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Full Length Research Paper

Antibacterial and anti-adhesion activity of the pentacyclic triterpenoids isolated from the leaves and edible fruits of *Carissa macrocarpa*

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Four pentacyclic oleanane triterpenes (β-amyrin, methyl oleanolate, oleanolic acid and 3β-hydroxyolean-11-en-28,13β-olide) were isolated from the fruits of *Carissa macrocarpa* whilst the ursane triterpene, ursolic acid, was isolated from the leaves. 3 β-hydroxyolean-11-en-28,13β-olide has only been found once previously (in the Lamiaceae) and its lactone ring, which contributes significantly to the expression of antibacterial activity in oleanane triterpenes, makes it an interesting molecule for synthetic and biological studies. The immune boosting properties of the triterpene rich edible fruits of *C. macrocarpa* are important, especially in South Africa, due to high incidences of Human immunodeficiency virus (HIV) and hepatitis in this country.

Key words: *Carissa macrocarpa*, amatungula, oleanane triterpenes, ursolic acid.

INTRODUCTION

The dependence on uncultivated resources in sub-Saharan Africa, where purchased food is substituted with indigenous or wild fruit and vegetable, is well documented (UNAIDS, 1999). The dependence on traditional medicine and medicinal plants by the rural poor for the treatment of HIV related infections such as venereal diseases, tuberculosis, diarrhea and appetite loss have also been reported (Bodeker et al., 2000; King, 2000). The reliance on wild foods and medicinal plants for nutrition, treatment and care is primarily a function of economic and physical accessibility. This can result in the decline of natural resources of commonly used medicinal plants as indicated by traditional healers at the 13th International Acquired immune deficiency syndrome (AIDS) Conference in Durban, South Africa, in 2000. This necessitates the identification of other natural resources, with the same nutritional and medicinal benefits. One such plant is *Carissa macrocarpa* 'Tomlinson' (Ecklon) A. DC, of the family Apocynaceae, which is indigenous to KwaZulu-Natal (KZN), South Africa (Botha and Botha, 1997). *C. macrocarpa* is a small, evergreen, twiggy shrub, found in abundance in KZN, with star-shaped white scented flowers, Y-shaped thorns and tasty red fruit (known as the Amatungula by the Zulu people of South Africa) that are enjoyed by both birds and children (Botha and Botha, 1997). The fruit is reputed to be rich in vitamin C, Ca, Mg and P (Wehmeyer, 1966). Leaves of *C. macrocarpa* are used by the Zulu people to treat diarrhea in livestock. Different morphological parts are used in South African folk medicine to treat coughs and venereal diseases (National Research Council, 2008).

Other *Carissa* species are also used in traditional systems of medicine in different parts of the world. Of these, *Carissa edulis* is used in African traditional medicine (Ibrahim et al., 2005); *Carissa carandas* in Ayurvedic systems of medicine (Hegde et al., 2009); and Codonopsis *lanceolata* by the Aboriginal communities of Western Australia (Lindsay et al., 2000). These *Carissa* species have received widespread scientific attention

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Abbreviations: HIV, Human immunodeficiency virus; **KZN,** KwaZulu-Natal; **AIDS,** acquired immune deficiency syndrome; **IR,** infrared; **DMSO,** dimethyl sulfoxide; **TMS,** tetramethylsilane; **ACN,** acetonitrile; **UKZN,** University of KwaZulu-Natal; **DCM,** dichloromethane; **MeOH,** methanol; **NMR,** nuclear magnetic resonance; **MICs,** minimum inhibitory concentrations; **TSA,** tryptic soy agar; **TSB,** triptone soy broth; **M-H,** mueller-hinton; **LB,** luria bertani; **OD,** optical density.

(Nedi et al., 2004; Siddiqui et al., 2002) whilst no phytochemical studies have been done on *Carissa macrocarpa* despite its role in South African traditional medicine. Furthermore, since the fruits are eaten by children, a phytochemical study to determine the types of compounds present in the fruit is essential. Thus far, a preliminary phytochemical analysis and pharmacological screening has been done on the crude extracts of *C. macrocarpa* and some ubiquitous triterpenoids (lupeol, β-sitosterol and ursolic acid) were identified using thin layer chromatography, a technique that is neither quantitative nor absolute (Wehmeyer, 1986; Zaki et al., 1981). In this study, the isolation, characterization and identification of the phytocompounds from the edible fruits and leaves of *C. macrocarpa* was undertaken. Compounds isolated from other *Carissa* species include *inter alia* cardiac glycosides (Rastogi et al., 1969), lignans (Achenbach et al., 1983), sesquiterpenes (Achenbach et al., 1985) and triterpenes (Siddiqui et al., 2002). The antimicrobial activities of selected isolated compounds were also determined to evaluate the plant's potential to control microbial manifestations on biotic or abiotic surfaces.

MATERIALS AND METHODS

General experimental procedure

Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum 100 FT-IR spectrometer with Universal ATR sampling accessory. NMR spectra were recorded in deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO) at room temperature on a Bruker Avance^{III} 400 spectrometer with tetramethylsilane (TMS) as internal standard. The ^{13}C-NMR spectral assignments were made by comparison of chemical shifts with literature data (Mahato and Kundu, 1994; Pereda-Miranda and Delgado, 1990), by analysis of DEPT spectra for the determination of primary, secondary and tertiary carbons and by use of 2D techniques (COSY, HSQC and HMBC). GC-MS data were recorded on an Agilent GC-MSD apparatus equipped with a DB-5SIL MS (30 m x 0.25 mm i.d., 0.25 µm film thickness) fused silica capillary column. He (2 mL/min) was used as a carrier gas and acetonitrile (ACN) was used to dissolve the sample. The injector was kept at 250°C whilst the transfer line was at 280°C. The column temperature was held at 50°C for 2 min, and then ramped to 280 at 20°C/min where it was held for 15 min. The MS was operated in the EI mode at 70 eV. Melting points were recorded on an Ernst Leitz Wetziar micro-hot stage melting point apparatus and are uncorrected.

