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Phytochemical and antimicrobial studies of *Pachypodium lamerei*

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Five compounds were reported for the first time in the tribe Malouetieae from the leaves and stems of *Pachypodium lamerei* viz., fatty acid methyl ester (stearic acid methyl ester 1), a mixture of two steroidal aglycones (β -sitosterol 2 and stigmasterol 3), a pentacyclic triterpene (ursolic acid 4) and a steroidal glucoside (β -sitosterol-3-O- β -D-glucopyranoside 5). The structure elucidation was based on comparison of their physical, chemical, chromatographic properties, spectral data with literature as well as direct comparison with authentic compounds. The petroleum ether fraction and the total ethanolic extract of (leaves and stems) exhibited minimum inhibitory concentrations (MICs) against *Pseudomonas aeruginosa* (1.06 and 2.52 μ g/ml), respectively. These results are comparable to that obtained by amikacin (MIC, 2.38 μ g/ml). Moreover, the petroleum ether fraction displayed the lowest MIC (16.45 μ g/ml) against *Candida albicans*. This result is greatly promising since the standard drug (ketoconazole) exhibited MIC (185.87 μ g/ml). This is the first time to estimate both antibacterial and antifungal activities of any of *Pachypodium* species.

Key words: *Pachypodium lamerei*, Apocynaceae, phytochemical, antimicrobial, antibacterial, antifungal.

INTRODUCTION

Family Apocynaceae is rich in many secondary metabolites with important biological and economic values, viz., triterpenes, cardenolides, sterols, saponins, and alkaloids (Seigler, 1998; Pelletier, 1996; Trease and Evans, 1991; Gunatilaka, 1986). Reviewing the available literature, it was evident that alkaloids of family Apocynaceae have been extensively reported (Raffauf and Flagler, 1960). Moreover, triterpenes and sterols are found to be widely distributed in family Apocynaceae and summarized in a review (El-Kashef et al., 2015a). The

different members of this family showed antimicrobial activities against different bacterial strains, viz., *Funtumia elastic* (leaves and barks) exhibited antibacterial effects against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* (Agyare et al., 2013). Furthermore, *Cynanchum acutum* (leaves and fruits) displayed certain bactericidal influences against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *Proteus vulgaris* and *E. coli* (Dehghani et al., 2012). In addition to, *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* were inhibited by root

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extracts of *Rhazya stricta* (Marwat et al., 2012). This family also displayed antifungal activities such as the aqueous leaf extracts of *Catharanthus roseus* and *Tabernaemontana divaricate*, which showed inhibitory effects against *Candida albicans* (Wankhede et al., 2013). Moreover, *F. elastic* (leaves and barks) exhibited a fungicidal activity against *C. albicans*, *Aspergillus flavus* and *Aspergillus niger* (Agyare et al., 2013). Additionally, *Aspergillus terreus*, *A. flavus* and *C. albicans* were inhibited by CHCl_3 and MeOH root extracts of *R. stricta* (Marwat et al., 2012). One of these plants belongs to family Apocynaceae is *Pachypodium lamerei* Drake. The unsaponifiable and saponifiable matters of *P. lamerei* leaves and stems were investigated by gas chromatography mass spectrometry (El-Kashef et al., 2014). Reviewing the available literature about this plant, nothing could be traced about the chemical and antimicrobial studies. This provoked us to carry out extensive studies of this plant, including phytochemical and antimicrobial investigations.

METHODOLOGY

Plant material

The leaves and stems of *P. lamerei* Drake were collected in May 2010 from the El-Orman Botanical Garden, Giza, Egypt. It was identified by Agricultural Engineer/Tereez Labib, consultant of plant taxonomy at the Ministry of Agriculture and ex. Director of El-Orman Botanical Garden. A voucher specimen was deposited at the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia University under registration number (Mn-Ph-Cog-006).

Taxonomy

P. lamerei Drake belongs to (Integrated Taxonomic Information System, 2014; Sennblad and Bremer, 2002): Kingdom: Plantae; Subkingdom: Viridaplantae; Infrakingdom: Steptophyta; Division: Tracheophyta; Subdivision: Spermatophytina; Infradivision: Angiospermae; Class: Magnoliopsida; Suborder: Asterales; Order: Gentianales; Family: Apocynaceae (dogbane, apocyns); Subfamily: Apocynoidae; Tribe: Malouetieae; Genus: *Pachypodium* Lindl. and Species: *P. lamerei* Drake.

