Journal of Diseases and Medicinal Plants
2018; 4(5): 128-132
http://www.sciencepublishinggroup.com/j/jdmp
doi: 10.11648/j.jdmp.20180405.12
ISSN: 2469-8202 (Print); ISSN: 2469-8210 (Online)

Fatty Alcohols Isolated from *Prosopis africana* and Evaluation of Antibacterial and Antituberculosis Activities

Nganso Ditchou Yves Oscar[1, *], Soh Desire[2], Ndogo Eteme Olivier[3], Mala Opono M. T. G.[4], Nyasse Barthelemy[3]

[1]Department of Chemistry, Faculty of Science, University of Maroua, Maroua, Cameroon

[2]Department of Organic Chemistry, Higher Teachers Training College, University of Bamenda, Bamenda, Cameroon

[3]Department of Organic Chemistry, Faculty of Science, University of Yaounde I, Yaounde, Cameroon

[4]Department of Biochemistry, Faculty of Science, University of Yaounde I, Yaounde, Cameroon

Email address:
nganso_yves@yahoo.fr (N. D. Y. Oscar)

[*]Corresponding author

To cite this article:
Nganso Ditchou Yves Oscar, Soh Desire, Ndogo Eteme Olivier, Mala Opono M. T. G., Nyasse Barthelemy. Fatty Alcohols Isolated from *Prosopis africana* and Evaluation of Antibacterial and Antituberculosis Activities. *Journal of Diseases and Medicinal Plants*. Vol. 4, No. 5, 2018, pp. 128-132. doi: 10.11648/j.jdmp.20180405.12

Received: October 6, 2018; **Accepted**: November 5, 2018; **Published**: December 3, 2018

Abstract: *Prosopis africana*, Family Leguminoceae, is used in ethnomedicine to treat different ailments including, diarrhoea, bacillary dysentery, malaria, male sterility and as a cardiotonic. This research aims at evaluating the antibacterial and antituberculosis activities of fatty alcohol. Heneicosanol (1), hexacosanol (2), nonacosanol (3), β-sitosterol (4), quercetin (5), β-sitosterol 3-O-β-D-glucopyranoside (6) and quercitrin (7) were isolated from the leaves of *Prosopis africana*. Their structures were elucidated on the basis of a spectroscopic analysis and a comparison of their data spectral with those reported in the literature. Heneicosanol (1), hexacosanol (2), nonacosanol (3) were isolated for the first time to this plant and was subject of antibacterial and antituberculosis activities. The results of this study suggest that the compounds 1, 2, 3 had an MBC a respective value of 1.56 µg/mL, 1.62 µg/mL, 0.12 µg/mL against *M. smegmatis*, *M. tuberculosis*. The study of fatty alcohols provides evidence of activity against clinical isolate of mycobacteria, significant anti-inflammatory and analgesic properties, and provides a basis for its possible use as an affordable and effective Antituberculosis agent from natural product.

Keywords: *Prosopis africana*, Fatty Alcohols, Antibacterial Activities, Antituberculosis Activities

1. Introduction

Woody plants have been known to produce many biologically active metabolites. In addition to the studies on chemical constituents of herbal medicinal plants, our phytochemical program also targets woody plants as potential sources of useful natural compounds. *Prosopis africana* is a woody plant that reaches up to 10 - 15 m in height. The bark is used to treat diarrhoea, bacillary dysentery, and inflammatory diseases [1, 2]. The study on *Prosopis africana* should be of interest since the constituents of *Prosopis* species have been demonstrated to possess antioxidant [3, 4], anti-inflammatory [5-8], anticancer [9, 10] and hepatoprotective effects [3, 11]. In this paper the isolation and structural elucidation of seven compounds, heneicosanol (1), hexacosanol (2), nonacosanol (3), β-sitosterol (4), quercetin (5), β-sitosterol 3-*O*-β-D-glucopyranoside (6), and quercitrin (7) (Figure 1) from the MeOH extract of the leaves of *Prosopis aficana.* Heneicosanol (1), hexacosanol (2), nonacosanol (3) were isolated from this plant for the first time. The goal of this study is evaluating the antibacterial and antituberculosis activities of the fatty alcohol isolated for the first time from the leaves of *Prosopis africana.*