Plant materials

The fruits and leaves from *C. macrocarpa* were collected from the University of KwaZulu-Natal (UKZN), Westville campus, South Africa, in May 2009. These were identified by taxonomist, Prof. A. Nicholas, from the School of Biological and Conservation Sciences, UKZN, Westville and a voucher specimen (Moodley, R1) was deposited in the ward herbarium at UKZN.

Extraction and isolation

Ground fruit (900 g) and ground leaves (450 g) were subjected to exhaustive extraction with hexane, dichloromethane (DCM) and methanol (MeOH) by maceration and continuous shaking on an orbital shaker at room temperature for 48 h. The solvent extracts were concentrated by use of a rotary evaporator and the crude extracts were stored at 4°C for further analysis. The crude extracts were subjected to column chromatography (Merck Kieselgel 60, 0.063-0.200 mm, 70-230 mesh ASTM) on suitably sized columns and the fractions monitored by TLC (Merck silica gel 60, 20 x 20 cm F254 aluminium sheets) which was analyzed under UV (254 nm) and visualized using anisaldehyde spray reagent (97: 2: 1; MeOH: conc. H_2SO_4: anisaldehyde). For the crude DCM extract from the fruits (3.0 g), a hexane: ethyl acetate step gradient was used on a 4 cm diameter column starting with 10% ethyl acetate in hexane. This was increased to 20%, then 50% ethyl acetate and finally 100% ethyl acetate. Twenty five fractions of 40 mL each were collected for each solvent system. Fractions 26 to 44 were combined to yield fraction A and fractions 71 to 82 were combined to yield fraction B. Fraction A was rechromatographed over silica gel in a 2 cm column with 100% hexane to yield compound 1 (12.65 mg) which eluted after 20 mL.

Fraction B was rechromatographed over silica gel in a 2 cm column with 100% DCM (50 mL) followed by 10% EtOAc in DCM (50 mL) and 20% EtOAc in DCM (50 mL). Fifteen fractions of 10 mL each were collected, with fractions 7 to10 yielding compound 5 (12.61 mg). Fractions 12 to15 were combined and recrystallisation with $CHCl_3$: MeOH (1:1) afforded compound 2 (40.52 g). The crude MeOH extract (10.0 g) from the fruits was dissolved in water and subjected to partitioning with an equal volume of EtOAc. The EtOAc fraction was then dried with anhydrous Na_2SO_4 and subjected to column chromatography on a 4 cm diameter column with 100% hexane (50 mL) followed by 100% DCM (100 mL). Ten fractions of 10 mL each were collected. Compound 3 (50.35 mg) eluted in fractions 6 to10 yielding white crystals upon evaporation. The crude DCM extract from leaves (8.85 g) was subjected to column chromatography on a 4 cm diameter column. For elution, a mobile phase consisting of a hexane: DCM and DCM: ethyl acetate gradient was used, starting with 100% hexane stepped to 50 and 100% DCM, which was further stepped to 20 and 40% ethyl acetate. Ten fractions of 50 mL each were collected in each step. Fractions 36 to 38 with similar TLC profiles were combined and purified on a 1.5 cm diameter column using 100% DCM (3 x 40 mL), stepped to 50% ethyl acetate (3 x 40 mL) and finally 100% ethyl acetate (3 x 40 mL). Compound 4 (1.78 g) was obtained in fractions 6 to 9.

The physical and spectroscopic data for compounds 1 to 4 matched those of Mahato and Kundu (1994). The Nuclear magnetic resonance (NMR) data for compound 5 matched those of Pereda-Miranda and Delgado for most, but not all resonances, therefore NMR data is provided (Pereda-Miranda and Delgado, 1990). 3β-hydroxyolean-11-en-28,13β-olide (5), colourless crystals, melting point 260-262ºC; EI-MS *m/z* : 454 [M^+], 345, 281, 207, 55, 43; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.76 (3H, s, Me-25), 0.86 (3H, s, Me-24), 0.89 (3H, s, Me-26), 0.95 (3H, s, Me-27), 0.96 (3H, s, Me-30), 1.03 (3H, s, Me-23), 1.22 (3H, s, Me-29), 3.20 (1H, dd, *J*=11.55, 4.77 Hz, H-3), 5.39 (1H, dd, *J*=10.35, 3.03 Hz, H-11), 6.02 (1H, d, *J*=10.35 Hz, H-12); ^{13}C-NMR ($CDCl_3$, 400 MHz): δ 180.00 (C-28), 135.83 (C-12), 126.92 (C-11), 89.80 (C-13), 78.83 (C-3), 54.78 (C-5), 53.21 (C-9), 50.54 (C-18), 44.03 (C-17), 41.60 (C-14), 41.41 (C-8), 38.92 (C-4), 38.26 (C-1), 37.34 (C-19), 36.35 (C-10), 34.37 (C-7), 33.26 (C-29), 31.42 (C-20), 31.16 (C-21), 29.68 (C-22), 27.75 (C-23), 27.16 (C-2), 27.01 (C-16), 25.39 (C-15), 23.54 (C-30), 18.96 (C-27), 18.27 (C-26), 17.93 (C-24), 17.65 (C-6) and 14.90 (C-25).