Extraction and isolation

The air-dried powdered (leaves and stems, 1.4 kg) of *P. lamerei* were extracted by maceration with EtOH (90%) till exhaustion (4x, 5 L each) and then concentrated under reduced pressure till dryness. (TLSEE, 265.3 g) was suspended in the least amount of distilled water (500 ml), transferred to a separating funnel and partitioned successively with petroleum ether and CHCl_3 . The fractions were concentrated under reduced pressure to afford: petroleum ether fraction (40.0 g) and CHCl_3 fraction (78.7 g). The aqueous mother liquor (126.0) was suspended in the least amount of distilled water (500 ml) then subjected to Diaion HP-20 CC ($\Phi=5$, $L=150$ cm) then eluted successively with demineralized water, 50% MeOH and MeOH to give three corresponding fractions. The aqueous fraction was excluded and the other two fractions were concentrated under reduced pressure to afford 50% MeOH (3.7 g) and MeOH (3.6 g). Petroleum ether fraction (20 g) was slurried with about (20 g) silica gel for column, dried, powdered and transferred to the top of a

glass column ($\Phi=4.5$, $L=80$ cm) packed with silica gel for column (600 g), slurried in petroleum ether. The column was eluted initially with petroleum ether, then with petroleum ether-EtOAc in the order of increasing polarities. The effluents were collected in fractions (2 L each). Each fraction was concentrated under reduced pressure and monitored by thin layer chromatography (TLC) plates. Similar fractions were combined together then concentrated. Five fractions were obtained (I-V). Fraction II (4 g, eluted by petroleum ether-EtOAc, 9:1) was further purified by silica gel column chromatography (CC) ($\Phi=2$, $L=80$ cm, 160 g silica), using petroleum ether-EtOAc gradient elution as a mobile phase to yield compound 1 [yellow oil, 100 mg, $R_f=0.64$, system; petroleum ether-EtOAc (98:2)]. Moreover, fraction III [7.1 g, eluted by petroleum ether-EtOAc, (85:15)] was further subjected to silica gel CC ($\Phi=3$, $L=80$ cm, 300 g silica) using petroleum ether-EtOAc gradient elution to give a mixture of two compounds 2 and 3 [white needle crystals, 2.3 g, $R_f=0.43$, system; petroleum ether-EtOAc (75:25)].

On the other side, the CHCl_3 fraction (78.7 g) was fractionated by VLC. The column ($\Phi=5$, $L=80$ cm) was packed with (800 g) silica gel for TLC and eluted initially with petroleum ether and the EtOAc concentrations were increased gradually to 100% EtOAc. The effluents were collected in fractions (2 L each). Each fraction was concentrated under reduced pressure and monitored by TLC plates. Similar fractions were combined together, then concentrated under reduced pressure. Seven fractions (I-VII) were collected. Compound 4 [white amorphous powder, 3.1 g, $R_f=0.64$, system; CHCl_3 -MeOH (98:2)] was obtained by precipitation from fraction V (10.8 g, eluted by petroleum ether-EtOAc, 60:40). Furthermore, fraction VII (20 g, eluted by EtOAc) was further purified by subjection again to silica gel CC ($\Phi=4.5$, $L=80$ cm). The column was filled with (800 g) silica gel for column. Elution started with 100% CHCl_3 and the MeOH concentrations were increased gradually till 100% MeOH. Eleven subfractions were afforded (VII-1 to VII-11). Subfraction VII-10 (950 mg) was further purified by precipitation to afford compound 5 [white amorphous powder, 17 mg, $R_f=0.36$, system; CHCl_3 -MeOH (90:10)]. Finally, the subterranean organs of *P. lamerei* were also powdered after drying in the shade to yield (280.5 g) fine powder. It was extracted by maceration with EtOH (90%) till exhaustion (4x, 3 L each) and then concentrated under reduced pressure till dryness to yield (51.9 g, TSOEE).

General experimental procedures

^1H - and ^{13}C -NMR spectrum was recorded on a JEOL JNM α -400 and Brüker Avance 200 MHz NMR (Germany) spectrometers with tetramethylsilane as an internal standard. Mass spectrum was taken on a JEOL JMS600 Mass Spectrometer (Japan).

