuum rotary evaporator. The concentrated fraction was tested for its antioxidant activity against DPPH radicals and FRAP radicals.

Table 1. Eluent gradient system.

Sample	Eluents		Weight (gram)
	n-Hexane (mL)	Ethyl Acetate (mL)	
Fraction A	100	0	0.62
	90	10	
Fraction B	80	20	3.75
	70	30	
Fraction C	60	40	5.63
	50	50	
Fraction D	40	60	9.43
	30	70	
Fraction E	20	80	11.02
	10	90	
	0	100	

Note: nonpolar fraction: A and B, semipolar fraction: C, polar fraction: D and E.

2.4. Measurement of Radical Scavenging Activity Using the DPPH Method

Radical scavenging activity is a chemical test based on the ability of samples to scavenge synthetic free radicals, using a variety of radical generating systems and for detection methods [15]. The radical scavenging activity of Patiwala leaves against DPPH radicals was carried out according to [16,17]. Briefly, 3 mL of 0.6 mM DPPH solution was mixed with 2 mL of sample solution (concentration series 3 µg/mL, 6 µg/mL, 9 µg/mL, 12 µg/mL, and 15 µg/mL), and then 3 mL of methanol was added. Furthermore, the mixture was incubated for 30 min, and the absorbance was measured using UV–Vis spectrophotometer at 517 nm. As a positive control, ascorbic acid was used. The percentage of radical scavenging activity was

$$\% \text{ inihibition} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\%.$$

The IC_{50} value is obtained by replacing the *y*-axis with 50% in the linear regression equation of y = bx + a; so that the value of x-value (IC_{50}) is obtained.

2.5. Determination of Reducing/Antioxidant Power (FRAP)

Reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable and unreactive species [15]. The measurement of antioxidant activity using the FRAP method was carried out according to [18] with a few modifications. A-1 mL of the sample was added with 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% $K_3Fe(CN)_6$ and incubated. Then, 1 mL of 10% TCA was added to the potassium ferricyanide complex centrifuged at 3000 rpm for 10 min to speed up the

precipitation process. After centrifugation, 1 mL of supernatant was pipetted into a test tube and added with 1 mL distilled water and 0.5 mL of 0.1% $FeCl_3$. The solution was allowed to stand for 10 min, and the absorbance was measured at 724 nm.

2.6. Determination of Total Phenolic Content

Determination of the total phenolic content (TPC) was carried out using the Folin-Ciocalteau method [19]. This method is an electron transfer-based assay and provides a reduction capacity expressed as phenolic content. TPC of the sample and the yield depends on the solvent selected for extraction. External calibration was carried out using different concentrations of gallic acid, namely, 10, 20, 30, 40, and 50 µg/mL. Briefly, 1 mL of the sample was placed in a test tube, and then 0.4 mL of Folin–Ciocalteau reagent was added, then allowed to stand for 5–8 min. Then, 4 mL of 7% Na_2CO_3 solution was added and then shaken until homogeneous. Then it was allowed to stand for 30 min at room temperature, and then the absorbance was measured using a UV-Vis spectrophotometer at 750 nm. TPC was calculated as milligram gallic acid equivalent (mg GAE)/g sample.

2.7. Determination of Total Flavonoid Content

Measurement of total flavonoid content (TFC) was carried out using the aluminum chloride method according to [20–22]. This method is based on the reaction of $AlCl_3$ with a C-4 keto group and a C-3 or C-5 hydroxyl group of flavones and flavonols to form a stable acid complex. In addition, it also forms labile acid complexes with ortho-dihydroxyl groups on rings A or B of flavonoids. The external calibration curve was prepared using a series of quercetin concentrations, namely, 20, 40, 60, 80, and 100 µg/mL. Briefly, 1 mL of the sample was added with 3 mL of methanol, 0.2 mL of 10% $AlCl_3$, and 0.2 mL of 1 M potassium acetate, and made up 10 mL with distilled water. The mixture was shaken until homogeneous and incubated at room temperature for 30 min. Then, the absorbance was measured using spectrophotometer at 417 nm. TFC was calculated as milligram of quercetin equivalent (mg QE/g sample).