Determination of minimum inhibitory concentrations (MICs)

MICs were determined by the broth microdilution method (Andrews, 2001). Three Gram-negative strains (*Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 35032 and

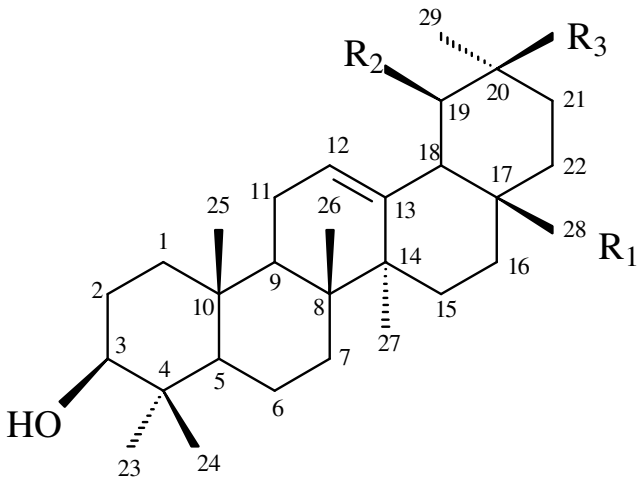


Figure 1. Chemical structures of compounds 1 to 4, β-amyrin (1): R_1=CH_3; R_2=H; R_3=CH_3, methyl oleanolate (2): R_1=$COOCH_3$; R_2=H; R_3=CH_3, oleanolic acid (3): R_1=COOH; R_2=H; R3=CH_3, ursolic acid (4): R_1=COOH R_2=CH_3; R_3=H.

Escherichia coli ATCC 25922) and four Gram-positive strains (*Staphylococcus saprophyticus* ATCC 35552, *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 43300 and *Enterococcus faecium* ATCC 19434) were selected for study. Bacterial strains used as stock cultures were grown on Tryptic soy agar (TSA) and kept at 4ºC throughout the study. For MIC determinations, cultures were inoculated by suspending one isolated colony from TSA plates in 3 mL Triptone Soy broth (TSB). After 18 h of growth at 37ºC, the suspensions were centrifuged and pellets were resuspended in sterile distilled water to obtain final inoculums of 5×10^8 cfu/mL, equivalent to a 0.5 McFarland standard (Andrews, 2001). Stock solutions of the isolated phytocompounds were at 10 mg/mL in 100% DMSO. The 96 well microtitre plates were prepared by dispensing 90 µL of Mueller-Hinton (M-H) broth into each well after which bacterial inoculum (10 µL) was added to the wells. Thereafter, appropriate volumes of the test compound stock solutions were dispensed into the wells to obtain two-fold dilutions of test concentrations ranging from 0.001 mg/mL to 2 mg/mL. This was done in triplicate for each test concentration. Tetracycline, at the concentration range of 0.04 to 32 µg/mL, was used as the standard antimicrobial agent for comparison. All plates contained a medium control to test for sterility as well as growth control wells which contained only medium and bacterial inoculum without any test compound. All plates were incubated at 37ºC for 24 h. The MIC for each test bacterium was considered as the lowest concentration of the test compound that prevented visible growth.

Bacterial adhesion

The anti-adhesion effect of oleanolic acid, ursolic acid and methyl oleanolate against *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 35032), *K. pneumoniae* (ATCC 700603), *E. faecium* (ATCC 19434), *S. aureus* (ATCC 25923), *S. aureus* (ATCC 43300) and *S. saprophyticus* (ATCC 35552) was determined using a modified microtitre plate protocol (Basson et al., 2008). Due to insufficient sample, β-amyrin and 3β-hydroxyolean-11-en-28,13β-olide could not be tested. Bacterial strains were cultured overnight in TSB, then washed and resuspended in sterile distilled water to a turbidity equivalent to a 0.5 McFarland standard. Each well of a sterile 96-well U-bottomed microtiter plate was filled with 90 µL Luria Bertani broth (LB) and 10 µL of the selected cultures. Based on pre-determined MICs for each test compound, the effect of MIC, sub-MIC (0.5×MIC) and supra-MIC (2×MIC) of oleanolic acid, ursolic acid and methyl oleanolate on bacterial adhesion was investigated. Plates were incubated aerobically at 37˚C for 24 h with shaking on an Orbit P4 microtiter plate shaker (Labnet). The supernatant in each well was aspirated and then washed three times with 250 µL of sterile distilled water to remove planktonic bacteria. Adherent bacteria were fixed with 200 µL of 99% methanol for 15 min.

Methanol was removed and plates were left to dry. Subsequently, 150 µL of 2% Hucker crystal violet was added and left to stand for 5 min to effect staining. Excess stain was rinsed off by thorough washing under running water and plates were left to air dry (Basson et al., 2008). Bound stain in each well was resolubilised with 150 µL of 33% (v/v) glacial acetic acid and the concentration of crystal violet was determined by measuring the optical density (OD) of destaining solution at 595 nm using a Fluoroskan Ascent F1 spectrophotometer (Thermolabsystems). All experiments were performed in triplicate, repeated twice and the data was averaged to produce final results (Stepanović et al., 2000). The negative control for both assays was un-inoculated LB to test for sterility and non-specific binding of media, while the positive control was LB with respective cell suspensions without oleanolic acid, ursolic acid or methyl oleanolate. OD_{595nm} values of treated cells were compared to untreated cells to investigate the increase or decrease in adhesion as a result of antimicrobial agent exposure. Treated and untreated samples were compared statistically using paired t-tests and Wilcoxon signed rank tests if normality failed (SigmaStat V3.5, Systat Software, Inc).

