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Strelitzia reginae Leaves: Phytochemical Elucidation and Antimicrobial Assessment on Clinical Isolates and Standard Strains

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Abstract: *Strelitzia reginae* is used in some African countries as a traditional medicine with very few phytochemical and biological assessments. The study aims to isolate and structure elucidate of *S. reginae* phytoconstituents using different chromatographic and spectral techniques. In addition to, antimicrobial assessment of ethyl acetate and *n*-butanol fractions on both clinical isolates and standard strains. *Strelitzia reginae* belongs to the family Strelitziaceae which is a rich source of biologically active phytoconstituents and has diverse therapeutic values. *Strelitzia reginae* leaves were fractionated into ethyl acetate and *n*-butanol, and five compounds were detected. Compounds were identified as kaempferol **(1),** quercetin **(2),** daucosterol **(3)**, vitexin **(4),** and rutin **(5)**. The isolated compounds were elucidated by their NMR and ESI-MS spectral data. Antimicrobial activity of *Strelitzia reginae* ethyl acetate and *n*-butanol fractions screened against six different bacterial strains (three Gram-positive, three Gram-negative, and one fungus isolate). Both fractions showed antimicrobial activities against *Klebsiella pneumonia* and *Staphylococcus aureus* which is methicillin-resistant (MRSA) strains. As well, the activity of antimicrobial was detected against *Proteus mirabilis* clinical isolate from urine and MRSA. On the other hand, both fractions showed inadequate antimicrobial activities against the *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* clinical isolates compared to reference drugs. The antimicrobial results were in accordance with previously reported folk uses and attributed to the isolated phytoconstituents.

Keywords: Antimicrobial; Clinical isolates; Flavonoids; MRSA; Sterols; *Strelitzia reginae*.

1. INTRODUCTION

Family Strelitziaceae is a flowering plant belonging to the order Zingiberales[1]. The family Strelitziaceae includes three genera (*Ravenala, Phenakospermum, and Strelitzia*) all occurring in tropical to subtropical regions. The genus *Strelitzia* includes five species in southern Africa; *S. alba* (White bird of paradise), *S. caudate*, *S. nicolai*, *S. reginae* (Bird of Paradise), and *S. juncea* [2-5]. Zingiberales order-specific phytochemical markers are flavonoids which have been detected in several species of Strelitziaceae [6]. Phenalenones are secondary metabolites containing the phenalenone nucleus which are a unique class of fused three-ring system of hydroxyl-perinaphthenones[7]. It confirmed many biological activities such as anti-fungal, anti-malarial, and cytotoxic activity [8]. Also, steroids, triterpenes, and fixed oils were reported [9, 10]. Antimicrobial resistance largely indiscriminate use of antimicrobials i.e. misuse, overuse, and agricultural use of antibiotics,

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especially in developing countries such as Egypt [11]. Various techniques to combat antibiotic resistance have been proposed recently [12]**.** Phytochemicals possess significant antibacterial properties, and several studies have employed natural compounds to combat bacterial resistance [13]**.** These compounds could be used alone or in combination with antibiotics to boost antibacterial activity against a variety of bacteria [14]**.** Some African nations employed the roots of *Strelitzia reginae* as a traditional medicine to cure diseases brought on by bacterial pathogens, particularly urinary tract disorders and sexually transmitted disorders. [15]. According to the literature review, very few studies on *Strelitzia reginae* leaves have been conducted [16]**.** The antibacterial activity of *Strelitzia reginae* leaf extracts (chloroform and methanol) using well diffusion method[16]. Among the test samples, chloroform extract demonstrated antibacterial efficiency against *Staphylococcus aureus*, *Mycobacterium tuberculosis*, and *Streptococcus mutans*, while methanol extract displayed antibacterial efficiency against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter aerogenes* [16]. In this study, ethyl acetate and *n*-butanol fractions of *Strelitzia reginae* leaves were screened for their antimicrobial activity against standard strains and multidrug-resistant clinical isolates from different biological samples (urine, vaginal, diabetic foot infection, and wound), which lacked in the previous studies.