2.8. UPLC-MS Measurement

The separation procedure was carried out by ultra-performance liquid chromatography (UPLC) using an Aquasil C18 column (Thermo Electron, Dreieich, Germany) with a column size (150 mm × 3 mm, particle size 3 µm). The mobile phase (A) formic acid 0.1% in water and (B) formic acid 0.1% in acetonitrile was used in gradient manner. The elution was 90% A: 10% B (0–20 min); 100% B (20–20.1 min); 90% A: 10% B (20.1–25 min), with running re-equilibration time every 5 min. The mobile phase flow rate was 0.4 mL/min, and the injection volume was 10 µL. The column temperature was maintained at room temperature.

LC-ESI-MS/MS analysis was performed using a triple quadrupole mass spectrophotometer (Waters, Acquity UPLC I-Class with Xevo G2-XF QTof) equipped with electrospray ionization (ESI). The mass spectrometer operated in positive ion mode, full scan spectrum from 100–1300, cone voltage 30 V, Capillary Voltage 3.0 kV, and Source temp 500 °C. All LC-MS data were processed, peaked, and analyzed using the UNIFI informatics platform. The intensity of each ion produces a matrix consisting of the m/z value, retention time (RT), and peak area. The variables of interest were then identified using the UNIFI software [23].

2.9. Statistical Analysis

Analysis of variance followed a post hoc test, namely, the Fisher LSD method was conducted to identify differences among means using Minitab® version 17 (Minitab Inc., State College, PA, USA). Statistical significance was declared at $p < 0.05$. All analyses were conducted in triplicate.

3. Results

3.1. Antioxidant Activities of Patiwala Leaves

Various studies have been conducted to find natural antioxidants such as flavonoid and phenolic compounds. The antioxidant activities of plant extracts cannot be measured using a single method due to the complex nature of the secondary metabolites present in plants [24,25]. Therefore, in this study, the measurement of radical scavenging activities was carried out to measure the ability of the sample to stabilize DPPH radicals.

DPPH is a stable free radical, purple in color, with a central nitrogen atom. When the DPPH radical binds to an antioxidant, the color will fade. The radical scavenging activity of DPPH has been widely used to evaluate the antiradical scavenging activity of natural materials [26,27].

The increased radical scavenging activity was indicated by a decrease in the measured absorbance value. The lower the measured absorbance value indicates an increase in the percentage of free radical inhibition. In addition, increased radical inhibition is characterized by a decrease in the color intensity of free radicals. The level of color change that occurs indicates the level of ability of the antioxidant compounds contained in the sample to donate hydrogen atoms that are owned by binding to free radicals. The ability of Patiwala (*Lantana camara*) to reduce DPPH can be used as material to study the ability of *Lantana camara* to scavenge free radicals from a stable free radical [27]. The antioxidant activity of the patiwala fraction leaf using the DPPH method is shown in Table 2. The measurement results show the IC_{50} value of each fraction is different, with IC_{50} values between 32.84 ± 0.09 μg/mL to 40.23 ± 0.18 μg/mL with ascorbic acid as a positive control.

Table 2. IC_{50} value of Patiwala (*Lantana camara*) leaf fraction using the DPPH and FRAP method.

Sample	IC_{50} Value (μg/mL)	
	DPPH	FRAP
Methanol extract	$24.80^{d} \pm 0.52$	$21.61^{b} \pm 0.26$
Fraction A	$34.65^{b} \pm 1.26$	$4.93^{f} \pm 0.22$
Fraction B	$40.23^{a} \pm 0.18$	$12.79^{e} \pm 0.09$
Fraction C	$40.22^{a} \pm 0.58$	$15.32^{d} \pm 0.32$
Fraction D	$34.69^{b} \pm 0.39$	$18.31^{c} \pm 0.40$
Fraction E	$32.84^{c} \pm 0.09$	$22.67^{a} \pm 0.23$
ascorbic acid	$4.96^{e} \pm 0.03$	$3.82^{g} \pm 0.02$

Note: nonpolar fraction: A and B; semipolar fraction: C; polar fraction: D and E; values (means with different index letter are significantly different $p < 0.05 \pm$ standard deviations)

Table 2 showed that the extract and fraction of *L. camara* leaves has very strong antioxidant activity against DPPH radicals. This is in line with previous studies which showed that the L. camara leaf showed strong activity as radical scavenging, including the *L. camara* purple variety extract leaves [4], and crude extract of *L. camara* leaves [3,28]. However, there have been no studies showing the antioxidant activity of fraction of the *L. camara* leaves.

