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Original Research Article

Phytochemical composition and antimicrobial properties of *Markhamia platycalyx* (Baker) Sprague leaf

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Abstract

Purpose: *To isolate new antimicrobial agents from the leaves of Markhamia platycalyx (Baker) Sprague and assess their phytochemical characteristics and antimicrobial activity.*
Methods: *Different chromatographic and spectroscopic techniques (NMR and ESI-MS) were applied for the identification of antimicrobial compounds. Agar-well diffusion technique was used for determination of antimicrobial activity. Anti-HCV effects were investigated using VITROS Anti-HCV assay.*
Results: *Eighteen compounds were isolated for the first time from this genus. These were phytol, n-octacosanoic acid (OCTA), tormentic acid and β-sitosterol-3-O-(6'-O-heptadecanoyl)-β-D-glucopyranoside. The other compounds were β-sitosterol, ursolic acid (URSA), oleanolic acids, pomolic acid (POMA), 2-epi-tormentic and β-sitosterol-3-O-β-D-glucopyranoside. However, stigmasterol and acteoside, which were seen in previous studies, were also present. Total ethanol extract (TEE) was the most effective against Escherichia coli, with the lowest minimum inhibitory concentration (MIC) of 1.0 µg/mL. Acteoside, URSA and 2-epi-tormentic acid showed the highest antibacterial effect on Pseudomonas aeruginosa while 2-epi-tormentic acid and acteoside produced the least MIC on Candida glabrata. These effects were superior to those produced by standard antibiotics. However, 2-epi-tormentic acid and β-sitosterol-3-O-β-D-glucopyranoside had no anti-HCV effects.*
Conclusion: *Due to the good antimicrobial properties of Markhamia platycalyx, it is a potential source of new antimicrobial drugs.*

Keywords: *Markhamia platycalyx, Dolichandrone platycalyx, Bignoniaceae, Phytochemical, Antimicrobial, Hepatitis C, Anti-HCV*

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INTRODUCTION

The *Bignoniaceae* family is rich in secondary metabolites. It comprises 104 genera and 860 species of high economic and therapeutic importance [1]. One of these species is *Markhamia platycalyx* Sprague (Syn. *Dolichandrone platycalyx* Baker). Some

researchers consider *M. platycalyx* as synonymous with *M. lutea*. However, a recent study on cultivated plants in Egypt classified both of them in two different lineages [2].

A review of phytochemical literature showed that sesamin and paulownin were isolated from *M. platycalyx* [3]. Moreover, acteoside (verbascoside), isoacteoside (isoverbascoside), apigenin, luteolin, caffeic acid and methyl caffeate have been isolated from this plant [3]. The antibacterial effects of leaves of the genus *M. tomentosa* have been investigated [4]. The antifungal effects of *M. obtusifolia* and *M. tomentosa* have been reported [5], and there are reports on the antimalarial and antileishmanial properties of *M. lutea* [6]. A survey of available literature revealed only two studies on the antimicrobial and anti-protozoal effects of *M. platycalyx.* The first one focused on the antimicrobial effect of volatile oil of from the leaves of *M. platycalyx* [7], while the second study reported its promising anti-protozoan properties against *Plasmodium falciparum* D_6, *P. falciparum* W_2 and *Leishmania donovani* amastigotes [7]. However, the petroleum ether fraction had no cytotoxic effect against VERO mammalian cells [7].

The present study was focused on extensive investigation of the phytochemical, antibacterial, antifungal and anti-hepatitis C virus effects of *M. platycalyx*.

EXPERIMENTAL

Chemicals

Authentic chemicals were obtained from the Pharmacognosy Department, Faculty of Pharmacy, Minia University, Egypt. Deuterated solvents were purchased from Sigma-Aldrich, Germany, and were used for NMR spectroscopic analyses.

Spectroscopic methods

BRÜKER AVANCE spectrometer was applied for ^{1}H and ^{13}C-NMR spectra, while LTQ Orbitrap XL MS (Japan) was used for ESI-MS. High Content Screening Operetta instrument (Perkin Elmer, USA) was used for analyses of the Anti-HCV plates, while antigen-positive cells were quantified using Coolsnap ES camera (Photometrix).