Column chromatographic techniques

Silica gel column

Silica gel CC was performed on silica gel 60 [(E. Merck, Darmstadt, Germany), 70-230 mesh]. The wet packing method was used in the columns using the stated solvents (Mikes and Chalmer, 1966). The concentrated solution of the plant extract or fraction to be fractionated was mixed with a small amount of the silica for column, dried and powdered. It was loaded to the top of the column. Then, the column was eluted with appropriate solvents either isocratic or gradient elution.

Diaion HP-20 column

The concentrated solution of the plant extract or fraction to be

fractionated was applied to the top of the column. The column was eluted with H₂O and MeOH in the order of decreasing polarities (H₂O, 50% MeOH and MeOH). Finally, the column is washed with acetone.

Vacuum liquid chromatography (VLC) technique

Dry packing was applied with continuous gentle shaking, using silica gel for TLC as the adsorbent. The initial zone was prepared by mixing the concentrated solution of the mixture to be fractionated with an equal amount of silica gel and the dried fine mixture was applied to the top of the column. The column was eluted with suitable solvents using gradient elution technique by the assistance of vacuum pump.

TLC technique

Precoated silica gel 60 F₂₅₄ plates (E. Merck; 0.25 mm in thickness) were used for TLC analyses, visualized by spraying with a H₂SO₄ (10%) solution in EtOH and heating to around 150°C on a hot plate.

Antimicrobial study

Microbial strains

The following are the tested organisms used in this study.

Bacterial strains: *S. aureus* (Gram-positive, Facultative anaerobic bacteria), *E. coli* and *Klebsiella pneumonia* (Gram-negative, Facultative anaerobic bacteria) and *P. aeruginosa* (Gram-negative, Facultative aerobic bacteria).

Fungal strain: *C. albicans* (Diploid fungus). All the bacterial and fungal strains used in the current study were clinical isolates obtained from Microbiology Department, Faculty of Pharmacy, Minia University. The bacterial strains were cultured on Müller Hinton agar and *C. albicans* was cultured on Sabouraud agar.

Evaluation of the antibacterial and antifungal activities using agar-well diffusion technique

For estimation of the antimicrobial activity, the bacterial cultures were adjusted to 0.5 ml of 1×10^6 CFU/ml (0.5 McFarland turbidity) and the fungal culture was adjusted to the concentration 10^6 CFU/ml (Doughari, 2007). Fifteen ml of sterile, molten and cooled media, Müller Hinton or Sabouraud agar, were added to the petri dishes. Then, the plates were rotated gently and allowed to congeal on a flat surface. The media were then inoculated with the microorganisms using a sterile swab to evenly distribute bacterial or fungal culture over the appropriate medium. The plates were allowed to dry for 15 min before use, then four equidistant and circular wells of 7 mm diameter were punched into the agar medium using a sterile cork borer (Delahaye et al., 2009; Ogbulie et al., 2007; Ahmad and Beg, 2001). The wells were then loaded with 100 µl of (1.25 mg/ml) of plant extracts, fractions, solvent blank, antibiotics or antifungal drug. Then, the plates were allowed to stand for 1 h to allow the prediffusion of the drug, then they were incubated overnight at 37°C after that the antimicrobial activity was evaluated by measuring the area of inhibition against the test organism (Delahaye et al., 2009; Ogbulie et al., 2007; Bennett et al., 1966). The results are interpreted according to CLSI (2011) and summarized in Tables 2 to 4 and 6.

Determination of MIC

The MIC values of *P. lamerei* extracts, fractions, antibiotics and antifungal drug were determined using two-fold serial dilution to prepare concentrations of 1.25, 2.5, 5, and 10 mg/ml. Equal volumes of the extracts, fractions and antibiotics were applied separately to each well using a micropipette (Delahaye et al., 2009; Ogbulie et al., 2007). After incubation, the plates were collected and the inhibition zones that developed were measured then the averages of inhibition zones (IZs) were calculated. MICs were calculated by plotting the natural logarithm of the drug concentration against IZ square. A regression line was drawn through the points. The antilogarithm of the intercept on the logarithm of concentration axis gave MIC values (Esimone et al., 1998). The results are shown in Tables 5 and 7.

RESULTS

Phytochemical study

Air-dried leaves and stems of *P. lamerei* were extracted with EtOH (90%) and concentrated under reduced pressure. It partitioned with different solvents of increasing polarity.