3.2. Reducing Power Assay (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) method is used to evaluate the antioxidant power of the Patiwala fraction leaf in reducing Fe^{3+} ions in an acidic medium to produce Fe^{2+} ions, characterized by a color change to dark blue, which can be measured at a wavelength of 587 nm and can be expressed as equivalent mg Fe^{2+} [16]. The results of the measurement of antioxidant activity using the FRAP method are shown in Table 2, with the order of: Fraction A (IC_{50} value 4.93 ± 0.22 μg/mL) > fraction B (IC_{50} value 12.79 ± 0.09 μg/mL) > fraction C (IC_{50} value 15.32 ± 0.32 μg/mL) > fraction D (IC_{50}

value 18.31 ± 0.40 μg/mL) > methanol extract (IC_{50} value 21.61 ± 0.26 μg/mL) > fraction E (IC_{50} value 22.67 ± 0.23 μg/mL), respectively. Ascorbic acid was used as a positive control.

3.3. Determination of Total Phenolic and Total Flavonoids Content

Determination of total phenolic content was carried out to strengthen the antioxidant activity test contained in the sample. The hydroxyl groups contained in phenolic compounds act as active sites as antioxidants from these phenolic compounds. Phenolic compounds have a unique structure and properties, namely, one or more hydroxy groups attached to an aromatic ring. As a result, these compounds are easily oxidized. The ability of phenolic compounds to form stable phenoxy in oxidation reactions causes them to be used as antioxidant compounds [9]. The increase in antioxidant activity was determined by the amount of hydroxyl contained in the compound. The more hydroxy groups, the stronger the antioxidant activity of the sample. Meanwhile, the antioxidant activity of flavonoid compounds is the hydroxy position on ring B. The hydroxy position that provides higher stability against free radical scavenging is at the 3′, 4′-O-dihydroxy position on ring B because the electrons are in the form of radicals at this position. The flavonoid structure will undergo a delocalization process [16,29].

Table 3 showed that fraction E of patiwala leaf methanol extract has a highest phenolic content compared to other fractions, with values of 98.81 ± 0.27 mg GAE/g sample (fraction E), 82.78 ± 0.16 mg GAE/g sample (fraction D), 75.76 ± 0.82 mg GAE/g sample (methanol extract), 60.43 ± 0.27 mg GAE/g sample (fraction C), 39.17 ± 0.56 mg GAE/g sample (fraction B), and 20.25 ± 0.41 mg GAE/g sample (fraction A), respectively. Meanwhile, the flavonoid content in the methanol extract fraction showed the flavonoid content of Fraction D > Fraction E > methanol extract > Fraction B > Fraction A, with values, 97.56 ± 0.62 > 82.04 ± 0.48 > 70.53 ± 0.63 > 61.31 ± 0.31 > 19.85 ± 0.65 mg QE/g sample, respectively.

Table 3. The total phenolic and flavonoid content of the methanol fraction of Patiwala (*Lantana camara*) leaves.

Sample	Phenolic Total Content (mg GAE/g Sample)	Flavonoid Total Content (mg QE/g Sample)
Methanol extract	$75.76^{c} \pm 0.80$	$70.53^{c} \pm 0.62$
Fraction A	$20.25^{f} \pm 0.41$	$19.85^{f} \pm 0.65$
Fraction B	$39.17^{e} \pm 0.56$	$38.19^{e} \pm 0{,}31$
Fraction C	$60.43^{d} \pm 0.27$	$61.31^{d} \pm 0.31$
Fraction D	$82.78^{b} \pm 0.16$	$82.04^{b} \pm 0{,}48$
Fraction E	$98.81^{a} \pm 0.27$	$97.56^{a} \pm 0.63$

Note: nonpolar fraction: A and B, semipolar fraction: C, polar fraction: D and E, values (means with different index letter are significantly different $p < 0.05 \pm$ standard deviations)