Plant material

Leaves of *M. platycalyx* Sprague were collected in May 2012 from El-Zohria botanical garden in Giza, and was authenticated by Dr M Shokry, Director of El-Zohria botanical garden. A voucher specimen (no. Mn-Ph-Cog-015) was deposited in the Pharmacognosy Herbarium of Faculty of Pharmacy, Minia University, Egypt.

Isolation of phytochemicals from *M. platycalyx* Sprague

Air-dried powdered leaves of *M. platycalyx* (5 kg) were extracted by maceration with 95 % ethanol (3 x, 5 L), and then concentrated under reduced pressure to give 0.5 kg of a viscous residue (TEE). It was suspended in 600 mL of H_2O and subjected to successive solvent partitioning using different organic solvents in ascending order of polarity *viz*. light petroleum ether (LPE), dichloromethane (DCM) and ethyl acetate (EtOAc) (3 x 350 mL for each solvent), resulting in 3 sub-fractions: LPE fraction (95 g), DCM fraction (19 g), and EtOAc fraction (30 g). The aqueous layer was concentrated to yield the aqueous fraction (250 g). A portion of the concentrated aqueous fraction (25 g) was gradually added to a flask containing methanol (1 L) with vigorous shaking and kept in refrigerator overnight, after which it was filtered through a Büchner funnel under reduced pressure. The residue obtained was dried in vacuum oven 40 °C to obtain 3 g of crude polysaccharide powder (CP).

The LBE fraction was gross-fractionated twice (25 g each) using VLC (250 g TLC silica gel). The column was subjected to gradient elution with LPE-EtOAc at volume ratios of 100:0, 90:10, 80:20, 70:30 and 60:40. The effluents were collected in fractions (3 L each) to yield five sub-fractions (PEI-PEV).

Subfraction PEII (14.3 g) was subjected to silica gel column chromatography (560 g), using gradient elution with LPE-EtOAc at volume ratios of 94:6, 92:8, 90:10 and 88:12. This resulted in 4 sub-fractions ($PEII_1$-$PEII_4$). Sub-fraction $PEII_2$ (300 mg) was re-chromatographed using silica gel column chromatography (15 g), with LPE-DCM gradient elution, to produce compound **1** [(transparent oil, 65 mg, R_f=0.53, with LPE-EtOAc (75:25, v:v)] and compound **2** (yellowish-white amorphous powder, 70 mg, R_f=0.50; eluted with LPE-EtOAc (75:25, v:v)]. Sub-fraction $PEII_3$ (400 mg) yielded a mixture compounds **3** and **4** [60 mg, R_f=0.40, system; eluted with LPE-EtOAc (75:25, v:v)] after re-crystallization in MeOH.

Furthermore, sub-fraction PEIV (2 g) was subjected to silica gel column chromatography and eluted with LPE-EtOAc at 3 different volume ratios (8:2, 75:25 and 7:3) to yield 3 sub-fractions

($PEIV_1$-$PEIV_3$). Sub-fraction $PEIV_1$ (300 mg) was re-chromatographed in silica gel column chromatography, using isocratic elution of LPE-EtOAc (82:18, v:v) to produce compound **5** (white flakes, 78 mg, R_f=0.50). The second sub-fraction $PEIV_2$ (700 mg) was purified with silica gel column chromatography and eluted with LPE-EtOAc gradient elution to give compound **6** [amorphous white powder, 15 mg, R_f=0.37; eluted with LPE-EtOAc (70:30, v:v)] and 3 mg of compound **7** [white amorphous powder, 3 mg, R_f=0.43, system; eluted with LPE-EtOAc (70:30, v:v)].

