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Antimicrobial activity of *Mystroxylon aethiopicum* leave extracts and isolated 3-O-acetyloleanolic acid

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Mystroxylon aethiopicum (Thumb.) Loes subsp. *schlechteri* (loes.) R. H. Archeris, a member of the Celastracea family, is widely used in traditional medicine to treat several diseases, such as hemorrhagic diarrhoea and respiratory tract infections. The study aimed to isolate the biologically active compounds from the leaves of *M. aethiopicum*. The leaves were ground to a fine powder and sequentially extracted with *n*-hexane, dichloromethane, acetone and methanol using serial exhaustive extraction (SEE) method. The bioautography and broth micro-dilution methods were used to analyse the antibacterial activities. The bioautography assay revealed that the compounds in the *n*-hexane, dichloromethane and acetone extracts have activity against all tested microorganisms. In contrast, the methanol extracts exhibited no antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli*. The *n*-hexane extract had the lowest average MIC of 0.08 mg/mL, followed by the acetone (0.71 mg/mL) and dichloromethane (0.82 mg/mL) extracts. The bioassay-guided fractionation of dichloromethane and acetone extracts led to the isolation of 3-O-acetyloleanolic acid. The structure of the isolated compound was elucidated using NMR data and comparison with literature data. This is the first report on the isolation of 3-O-acetyloleanolic acid from the leaves of *M. aethiopicum*.

Keywords: Antibacterial activity, Minimum inhibitory concentration, *Mystroxylon aethiopicum*, 3-O-acetyloleanolic acid

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Introduction

The abrupt population growth in urban areas has initiated a massive demand for medicinal plants collected and supplied to urban areas to be merchandised in shops and informal markets. This can be observed in Gauteng Province, where 25.4% of the population in South Africa lives[1]. In this province, health practitioners assemble to sell and market their medicine at the Faraday *Muthi* Market. It has been reported that about 60% of the population in South Africa consults traditional health practitioners since herbs are affordable, readily available and contain fewer side effects[2]. Medicinal plants are also sold in shops and informal markets[3]. This is a clear indication that traditional health practitioners play a crucial role in the lives of people, and due to the lack of formal records of the traditional medicine systems, the information is passed on verbally from one generation to the next, which is why it is important to conserve this knowledge[4]. Using medicinal plants gathered over the years has empowered scientists to isolate different drugs such as aspirin, morphine, and quinine[5]. Plant extracts have also been explored for antimicrobial agents[6,7].

Mystroxylon aethiopicum (Thumb.) Loes subsp. *schlechteri* (loes.) R.H. Archer, commonly known as bushveld kooboob berry (English) and a member of the Celastracea family, is widely distributed in the highlands of Arusha and Kilimanjaro, where it is locally known in the Maasai language as Oldonyanangui. In Kenya, the stem barks of the plant are boiled and used separately or with meat soup for health benefits and as treatment of various ailments[8]. The root bark extract of this plant is also reported to be used in Kenya for making tea, consumed by children as stomach medicine[9]. The stem barks of this plant are commonly used in Madagascar and are valued specifically for their beneficial effect on the stomach[10].

Moreover, the plant leaves are traditionally used in Uganga to treat helminthosis[11]. The extracts of

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M. aethiopicum exhibited antibacterial activities against *P. aeruginosa*, *K. oxytoca*, *P. mirabilis*, *K. pneumoniae*, *S. kisarawe*, *S. typhi* and *E. coli*[12]. The toxicity study by Kilonzo *et al*.[13] on the root bark aqueous extract tested in albino mice revealed that the plant is safe when administered orally up to 5000 mg/kg. However, the root bark extracts were found to be toxic against brine shrimp larvae[14]. There are no reports on the isolation of compounds from *M. aethiopicum*. Therefore, this study was aimed at the bio-assay-guided isolation of the biologically active compounds from the leaves of *M. aethiopicum* that could be used as novel antibacterial agents.