2. Experimental

2.1. General Procedure

Electron-impact (EI) mass spectra (70eV) were measured

on a Hewlett-Packard 5989B mass spectrometer. Electrospray Ionization (ESI) mass spectra were recorded on a LC/MSD Trap Agilent Series 1100 system with an ESI source. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) with DEPT program spectra were obtained on a Bruker Avance 500 NMR spectrometer. Tetramethyl silane (TMS) was used as zero reference. Silica gel 60 (63-200 µm, Merck, Darmstadt, Germany) was used for open column (CC) and silica gel 60 (15-40 and 40-63 µm, Merck, Darmstadt, Germany) for flash column (FC) chromatography. TLC was performed on precoated DC Alufolien 60 F_{254} sheets (Merck, Darmstadt, Germany) and detected by UV light (λ 254 nm) or by spraying with 1% vanillin in conc H_2SO_4.

2.2. Plant Material

The fresh leaves of *Prosopis africana* were identified and collected by Dr. Froumsia Moskia Department of Biology Science, Faculty of Science, University of Maroua, Cameroon.

2.3. Extraction and Isolation

The air-dried leaves of *Prosopis africana* (232 g) were oven-dried at 40°C, then powdered and extracted with MeOH by percolation (6 times) at room temperature. The combined MeOH extract was concentrated under reduced pressure. The resultant MeOH extract was suspended in H_2O and partitioned successively with *n*-hexane, CH_2Cl_2 and EtOAc. Part of the *n*-hexane-soluble fraction (26g) was chromatographed on silica gel CC using a gradient *n*-hexane-EtOAc solvent system (*n*-hexane; *n*-hexane-EtOAc 7:1, 4:1, 2:1, and 1:1; and EtOAc). Four pooled fractions were collected on the basis of the volumes of eluents and TLC analysis; fraction A (0.78 g) was eluted with *n*-hexane; fractions B (0.54 g), C (0.66 g), and D (0.84 g) with *n*-hexane-EtOAc 7:1;

Separation of fraction A on silica gel CC (*n*-hexane-EtOAc 90:1) gave 1 (21.3 mg), 2 (22 mg) after recrystallization from *n*-hexane-EtOAc 9:1, and 3 (30 mg).

Fraction B was chromatographed by silica gel CC (gradient *n*-hexane-EtOAc 30:1, 15:1, 7:1 and 4:1) and one of the fractions obtained was recrystallized from CH_2Cl_2 to give 4 (8 mg), 5 (8.8mg), 6 (7.6mg) and 7 (11.4mg).

Heneicosanol (1): 21.3 mg. Crystalline white solid. M.p. 68°C (hexane/AcOEt). FT-IR: 3442, 2919, 2850, 1735, 1466, 724 cm^{-1}. The ^{1}H NMR spectrum displayed the typical signals 0.88 (3H, t, J=7 Hz, CH_3), 1.25 (2H, m, (CH_2)n), 1.57 (2H, t, J=6.5 Hz, CH_2), 3.65 (2H, t, J=6.5 Hz, CH_2-OH). In the ^{13}C NMR spectrum showed different C-atom 14.1 (CH_3), 22.7 (CH_2), 25.7 (CH_2), 29.4 (CH_2), 29.6 (CH_2), 31.9 (CH_2), 32.8 (CH_2), 63.1 (CH_2). EI-MS (70 eV): 312(3, M^+), 227(5), 125(9), 111(18), 97(35), 83(48), 69 (52), 57(77), 43 (100).

Hexacosanol(2): ESIMS showed $[M+1]^+$ at m/z 383.2057 indicating a molecular formula of $C_{26}H_{54}O$. ^{13}C NMR ($CDCl_3$, 100 MHz): d_C 63.1 (CH_2-OH), 32.7 (CH_2), 31.9

(CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 25.4 (CH_2), 22.7 (CH_2), 14.2 (CH_3) indicating unsaturated primary fatty alcohol.

Nonacosanol(3): White amorphous powder. Rf 0.5 (n-hexane-EtOAc 4:1). EI-MS: m/z 364 (M+., $C_{29}H_{60}O$, − 60). ^{1}H NMR ($CDCl_3$): δ0.88 (3H, t, J = 7.0 Hz, H-29), 1.26 50H, br s), 1.58 (4H, m (2H-2 → 2H-28), 3.64 (2H, t, J = 6.5 Hz, H-1).

β-Sitosterol (4): White amorphous powder. R_f 0.37 (n-hexane-EtOAc 4:1). The Co-TLC analysis is superimposable with that of our authentic sample.