The previous studies revealed that phenolic content of 80% methanol extract and absolute methanol of *L. camara* leaves were of 18.37 ± 0.49 g/100 gDW and 12.48 ± 0.49 g/100 gDW, respectively [30]. Another study also showed that the phenolic and flavonoid content of *L. camara* leaf extract was 563.57 ± 2.49 mg GAE/g and 243.89 ± 1.30 mg QE/g, respectively [28]. The fact that different units were used in these studies makes it difficult to correlate these results with our data. Moreover, these differences were due to some environmental factors including plants grow, soil pH, climate, humidity, and intensity of sunlight.

3.4. Qualitative Characterization of Compounds Present in the Methanol Fraction of Lantana camara Leaves

LC-ESI-MS/MS analysis was carried out in positive ionization modes to obtain maximum information about the composition of the polar fraction of the methanol extract of Patiwala (*Lantana camara*) leaf. Compounds were characterized based on their mass spectra, using precursor ions, fragment ions, and comparison of fragmentation patterns with molecules described in the literature.

Figure 2 shows the total ion chromatogram (TIC) of the nonpolar fraction of the leaf extract of *Lantana camara* obtained in the positive ion mode. Peak 1 with a retention time (tr) of 0.349 min (Table 4 and Figure 1) was proposed as a Resveratrol dimer that produces $[M + H]^+$ ions at m/z 453. In addition, fragment ions at m/z 397, m/z 290 and at m/z 274 were observed in the ESI-MS/MS spectrum.

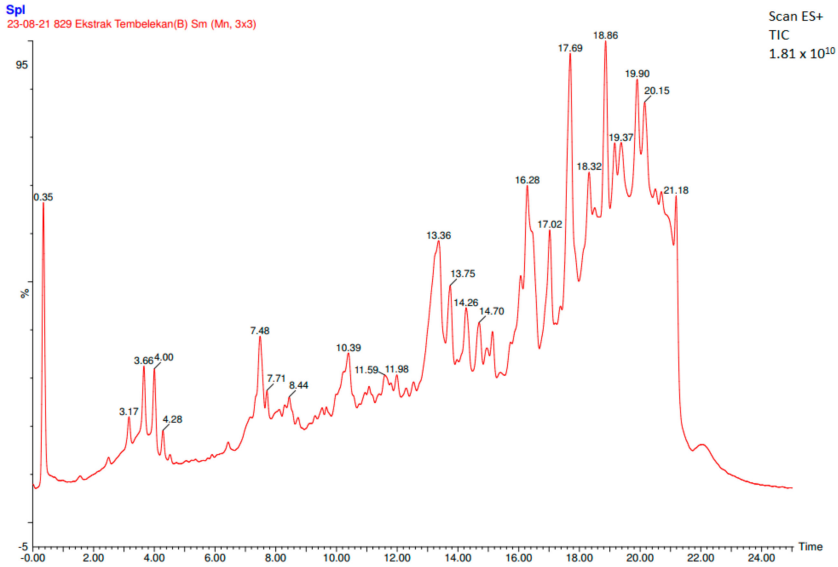

Figure 2. Chromatogram of chemical constituents from fraction B of Patiwala extract.

Peak 2 and 3 at (Rt) 3.166 min and 3.66 min gave the molecular ion $[M + H]^+$ at 701 (Figure 2, Table 4). When subjected to MS/MS, compounds (2) and (3) yielded fragment ions at m/z 588, 397, and 340, which then correspond to Oleuropein glucoside and its isomers (Figure 3a). This has also been reported in olive pulp and olive pomace species [31]. The ion spectrum production of compound 4 at m/z 464 (Rt 4.281 min) as shown in (Figure 2, Table 4) resulted in a fragmentation pattern of m/z 302 $[M + H-162]^+$ due to loss of glucoside and m/z 274 $[M + H-190]^+$, due to cleavage of the carbonyl group moiety. A similar compound was obtained in the genus Prunus in a different pattern (at m/z 303 and 269), called quercetin galactoside [30]. Based on the pattern of the fragments, the compound is temporarily referred to as Quercetin-3-O-glycoside (Figure 3b).