The last sub-fraction of LPE, PEV (2 g) was applied to silica column chromatography using LPE-EtOAc at volume ratios of 80:20, 75:25, 70:30 and 65:35 to generate 4 sub-fractions (PEV_1-PEV_4). Sub-fraction (PEV_1) yielded compound **8** [white amorphous powder, 9 mg, R_f=0.44; eluted with LPE-EtOAc (35:65, v:v)], while PEV_2 produced compound **9** [white amorphous powder, 70 mg, R_f=0.54; eluted with LPE-EtOAc (40:60, v:v)]. The third subfraction PEV_3 (70 mg) was re-subjected to silica gel column chromatography with isocratic elution using LPE-EtOAc (67:33, v:v) to yield compound **10** [white amorphous powder, 40 mg, R_f=0.48; eluted with DCM-MeOH (90:10, v:v)].
The DCM fraction (14 g) was gross-fractionated with VLC (140 g silica for TLC). The column was subjected to gradient elution with LPE-EtOAc at volume ratios of 90:10, 70:30, 60:40 and 25:75. The eluents were collected in fractions (1.5 L each) and concentrated to yield 4 sub-fractions (DI-DIV). Sub-fraction DIV (300 mg) was subjected to silica gel column chromatography, using DCM-MeOH at increasing polarities, to yield compound **11** [yellow oil, 44 mg, R_f=0.50; eluted with DCM-MeOH (90:10, v:v)].

Finally, the EtOAc fraction (25 g) was gross-fractionated with VLC and eluted with DCM-MeOH at volume ratios of 95:5, 90:10, 80:20 and 60:40. The eluents result gave rise to 4 sub-sets (EtI-EtIV). Sub-fraction EtIII (14 g) was fractionated with column chromatography in silica gel, using EtOAc-MeOH at increasing polarities, to yield 3 sub-fractions ($EtIII_1$ - $EtIII_3$). When $EtIII_1$ (5 g) was further subjected to silica gel column chromatography and elution was done with EtOAc-MeOH (97:3, v:v), compound **12** was obtained as a yellow amorphous powder [130 mg, R_f=0.47; EtOAc-MeOH (80:20 v:v)].

Antimicrobial studies

The microbial strains were maintained either on Müller Hinton agar (bacteria) or Sabouraud agar (fungi). The bacterial strains comprised Gram positive, facultative anaerobic strain (*Staphylococcus aureus,* ATCC 6538); Gram negative, facultative anaerobic strains (*Escherichia coli,* ATCC 10145 and *Klebsiella pneumoniae*, ATCC 10031), and Gram negative, aerobic bacteria (*Pseudomonas aeruginosa,* ATCC 10145). The fungi were standard strains of *Candida krusei, Candida glabrata* and *Candida albicans.* The TEE extract, fractions and pure compounds were weighed and dissolved in DMSO to obtain desired concentrations.

The antimicrobial effects of all tested samples were investigated with agar-well diffusion method. The wells contained 100 µL of tested samples or standard drug. The TEE and different fractions were each screened for antibacterial effects at a concentration of 2.5 mg/mL, while 1.0 mg/mL of each isolated compound was investigated. Antimicrobial effect was calculated by measuring the diameters of zones of inhibition (ZIs) against the tested organisms, and the results were expressed in mm.

Moreover, TEE and different fractions (1.25 mg/mL) were screened for their antifungal effects. The MIC was measured using two-fold serial dilutions. The initial concentrations of TEE and fractions were 10000 and 1000 µg/mL of pure compounds, respectively. Equal volumes were put in each well, and after overnight incubation at 37 °C, the IZs were measured. The MIC for agar-well diffusion method was calculated as described earlier [8].

Determination of anti-HCV activity

Infection of 6.000 Huh-7 cells with HCVcc during exposure to the compounds (50 or 25 µg/mL) was carried out in multiples of 0.70. Viral cell culture of HCV (HCVcc) was produced using a modification of plasmid encoding JFH1 genome (Wakita, Tokyo). The production of HCVcc was carried out in Huh-7 cells subjected to electroporation using *in vitro*-transcribed RNA of JFH1 containing JFH1-Luc and titer-enhancing mutations. The JFH1 stocks were generated through amplification in Huh-7 cells. The suppressive potential of drugs was assessed by measuring luciferase activity in cell lysates or determination of degree of infection through indirect immunofluorescence with anti-E1 MAb. In addition, HCV pseudo-typed retroviral particles (HCVpp) expressing firefly luciferase reporter gene were produced in HEK-293T [9].

Statistical analysis

Results are expressed as mean ± SEM. Statistically significant differences were

determined using one-way analysis of variance (ANOVA), followed by Dunnett's test. All statistical analyses were done with GraphPad Prism 5 (GraphPad Software, San Diego California, USA). Values of $p < 0.05$ were taken to indicate statistical significance.