2. METHODS

2.1. Plant material

Strelitzia reginae leaves accumulated in November 2019 from El-zohria garden, Egypt. Material of plant identified thankfully by Dr. Ahmed Wahba, Horticulture Research Institute, Egypt. Voucher specimen (November 2019, SR-19) preserved in the Faculty of Pharmacy (Girls) at Al-Azhar University's Department of Pharmacognosy and Medical Plant herbarium.

2.2. Pure compounds extraction and isolation

The air-dried powdered leaves of *Strelitzia reginae* (3 kg) exhaustively extracted with 70% aqueous methanol (3 × 6 L). The methanol extract was evaporated by vacuum at 45 °C until dryness (190 g). The methanol extract was suspended in distilled water (500 ml) and the water-soluble component partitioned to afford; *n*-hexane (60 g), ethyl acetate (10 g), and *n*-butanol (45 g) fractions. The fraction of ethyl acetate was applied over the silica gel column chromatography (100 cm x 3.5 cm) (Si gel CC) 60 mesh (Merk)® and eluted with *n*-hexane - ethyl acetate (100:0→10:90). Similar fractions collected, evaporated, and concentrated under vacuum to afford three subfractions. Subfraction I (1 g) was chromatographed by successive sephadex LH_{20} CC (Pharmacia) (100 gm each) by isocratic elution with methanol to give compound **1 (20 mg)** and compound **2 (30 mg)**. Subfraction II (200 mg) rechromatographed by successive sephadex LH_{20} CC (70 g each) by isocratic elution with methanol to afford compound **3 (20 mg)**. Sub fraction III (400 mg) was rechromatographed by successive sephadex LH_{20} CC (100 mg each) by isocratic elution with methanol to afford compound **4 (40 mg)**. Similarly, the *n*-butanol fraction chromatographed by polyamide CC (130 cm x 5 cm) (250 g) by gradient elution using water-methanol (100:0→0:100). The effluent was monitored using TLC (Merk)® to afford six sub fractions. Sub fraction III (800 mg) was subjected to repeated sephadex LH_{20} CC (100 g each) using methanol to afford compound **5 (35 mg).**

2.3. Structural elucidation

2.3.1. Nuclear magnetic resonance (NMR) analysis

The Bruker spectrometer was used to record the NMR spectra, which were calibrated in parts per million (ppm) of downfield from the internal standard tetramethylsilane (TMS) at (400 MH for ^{1}H and 100 MH for ^{13}C). Resonances given in δ ppm (TMS, δ 0.0) and the acronyms listed below have been employed: the singlet (s), the doublet (d), the triplet (t), the multiplet (m), the broad singlet (brs), the coupling constant *(J*) in Hertz (Hz). The analysis done at the NMR unit of the Faculty of Pharmacy at Cairo University in Egypt.

2.3.2. Electrospray ionization-mass spectroscopy (ESI-MS) analysis

The Faculty of Pharmacy at Ain Shams University in Egypt used an (XEVO TQD) triple quadruple instrument "Milford, Waters Corporation, and MA01757 U.S.A." to perform positive ion mode ESI-mass spectrometry. Using Acetonitrile including 0.1% formic acid as a

carrier, samples were dissolved in 10 µL of this solution before being injected at a flow rate of 0.2 µL/min. The electrospray positive voltage was set at + 4.5 kv and nitrogen (30 psi) was used as sheath gas. Scans performed from *m/z* 50 to m/z 2000 in 2 seconds, and the spectra acquired were the average of a 2 minutes period. The multiplier voltage set at 1200V.