The quasi-molecular ion of the protonated compound 5 at m/z 319 $[M + H]^+$ (Rt) 7481 min as shown in Figure 2, Table 4. When the compound was subjected to MS/MS, compound (5) underwent fragmentation at m/z 274, caused by cleavage of the methyldehida (C_2H_4O) molecule, and fragmentation at m/z 230, due to cleavage of the CO_2 molecule. Similar compounds were also obtained in Grapevine leaves [31]. Compound 5 is tentatively declared as myricetin (Figure 3c) based on the argument above.

The proposed fragmentation pattern of the compound shown in compound 6, appearing at retention time (tR) 7.71 min has a molecular ion peak $[M + H]^+$ at m/z 379 (Figure 2, Table 4), and ion fragmentation observed at m/z 362 $[M\text{-}17 + H]^+$, due to loss of hydroxyl ions. Further, fragmentation resulted in precursor ion yielding daughter ions at m/z 290 $[M\text{-}17\text{-}72 + H]^+$ indicating a loss of 2-butene-1-ol (C_4H_7OH) molecule. This fragmentation pattern resembles the fragmentation pattern of compounds obtained from olive leaf extracts [32]. Based on these data, compound 6 is predicted to be Oleuropein aglycon (Figure 3d).

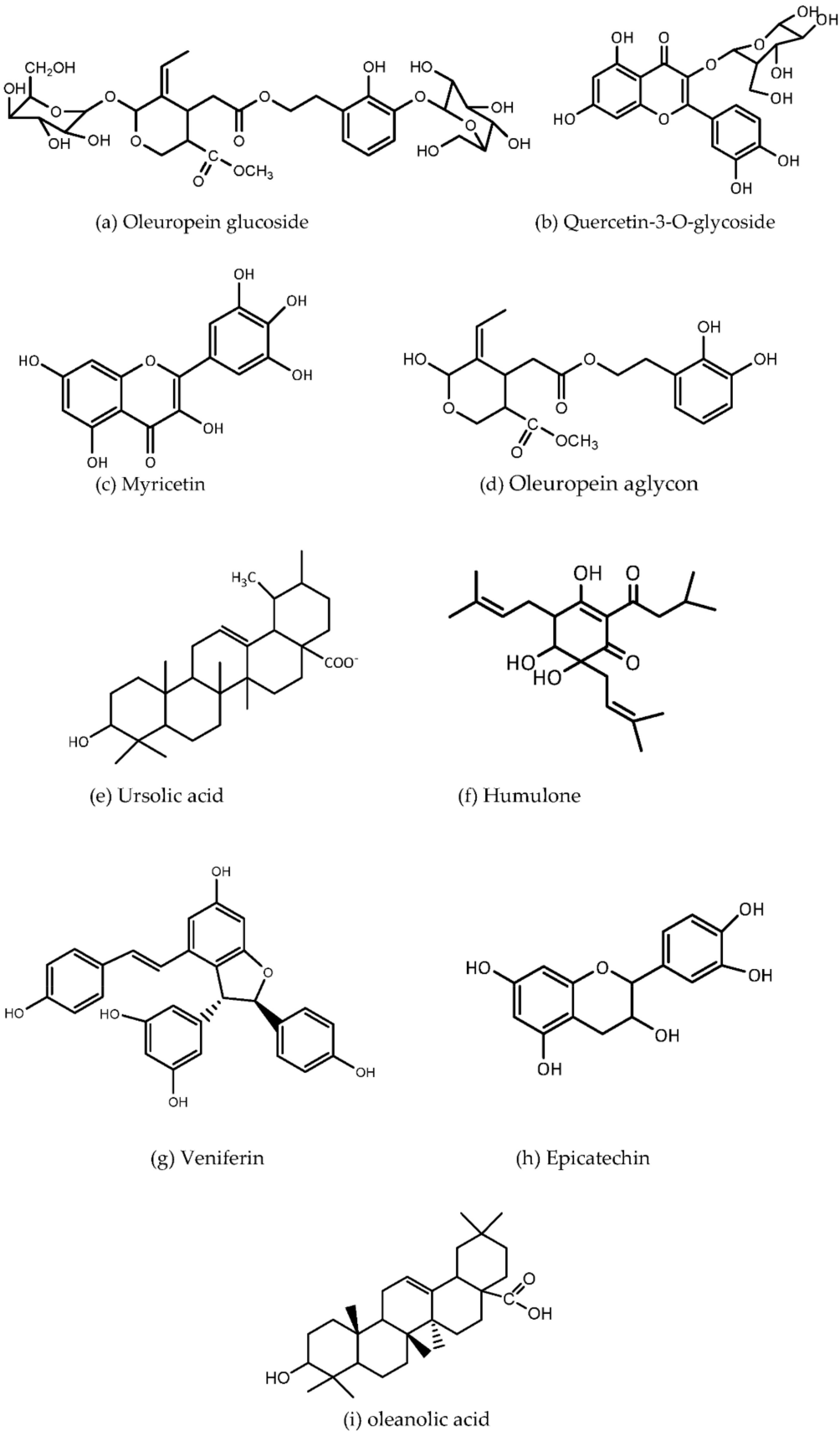

Figure 3. Molecular structure of the nonpolar fraction of *Lantana camara*.

Peaks 7, and 10 (Figure 2, Table 4) show the terpenoid group of compounds, where peak 7 (tR) 8443 min, produces cation molecules at m/z 365 and diterpene MS fragments at m/z 288 and MS/MS at m/z 244 and 203, indicating the presence of 12-deoxy-16-hydroxy-phorbol [33]. Peak 10 yields a pseudomolecular at m/z 455 (Figure 2, Table 4), further resulting in fragmentation at m/z 363 which caused by the loss of 2-butanol molecules and water molecules ($C_4H_{10}O$ and H_2O), then MS/MS fragmentation at m/z 347 was caused by loss of methyl molecules and H+ ions ($CH_3 + H^+$). Furthermore, it underwent further fragmentation at m/z 277 indicating a loss of the 3-metyhl-1-butene molecule and methyl