RESULTS

Phytochemical composition

Twelve compounds were isolated from TEE *M. platycalyx* leaves. These were phytol (**1**) [10], OCTA (**2**) [11], *β*-sitosterol (**3**) and stigmasterol (**4**) [12]; URSA (**5**) and oleanolic acid (**6**) [13]; POMA (**7**) [14], tormentic acid (**8**) [15], 2-epi-tormentic (**9**) [16], *β*-sitosterol-3-*O*-(6'-*O*-heptadecanoyl)-*β*-D-glucopyranoside (**10**) [17], *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**11**) [18] and acteoside (**12**) [19]. Identification was done by comparing their physical, chemical and chromatographic properties, and spectral results shown in Table 1 with published reports, and reference to standards. Figure 1 shows the structures of these compounds.

Phytol (1): $C_{20}H_{40}O$, ^{1}H-NMR (600 MHz, $CDCl_3$): δ_H 5.38 (1H, td, 6.6, 1.2 Hz, H-2), 4.13 (2H, d, 6.6 Hz, H-1),1.64 (3H, s, H-20), 0.85 (3H, d, 6.6 Hz, H-19), 0.84 (3H, d, 6.6 Hz, H-18), 0.83 (3H, d, 4.2 Hz, H-17) and 0.82 (3H, d, 4.2 Hz, H-16).

***n*-Octacosanoic acid (2):** ^{1}H-NMR (600 MHz, $CDCl_3$): δ_H 2.26 (2H, t, 7.5 Hertz, H-2), 1.53 (2H, m, H-3), 1.19 (br. s, other CH_2 protons) and 0.80 (3H, t, 7.1 Hz, H-28).

Spectral properties of selected isolated compounds

Tormentic acid (2α-3β-19α-Trihydroxyurs-12-en-28-oic acid) (8)

^{1}H-NMR (600 MHz, C_5D_5N): δ_H 5.57 (1H, br.s., H-12), 4.11 (1H, m, H-2*β*), 3.38 (1H, d, 9.6 Hz, H-3α), 3.06 (1H, s, H-18), 1.72 (3H, s, H-27), 1.44 (3H, s, H-29), 1.20 (3H, s, H-23), 1.11 (3H, d, 5.4 Hz, H-30), 1.09 (3H, s, H-26), 1.04 (3H, s, H-24) and 1.02 (3H, s, H-25).

β-sitosterol-3-O-(6'-O-heptadecanoyl)-β-D-glucopyranoside (10)

^{1}H-NMR (600 MHz, $CDCl_3$): δ_H 5.30 (1H, br.s., H-6), 4.5 (1H, m, H-3), 4.33 (1H, d, 7.6 Hz, H-1' Glucose), 2.27 (2H, t, 7.6 Hertz, H-2'' Fatty acid), 1:2 (br s, $(-CH_2-)_{14}$ Fatty acid), 0.95 (3H, s, H-19), 0.87 (3H, d, 6.4 Hz, H-21), 0.84 (3H, t, 7.1 Hz, H-17'' Fatty acid), 0.80 (3H, t, 7.7 Hz, H-29), 0.79 (3H, d, 6.8 Hz, H-29), 0.76 (3H, d, 6.8 Hz, H-26) and 0.63 (3H, s, H-18).

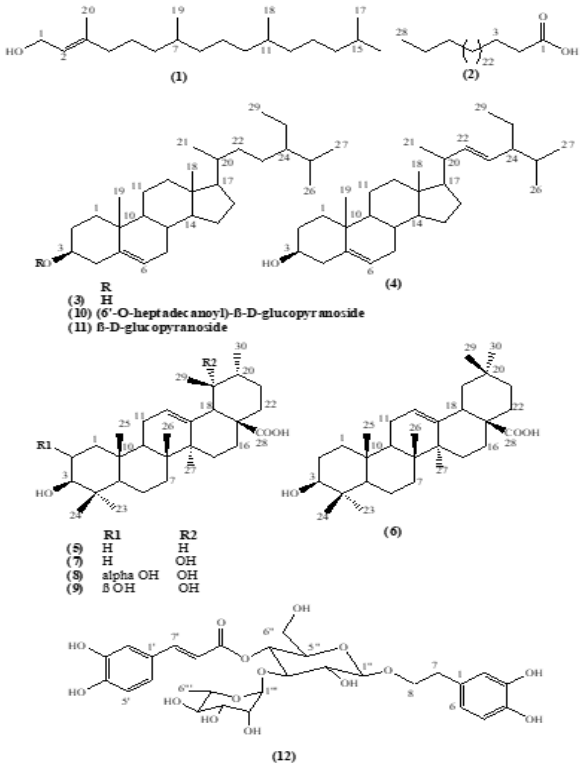

Figure 1: Structures of isolated compounds (**1**-**12**)