2.4. Antimicrobial activity

The Clinical and Laboratory Standards Institute (CLSI M100-S28) for antimicrobial susceptibility testing was used to estimate the minimum inhibitory concentration (MIC) by assessing the antimicrobial properties of *Strelitzia reginae's* ethyl acetate and *n*-butanol fractions [17, 18]. MHB (Muller-Hinton broth, 100 µl) (Oxoid® Limited, Basingstoke, UK) was dispersed in all wells of 96 multi-well microtiter plate (TPP-Swiss), and a volume of 100 µl from each ethyl acetate and *n*-butanol fractions was poured into each well of the microtiter plate's first row. Well-executed serial dilution from the first to the twelfth; this resulted in serial dilutions done twice. Lastly, 7 µl of freshly made bacterial suspension (1.5 x10^8 cfu/mL) with a matched turbidity of 0.5 McFarland added to each well. For each bacterial strain, positive and negative controls carried out. Plates were incubated for 18 to 24 hours at 37 ˚C. The MIC was calculated, in comparison to control wells, showed no discernible bacterial growth. Duplicates of each test were using; Amoxicillin, Ciprofloxacin, or Nystatin were available from (Sigma Aldrich, St. Louis, USA) which used as standard reference drugs. Antimicrobial activity was screened against the following microorganisms:

Gram-positive bacteria:

1. *Staphylococcus aureus* clinical isolate (isolated from wound infection)
2. *Staphylococcus aureus* that is methicillin-resistant (MRSA) (ATCC 43300)
3. Methicillin resistant *Staphylococcus aureus* (MRSA) clinical isolate (isolated from diabetic foot infection).

Gram-negative bacteria:

1. *Klebsiella pneumonia* (ATCC 700603).
2. *Pseudomonas aeruginosa* clinical isolate (isolated from wound infection)
3. *Proteus mirabilis* clinical isolate (isolated from urine sample)

Fungi:

1. *Candida albicans* clinical isolate (isolated from vaginal candida infection)

All standard strains were acquired from Al-Azhar University's Regional Center for Mycology and Biotechnology in Cairo, Egypt. The clinical isolates were obtained from the Al-Hussein hospital's Microbiology Laboratories at Al-Azhar University in Cairo, Egypt.

3. RESULTS

3.1. Spectral data

Compound 1: it was purified in the form of a yellow amorphous powder. ^{13}C NMR with predefined significant correlation with ^{1}H NMR were tabulated in (Table 1), compared with previously reported/published data[19]. The ESI-MS displayed pseudo molecular ion peak at *m/z*: 287 $[M+H]^+$ compatible with molecular formula $C_{15}H_{10}O_6$.

Compound 2: it was obtained as a yellow amorphous powder. ^{13}C NMR with predefined significant correlation with ^{1}H NMR were tabulated in (Table 1), compared with previously reported/published data[20]. The ESI-MS showed pseudo-molecular ion peak at *m/z* 303 $[M+H]^+$ compatible with the molecular formula $C_{15}H_{10}O_7$.

Compound 3: it was isolated as a whitish, amorphous powder. ^{13}C NMR with predefined significant correlation with ^{1}H NMR were tabulated in (Table 2), compared with previously reported/published data [21,22]. The positive ion mode of ESI-MS revealed a $[M+H]^+$ ion at *m/z* 577 that is compatible with the molecular formula $C_{35}H_{60}O_6$.

Compound 4: It was observed as an amorphous yellow powder. ^{13}C NMR with a predefined significant correlation with ^{1}H NMR were tabulated in (Table 1), compared with previously reported/published data [23-26]. The ESI-MS revealed molecular ion peaks corresponding with the molecular formula $C_{21}H_{20}O_{10}$ at *m/z* 433 $[M+H]^{+\cdot}$

Compound 5: it was obtained from *n*-butanol fraction as yellowish-white, amorphous powder. ^{13}C NMR with a predefined significant correlation with ^{1}H NMR were tabulated in (Table 1), compared with previously reported/published data [27, 28]. The pseudo molecular ion peak at *m/z* 611 $[M+H]^+$ was visible in the positive ion mode of the ESI-MS is compatible with the molecular formula $C_{27}H_{30}O_{16}$.

Table 1. [1]H NMR (400 MHz) and [13] C NMR (100 MHz), (DMSO-d_6) spectral data of compound 1, 2, 4, and 5.