The petroleum ether and CHCl₃ fractions were subjected to various chromatographic procedures including silica gel CC to afford five compounds (1-5). The structures of compounds were elucidated to be stearic acid methyl ester 1 (Basumatary and Deka, 2012; Gua et al., 2005), a mixture of two steroid aglycones, viz., β -sitosterol 2 and stigmaterol 3 (Maima et al., 2008), ursolic acid 4 (Babalola and Shode, 2013; Kontogianni et al., 2009; Lee et al., 2005) and β -sitosterol-3-O- β -D-glucopyranoside 5 (Kojima et al., 1990) by a comparison of their physical, chemical, chromatographic properties, spectral data with literature as well as direct comparison with authentic compounds (Figure 1 and Table 1).

Compound 1: Stearic acid methyl ester (Octadecanoic acid methyl ester)

¹H-NMR (400 MHz, CDCl₃): 3.61 (3H, s, H-1'), 2.25 (2H, t, J=7.9 Hz, H-2), 1.56 (2H, m, H-3), 1.19-1.24 (2H, m, H-4:H-17) and 0.84 (3H, t, J=7.9 Hz, H-18). ¹³C-NMR (100 MHz, CDCl₃): 51.20 (C-1', q), 173.49 (C-1, s), 34.01 (C-2, t), 31.90 (C-3, t), 29.12-29.66 (C-4 - C-15, t), 24.89 (C-16, t), 22.65 (C-17, t) and 14.05 (C-18, q).

El-MS analysis (positive mode) was given in (Figure 2) showed significant peaks at m/z: 298, 283, 269, 255, 241, 227, 199, 171, 143, 129, 101, 87 and 74.

Compound 4: Ursolic acid (3- β -hydroxy-urs-12-ene-28-oic-acid)

¹H-NMR (400 MHz, C₅D₅N): 3.45 (1H, dd, J=5.7, 9.9 Hz, H-3), 5.49 (1H, br.s, H-12), 2.63 (1H, d, J=11.2 Hz, H-18), 1.24 (3H, s, H-23), 0.90 (3H, s, H-24), 1.05 (3H, s, H-25), 1.03 (3H, s, H-26), 1.23 (3H, s, H-27), 1.00 (3H, br.s, H-

Table 1. ^{13}C -NMR spectral data of compounds (1-5).

No.	δ_{C} , Multiplicity					No.	δ_{C} , Multiplicity				
	(1) ¹	(2) ²	(3) ²	(4) ³	(5) ³		(2) ²	(3) ²	(4) ³	(5) ³	
C-1'	51.20, q	-	-	-	-	C-19	19.00, q	19.00, q	40.00, d	19.50 ^d , q	
C-1	173.49, s	37.68, t	37.68, t	39.20 ^a , t	37.60, t	C-20	36.25, d	36.25, d	39.50, d	36.40, d	
C-2	34.01, t	29.18, t	29.18, t	28.20 ^b , t	30.30, t	C-21	21.15, q	21.15, q	31.10, t	19.30 ^d , q	
C-3	31.90, t	71.12, d	71.12, d	78.20, d	78.20 ^a , d	C-22	34.04, t	138.69, d	37.50, t	34.30, t	
C-4	-	-	-	-	39.40, t	C-23	26.28, t	129.27, d	28.80, q	26.60, t	
C-4-C15	29.12-29.66, t	-	-	-	-	C-24	46.00, d	46.10, d	16.60, q	46.20, d	
C-5	-	141.81, s	141.81, s	55.90, d	141.00, s	C-25	29.28, d	29.28, d	15.70, q	29.60, d	
C-6	-	121.07, d	121.07, d	18.80, t	121.90, d	C-26	18.85, q	18.85, q	17.50 ^c , q	20.00, q	
C-7	-	32.49, t	32.49, t	33.60, t	32.20, t	C-27	19.45, q	19.45, q	24.00, q	19.10 ^d , q	
C-8	-	32.01, d	32.01, d	39.50 ^a , s	32.20, d	C-28	23.22, t	22.24, t	179.90, s	23.50, t	
C-9	-	50.32, d	50.32, d	48.10, d	50.50, d	C-29	11.85, q	12.38, q	17.50 ^c , q	12.20 ^c , q	
C-10	-	36.75, s	36.75, s	37.30, s	37.00, s	C-30	-	-	21.40, q	-	
C-11	-	21.21, t	21.21, t	23.70, t	21.40, t	Glc.	-	-	-	-	
C-12	-	43.37, t	43.37, t	125.70, d	40.00, t	C-1'	-	-	-	102.70, d	
C-13	-	42.22 ^b , s	42.35 ^b , s	139.30, s	42.60, s	C-2'	-	-	-	75.40, d	
C-14	-	56.85 ^c , d	57.00 ^c , d	42.60, s	56.90 ^b , d	C-3'	-	-	-	78.60 ^a , d	
C-15	-	24.40, t	24.40, t	28.70 ^b , t	24.60, t	C-4'	-	-	-	71.80, d	
C-16	24.89, t	28.40, t	28.40, t	25.00, t	28.60, t	C-5'	-	-	-	78.50 ^a , d	
C-17	22.65, t	55.910, d	55.91, d	48.10, s	56.40 ^b , d	C-6'	-	-	-	63.00, t	
C-18	14.05, q	12.00, q	12.00, q	53.60, d	12.00 ^c , q	-	-	-	-	-	