Materials and Methods

Plant collection, identification and extraction

The leaves of *Mystroxylon aethiopicum* (UNIN 121992) were collected during the summer (February 2019) at the University of Limpopo, South Africa and identified by Dr. B. Egan of the Larry Leach Herbarium (UNIN, South Africa). The leaves were dried and ground to a fine powder using a blender. The mass of 700 g of the ground leaf material was extracted with 7 L of each solvent (*n*-hexane, dichloromethane, acetone and methanol) in order of increasing polarity in a glass bottle. The mixture was vigorously shaken using a shaker (Thermo Scientific MaxQ 3000) at 200 rpm. Shaking was done thrice (overnight and twice in 3 h intervals). The supernatant was filtered, concentrated using a rotary evaporator (Buchi R-114) at 50°C and transferred into pre-weighed labelled beakers (250 mL). The remaining solvents were evaporated under a stream of cold air at room temperature, and the masses of the crude extracts were determined. The extracts were then reconstituted using acetone to a final concentration of 10 mg/mL.

Phytochemical analysis by TLC

Chemical constituents of each of the extracts obtained were analysed using aluminium-backed Thin Layer Chromatography (TLC) plates (ALUGRAM® SILg/ UV254-MACHEREY-NAGEL, Merck) that were developed in either one of the three eluent solvent systems developed by Kotze and Eloff[15], ethyl acetate: methanol: water: 40:5.4:5 [EMW] (polar), chloroform: ethyl acetate: formic acid:10:8:2 [CEF] (intermediate polarity: acidic), benzene: ethanol: ammonium hydroxide:18:2:0.2 [BEA] (nonpolar/basic). Exactly 10 µL of the extracts were loaded on the TLC plate and developed without delay to minimise the possibility of photo-oxidative change. The separated components were visualised under visible and ultraviolet light (245 and 365 nm, Camec Universal UV lamp TL-600). For the detection of chemical compounds not visible on UV light, the chromatograms were sprayed with vanillin-sulphuric acid reagent [0.1 g vanillin (Sigma®): 28 mL methanol: 1 mL concentrated sulphuric acid] and heated at 110°C for 1–2 min for colour development.

Antibacterial activity assays

Microorganisms used in the study

The four bacterial species selected were: Gram-positive (*Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212) and Gram-negative (*Psuedomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922). These microorganisms were selected based on the recommendation of the Clinical Laboratory Standards Institute (CLSI)[16]. The bacterial species were maintained on nutrient agar at 4°C, later inoculated in nutrient broth, and incubated at 37°C for 12 h before the screening procedures.

Qualitative antibacterial assay by bioautography method

The qualitative analysis of the number of antimicrobial compounds found in the extracts was determined by the bioautographic method[17]. The TLC plates were loaded with 20 µL of each extract (10 mg/mL) and developed in EMW/ CEF/ BEA. The plates were then dried at room temperature under air for 5 days to remove the remaining solvent. The developed TLC plates were sprayed with a concentrated suspension of bacterial cultures of *S. aureus*, *E. faecalis*, *P. aeruginosa*, and *E. coli*, which were grown overnight in nutrient broth at 37°C until completely moist. This process was carried out in a laminar flow cabinet (Labotec). After that, the plates were incubated overnight at 37°C in 100% humidity. The plates were then sprayed with a 2 mg/mL solution of *p*-iodonitrotetrazolium violet (INT) (Sigma-Aldrich) and further incubated for 2–6 h. Bacterial growth led to a purple-pink colour resulting from the reduction of INT into the corresponding formazan salt. White bands indicated the inhibition of the bacteria by the active compounds present.