RESULTS AND DISCUSSION

Structure elucidation

The DCM extract from the fruits of *C. macrocarpa* yielded three compounds, β-amyrin (1), methyl oleanolate (2) and 3β-hydroxyolean-11-en-28,13β-olide (5) whilst the methanol extract yielded one compound, oleanolic acid (3). The DCM extract from the leaves of *C. macrocarpa* yielded ursolic acid (4) only. EI-MS, IR spectroscopy, 1D-NMR (^{1}H, ^{13}C-NMR and DEPT) and 2D-NMR spectroscopy (COSY, HSQC and HMBC) together with data published in the literature were used to identify the compounds. Compounds 1 to 4 (Figure 1) are common triterpenes found widely in many plant species; however compound 5 (Figure 2) has only been isolated previously from *Hyptis albida* in the Lamiaceae family (Pereda-Miranda and Delgado, 1990). For compounds 1 to3, the ^{1}H-NMR spectrum showed characteristic resonances at δ_H 2.81 (H-18), a triplet at δ_H 5.26 (H-12) and a doublet at δ_H 3.21 (H-3). The only structural difference in these compounds occurred at C-28 and therefore the resonances of H-3, H-12 and H-18 are similar. The H-3 and H-12 resonances of 4 are also similar; however the H-18 resonance occurred at □$_H$ 2.25, consistent with that of ursolic acid and the methyl group at C-19 instead of the tertiary carbon atom with two methyl groups at C-20. The ^{13}C-NMR and DEPT spectra for compounds 1 to 4 had the required methyl, methylene, methine and quaternary carbon resonances for β-amyrin, methyl oleanolate, oleanolic acid, and ursolic acid and the

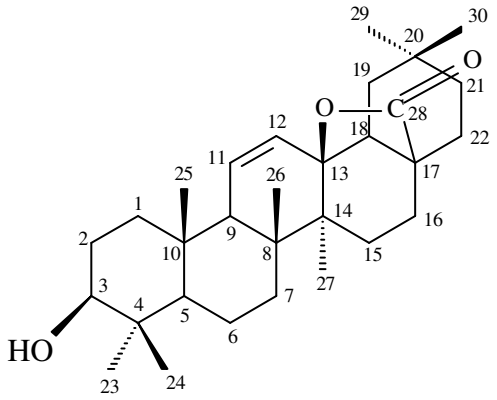


Figure 2. Chemical structure of 3β-hydroxyolean-11-en-28,13β-olide (5).

molecular ion peaks were observed at *m/z* 426 for 1, 470 for 2 and 456 for 3 and 4, respectively.

The NMR data compared well with the data in the literature for these compounds (Mahato and Kundu, 1994). The ^{1}H-NMR spectrum for compound 5 showed a resonance at δ_H 3.21, for H-3, similar to the other four compounds. Two olefinic resonances at δ_H 5.39 (1H, dd, *J*=10.35, 3.03 Hz, H-11) and δ_H 6.02 (1H, d, *J*=10.35 Hz, H-12) were also observed. The ^{13}C-NMR spectrum for compound 5 resolved 30 carbon resonances comprising 7 methyl, 9 methylene, 6 methine, 7 quaternary and 1 carbonyl resonance, identified using the DEPT spectrum. The carbon resonances in the A and B rings were similar to compounds 1, 2 and 3. This is typical of oleanane triterpenes where the geometry of the D/E ring junction does not cause significant alterations in the shielding of carbons in the A/B rings (Mahato and Kundu, 1994). The C-11 and C-12 resonances occurred at δ_C 126.90 and δ_C 135.80, respectively and the carbonyl resonance at δ_C 180.00 was assigned to C-28 which correlated to H-18 in the HMBC spectrum. The C-13 resonance occurred at δ_C 89.80. The data above compared well with the data in literature for 3β-hydroxyolean-11-en-28,13β-olide (Pereda-Miranda and Delgado, 1990) as confirmed by the molecular ion peak, M^+ at *m/z* 454, corresponding to the formula, $C_{30}H_{46}O_3$.

Antimicrobial activity

The pentacyclic triterpenoids, oleanolic acid and ursolic acid, and their derivatives show appreciable antibacterial activity (Krystyna et al., 2010) with the position of the hydroxyl group influencing the activity of the compound (Djoukeng et al., 2005). The present study shows that the pentacyclic triterpenes, β-amyrin (1), methyl oleanolate (2), oleanolic acid (3) and ursolic acid (4), have moderate antibacterial activity (MICs in the range 0.12 to 1.0 mg/mL) whilst the oleanolic acid derivative, 3β-hydroxyolean-11-en-28,13β-olide (5), has good antibacterial activity (MICs in the range 0.06 to 0.12 mg/mL) for all bacterial strains studied (Table 1). The addition of a lactone ring contributes significantly to the expression of antibacterial activity in oleanane triterpenes. No difference in the extent of activity between Gram-positive and Gram-negative bacteria was observed indicating that the mode of antibacterial activity of these pentacyclic triterpenes is unaffected by the cell wall structural differences between these organisms. Most of the isolated compounds have a bacteriostatic (bacteria-inhibiting) effect on the studied microorganisms which is favoured in some cases especially when treating immuno-competent patients with urinary tract infections (Gleckman, 1975). Increased adhesion to abiotic surfaces was observed for 100% of bacterial strains following all three oleanolic acid (3) exposures (Figure 3a), of which the increases observed following MIC and Supra-MIC exposures were statistically significant. Oleanolic acid (3) which increases the adhesion of Gram-positive and Gram-negative bacteria may be used in applications that need to limit the migration of pathogenic bacteria like in groundwater aquifers (Li and Logan, 1999).