Acteoside (Verbascoside or Kusaginin) (12)

^{1}H-NMR (400 MHz, CD_3OD): δ_H 7.61 (1H, d, 16.0 Hz, H-7'), 7.07 (1H, br.s., H-2'), 6.97 (1H, d, 8.0 Hz, H-6'), 6.80 (1H, d, 8.0 Hz, H-5), 6.72 (1H, d, 16.0 Hz, H-8'), 6.71 (1H, br.s., H-2), 6.70 (1H, d, 8.0 Hz, H-5'), 6.57 (1H, d, 8.0 Hz, H-6), 5.18 (1H, br.s., H-1'''), 4.38 (1H, d, 8.0 Hz, H-1''), 4.02 (2H, m, H-8), 3.61 (2H, m, H-7) and 1.09 (3H, d, 8.0 Hz, H-6'''). ^{13}C-NMR (100 MHz, CD_3OD): (δ_C) = C-1 to C-8: 131.4, 117.1, 144.5, 146.0, 116.3, 121.3, 36.4 and 72.2, respectively. Caffeoyl: C-1' to C-9': 127.5, 115.2, 149.7, 146.7, 116.5, 123.2, 63.2, 114.6 and 168.1, respectively. Glucose: C-1'' to C-6'': 104.0, 76.1, 81.6, 70.3, 75.8 and 62.2, respectively. Rhamnose: C-1''' to C-6''': 102.9, 72.2, 71.9, 73.6, 70.4 and 18.5, respectively.

Antibacterial effect

The antibacterial effects (in terms of IZs and MICs) are summarized in Table 3 and Table 4.

Antifungal effects

The antifungal properties (IZs and MICs) are shown in Table 4 and Table 5.

Anti-HCV activity

No anti-HCV effect was observed with 2-epi-tormentic acid and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside regarding quantity of infected Huh-7 cells calculated with and without addition of drug.

DISCUSSION

Although antibacterial agents are indispensable in the treatment of infections, the use of many antibiotics is associated with adverse side reactions and bacterial resistance.