Quantitative antibacterial assay by serial broth microdilution method

A serial micro dilution assay[18] was used to determine the extracts Minimum Inhibitory Concentration (MIC), using INT reduction as an indicator. This was determined against Gram-positive *S. aureus*, *E. faecalis* and Gram-negative *P. aeruginosa* and *E. coli*. Aliquots of the extracts were dissolved in acetone to a final concentration of 10 mg/mL. Two-fold serial dilutions of extracts (2.5-0.02 mg/mL) were prepared in a 96-well microtitre plate, and 100 µL of the microbial culture was added to each well. Chloramphenicol (Sigma-Aldrich) was used as a positive control, and acetone as a negative control. As the indicator of growth, 40 µL of 0.2 mg/mL *p*-iodonitrotetrazolium violet (INT) (Sigma-Aldrich) was added to the microtitre wells. The covered microtitre plates were incubated and examined after 30 min at 37°C at 100% relative humidity. Where bacterial growth was inhibited, the solution in the well showed a marked reduction in the intensity of the colour after incubation with INT. Where bacterial growth occurred, it was seen by the presence of a pink/purple colour on the wells. The MIC was recorded as the lowest concentration of the extract that inhibited bacterial growth. All samples were assayed in triplicates.

Isolation of antibacterial compounds by bioassay-guided fractionation

The dichloromethane and acetone extracts were selected to be subjected to open-column chromatography, as they exhibited high antibacterial activity. An open column (35 cm × 4 cm) was packed with silica gel 60 (Fluka) using 100% *n*-hexane. The extracts (130.17 g) were eluted using 2 L of the solvent systems of increasing polarity of *n*-hexane/ethyl acetate and ethyl acetate/methanol. A total of 15 fractions were collected and concentrated using a rotary evaporator (Buchi R-114). The fractions were then analysed on TLC and tested for antibacterial activity. The fractions 4-9 [*n*-hexane (70, 50, 30 and 10%) and ethyl acetate (100 and 90%)] were further fractionated on another column (63 cm × 4 cm) and eluted using 80% chloroform in ethyl acetate. The phytochemical profile of the fractions was analysed on the TLC and then tested for antibacterial activity. The fractions were grouped into 5 groups that were then combined since they had similar TLC profiles and biological activities. The combined fractions were labelled as compound 1. The structure of the isolated compound was elucidated from the data obtained from ^{1}H- and ^{13}C-NMR spectra (Supplementary Figs. 1-3). This is in agreement with the reported data by Endo *et al*.[19].

Results and Discussion

The extracts from Serial Exhaustive Extraction were spotted on the TLC plates and developed in BEA, CEF, and EMW mobile phases for phytochemical analysis. The MIC values ranged from 0.08-2.5 mg/mL (Table 1). The *n*-hexane (H1) extract had the lowest average MIC of 0.08 mg/mL, followed by acetone (A1) (0.71 mg/mL) and dichloromethane (D1) (0.82 mg/mL). The *n*-hexane, dichloromethane and acetone extracts had high activity against all tested microorganisms, whereas the methanol extracts had the least activity.

In literature, MIC values of ≤ 0.5 mg/mL are considered to be exhibited by strong microbial inhibitors[20]. Therefore, dichloromethane (D1-3) and acetone (A1) extracts were selected to isolate antibacterial compounds. The combined dichloromethane and acetone extracts (24.28 g) were subjected to column chromatography, where they were eluted with solvents of varying polarities. Of the fifteen fractions collected, Fraction 4 had the lowest average MIC against all tested microorganisms (0.65 mg/mL), followed by fraction 8 (0.94 mg/mL) and fraction 9 (1.56 mg/mL).