^a, ^b, ^c, ^d Assignments may be interchangeable. Glc.: Glucose. ¹(CDCl₃, 100 MHz), ²(C₅D₅N, 50 MHz) and ³(C₅D₅N, 100 MHz).

Table 2. IZs of *P. lamerei* and antibiotics against the tested organisms.

Microorganism	IZ (mm)										
	Amp.	Gen.	Clin.	Amik.	Unic.	Aug.	TSOEE	TLSEE	Pet. ether fr.	CHCl ₃ fr.	Aqu. fr.
<i>S. aureus</i>	60, S	50, S	70, S	50, S	70, S	62, S	35	10	30	32	17
<i>E. coli</i>	40, S	40, S	NT	40, S	43, S	40, S	27	29	20	22	21
<i>K. pneumoniae</i>	15, I	50S	NT	50, S	50, S	54, S	22	21	28	26	28
<i>P. aeruginosa</i>	NT	32	NT	37, S	10, R	10, R	31	25	28	22	22

NT: Not tested; S: Susceptible>35, R: Resistant<10, I: Intermediate (25>I>15).

29) and 0.96 (3H, d, J=5.8 Hz, H-30).

Compound 5: β -sitosterol-3-O- β -D-glucopyranoside

¹H-NMR (400 MHz, C₅D₅N): 3.96 (1H, m, H-3), 5.36 (1H, br.s, H-6), 0.68 (3H, s, H-18), 0.95 (3H, s, H-19), 1.28 (3H, br.s, H-21), 0.91 (3H, d, J=6.8 Hz, H-26), 0.87 (3H, br.s, H-27), 0.89 (3H, br.s, H-29) and 5.02 (1H, d, J=7.5 Hz, H-1').

Antimicrobial study

Antibacterial activity

Evaluation of the antibacterial activity of *P. lamerei* was

performed using agar-well diffusion technique. The petroleum ether fraction exhibited the lowest MIC (1.05 $\mu\text{g/ml}$) against *K. pneumoniae* among all tested extracts, fractions and antibiotics; even the broad spectrum antibiotics (Unictam[®] and Augmentin[®]) as shown in Table 5.

Although, *P. aeruginosa* has the ability to develop resistance to many classes of antibiotics (Lister et al., 2009), petroleum ether fraction succeeded to inhibit the growth of the opportunistic pathogen. MIC given by the petroleum ether fraction shown in Table 5 (1.06 $\mu\text{g/ml}$). TLSEE also showed low MIC (2.52 $\mu\text{g/ml}$) against *P. aeruginosa*, which was comparable to that obtained by the amikacin (2.38 $\mu\text{g/ml}$) as demonstrated in Table 5. The different extracts and fractions of *P. lamerei* were found to possess antibacterial activity against different