Position	Compound 1	Compound 2		Compound 4		Compound 5		
	^{1}H-NMR[a]	^{1}H-NMR[a]	^{13}C-NMR[a]	^{1}H-NMR[a]	^{13}C-NMR[a]	^{1}H-NMR[a]	APT ^{13}C-NMR[a,b]	
2	-	-	147.19	-	164.37	-	156.87	C
3	-	-	136.20	6.78, s	102.90	-	133.75	C
4	-	-	176.27	-	182.53	-	177.82	C
5	12.40, s (OH)	12.49, s (OH)	161.16	13.17, s (OH)	161.63	12.6, s (OH)	161.66	C
6	6.16, d, *J*= 2.0	6.18, d, *J*=1.8	98.70	6.27, s	98.65	6.19, d, *J*=2.0	99.13	CH
7	-	-	164.58	10.45, s (OH)	163.23	10.86, s (OH)	164.52	C
8	6.40, d, *J*= 2.0	6.40, d, *J*=1.8	93.83	-	105.09	6.38, d, *J*=2.0	94.04	CH
9	-	-	156.60	-	156.47	-	157.06	C
10	-	-	103.37	-	104.42	-	104.42	C
1`	-	-	122.41	-	122.06	-	121.62	C
2`	8.04, d, *J*=8.7	7.67, d, *J*=2.1	116.06	8.03, d, *J*=8.2	129.42	7.55, d, *J*=2.1	116.72	CH
3`	6.92, d, *J*=8.7	9.54, s (OH)	145.53	6.89, d, *J*=8.2	116.29	9.74, s (OH)	145.20	C
4`	-	9.42, s (OH)	148.18	-	160.86	9.13, s (OH)	148.86	C
5`	6.92, d, *J*=8.7	6.88, d, *J*=8.4	115.48	6.89, d, *J*=8.2	116.29	6.84, d, *J*=8.2	115.68	CH
6`	8.04, d, *J*=8.7	7.55, dd, *J*=8.4,2.1	120.41	8.03, d, *J*=8.2	129.42	7.53, dd, *J*=8.2,2.1	122.04	CH
1``	-	-	-	4.69, d, *J*=9.7	73.85	5.35, d, *J*=7.3	101.63	CH
2``	-	-	-	3.82, t, *J*=9.2	71.32	3.05-3.35, m	74.52	CH
3``	-	-	-	3.26, m	79.13	3.05-3.35, m	76.35	CH
4``	-	-	-	3.49, m	71.00	3.05-3.35, m	70.82	CH
5``	-	-	-	3.76, d, *J*=11.3	82.31	3.05-3.35, m	76.89	CH
6``	-	-	-	3.50, m	61.75	3.72, m	67.44	CH_2
1```	-	-	-	-	-	4.38, brs	101.19	CH
2```	-	-	-	-	-	3.05-3.35, m	71.01	CH
3```	-	-	-	-	-	3.05-3.35, m	70.45	CH
4```	-	-	-	-	-	3.05-3.35, m	72.29	CH
5```	-	-	-	-	-	3.05-3.35, m	68.69	CH
6```	-	-	-	-	-	0.99, d, *J*=6.1	18.19	CH_3

δ value (ppm) and *J* value (Hz), [b] Hydrogenation pattern

3.2. Antimicrobial activity

In this study, *in vitro* screening of the potential antimicrobial activity of *Strelitzia reginae* fractions of ethyl acetate and *n*-butanol against several Gram-negative, Gram-positive bacteria, and one fungus isolates was carried out. The antimicrobial activity MICs, described in (Table 3). Both ethyl acetate fraction and *n*-butanol fraction have equipotent antimicrobial activity against Gram-negative bacteria, *Klebsiella pneumonia* (ATCC 700603), *Pseudomonas aeruginosa* and *Proteus mirabilis* clinical isolates. Both fractions were more potent than amoxicillin reference standard antibiotic, for *Klebsiella pneumonia* strain (ATCC 700603) and

Proteus mirabilis clinical isolate while their activities were equipotent to amoxicillin reference standard antibiotic against *Pseudomonas aeruginosa* clinical isolate. Regarding gram-positive bacteria, both fractions have equipotent activity against MRSA strain (ATCC 43300) and MRSA clinical isolate respectively, which is more potent than amoxicillin reference standard. Regarding ciprofloxacin reference standard antibiotic, both fractions were less potent against all tested strains and isolates. The MIC of both fractions against *Candida albicans* clinical isolate was 1.25 mg/ml which is greater than the standard antifungal nystatin MIC = 0.0156 mg/ml as mentioned in (Table 3).