The results obtained from first-column chromatography showed that the targeted biologically active compound was nonpolar. Therefore, bioautography of *M. aethiopicum* fractions was done using different nonpolar solvent combinations as mobile phases as guidance to determine the combination that best separates the targeted compound from the other compounds. The 80% chloroform in ethyl acetate combination separated the compound of interest and was used as the eluent system for the second column chromatography. The fractions 4-9 [*n*-hexane (70, 50, 30, and 10%) and ethyl acetate (100 and 90%)] were subjected to second column

Table 1 — The MIC values of *M. aethiopicum* plant extracts (mg/mL) against the test microorganisms

Microorganisms	H1	H2	H3	D1	D2	D3	A1	A2	A3	M1	M2	M3	Avg	PC
S. aureus	0.08	0.31	0.31	0.16	0.16	0.31	0.31	2.5	0.63	1.25	2.5	2.5	0.92	0.02
E. faecalis	1.25	2.5	2.5	0.63	0.63	0.63	0.63	2.5	1.25	2.5	2.5	2.5	1.67	0.02
P. aeruginosa	0.63	0.63	0.63	1.25	1.25	2.5	1.25	1.25	2.5	2.5	2.5	2.5	1.62	1.25
E. coli	1.25	2.5	2.5	1.25	0.63	1.25	0.63	2.5	0.63	2.5	2.5	2.5	1.72	2.5
Average	0.80	1.49	1.49	0.82	0.67	1.17	0.71	2.19	1.25	2.19	2.5	2.5		

Keywords: H= *n*-Hexane, D= Dichloromethane, A= Acetone, M= Methanol, Avg= Average, PC= Positive control (Chloramphenicol)

Table 2 — ^{1}H and ^{13}C NMR ($CDCl_3$) data of the isolated compound.

Position	^{1}H	^{1}H^{a}	^{13}C	^{13}C^{a}
1	1.24 m and * m	* and ***	38.3	38.1
2	** m and 1.96 m	*** and 1.87-1.90 m	23.3	23.5
3	3.21 *dd* (*J*= 4, 10 Hz)	4.49 m	78.9	80.9
4	-	-	38.7	37.7
5	0.84 m	0.83-0.88	55.1	55.3
6	1.24 m and **	** and ***	18.2	18.2
7	***	**	32.8	32.6
8	-	-	39.1	39.3
9	*	***	47.5	47.6
10	-	-	37.0	37.0
11	** and *	** and 1.87-1.90 m	22.8	23.4
12	5.26 brs	5.28 t (*J*=3.5 Hz)	122.5	122.6
13	-	-	143.5	143.6
14	-	-	41.5	41.6
15	1.24 m and †	*and***	27.6	27.7
16	*** and 1.96 m	**and 1.99 dt (*J*=13.6, 13.6,3.9 Hz)	22.8	22.9
17	-	-	46.4	46.5
18	2.78 *dd* (*J*=4, 9.6 Hz)	2.82 *dd* (*J*=13.6, 4.1 Hz)	40.9	41.0
19	1.24 m and †	* and ***	45.8	45.9
20	-	-	30.7	30.7
21	** and ***	**	33.7	33.8
22	** and †	***	32.5	32.4
23	0.88 s	0.85 s	15.5	16.7
24	0.89 s	0.87 s	28.1	28.0
25	0.87 s	0.94 s	15.3	15.4
26	0.75 s	0.76 s	17.0	17.1
27	1.11 s	1.13 s	25.9	25.9
28	-	-	183.1	182.7
29	0.97 s	0.93 s	23.5	23.6
30	0.90 s	0.91 s	33.0	33.1
acetyl CH_3	2.16	2.05 s	21.1	21.3
acetyl C=O	-	-	171.2	171.0

^{a}Endo *et al*., 2019; *1.00-1.13 ppm; **1.17-1.48 ppm; ***1.52-1.81 ppm; *1.85-1.88 m; **1.51-1.64 m; ***1.32-145 m; †1.68-1.78 m

chromatography using 80% chloroform in ethyl acetate as the eluent system. A total of 440 fractions were collected in test tubes and spotted on TLC plates to determine their phytochemical profile. The fractions with similar phytochemical profiles were combined. The groups showed activity against *E. faecalis*. The average MIC of the fractions against *E. faecalis* was 125 µg/mL, compared to the control, chloramphenicol, which exhibited a similar MIC value. The five groups of fractions were combined to form one compound since they had a similar phytochemical profile and biological activities, suggesting they have the same compound. The compound was further characterised using Nuclear Magnetic Resonance (NMR) data for structural elucidation.