Table 1: ^{13}C-NMR spectra of compounds (**1-11)**

No.	1	2	3	4	5	6	7	8	9	10	11
1	59.6	180.3	37.2	37.2	39.7	39.4	38.8	47.9	45.5	37.5	37.9
2	123.3	34.3	31.8	31.8	28.5	28.5	27.9	68.9	71.9	29.7	30.6
3	140.5	32.1	71.7	71.7	78.5	78.5	78.0	84.2	78.8	80.0	78.5
4	40.1	---	42.1	42.1	39.5	39.8	39.2	40.2	39.2	39.16	39.7
5	25.4	---	140.6	140.6	56.2	56.2	55.6	56.4	56.5	140.6	141.3
6	36.9	---	121.6	121.6	19.2	19.2	18.7	19.4	19.2	122.3	122.3
7	32.9	---	31.8	31.8	34.0	33.6	33.4	33.9	34.1	32.6	32.5
8	37.6	---	31.8	31.8	40.4	40.2	40.1	40.8	40.9	32.1	32.4
9	24.7	---	50.0	50.0	48.4	48.5	47.6	48.2	48.6	50.4	50.7
10	37.6	---	36.4	36.4	37.7	37.7	37.1	39.1	37.8	36.9	37.3
11	33.0	---	21.0	21.0	24.0	24.0	23.8	24.5	24.6	21.3	21.7
12	37.5	---	39.7	39.6	126.0	123.0	127.8	128.3	128.6	40.0	40.3
13	25.0	---	42.2	42.2	139.6	145.0	139.8	140.4	140.4	42.6	42.9
14	39.6	---	56.7	56.7	42.9	42.6	41.9	42.5	42.7	57.0	57.3
15	28.2	---	24.3	24.2	29.1	28.7	29.1	29.7	29.7	24.5	24.9
16	22.8	---	28.1	28.8	25.3	24.2	26.2	26.8	26.9	28.5	28.9
17	22.9	---	56.0	55.9	48.7	47.1	48.1	48.7	48.8	56.4	56.6
18	19.9	---	11.8	11.9	53.9	42.4	54.4	55.0	55.1	12.1	12.4
19	20.0	---	19.3	19.3	39.9	46.9	72.5	73.1	73.1	19.6	19.6
20	16.4	---	36.0	40.4	39.8	31.4	42.2	42.8	42.8	36.4	36.8
21	---	---	18.9	21.1	31.5	34.6	26.7	27.3	27.4	19.3	19.4
22	---	---	33.9	138.2	37.8	33.6	38.3	38.9	38.9	34.2	34.6
23	---	---	26.0	129.2	29.2	29.1	28.6	29.7	30.7	26.4	26.8
24	---	---	45.7	51.1	16.9	16.9	16.6	18.0	18.6	46.1	46.5
25	---	---	29.1	31.8	16.1	16.0	15.4	17.3	17.1	29.4	29.9
26	---	24.9	19.7	19.7	17.8	17.9	17.0	17.6	17.7	19.0	19.8
27	---	22.9	18.9	18.9	24.3	26.6	24.5	25.1	25.2	20.0	20.4
28	---	14.3	23.0	25.3	180.2	180.3	ND	181.0	181.1	23.3	23.8
29	---	---	11.9	12.3	17.9	34.0	26.9	27.5	27.6	12.2	12.6
30	---	---	---	---	21.8	24.1	16.3	17.2	17.2	---	---

Table 2: ^{13}C-NMR spectral data of compounds (**1-11**) (*contd*)

No.	1	2	3	4	5	6	7	8	9	10	11
1'	---	---	---	---	---	---	---	---	---	101.5	102.9
2'	---	---	---	---	---	---	---	---	---	73.6	75.7
3'	---	---	---	---	---	---	---	---	---	76.4	79.0
4'	---	---	---	---	---	---	---	---	---	70.1	72.1
5'	---	---	---	---	---	---	---	---	---	73.9	78.8
6'	---	---	---	---	---	---	---	---	---	63.8	63.2
1"	---	---	---	---	---	---	---	---	---	174.5	---
2"	---	---	---	---	---	---	---	---	---	34.5	---
3"	---	---	---	---	---	---	---	---	---	25.2	---
4"	---	---	---	---	---	---	---	---	---	---	---
5"	---	---	---	---	---	---	---	---	---	---	---
6"	---	---	---	---	---	---	---	---	---	---	---
16"	---	---	---	---	---	---	---	---	---	22.9	---
17"	---	---	---	---	---	---	---	---	---	14.3	---

*150 MHz, $CDCl_3$ (**1**, **2** and **10**), 100 MHz, $CDCl_3$ (**3** and **4**), 150 MHz, C_5D_5N (**5**, **6**, **8**, **9** and **11**) and 100 MHz, C_5D_5N (**7**). ND: not detected

Table 3: Antibacterial effects (IZs) of *M. platycalyx* leaves

Extract/fraction/compound	Zone of inhibition (mm)			
	S. aureus	***E. coli***	***K. pneumoniae***	***P. aeruginosa***
TEE	16±0.20	17±0.30	19±0.10	15±0.41
LPE fraction	20±0.3	16±0.23	16±0.30	20±0.26
DCM fraction	NA	17±0.17	18±0.11	13±0.35
EtOAc fraction	15±0.06	15±0.26	16±0.29	17±.05
Aqueous fraction	NA	NA	NA	13±0.1
CP	NA	NA	NA	NA
Acteoside	15±0.15	16±0.11	NA	15±0.15
Ursolic acid	16±0.06	15±0.06	NA	17±0.11
2-Epi-tormentic acid	14±0.16	14±0.10	NA	16±0.26