For methyl oleanolate (2), variable effects were observed (Figure 3b), with 57% of bacterial strains displaying decreased adhesion.

The decreased adherence for *E. coli, S. aureus* and *S. saprophyticus* indicated that methyl oleanolate (2) interfered with the ability of these microorganisms' to adhere to polystyrene surfaces. Increased adhesion was observed for the remaining three microorganisms demonstrating the ability of this compound to promote their biofilm formation. Decreased adherence was also observed for *E. coli* and *S. aureus* bacterial strains with all three concentrations of ursolic acid (4) (Figure 3c). For *P. aeruginosa*, decreased adhesion was observed only with sub-MIC and MIC exposures, whilst increased adhesion was observed at supra-MIC exposure. In contrast, *S. saprophyticus* adhesion was increased at sub-MIC exposure but decreased following MIC and supra-MIC exposures. Of the three compounds tested, ursolic acid (4) demonstrated the greatest ability to prevent bacterial colonization, with 71% of bacterial strains showing decreased adhesion at most ursolic acid (4) exposures. It was interesting to note that the adhesion of *K. pneumonia,* the gram-negative opportunistic pathogen most frequently implicated in nosocomial infections, was increased following exposure to all three compounds at all concentrations. The reason for this is unclear.

Importance of the phytochemical constituents in the edible fruits

Oleanolic acid is known for its hepatoprotective effects

Table 1. Minimum inhibitory concentrations (MICs), in mg/mL, of compounds 1-5 isolated from *Carissa macrocarpa*, against Gram-positive and Gram-negative bacteria.

	MIC (mg/mL)					
	1	**2**	**3**	**4**	**5**	**T[a]**
S. aureus (ATCC 25923)	0.25	0.25	0.25	0.25	0.06	1
S. aureus (ATCC 43300)	0.25	0.25	0.25	0.25	0.06	2
E. faecium (ATCC 19434)	0.25	0.25	0.25	0.5	0.12	8
S. saprophyticus (ATCC 35552)	0.25	0.25	1.0	0.25	0.12	4
E. coli (ATCC 25922)	0.12	0.25	1.0	0.25	0.06	2
K. pneumonia (ATCC 700603)	0.5	0.5	1.0	0.5	0.12	8
P. aeruginosa (ATCC 35032)	1.0	1.0	0.5	0.5	0.06	32

[a] Reference antibiotic in µg/mL, T: Tetracycline; 1: β-amyrin, 2: methyl oleanolate, 3: oleanolic acid, 4: ursolic acid, 5: 3 β-hydroxyolean-11-en-28,13β-olide.