Quercetin (5): Yellow needles. R_f 0.54 (CH_2Cl_2-$(CH_3)_2CO$ 2:1). ESI-MS: m/z 302.9 $[M+H]^+$ (positive mode), m/z 301.0 $[M-H]^-$ (negative mode). ^{1}H NMR (CD_3OD): δ 6.2 (1H, d, *J* = 2.0 Hz, H-8), 6.4 (1H, d, *J* = 2.0 Hz, H-6),

6.90 (1H, d, *J* = 8.5 Hz, H-5′), 7.64 (1H, dd, *J* = 8.5 Hz, 2.5 Hz, H-6′), 7.75 (1H, d, *J* = 2.5 Hz, H-2′). ^{13}C-NMR (CD_3OD): δ 94.4 (d, C-8), 99.2 (d, C-6), 104.5 (s, C-10), 116.0 (d, C-2′), 116.2

(d, C-5′), 121.7 (s, C-6′), 124.1 (s, C-1′), 137.2 (s, C-3), 146.2 (s, C-3′), 148.0 (s, C-2), 148.7 (s, C-4′), 158.2 (s, C-9), 162.5 (s, C-5), 165.5 (s, C7), 177.3 (s, C-4).

β-Sitosterol-3-O-β-D-glucopyranoside (6): White amorphous powder. R_f 0.54 ($CH_2Cl_2(CH_3)_2CO$ 1:3). The ^{1}H NMR (CD_3OD) is identical with that of our authentic sample.

Quercitrin (7): Yellow needles. R_f 0.57 (CH_2Cl_2-$(CH_3)_2CO$ 1:3). EI-MS: m/z 302 ($M^{+\cdot}$, $C_{21}H_{20}O_{11}$, − 146). ^{1}H-NMR (CD_3OD): δ 0.96 (3H, d, *J* = 6.0 Hz, 5″-CH_3), 3.33 (1H, m, H-4″), 3.44 (1H, m, H-3″), 3.77 (1H, dd, *J* = 8.0 Hz, 3.5 Hz, H-2″), 4.24 (1H, m, H-5″), 5.37 (1H, d, *J* = 1.0 Hz, H-1″), 6.22 (1H, d, *J* = 2.0 Hz, H-8), 6.39 (1H, d, *J* = 2.0 Hz, H-6), 6.93 (1H, d, *J* = 8.5 Hz, H-5′), 7.32 (1H, dd, *J* = 8.5 Hz, 2.0 Hz, H-6′), 7.36 (1H, d, *J* = 2.0 Hz, H-2′). ^{13}C NMR

(CD_3OD): δ 17.6 (q, C-6″), 71.9 (d, C-5″), 72.0 (d, C-3″), 72.1 (d, C-2″), 73.3 (d, C-4″), 94.7 (d,

C-8), 99.8 (d, C-6), 103.6 (d, C-1″), 105.9 (s, C10), 116.4 (d, C-2′), 117.0 (d, C-5′), 122.9 (s, C6′), 123.0 (s, C-1′), 136.3 (s, C-3), 146.4 (s, C-3′), 149.8 (s, C-4′), 158.5 (s, C-2), 159.3 (s, C-9), 163.2 (s, C-5), 165.8 (s, C-7), 179.6 (s, C-4).

2.4. Antibacterial Activity

2.4.1. In Vitro Evaluation of the Antibacterial Activity of the Crude Extracts

7.6 g of Mueller Hinton Agar (MHA) was dissolved in 200 mL of distilled water and then heated on autoclave at 121 °C for 30 min. After cooling the mixture was poured into the petri dishes near the beak of Bunsen burner [12].

2.4.2. Liquid Medium

13.65 g of Mueller Hinton Broth (MHB) were dissolved in 650 mL of distilled water. A part of this medium was distributed in tubes of 15mL (10.853mL per tube which will be used for inocula). Another part was distributed in the 2 mL tubes (1.7 mL per tube for the dilution of the extracts). These tubes and the rest of medium were heated in an autoclave at 121°C for 30 min [13].

2.4.3. Culture of Bacterial Strains

The different bacterial strains were subcultured by the

method of the streaks on MHA agar medium poured into the Petri dishes. The petri dishes were introduced into the incubator at 37°C. For 18 hours in order to obtain a young culture and isolated colonies. The isolated colonies were used to prepare the inoculum [13, 14].