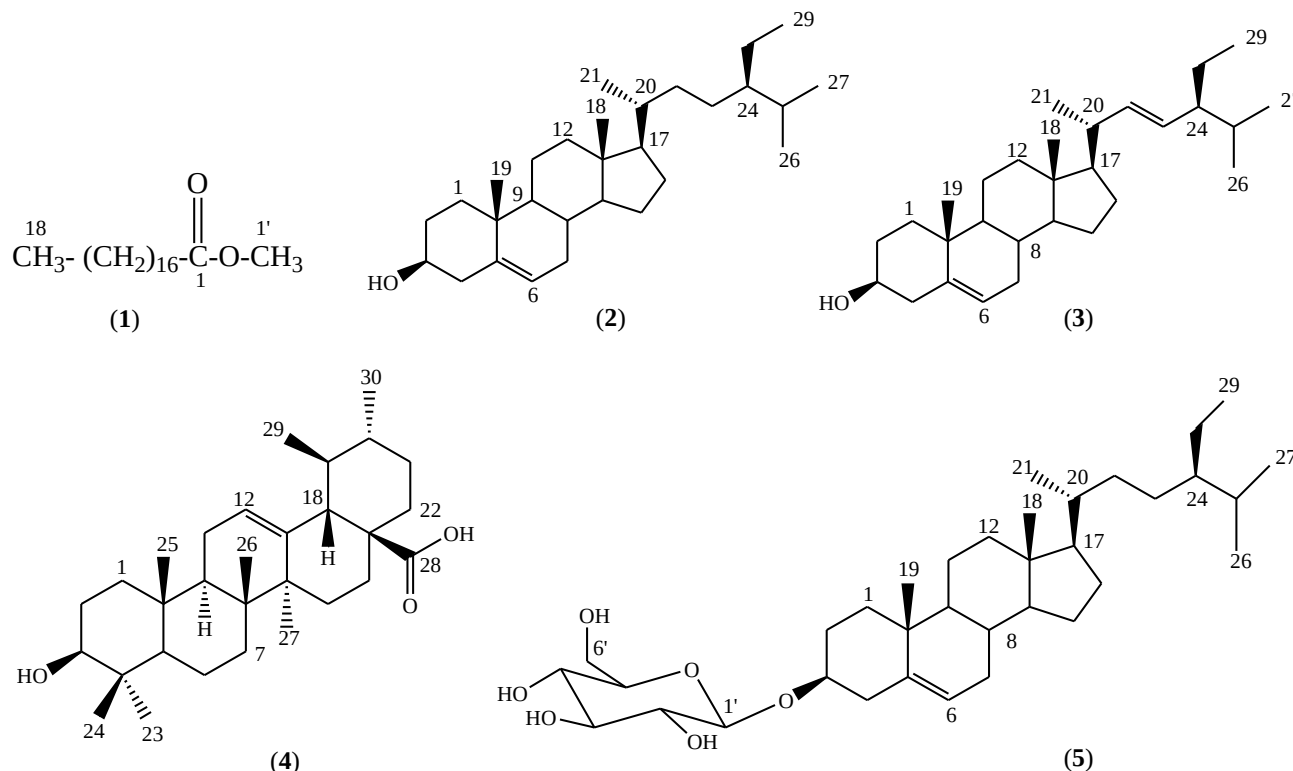


Figure 1. The structures of the isolated compounds (1-5).

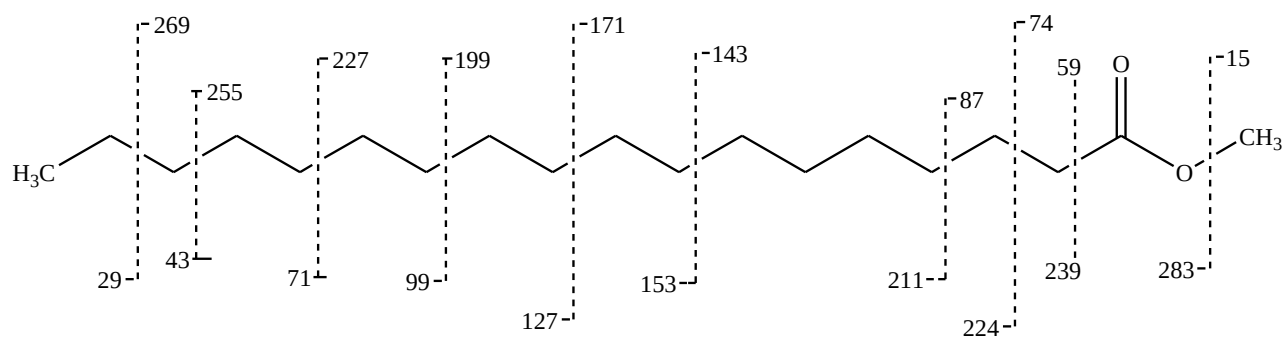


Figure 2. Fragmentation pattern of compound (1).

strains of bacteria. The TSOEE and aqueous fraction of *P. lamerei* showed different bacterial inhibition zones against *E. coli* and *K. pneumoniae*.