The ^{13}C and ^{1}H chemical shift values from the NMR spectra of the isolated compound were in agreement with the reported values (Table 2). The isolated compound was found to be 3-O-acetyloleanolic acid (OAA) (Fig. 1), an oleanane triterpenoid.

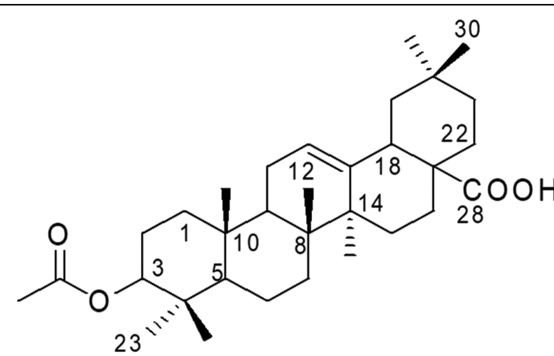

Fig. 1 — 3-O-acetyloleanolic acid isolated from *Mystroxylon aethiopicum*.

This compound has been isolated from various plants, including *Vigna angularis*[21], *Phaseolus angularis* seeds[22] and *Quercus crispula* Blume outer bark[19]. Many studies have shown the different pharmacological activities of OAA. The extracts from *V. angularis* inhibited IL-6-induced cellular signalling, including STAT3 activation[21], OAA inhibited atopic dermatitis (AD) and allergic contact dermatitis (ACD) symptoms, suggesting that OAA may be effective in treating allergic skin disorders[23], OAA mitigated the inflammatory response and development of pulmonary fibrosis in the lungs of mice treated with PHMG-P[24,25], OAA was shown to reduce the expression of several key regulatory genes through IKKα/β suppression in TLR3-mediated NF-κB activation[26] and Endo *et al*.[19], demonstrated the anti-toxoplasma activity and selective toxicity of OOA against *Toxoplasma gondii*. OAA also inhibited the proliferation of keloid fibroblasts and the expression of extracellular matrix-related proteins in its evaluation for keloid efficacy[27]. Furthermore, the *n*-hexane, ethyl acetate and acetone extracts of *M. aethiopicum* were reported to exhibit significant activities against *S. aureus*, *E. faecalis*, *P. aeruginosa* and *E. coli* with MIC ranges of 0.31–1.25 mg/mL[28]. The 1:2 combination of *Viscum rotundifolium* and *M. aethiopicumn*-hexane leaf extract lowered the MIC against *S. aureus* from 0.63 to 0.16 mg/mL. A T-test performed on the *Psidium guajava* and *M. aethiopicum* leaves petroleum ether, and methanol extracts synergistic activity showed no significant difference between the individual and combined extracts antibacterial activities[29]. The current report identifies one of the compounds responsible for the *M. aethiopicum* leaf extract's antibacterial activity. Further probing of its mode of action and synergistic activity with other compounds[30–33] may enable it to be characterised as a potential antimicrobial agents[7].

Conclusion

The bioassay-guided fractionation enabled the isolation of the bioactive compound from *M. aethiopicum* leaves using preparative TLC and column chromatography. The compound isolated from *M. aethiopicum* was identified as 3-O-acetyloleanolic acid acetate. The spectroscopic data obtained from NMR compared with the data obtained from the literature led to the identity of this compound. The compound has already been isolated from various plants; however, this is the first report on the isolation of 3-O-acetyloleanolic acid acetate from the leaves of *M. aethiopicum*. The data reported in this paper shows that the leaves n-hexane extract had the lowest MIC values compared to the dichloromethane, acetone and methanol extracts. The plant extracts and the isolated compounds have the potential in the development of antibacterial drugs due to the antibacterial activity observed in this study.