Table 2. ^{1}H NMR (400 MHz) and ^{13} C NMR (100 MHz), (DMSO-d_6) spectral data of compound 3.

Position	**^{1}H-NMR**[a]	**APT ^{13}C-NMR**[a,b]		Position	**^{1}H-NMR**[a]	**APT ^{13}C-NMR**[a,b]	
1	1.47, brs	37.28	CH_2	19	0.96, s	19.38	CH_3
2	2.13, d, *J*=11.9 1.08, brs	29.46	CH_2	20	1.47, brs	35.93	CH
3	3.48 m	77.43	CH	21	0.91, d, *J*=6.1	19.06	CH_3
4	2.89, m 2.5, m	38.75	CH_2	22	1.23, m	33.80	CH_2
5	-	140.91	C	23	1.90, brs 1.28, brs	25.88	CH_2
6	5.32, m	121.68	CH	24	1.08, brs	45.59	CH_3
7	1.98, d, *J*=12 1.95, d, *J*=16.7	31.88	CH_2	25	1.81, m	29.16	CH
8	1.47, brs	31.83	CH	26	0.81, d, *J*=7.1	20.16	CH_3
9	0.96, s	50.06	CH	27	0.82, d, *J*=7.1	19.55	CH_3
10	-	36.66	C	28	1.23, m	23.06	CH_2
11	1.47, s	21.05	CH_2	29	0.79 d, *J*= 7.1	12.11	CH_3
12	1.98, d, *J*=12 1.16, d, *J*=7.5	38.75	CH_2	1`	4.22 d, *J*=7.7	101.22	CH
13	-	42.30	C	2`	3.07, m	73.92	CH
14	0.96, s	56.64	CH	3`	2.89, m	77.43	CH
15	1.50, brs	24.31	CH_2	4`	3.05, m	70.55	CH
16	1.30, brs	29.71	CH_2	5`	3.12, m	77.18	CH
17	1.16, d, *J*=7.5	55.88	CH	6`	3.42, b, dd, *J*=11.9, 5.5 3.65, a, dd, *J*=11.2, 5.8	61.54	CH_2
18	0.79, s	12.23	CH_3				

[a] δ value (ppm) and *J* value (Hz), [b] Hydrogenation pattern

Table 3. Antimicrobial activities of both *Strelitzia reginae* ethyl acetate and *n*-butanol fractions (**MIC (mg/ml).**

Gram-negative bacterial isolates and/or strains	**Ethyl acetate fraction**	***n*-Butanol fraction**	**Amoxicillin (Standard)**	**Ciprofloxacin (Standard)**
***Klebsiella pneumonia* strain (ATCC 700603)**	2.5	2.5	> 4	0.125
***Pseudomonas aeruginosa* clinical isolate**	0.625	0.625	0.5	≤ 0.0078
***Proteus mirabilis* clinical isolate**	1.25	1.25	> 4	0.03125
Gram-positive bacterial isolates and/or strains	**Ethyl acetate fraction**	***n*-Butanol fraction**	**Amoxicillin (Standard)**	**Ciprofloxacin (Standard)**
***Staphylococcus aureus* clinical isolate**	1.25	2.5	0.0156	≤ 0.0078
MRSA strain (ATCC 43300)	1.25	1.25	> 4	-
MRSA clinical isolate	2.5	2.5	> 4	0.125
Fungi	**Ethyl acetate fraction**	***n*-Butanol fraction**	**Nystatin (Standard)**	
***Candida albicans* clinical isolate**	1.25	1.25	0.0156	

4. DISCUSSION

4.1. Interpretation of isolated phytoconstituents

Kaempferol (Figure 1)