Antifungal activity

The inhibition zones of the different extracts and fractions of *P. lamerei* compared to the standard antifungal drug (ketoconazole) at a concentration of 1.25 mg/ml are determined and the results are interpreted according to CLSI (Pfaller, 1997) and summarized in Tables 6 and 7.

The highest IZ against *C. albicans* was exhibited by the CHCl_3 fraction (34 mm), which is very significant compared to that of ketoconazole. Furthermore, the petroleum ether fraction, TLSEE, aqueous fraction and TSOEE showed IZs 30, 27, 24 and 20 mm against the same fungus, respectively as shown in Table 6. The petroleum ether fraction showed the lowest MIC against *C. albicans* (16.45 $\mu\text{g/ml}$), which is very significant compared to that of the standard drug (185.87 $\mu\text{g/ml}$). Moreover, the TLSEE and the TSOEE also showed low MICs (69.48 and 170.39 $\mu\text{g/ml}$, respectively) as illustrated in Table 7. The CHCl_3 fraction exhibited low MIC (201.2

Table 3. IZs of the tested antibiotics according to CLSI.

Microorganism	IZ (mm)								
	Amp. (10 µg/ml)			Gen. (10 µg/ml)			Clin. (2 µg/ml)		
	S	I	R	S	I	R	S	I	R
<i>S. aureus</i>	≥ 29	-	≤ 28	≥ 15	13-14	≤ 12	≥ 21	15-20	≤ 14
<i>E. coli</i> and <i>K. pneumoniae</i>	≥ 17	14-16	≤ 13	≥ 15	13-14	≤ 12	-	-	-
<i>P. aeruginosa</i>	-	-	-	≥ 15	13-14	≤ 12	-	-	-

S: Susceptible; R: Resistant, I: Intermediate.

Table 4. IZs of the tested antibiotics according to CLSI.

Microorganism	IZ (mm)								
	Amik. (30 µg/ml)			Unic. (10 µg/ml)			Aug. (10 µg/ml)		
	S	I	R	S	I	R	S	I	R
<i>S. aureus</i>	≥17	15-16	≤14	≥15	12-14	≤11	≥20	-	≤19
<i>E. coli</i> and <i>K. pneumoniae</i>	≥17	15-16	≤14	≥15	12-14	≤11	≥18	14-17	≤13
<i>P. aeruginosa</i>	≥17	15-16	≤14	≥18	-	≤17	≥18	-	≤17

S: Susceptible; R: Resistant, I: Intermediate.

µg/ml) comparable to that of the antifungal drug ketoconazole as demonstrated in Table 7.

DISCUSSION

Phytochemical study

The isolated five compounds were reported for the first time in the tribe Malouetieae from the leaves and stems of *P. lamerei*.

Antimicrobial study

It is worth mentioning that this is the first time to evaluate the antibacterial and antifungal activities of the genus *Pachypodium* and to determine its MIC.

Antibacterial study

Although, the antibiotics are one of the most important weapons in fighting bacterial infections and greatly enhance the health-related quality of human life since their introduction, many of them produce toxic reactions. Also, the extensive use of antibiotics results in the emergence of drug-resistant strains of microorganisms. So, it is important to investigate newer drugs with lesser resistance (Kumar et al., 2012; Bhalodia and Shukla, 2011) and search for natural products having antimicrobial activity with less or no toxic reactions. Therefore, the authors of the present study determined

their aim. The authors evaluated the antibacterial activity of the different extracts and fractions of *P. lamerei*. The results were compared to that obtained by different narrow and broad spectrum antibiotics. This includes determining IZs and the MICs of the extracts, fractions and antibiotics using the agar-well diffusion technique as shown in Tables 2 and 5.

The antibacterial activity of *P. lamerei* may be attributed to the presence of many phytoconstituents such as sterols, tannins and triterpenes (El-Kashef et al., 2015b; Singh et al., 2012; Ogueke et al., 2007; Djoukeng et al., 2005). Moreover, ursolic acid has been reported extensively to exhibit antibacterial activity (Babalola and Shode, 2013; Liu, 1995).

Antifungal study

C. albicans is a member of the human's oral and gastrointestinal pathogens. Due to the presence of resistance to the antifungal drug ketoconazole, the search for new antifungal agents become necessary (Rathod et al. 2012; LaFleur, 2011).