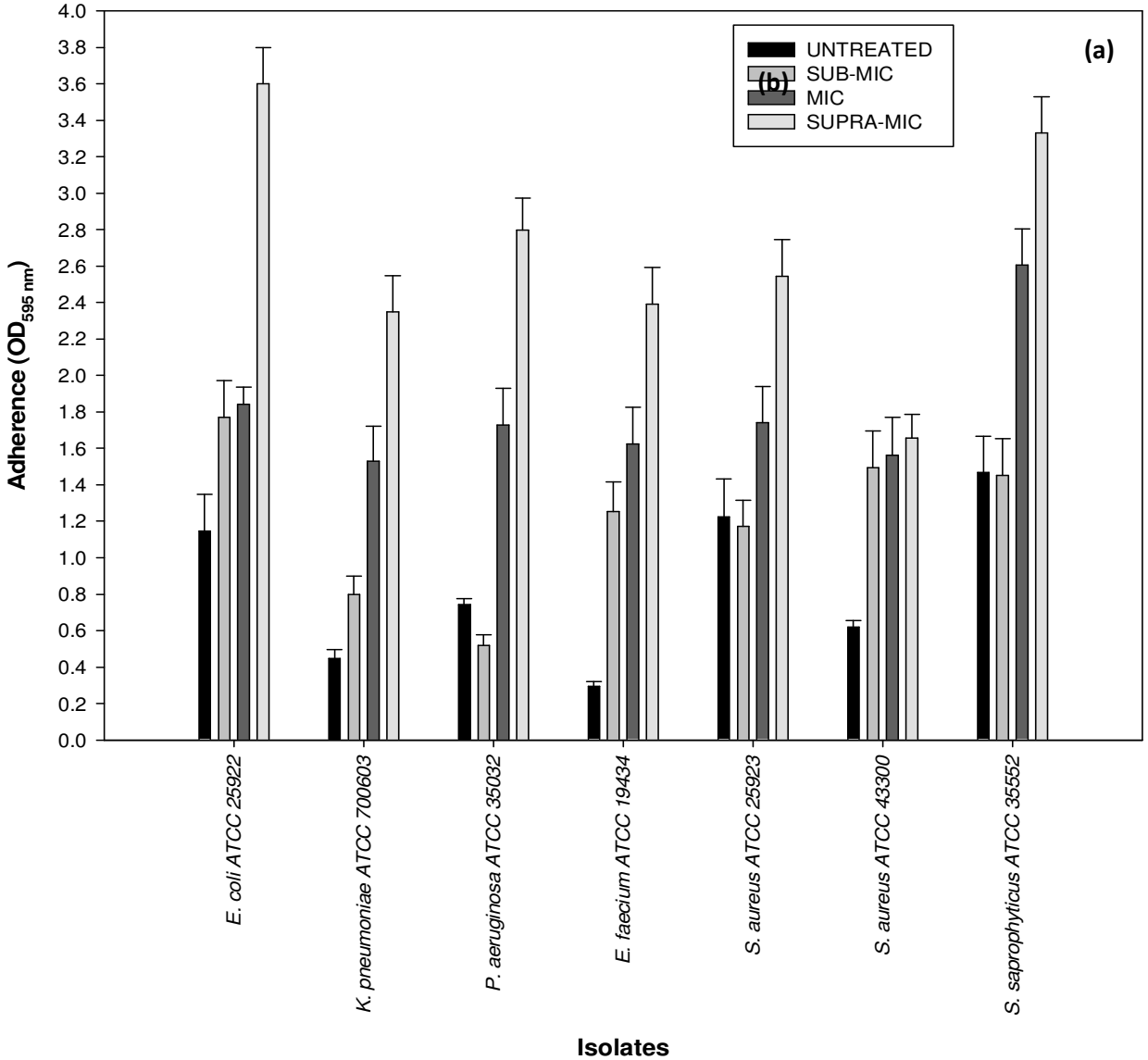
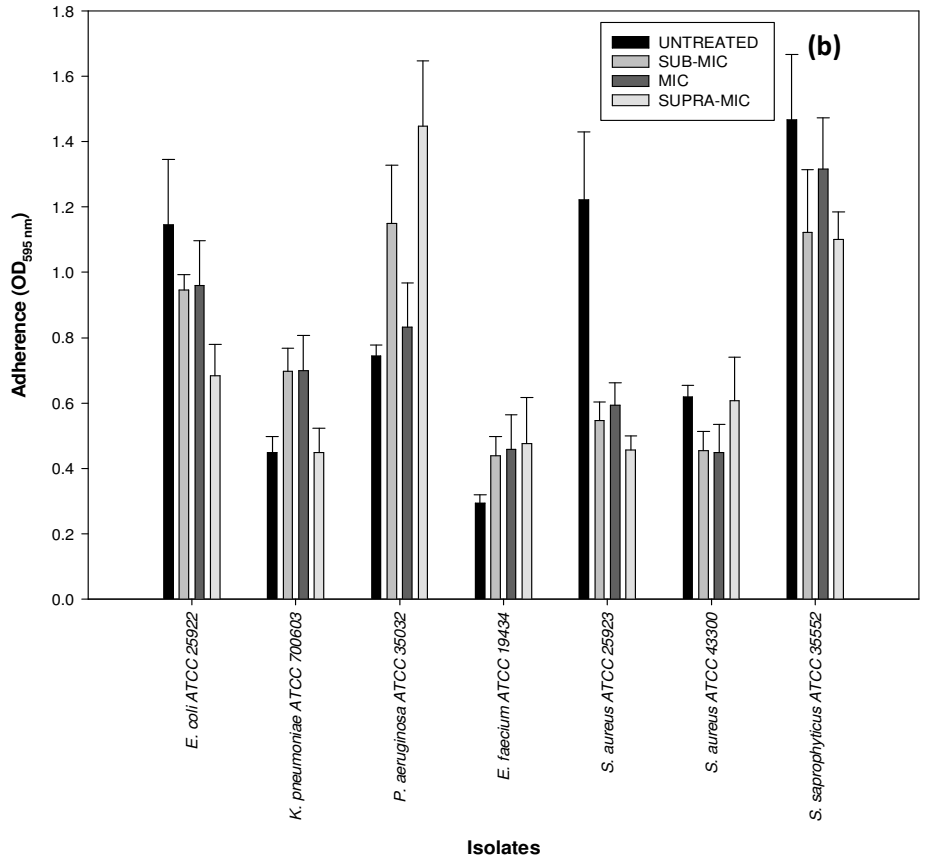


Figure 3. Alterations in adhesion profiles of Gram-positive and Gram-negative bacteria following sub-MIC, MIC and supra-MIC exposures of (a) oleanolic acid (b) methyl oleanolate and (c) ursolic acid.