The compound's ^{1}H NMR spectrum revealed a clear Flavonol-type pattern. The presence of two meta-coupled signals for proton at δH 6.16 (d, *J*=2.0 Hz for H-6) and δH 6.40 (d, *J*=2.0 Hz for H-8), together with H-3 proton signal absence represent flavonol skeleton. ^{1}H NMR spectral data demonstrated the AA'BB system's presence, characteristic of the 4`-oxygenated aromatic B ring (comprising the protons at C-3`, -5` and C-2`, -6`) which showed; the four-peak pattern of two doublets at δH 6.92 (H-3`, -5`) and δH 8.04 (H-2`, -6`) (each 2H, *J*= 8.7 Hz). These are typically ortho-related protons, indicating only one substituent at C-4`. From the above-mentioned data and from the published data[19]**;** compound **1** was recognized as kaempferol.

Quercetin (Figure 1)

The compound's ^{1}H NMR spectrum revealed a clear Flavonol-type pattern. Aromatic proton signals are present; two meta-coupled protons at δH 6.18 (d, *J*= 1.8 for H-6) and δH 6.40 (d, *J*= 1.8 for H-8), together with H-3 proton signal absence explain the above mentioned flavonoid skeleton. As well ^{1}H NMR spectral data demonstrated the ABX system's presence indicative of a 3`,4`-substituted B ring at δH 7.67 (d, *J*,= 2.1 for H-2`), 6.88 (1H, d, *J*= 8.4 Hz for H-5`) and 7.55 (dd, *J*,=8.4, 2.1 for H-6'). The ^{13}C NMR spectral data showed significant fifteen carbon signals which were assigned for fifteen carbons indicative of a 3`,4`-substituted B ring. Seven signals were observed in oxygenated carbons (C-2, -3, -5, -7, -9, -3`, -4`), seven signals were observed in the region (93.83-122.41) revealed the presence of seven aromatic non-oxygenated carbons (C-6, -8, -10, -1`, -2`, -5`, -6`), and one carbonyl signal (C-4) at (δC 176.27). The observed spectroscopic data together with literature spectroscopic data comparison; compound 2 established to be quercetin [20].

Daucosterol (Figure 1)

The compound's ^{1}H NMR spectrum revealed two tertiary singlets of methyl at δH 0.79 for (Me-18) and 0.96 for (Me-19), three secondary of methyl doublets at δH 0.82 (*J*= 7.1 Hz for Me-27), 0.81 (*J*= 7.1 Hz for Me-26) and 0.91 (*J* = 6.1 Hz for Me-21) and a primary of methyl triplet at δH 0.79 (*J*= 7.1 Hz for Me-29). Furthermore, the ^{1}H NMR spectrum displayed a one-proton multiplet at δH 5.32 corresponding to H-6 of the trisubstituted olefinic bond. The oxygenated methine H-3 proton was assigned a one-proton multiplet at δH 3.48. The presence of an anomeric proton at δH 4.22 (d, *J*= 7.7 Hz for H-1`) in the molecule proved the existence of a sugar moiety, methylene-6` protons at δH 3.42 (dd, *J*= 11.9, 5.5 for H-6b) and δH 3.65 (dd, *J*= 11.2, 5.8 Hz for H-6a) and other sugar-protons at δH 2.89-3.12 (H-2`, -3`, -4`, -5`). The ^{13}C NMR spectral data showed 35 signals, 29 were provided with the aglycone, and 6 were assigned for the sugar moiety. It showed signals for the sp2 olefinic carbons at δC 140.91 (C-5) and 121.68 (C-6), carbinol carbon at δC 77.43 (C-3`), methyl carbons at δC 12.11 (C-29), 12.23 (C-18), 20.16 (C-26), 19.38 (C-19), 19.55 (C-27) and 19.06 (C-21). In addition, one anomeric carbon at

δC101.22 (C-1`) was observed for the glucose moiety. The observed downfield shift of the C-3 signals corroborated the location of the glucosyl residue at C-3 of the aglycone. (Δδ 5 ppm) and the up field shift of the C-2, -4 signals (Δδ 2 and 3 ppm, respectively) respect to their associated *β*-sitosterol values [24]**.** Based on these spectral data and by comparison to the published data; compound 3 established to be *β*-sitosteryl-3-O-*β*-D-glucopyranoside (daucosterol) [21,22].