From the results listed in Table 7, it was obvious that the petroleum ether fraction showed the lowest MIC (16.45 µg/ml). This result is highly promising since ketoconazole exhibit MIC (185.87 µg/ml), which means that the activity of petroleum ether fraction is nearly eleven times more than that of ketoconazole as shown in Table 7.

Sterols, triterpenes and tannins are reported in the plant (El-Kashef et al., 2015b). Moreover, Stigmasterol and

Table 5. MIC of *P. lamerei* and antibiotics against the tested organisms.

Microorganisms	MIC (µg/ml)										
	Amp.	Gen.	Clin.	Amik.	Unic.	Aug.	TSOEE	TLSEE	Pet. ether fr.	CHCl ₃ fr.	Aqu. fr.
<i>S. aureus</i>	2.06, R	16.45, R	15.05, R	1.73, S	1.3, S	79.4, R	162.07	967.5	42.46	9.21	114.36
<i>E. coli</i>	34.94, R	26.44, R	NT	28.96, S	1.2, S	1.13, S	3.35	105.19	91.62	52.29	3.46
<i>K. pneumoniae</i>	1060, R	37.6, R	NT	9.59, S	2.18, S	2.18, S	3.34	498.32	1.05	62.96	2.72
<i>P. aeruginosa</i>	NT	5.34, R	NT	2.38, S	181.7, R	1279.03, R	4.669	2.52	1.06	200.43	11.03

Table 6. IZ of *P. lamerei* and ketoconazole against *C. albicans*.

Microorganism	IZ (mm)					
	Ketoconazole	TSOEE	TLSEE	Pet. ether fr.	CHCl ₃ fr.	Aqu. fr.
<i>C. albicans</i>	40 S	20	27	30	34	24

IZ of ketoconazole according to CLSI. S: Susceptible; R: Resistant, I: Intermediate.

Table 7. MIC of *P. lamerei* and ketoconazole against the tested organisms.

Microorganism	MIC (µg/ml)					
	Ketoconazole	TSOEE	TLSEE	Pet. ether fr.	CHCl ₃ fr.	Aqu. fr.
<i>C. albicans</i>	185.87, S	170.39	69.48	16.45	201.2	378.03

MIC of ketoconazole according to CLSI. S: Susceptible; R: Resistant, I: Intermediate.

β -sitosterol are known to possess antifungal activity (Mbambo et al., 2012). The antifungal activity of *P. lamerei* may be attributed to the presence of these secondary metabolites (Bisoli et al., 2008; Lim et al., 2006; Smania et al., 2003).

Conclusion

Five compounds were report for the first time in the tribe Malouetieae from the leaves and stems

of *P. lamerei*. This is the first time to estimate both antibacterial and antifungal activities of any of *Pachypodium* species. The significant results of the antibacterial and antifungal activities make this plant a good candidate for the cytotoxic studies that are strongly recommended.

Conflict of Interests

The authors have not declared any conflict of interests.

Abbreviations

Amik., Amikacin; **Amp.**, ampicillin; **Aqu.**, aqueous; **A. flavus**, *Aspergillus flavus*; **A. niger**, *Aspergillus niger*; **A. terreus**, *Aspergillus terreus*; **Aug**, augmentin; **B. subtilis**, *Bacillus subtilis*; **C. albicans**, *Candida albicans*; **CHCl₃**, chloroform; **Clin.**, clindamycin; **CLSI**, Clinical and Laboratory Standards Institutes; **CC**, column chromatography; **E. coli**, *Escherichia coli*; **EtOH**, ethanol; **EtOAc**, ethyl acetate; **fr.**, fraction; **Gen.**,

gentamicin; **IZ**, inhibition zone; **K. pneumonia**, *Klebsiella pneumonia*; **MeOH**, methanol; **MIC**, minimum inhibitory concentration; **P. lamerei**, *Pachypodium lamerei*; **pet.**, petroleum; **P. vulgaris**, *Proteus vulgaris*; **P. aeruginosa**, *Pseudomonas aeruginosa*; **S. aureus**, *Staphylococcus aureus*; **TLC**, thin layer chromatography; **TLSEE**, total leaves and stems ethanolic extract; **TSOEE**, total subterranean organs ethanolic extract; **Unic.**, unictam and **VLC.**, vacuum liquid chromatography.

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