molecule (C_5H_{10}). Based on these data, this compound was predicted to be an ursolic acid compound (Figure 3e). Similar compounds were also found in Chinese herbs although with slightly different fragmentation patterns (m/z 455; 407; 377; and 363) [34]. Peak 8 (Rt 10.392 min) with $[M + H]^+$ at 541 is the molecular weight of aloeresin A. The daughter fragment at m/z 393 is due to a molecular loss of 147 Da, indicating the loss of p-coumaroyl groups attached to the Aloresin A glycosides. Similar compounds were also obtained in methanol extract of Aloe species [35].

Table 4. Qualitative and quantitative phytochemical analysis of fraction B from methanol extract of *Lantana camara* leaves.

Peak	t_R (min)	[M + H] (m/z)	Main Fragment Ions (m/z)	Compound Prediction	References
1.	0.35	453	397; 290; 274	Resveratrol dimer (phenolic groups)	[43]
2.	3.16	701	588; 397; 340	Oleuropein glucoside (phenolic groups)	[31]
3.	3.66	701	588; 397; 340	Oleuropein glucoside isomer (phenolic groups)	[31]
4.	4.28	464	302; 274	Quercetin-3-O-glycoside (Flavonoid)	[44]
5.	7.48	319	274; 230	Myricetin (Flavonoid)	[37]
6.	7.71	379	363; 290; 274	Oleuropein (Phenolic)	[32]
7.	8.44	365	288; 244; 203	12-deoxy-16-hydroxy-phorbol (diterpenes)	[35]
8.	10.39	541	457; 411; 393	Aloeresin A (phenolic groups)	[35]
9.	11.03	363	347; 277; 203	Humulones (Phenolic group)	[36]
10.	11.31	455	363; 347; 277	Ursolic acid (triterpenoids)	[34]
11.	11.57	363	347; 275; 203	Iso-humolones	[36]
12.	11.98	453	279; 258; 205	Viniferin	[37]
13.	13.74	291	275; 257	epicatechin	[39]
14.	14.24	439	333; 293; 261	oleanolic acid	[40–42]
15.	14.70	331	309; 83	5-hydroxy-3′,4′,7-trimerthoxy-flavanone (Flavonoid)	[45]
16.	16.28	455	437; 291	Apigenin-6,8-di-C-β-D-glucoside (Flavonoid)	[46]
17.	17.31	575	453; 407; 295	Procyanidin A2 (phenolic groups)	[47]
18.	18.33	341	276; 243; 153	Caffeoyl-O-hexoside	[38]
19.	18.86	295	263; 245; 165	Tansihnone IIA	[48]
20.	19.90	557	407; 166; 152	phillyrin	[48]