Vitexin (Figure 1)

The ^{1}H NMR spectral data revealed an AA`BB` spin-system at δH 6.89 (2H d, *J* = 8.2 Hz for H-3`, H-5`) and δH 8.03 (2H d, *J* = 8.2 Hz for H-2`, H-6`) indicated that the B-ring was substituted at C-4`. The observation of two sharp aromatic proton singlets at δH 6.27 and 6.78 for H-6 and H-3, respectively, along with the absence of the H-8 proton signal, indicated H-8 substitution by C-glucosyl moiety. The one anomeric proton signal was found at δH 4.69 (1H, d, *J* = 9.7 Hz for H-1``) characteristic for C-glucoside. ^{13}C NMR spectral data displayed 19 significant signals of carbon from which 13 carbon signals assigned to the apigenin moiety. The aglycone signals assigned for one carbonyl carbon δC (182.53), five oxygenated aromatic carbons at δC 156.47 (C-9), 160.86 (C-4`), 161.63 (C-5), 163.23 (C-7) and 164.37 (C-2) and seven non-oxygenated aromatic carbons at δC 98.65 (C-6), 102.90 (C-3), 104.42 (C-10), 105.09 (C-8),116.29 (C-3`, -5`), 122.06 (C-1`) and 129.42 (C-2`, -6`). Given the fact that C-glycosylation of a flavonoid nucleus shifts the signal of the corresponding aglycone carbon downfield while the ortho carbons up field shifted and the other carbon signals are not significantly affected [23]. Therefore, comparing the carbon shifts of compound 4 to those showed in published apigenin data [24] revealed a downfield shift of C-8 (Δδ 11 ppm) and up filed shift of C-7 (Δδ 1 ppm) which indicated that the glucose substitution occurred at C-8 [25]**.** From the above-mentioned data, compound 4 was recognized as apigenin-C-8-*β*-D-glucopyranoside (vitexin) and is in accordance with the published data [26].

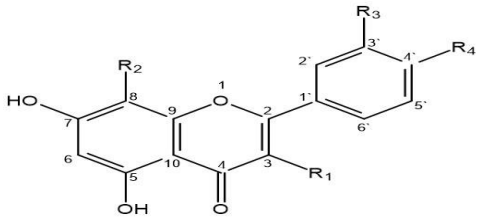
Rutin (Figure 1)

The ^{1}H and ^{13}C NMR spectra of compound 5 were similar to those of compound 2 with the exception of changes relating to sugar moiety. The ^{13}C NMR spectral data of compound 5 displayed 27 carbon signals including fifteen carbon signals for the aglycone, identical with those reported for quercetin together with twelve oxygenated aliphatic carbon signals at δC 67.44 - 76.89 for the bi-glycosidic sugar moiety. The C-3 glycosylation of compound 5 indicated by the up field shift of C-3 (Δδ 2 ppm) and the downfield shift of C-2 (Δδ 9 ppm) in comparison with compound 2 (quercetin) carbon shifts. The bi-glycosidic sugar moiety attested due to presence of two signals of anomeric protons at δH 5.35 (d, *J*=7.3 for H-1``) with δC 101.63 ppm (glucose), and at δH 4.38 (brs for H-1```) with δC 101.19 ppm (rhamnose). The appearance of the intense sharp signal at δH 0.99 (3H, d, *J*= 6.1 Hz) with δC 18.19 in the ^{1}H- and ^{13}C NMR spectra respectively; recognized the rhamnose unit. The downfield shift of C-6´´ of glucose; δC 67.44 indicated the attachment of rhamnose unit to C-6´´ of glucose (effect of rhamnosylation) [27]. Furthermore, the downfield- shift of glucose anomeric proton; δH 5.35 compared to that of rhamnose; δH 4.38 indicated that the glucose unit must be linked to the aglycone directly. As a result of the above mentioned data and comparison to the published data [28]; compound 5 was recognized as quercetin-3-O-rutinoside (rutin).