2.5. Antituberculosis Activity

2.5.1. Preparation of the Inoculums

Using a sterile platinum loop, a few colonies of bacteria from each strain were taken from the activation medium and each introduced into a tube containing a sterile physiological solution (0.9% NaCl). The contents of each tube were homogenized using the vortex in order to obtain a turbidity comparable to the standard scale of Mc Farland (Table 1) corresponding to the concentration of 1.5. 108CFU/mL. Subsequently, 147 µL of the resulting suspension was removed and introduced into 10.85 mL of MHB for a volume of 11000 mL of an inoculated medium at 2.10^6 CFU/mL [15].

2.5.2. Antitubercular Rapid Radiometric Assay Using M. Tuberculosis

A sensitive strain of *M. tuberculosis*, the H37Rv references strain, was used in the screening procedure. A standard inoculum was prepared for the sensitive strain in Middlebrook Dubos 7H9 broth containing 0,5% Tween 80 to obtain a concentration of 1.0 mg/mL (wet mass). The bacterial cultures, which were used to prepare the standard inoculum, were maintained on Lowenstein-Jensen medium. A representative amount of growth was taken from the cultures by using a sterile applicator stick. This sample was transferred to a sterile 16 x 125 mm screw capped round tube containing six to eight glass beads (1 – 2 mm) and 3.0 – 4.0 mL of the diluting fluid (0,1% Tween). A homogenous suspension was obtained by placing the tube on the Vortex mixer for five minutes and then left for 15 minutes to allow the particles to settle. After the large particles had settled, the supernatant, a homogeneous suspension was transferred into a separate sterile test tube and more Tween was added and adjusted approximately to McFarland no 1 turbidity standards [16].

2.5.3. Antituberculosis Activity Against M. Smegmatis and M. Tuberculosis

Solutions of all the extracts were prepared in DMSO to obtain a stock concentration of 500.0 mg/mL. Control experiments showed that a final concentration of DMSO (1%) in the medium had no adverse effect on the growth of *M. tuberculosis*. Isoniazid (INH; Sigma-Aldrich) at a final concentration of 0.2 µg/mL served as the drug-control in our bioassay. All the extracts were tested at concentrations ranging from 5.0 to 0.1 mg/mL. A homogenous culture (0.1 mL of *M. tuberculosis*, yielding 1 x 10^4 to 1 x 10^5 CFU/mL), was inoculated into the vials containing the extracts, as well as in the control vials. Three extract-free vials were used as controls (medium + 1% DMSO), two vials (V1) were inoculated in the same way as the vials containing the extracts, and one (V2) was inoculated with a 1:100 dilution of the inoculum (1:100 control) to produce an initial concentration representing 1% of the bacterial population (1 x 10^2 to 1 x 10^3 CFU/mL). The MIC was defined as the lowest concentration of the extract that inhibited > 99% of the bacterial population. When Mycobacterium grows in 7H12 medium containing a 14C-labelled substrate (palmitic acid), they use the substrate and $14CO_2$ is produced. The amount of $14CO_2$ detected reflects the rate and amount of growth occurring in the sealed vial and is expressed in terms of the growth index [16]. Inoculated bottles were incubated at 370°C and each bottle was assayed at 24 hours intervals at about the same time until cumulative results were interpretable to measure the GI. The difference in the GI values of the last two days was designated as ΔGI. The GI readings of the vials containing the test extracts were compared with the control vials (V2). Readings were taken until the control vials, containing a 100 times lower dilution of the inoculum than the test vials, reached a GI of 30 or more. If the ΔGI values of the vials containing the test extracts were less than the control vials, the population was reported to be susceptible to the compound and if it was equal to or greater than that in the control vials, the test organisms were considered to be resistant to the drugs. Each test was replicated three times [16-17].

3. Results and Discussion

The dried leaves of *Prosopis africana* were extracted with MeOH, and the resultant MeOH extract was partitioned between H_2O and solvents of increasing polarity to give *n*-hexane and EtOAc-soluble fractions. Fractionation of the *n*-hexane and EtOAc-soluble fractions by silica gel open column (CC) and flash column (FC) chromatography resulted in the isolation of seven compounds 1-7 (Figure 1).