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Conflicts of interest

The authors declare no conflict of interest.

References

1 https://www.statssa.gov.za (Accessed 09 March 2021).
2 McGaw L T and Eloff J N, Ethnoveterinary use of Southern African plants and scientific evaluation of their medicinal properties, *J Ethnopharmacol*, 2008, **119**(3), 559–574.
3 Rasethe M T, Semenya S S and Maroyi A, Medicinal plants traded in informal herbal medicine markets of the Limpopo province, South Africa, *Evid Based Complement Altern Med*, 2019, **2019**, 2609532, doi: org/10.1155/2019/2609532.
4 van Wyk B E, van Oudsthoorn B and Gericke N, *Medicinal plants of South Africa*, 2nd edn, (Pretoria, Briza Publications), 2000.
5 Butler M S, The role of natural product chemistry in drug discovery, *J Nat Prod*, 2004, **67**(12), 2141–2153.
6 Akinduti P A, Emoh-Robinson V, Obamoh-Triumphant H F and Banlo T T, Antibacterial activities of plant leaf extracts against multi-antibiotic resistant *Staphylococcus aureus* associated with skin and soft tissue infections, *BMC Complement Med Ther*, 2022, **22**(1), 47, doi: org/10.1186/s12906-022-03527-y.
7 Vaou N, Stavropoulou E, Voidarou C, Tsigalou C and Bezirtzoglou E, Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives, *Microorganisms*, 2021, **9**(10), 2041.
8 Onyango C A, Gakuya L W, Mathooko F M, Maina J M and Nyaberi M O, Phytochemical studies on herbal plants commonly used for processing and preserving meat and milk, *J Appl Biosci*, 2014, **73**, 5942–5948.
9 Kokwaro J O, *Medicinal plants of East Africa*, 2nd edn, (Kenya literature bureau, Nairobi Kenya), (1993), 176.
10 Burkil H, *The useful plants of west tropical Africa*, (Royal Botanic Gardens, Kew), 2004, 12-15.
11 Ndukui J G, Mugisha K, Owiny D and Waako P, Effects of crude aqueous leaf extracts of *Citropsis articulata* and *Mystroxylon aethiopicum* on sex hormone levels in male albino rats, *Int J Pharm Sci Invent*, 2014, **3**(1), 5-17.
12 Kilonzo M, Ndakidemi P and Chacha M, *In vitro* antibacterial activity of selected Tanzania medicinal plants, *Herb Med J*, 2016, **2**(2), 1–6.
13 Kilonzo M, Ndakidemi P A and Chacha M, Acute oral toxicity study of *Mystroxylon aethiopicum* root bark aqueous extract in albino mice, *J Med Plant Res*, 2016, **10**(37), 656–661.
14 Kilonzo M, Ndakidemi P A and Chacha M, *In vitro* antifungal and cytotoxicity activities of selected Tanzanian medicinal plants, *Trop J Pharm Res*, 2016, **15**, 2121–2130.
15 Kotze M and Eloff J N, Extraction of antibacterial compounds from *Combretum microphyllum (*Combretaceae), *S Afr J Bot*, 2002, **68**(1), 62–67.

16 National Committee for Clinical Laboratory Standards, Performance standards for antimicrobial susceptibility testing: fourth information supplement. NCCLS, Pennsylvania, USA (M100-S4), (1990).

17 Begue W J and Kline R M, The use of tetrazolium salts in bioautographic procedures, *J Chromatogr*, 1972, **64**, 182.

18 Eloff J N, A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria, *J Med Plant Nat Prod*, 1998, **64**, 711.

19 Endo M, Shigetomi K, Mitsuhashi S, Igarashi M and Ubukata M, Isolation, structure determination and structure-activity relationship of anti-toxoplasma triterpenoids from *Quercus crispula* Blume outer bark, *J Wood Sci*, 2019, **65**, 3.