Peaks 9 and 11 (Figure 2, Table 4) with a retention time (Rt) of 11.073 min and 11.567 min were the peaks of the hop bitter acid group. These compounds have a $[M + H]^+$ fragment at m/z 363, which is the molecular weight of the Humulones compound (Figure 3f) and its isomer [36]. When this compound is subjected to MS/MS, it undergoes fragmentation at m/z 347, which suggests a loss of molecular oxygen (O), m/z 275, which indicates a loss of iso-butenyl (C_4H_7) and hydroxy compounds (OH), and m/z 203 which indicates the loss of iso-butenyl (C_4H_7) and hydroxy compounds (OH) molecules has occurred again. However, there was a slight difference with the compounds that came out during the retention time (tR 11.073 min), namely, MS/MS at m/z 277, which indicated a loss of C_4H_6O molecules. Similar compounds were also obtained from various classes of Czech beer and hop extracts [36].

Peak 12 (Rt 11.984 min) with $[M + H]^+$ at 453 (Figure 2, Table 4) represents the molecular weight of veniferin. The daughter fragment at m/z 258 was due to a molecular loss of 195 Da, indicating the loss of the O-glycoside compound bound to the flavonoid aglycone and accompanied by the loss of oxygen atoms in the C ring. Subsequently, fragmentation was again at m/z 205. This occurred because of the loss of 53 Da, indicating a loss of C_3H_2OH molecules in ring A. Therefore, the compound at peak 12 was predicted as a veniferin compound (Figure 3g). The pattern of fragmentation of this compound is similar to the pattern of fragmentation of compounds obtained from Grapevine Leaves [37].

Peak 13 with a quasi-molecular ion at m/z 291 shows the diagnostic product ion at m/z 257 and 203 (Figure 2, Table 4), which originates after the splitting of an oxygen atom and an H_2O

molecule in ring C. The presence of two ions: m/z 257 $[M + H\text{-}16]^+$ and 203 $[M + H\text{-}34]^+$ indicated the cleavage of the ether group and the hydroxy group contained in ring C, which was a marker of flavan-3-ol. Based on the above, compound 13 is predicted to be epicatechin (Figure 3h). This compound is also obtained from the plant male flowers of Phoenix dactylifera [38], dan green tea [39].

The spectrum of peak 14 (Rt 14.239 min) indicates the spectrum of saponin compounds with the parent nucleus producing characteristic fragment ions at m/z 439 (Figure 2, Table 4). With a positive ESI spectrum, this compound produces characterization fragment ions at m/z 333, m/z 293, m/z 261. Previous studies [40–42] have also reported that oleanolic acid is the parent that produces fragment ions characteristics at m/z 439. Saponins with different parent nuclei produce different characteristic ions. Thus the compound is characterized as an oleanolic acid compound (Figure 3i).

4. Conclusions

This study identified nineteen compounds in the nonpolar fraction of Patiwala (*Lantana camara*) leaves, which were determined using Ultra Performance Liquid Chromatography tandem mass spectrometry (UPLC MS/MS). The nineteen proposed compounds are resveratrol dimer, iso-humolones, oleuropein glucoside, quercetin-3-O-glycoside, myricetin, oleuropein, 12-deoxy-16-hydroxy-phorbol, oleoresin A, humulones, ursolic acid, viniferin, epicatechin, oleanolic acid, 5-hydroxy-3′,4′,7-trimethoxy-flavanone, apigenin-6,8-di-C-β-D-glucoside, procyanidin A2, caffeoyl-O-hexoside, tanshinone IIA, and phillyrin. This study also showed that extract and fractions of *Lantana camara* had very strong antioxidant activity so that it could be used as a source for natural antioxidants.

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