Compound 1 was a saturated fatty alcohol (hexacosanol). The 1D and 2D-NMR spectra allowed inferring the presence of one Me and twenty CH_2 groups. The ^{1}H NMR spectra displayed the typical signals assigned to δ (H) 0.88 (t, J= 7, Me-(C_{21})) and δ (H) 3.65 (t, J= 6.5, H(C_1)). The presence of the OH group was deduced from the FT-IR absorption at 3492 cm^{-1} and by the signal at 63.13 ppm in the ^{13}C NMR spectrum. The location of the OH group at C(1) was established by correlation cross-peaks between H-C(1) and H-C(2) in the ^{1}H-^{1}H- COSY spectrum.

Compound 2, heneicosanol was isolated by column chromatography (CC). Spectroscopic techniques such as 1D and 2D-NMR as well as EI-MS were used for structure elucidation. The EI-MS analysis showed an ion peak at m/z 312 (M+), concordant with the molecular formulae $C_{21}H_{44}O$ (Figure 1). The 1D and 2D-NMR spectra allowed inferring the presence of one Me and twenty CH_2 groups. The ^{1}H NMR spectra displayed the typical signals assigned to δ (H) 0.88 (t, J= 7, Me-(C_{21})) and δ (H) 3.65 (t, J= 6.5, H(C_1)). In the HMBC spectrum, correlations were observed between the H-atom resonance of Me (21) (δ(H) 0.88) and that of C(20); the resonance of H-C(1) (δ(H) 3.65) and that of C(2), and the

resonance of H-C(2) (δ(H) 1.57) and that of C(4). The presence of the OH group was deduced from the FT-IR absorption at 3492 cm^{-1} and by the signal at 63.13 ppm in the ^{13}C NMR spectrum. The location of the OH group at C(1) was established by correlation cross-peaks between H-C(1) and H-C(2) in the ^{1}H-^{1}H-COSY spectrum.

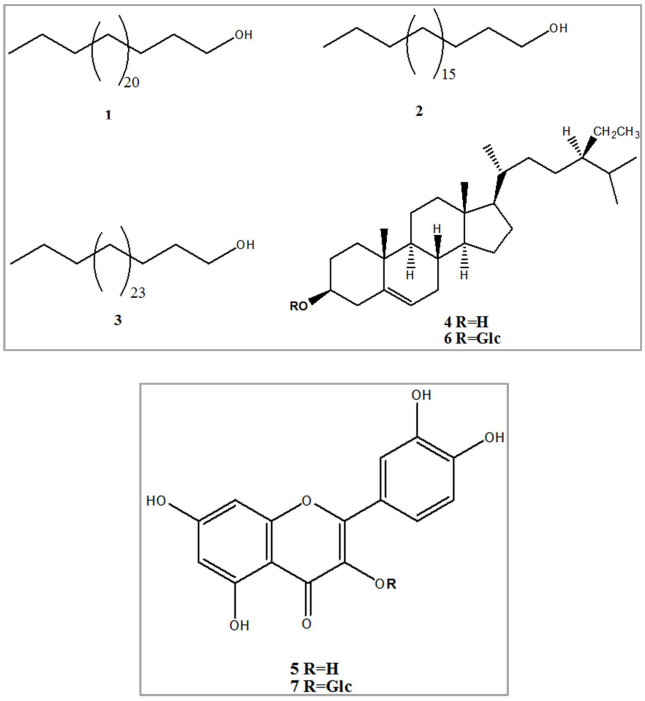

Figure 1. *Structure of compounds 1 to 7 isolated from Prosopis africana.*

Compound 3 was isolated as a white amorphous powder [R_f 0.5 (*n*-hexane-EtOAc 4:1)] from the *n*-hexane-soluble fraction. 3 was determined to be nonacosanol from its ^{1}H NMR spectroscopic data. In the ^{1}H NMR spectrum of 3 the terminal methyl group δ_H 0.88 (3H, t, J = 7.0 Hz)], methylene chains [δ_H 1.26 (50 H, br s) and 1.58 (4H, m)], and a methylene group bearing a hydroxy group [δ_H 3.64 (2H, t, J = 6.5 Hz)] were observed. The number of methylene groups was deduced to be 28 from the ^{1}H NMR integration. The EI-MS spectrum of 3 showed the highest peak at *m/z* 364, which was probably derived from simultaneous loss of H_2O and ethylene, and a methylene group ($M^{+\cdot}$, $C_{29}H_{60}O$, − 18 − 28 − 14). Nonacosanol was found as constituent of several species, *Agave*, *Sisalana*, *Citrulus*, and *Rhizophora* [18, 19].