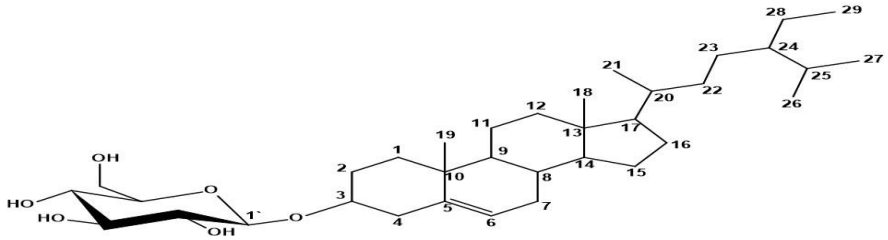
4.2. Antimicrobial activity

Antibacterial medications have become less effective, or useless, because of resistance development [29]. Interestingly, traditional South African medicine used *strelitzia reginae* to treat illnesses brought on by bacterial pathogens. [16]. This is directed our mind to explore its antimicrobial potentials especially against drug resistant strains. In this study, *in vitro* screening of the potential antimicrobial activity of *Strelitzia reginae* leaves ethyl acetate and *n*-butanol fractions against several Gram-positive, Gram-negative bacteria, and one fungal isolates was carried out via micro- broth dilution test for MIC measurement. Both ethyl acetate and *n*-butanol fractions showed antimicrobial activities against MRSA (ATCC 43300) strain and MRSA isolate from diabetic foot infection with MIC ranges from 1.25 - 2.5 mg/ml compared to reference drug amoxicillin (MIC > 4 mg/ml) and ciprofloxacin (MIC = 0.125 mg/ml). MRSA identified as a major nosocomial pathogen and designated as "priority pathogen" by the global health organization (WHO). It has effective clones and transmit rapidly along with being resistant to many antibiotics [30]. Additionally, both fractions showed activities against Gram negative bacterial strain; *Klebsiella pneumonia* (ATCC 700603), *and Proteus mirabilis* urine clinical isolate with MIC

ranges from 1.25 - 2.5 mg/ml compared to reference drugs amoxicillin (MIC > 4 mg/ml) and ciprofloxacin (MIC = 0.03125, 0.125 mg/ml, respectively). On the other hand, both fractions showed weak antimicrobial efficacy against Gram-positive (*Staphylococcus aureus*) Gram-negative (*Pseudomonas aeruginosa*) wound clinical isolates compared to reference drugs amoxicillin, and ciprofloxacin along with fungus (*Candida albicans*) vaginal clinical isolate, compared to reference drug nystatin as shown in (Table 3). These results were in good agreement with the reported traditional uses especially against bacterial pathogens causing urinary tract infections. Given the fact that flavonoids and daucosterol have antimicrobial potentials may be attributed to the isolated phytoconstituents (kaempferol**,** quercetin**,** daucosterol, vitexin, and rutin) [31, 32].



Compounds	R_1	R_2	R_3	R_4
Kaempferol	OH	H	H	OH
Quercetin	OH	H	OH	OH
Rutin	O-Rutinose	H	OH	OH
Vitexin	H	Glucose	H	OH



β-sitosteryl-3-*O*-*β*-D-glucopyranoside (Daucosterol)

Figure 1. Chemical structures of isolated phytoconstituents of *Strelitzia reginae* leaves extract.

5. CONCLUSIONS

Finally, the therapeutic antimicrobial potential of *Strelitzia reginae* requires further assessment with different microorganisms. Regarding, the above results along with early *in vitro* studies in addition to antimicrobial phytoconstituents recognition, we can conclude that *Strelitzia reginae* considered as promising antimicrobial drug after further safety studies to encourage its medicinal use.

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Shaimaa M. Azzam; data curation, review, editing, and supervision.

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