20 Mogana R, Adhikari A, Tzar M N, Ramliza R and Wiart C, Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* Miq. against bacterial clinical isolates, *BMC Complement Med Ther*, 2020, **20**, 55.

21 Oh H M, Lee S W, Yun B R, Hwang B S and Kim S N, *Vigna angularis* inhibits IL-6-induced cellular signalling and ameliorates collagen-induced arthritis, *J Rheumatol*, 2013, **53**, 64.

22 Hwang J T, Oh H M, Kim M H, Jeong H J and Lee C, Simultaneous quantitation and validation of triterpenoids and phytosteroids in *Phaseolus angularis* seeds, *J Mol*, 2014, **19**, 10319.

23 Choi J K, Oh H M, Lee S, Park J W and Khang D, Oleanolic acid acetate inhibits atopic dermatitis and allergic contact dermatitis in a murine model, *Toxicol Appl Pharmacol*, 2013, **269**, 72.

24 Kim M S, Han J Y, Kim SH, Jeon D and Kim H Y, Oleanolic acid acetate attenuates polyhexamethylene guanidine phosphate-induced pulmonary inflammation and fibrosis in mice, *Respir Physiol Neurobiol*, 2018, **252**, 1-9.

25 Wei L, Guo Y, Ding X, Guo C, Ge N, *et al*., The liver X receptor agonist T0901317 reduces the inflammation of alveolar epithelial cells induced by polyhexamethylene guanidine through inhibition of the NFκBsignaling pathway, *Ann Transl Med*, 2021, **9**, 1795.

26 Lim H J, Jang H J, Kim M H, Lee S and Lee S W, Oleanolic acid acetate exerts anti-inflammatory activity via IKKα/β suppression in TLR3-mediated NF-κB activation, *Molecules*, 2019, **24**, 4002.

27 Luo Y, Wang D, Yuan X, Jin Z and Pi L, Oleanolic acid regulates the proliferation and extracellular matrix of keloid fibroblasts by mediating the TGF-β1/SMAD signaling pathway, *J Cosmet Dermatol*, 2023, **22**, 2083.

28 Malada P M, Mogashoa M M and Masoko P, The evaluation of cytotoxic effects, antimicrobial activity, antioxidant activity and combination effect of *Viscum rotundifolium* and *Mystroxylon aethiopicum*, *S Afr J Bot*, 2022, **147**, 790.

29 Simugomwa C, Izere C, Habyarimana T and Niyonzima F N, Antimicrobial activity of *Mystroxylon aethiopicum* and *Psidium guajava* leaves on selected pathogenic bacteria, *Int J Health Sci*, 2022, **6**, 10817.

30 Ranjutha V, Chen Y, Al-Keridis L A, Patel M, Alshammari N, *et al*., Synergistic antimicrobial activity of ceftriaxone and *Polyalthia longifolia* methanol (MEPL) leaf extract against Methicillin-Resistant *Staphylococcus aureus* and Modulation of *mecA* gene presence, *Antibiotics*, 2023, **12**, 477.

31 Gharbani P, Jam N, Doshmanfekan H, Doshmanfekan H and Mehrizad A, Optimisation of synergic antibacterial activity of *Punica granatum* L. and *Areca nut* (P.G.L.A.N) extracts through response surface methodology, *Sci Rep*, 2023, **13**, 6098.

32 Atta S, Waseem D, Fatima H, Naz I, Rasheed F, *et al*., Antibacterial potential and synergistic interaction between natural polyphenolic extracts and synthetic antibiotic on clinical isolates, *Saudi J Biol Sci*, 2023, **30**(3), 103576.

33 Basavegowda N and Baek K H, Combination strategies of different antimicrobials: An efficient and alternative tool for pathogen inactivation, *Biomed*, 2022, **10**(9), 2219.