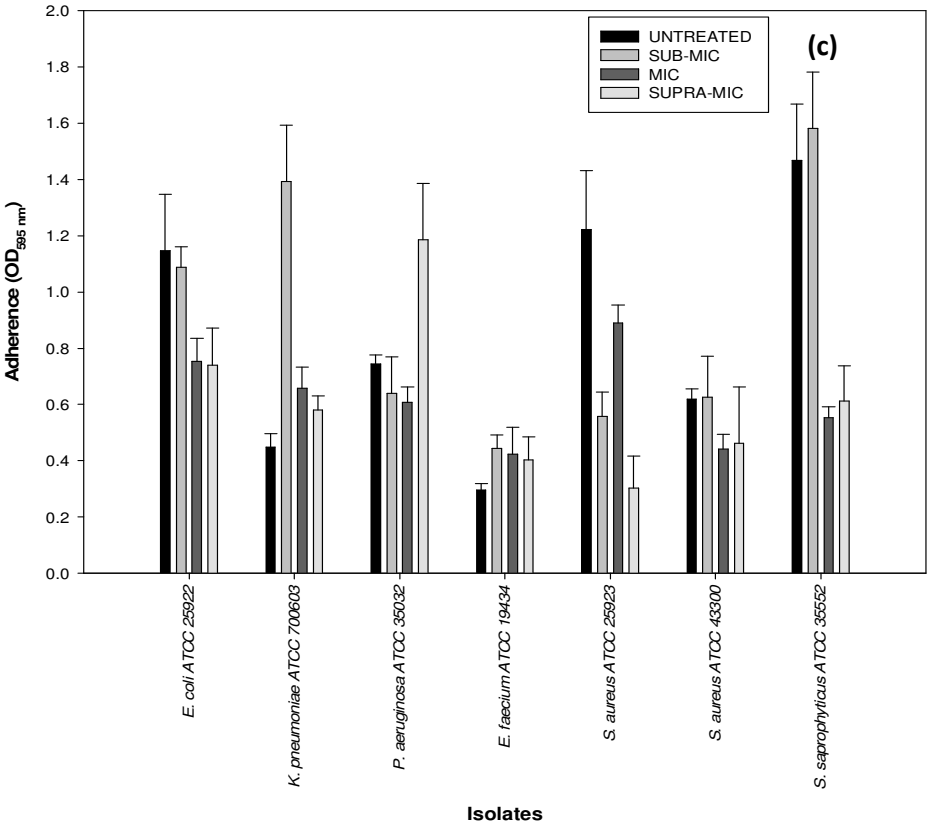


Figure 3. Contd.

(Oliveira et al., 2005). Consequently, it is used to treat acute and chronic hepatitis in China (Liu, 1995). In South Africa, due to the number of people living in informal settlements, the spread and prevalence of the hepaptitis virus is high (Tucker et al., 1996). Consumption of the easily accessible Amatungula fruit that has hepatoprotective effects should therefore be encouraged. Oleanolic acid is known for its anti-inflammatory effects without causing ulcerations, unlike Aspirin (Singh et al., 1992); β-amyrin is more potent than Aspirin in inhibiting platelet aggregation (Ching et al., 2010) therefore consumption of the Amatungula fruit that is rich in oleanolic acid and β-amyrin can be a natural alternative to Aspirin, which is not readily available in the rural parts of South Africa, and may promote the health and well-being of the local people. Children eating the Amatungula fruit also benefit from the anti-cariogenic properties of the compounds that it holds (Hada et al., 1990). Oleanolic acid also exhibits hypoglycemic (Hao et al., 1989), anti-lipidemic (Liu et al., 1987) and anti-cancer activities (Li et al., 2002) so the benefits of ingestion of the Amatungula fruit are numerous.

Conclusion

Five pentacyclic triterpenes were isolated from the leaves and fruits of *C. macrocarpa*. All four of the triterpenes isolated from the fruits of *C. macrocarpa* had the oleanane skeleton and appear to be derived biosynthetically from oleanolic acid whilst only the ursane triterpene, ursolic acid, was isolated from the leaves. MICs show that the addition of a lactone ring to the oleanane backbone contributes significantly to the expression of antibacterial activity in pentacyclic oleanane triterpenes. For most microorganisms studied, oleanolic acid tended to promote adhesion to abiotic surfaces whilst methyl oleanolate and ursolic acid tended to inhibit it.

The anti-adhesion effect of the three studied compounds were in the increasing order of oleanolic acid < methyl oleanolate < ursolic acid. The immune boosting properties of the compounds contained in the fruits of *C. macrocarpa* are important, especially for children in KZN, due to high incidences of HIV and hepatitis in this area. The cost of immune boosting supplements is out of reach for the indigenous people of South Africa therefore freely available fruit such as the Amatungula is a welcome source of these compounds. This study lends scientific credence and validity to the ethnomedicinal use of *C. macrocarpa* and highlights the medicinal benefits of consuming the indigenous edible fruit.

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REFERENCES

Achenbach H, Waibel R, Addae-Mensah I (1983). Lignins and other constituents from *Carissa edulis*. Phytochemistry, 22: 749-753.

Achenbach H, Waibel R, Addae-Mensah I (1985). Sesquiterpenes from *Carissa edulis*. Phytochemistry, 24: 2325-2328.

Andrews JM (2001). Determination of inhibitory concentrations. J. Antimicrob. Chemother., 48: 5-16.

Basson A, Flemming LA, Chenia HY (2008). Evaluation of adherence, hydrophobicity, aggregation and biofilm development of Flavobacterium johnsoniae-like isolates. Microb. Ecol., 55: 1-14.

Bodeker G, Kabatesi D, King R, Homsy J (2000). A regional task force on Traditional Medicine and AIDS. Lancet, 355: 1284.

Botha C, Botha J (1997). Bring Nature back to your garden. Durban: Natal Region of the Wildlife and Environment Society.

Ching J, Chua TK, Chin LC, Lau AJ, Pang YK, Jaya JM, Tan CH, Koh HL (2010). β-amyrin from *Ardisia elliptica* Thunb. is more potent than aspirin in inhibiting collagen-induced platelet aggregation. Indian J. Exp. Biol., 48: 275-279.