NA: not active

Table 3: Antibacterial effect (MICs) of *M. platycalyx* leaves

Extract/fraction/compound	***S. aureus***	***E. coli***	***K. pneumoniae***	***P. aeruginosa***
TEE	11.6	1.0	40.4	3.5
LPE fraction	23.7	34.2	11.6	4.1
DCM fraction	NA	11.57	NA	NA
EtOAc fraction	63.4	10.9	34.2	NA
Aqueous fraction	NA	NA	NA	2.8
Acteoside	2.3	4.1	NA	1.6
Ursolic acid	12.2	2.3	NA	1.2
2-Epi-tormentic acid	NA	3.2	NA	2.3
Ampicillin	2.1	34.9	NA	NA
Gentamycin	16.5	26.4	37.6	5.3
Amikacin	1.7	28.9	9.6	2.4
Augmentin	79.4	1.1	2.2	NA

NA: not active

Table 3: Antifungal effects (IZs) of *M. platycalyx* leaves

Extract/fraction/compound	Inhibition zone (mm)		
	C. krusei	***C. glabrata***	***C. albicans***
TEE	14±0.01	NA	22±0.08
LPE fraction	17±0.05	17±0.09	20±0.13
DCM fraction	18±0.10	14±0.09	16±0.10
EtOAc fraction	14±0.12	20±0.10	20±0.05
Aqueous fraction	20±0.03	17±0.06	21±0.10
CP	21±0.05	20±0.08	16±0.02
Acteoside	27±0.03	17±0.06	20±0.03
Ursolic acid	18±0.02	20±0.04	19±0.03
2-Epi-tormentic acid	19±0.04	16±0.03	18±0.02

NA: not active

Table 5: Antifungal effect (MICs) of *M. platycalyx* leaves

Extract/Fraction/Compound	*C. krusei*	*C. glabrata*	*C. albicans*
TEE	23.5	NA	2.9
LPE fraction	32.1	10.8	51.2
DCM fraction	NA	27.6	25.4
EtOAc fraction	NA	84.3	5.8
Aqueous fraction	72.6	7.4	1.1
CP	4.1	NA	NA
Acteoside	1.7	1.2	1.9
Ursolic acid	1.9	5.1	1.4
2-Epi-tormentic acid	2.6	1.2	10.6
Ketoconazole	1.0	1.58	2.6

NA: not active

Therefore, there is need to evolve novel antibiotics that are free from these drawbacks [20]. Previous preliminary phytochemical screening of TEE of *M. platycalyx* leaves showed presence of carbohydrates and/or glycosides, flavonoids, unsaturated (sterols and/or triterpenes), and anthraquinones. This screening showed many secondary metabolites, indicating various expected biological activities [21].

Twelve compounds in different structural groups were isolated from *M. platycalyx* leaves. This is the first report on the isolation of compounds **1**, **2**, **8** and **10** from *M. platycalyx*, while six compounds (**3**, **5**, **6**, **7**, **9** and **11**) have not been reported before now in *M. platycalyx*.

The TEE was the most active among all tested samples against *E. coli,* while URSA, acteoside and 2-epi-tormentic acid produced the highest effect on *P. aeruginosa*. These aforementioned results were higher than those from positive control drugs. It is likely that the potent antibacterial effect of TEE was due to the synergistic effect of its bioactive components, especially ursolic acid, which is a major compound in the plant [21]. Moreover, acteoside was previously reported to have antibacterial activity [22].

Ketoconazole is the most frequently applied antifungal drug. However, resistance against the drug is a significant drawback. Hence, it became necessary to develop newer antifungal agents [23]. The aqueous fraction, ursolic acid, 2-epi-tormentic acid and acteoside have lower MICs against *C. albicans* than ketoconazole. In addition, acetoside and 2-epi-tormentic acid exhibited the least MICs against *C. glabrata*. This is probably due to triterpenes and sterols which are known to exert antifungal activity [24]. Moreover, ursolic and 2-epi-tormentic acids were previously reported to possess antifungal activities [5], in addition to anti-candidiasis effect of acteoside [25].

CONCLUSION

Twelve compounds have been isolated and elucidated using different spectroscopic techniques. Total ethanol extract (TEE) produced the highest antibacterial effect against *Escherichia coli*, with minimum inhibitory concentration of 1.0 µg/mL, while URSA, 2-epi-tormentic acid and acteoside exhibit the highest activities against *Pseudomonas aeruginosa.* Therefore, this plant is considered a good source of potential new natural drugs.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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