Djoukeng JD, Abou-Mansour E, Tabacchi R, Tapondjou AL, Bouda H, Lontsi D (2005). Antibacterial triterpenes from *Syzygium guineense* (Myrtaceae). J. Ethnopharmacol., 101: 283-286.

Gleckman RA (1975). Trimethoprim-sulfamethoxazole vs. ampicillin in chronic urinary tract infections: a double-blind multicentre cooperative controlled study. J. Am. Med. Assoc., 233: 427-431.

Hada S, Hattori T, Namba T (1990). Dental caries prevention by traditional medicines. Effect of components of *Ganoderma lucidum* on glucosyltransferase from *Streptococcus mutans*. Chem. Abstracts, 113: 91423f.

Hao Z, Hang B, Wang Y (1989). Hypoglycemic effect of oleanolic acid. Zhougguo Yaoke Daxue Xuebao, 22: 210-212.

Hegde K, Thakker SP, Joshi AB, Shastry CS, Chandrashekhar KS (2009). Anticonvulsant activity of *Carissa carandas* Linn. root extract in experimental mice. Trop. J. Pharm. Res., 8: 117-125.

Ibrahim H, Bolaji RO, Abdurahman EM, Shok M, Ilyasand N, Habib AG (2005). Preliminary phytochemical and antimicrobial studies of the leaves of *Carissa edulis* VAHL. Chem. Class J., 2: 15-18.

King R (2000). Collaboration with traditional healers in HIV/AIDS prevention and care in sub-Saharan Africa. Geneva, Switzerland: UNAIDS.

Krystyna IW, Grudniak AM, Fiecek B, Kraczkiewicz-Dowjat A, Kurek A (2010). Antibacterial activity of oleanolic and ursolic acids and their derivatives. Cent. Eur. J. Biol., 5: 543-553.

Li J, Guo WJ, Yang QY (2002). Effects of ursolic acid and oleanolic acid on human colon carcinoma cell line HCT15. World J. Gastroenterol., 8: 493-495.

Li Q, Logan BE (1999). Enhancing bacterial transport for bioaugmentation of aquifers using low ionic strength solution and surfactants. Water Resour., 33: 1090-1100.

Lindsay EA, Berry Y, Jamie FJ (2000). Antibacterial compounds from *Carissa lanceolata* R. Br. Phytochemistry, 55: 403-406.

Liu J (1995). Pharmacology of oleanolic acid and ursolic acid. J. Etnopharmacol., 49: 57-68.

Liu J, Chen XF, Xia L, Geng XZ, Li ZS (1987). Effect of oleanolic acid on serum glyceride cholesterol and β-lipo-proteins in normal and experimental hyperlipedermia rats. Chin. Pharmacol. Bull., 4: 14-15.

Mahato SB, Kundu AP (1994). ^{13}C NMR spectra of pentacyclic triterpenoids – A compilation and some salient features. Phytochemistry, 37: 1517-1575.

National Research Council (2008). Lost Crops of Africa, Vol III: Fruits. Washington, D.C.: The National Academies Press.

Nedi T, Mekonnen N, Urqa K (2004). Diuretic effect of the crude extract of *Carissa edulis* in rats. J. Ethnopharmacol., 95: 57–61.

Oliveira FA, Chaves MH, Almeida FRC, Lima RCP, Silva RM, Maia JL, Brito GA, Santos FA, Rao VS (2005). Protective effect of α- and β-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March trunk wood resin, against acetaminophen-induced liver injury in mice. J. Ethnopharmacol., 98: 103-108.

Pereda-Miranda R, Delagdo R (1990). Triterpenoids and flavonoids from *Hyptis albida*. J. Nat. Prod., 53: 182-185.

Rastogi RC, Kulshreshtha DK, Rastogi RP (1969). Cardioactive constituents from *Carissa spinarum*. Indian J. Chem., 7: 1102-1104.

Siddiqui BS, Ghani U, Ali ST, Usmani SB, Begum S (2002). Triter penoidal constituents of the leaves of *Carissa carandas*. Nat. Prod. Res., 17: 153-158.
Singh GB, Singh S, Bani S, Gupta BD, Banerjee SK (1992). Anti-inflammatory activity of oleanolic acid in rats and mice. J. Pharm. Pharmacol., 44: 456-458.
Stepanović S, Vuković D, Dević I, Savić B, Švabić-Vlahović M (2000). A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J. Microbiol. Meth., 40: 175-179.
Tucker TJ, Kirsch RE, Louw SJ, Isaacs S, Kannemeyer J, Robson SC (1996). Hepatitis E in South Africa: evidence for sporadic spread and increased seroprevalence in rural areas. J. Med. Virol., 50: 117-119.
UNAIDS (1999). A review of household and community responses to the HIV/AIDS epidemic in the rural areas of sub-Saharan Africa. Geneva, Switzerland: UNAIDS.
Wehmeyer AS (1986). Edible wild plants of Southern Africa: data on nutrition contents of over 300 species. Pretoria: Council for Scientific and Industrial Research.
Wehmeyer AS (1966). The nutrient composition of some edible wild fruits found in the Transvaal. S. Afr. Med. J., 40: 1102-1104.
Zaki AY, El-Tohamy SF, El-Fattah SA (1981). Study of lipid content and volatile oil of the different organs of *Carissa carandas* Lin. and *Carissa grandiflora* Dc. growing in Egypt. Egypt J. Pharm. Sci., 22: 127-141.