3.1. Antimicrobial and Antituberculosis Activities

The antimicrobial tests carried out on compounds 1 to 3 led to the following results: Compounds 1 to 3 exhibited antimicrobial activity against *Candida albicans* and *Candida krusei*, with MIC values of 250 µg / mL for both yeast species. They were also tested against two Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa)* and Gram-positive bacteria (*Staphylococcus aureus*) and the results obtained from IC_{50} are respectively: 65.0 ± 4.1, 59.0 ± 6.8, 57.0 ± 1.7 (µg / mL), for a concentration of 3.20 µg / mL; 4.8 µg / mL 5.2 µg / mL. This suggests that these compounds are active on these elements. The anti-tuberculosis tests were also performed and the results are recorded in Table 1 below.

Table 1. *Antimycobacterial activity of the compounds 1, 2 and 3 against M. smegmatis and M. tuberculosis.*

Tested samples	M. smegmatis		M. tuberculosis	
	MIC(µg/mL)	MBC(mg/mL)	MIC(mg/mL)	ΔGI
Compound 1	0.78	1.56	1.20(S)	0.0 ± 0.0
Compound 2	0.80	1.62	1.3 (S)	0.0 ± 0.0
Compound 3	0.03	0.12	0.10 (S)	8.0 ± 2.8
Ciprofloxacin (positive drug control for *M. smegmatis*)	0.15	0.31	nd	nd
Isoniazid (positive drug control for *M. tuberculosis*)	nd	nd	2 x 10^{-4} (S)	13.0 ± 0.7

MIC: Minimum inhibitory concentration.
MBC: Minimum bactericidal concentration.
ΔGI: value (mean ± SD) of the control vial (10), 38.0 ± 3.8 for the sensitive strain.
S: Susceptible.
nd: Not determined.

In the present study, compounds 1, 2 and 3 were subject to antituberculosis activities. Compound 1 was found to be active at 0.78 µg/mL and 1.20 µg/mL against *M. smegmatis*and *M. tuberculosis* respectively. Compound 1 had an MBC of 1.56 µg/mL against *M. smegmatis* (Table 1). Compound 2 was found to be active at 0.80 µg/mL and 1.30 µg/mL against *M. smegmatis* and *M. tuberculosis* respectively. Compound 2 had an MBC of 1.62 µg/mL against *M. smegmatis*. Compound 3 was found to be active at 0.03 µg/mL and 0.10 µg/mL against *M. smegmatis* and *M. tuberculosis* respectively. Compound 3 had an MBC of 0.12 µg/mL against *M. smegmatis;* The reactivity of these fatty alcohols could be due to the presence of the hydroxyl functional group in their chemical structure, or linked to the length of their molecular chain.

4. Conclusion

The results provide a rationale for the use of *Prosopis africana* in traditional medicine to treat various diseases. This study could be considered as a prelude to the discovery of new antimicrobial agents for bacteria and antituberculosis activities. In addition, the broad-spectrum activity of compounds 1, 2, and 3 revealed that compound 1 is active at 0.78 µg / mL and 1.20 µg /mL against *M. smegmatis* and *M. tuberculosis*, respectively. Compound 2 is active at 0.80 µg/mL and 1.30 µg / mL against *M. smegmatis* and *M. tuberculosis* respectively. Compound 3 is active at

0.03µg/mL and 0.10µg/mL against *M. smegmatis* and *M. tuberculosis* respectively. These results provide the opportunity to discover new and effective components for downstream clinical development. Many studies must be conducted to better understand its mode of action and anti-tuberculosis activity.

Acknowledgements

Dr. Nganso Ditchou Yves Oscar of the Department of Chemistry of the Faculty of Sciences of the University of Maroua, thanks Mr. Ndogo Eteme Olivier of the University of Yaounde I of the Department of Organic Chemistry for his contribution to this work. Mrs Mala Opono MT G of the laboratory of Biochemistry of the University of Yaounde I for the antimicrobial tests and Dr. Froumsia Moskia of the Department of Biological Sciences of the Faculty of Sciences of the University of Maroua for the harvest of *Prosopis africana*.

References

[1] Kim ST, Kim JD, Ahn SH, Ahn GS, Lee YI, Yeong YS (2004). *Phytother. Res*, 18: 971 – 975.

[2] LAOUALI A, GUIMBO ID, LARWANOU M, INOUSSA MM, MAHAMANE A (2014). Utilisation de *Prosopis africana (G. et Perr.)* Taub dans le sud du département d'Aguié au Niger: les différentes formes et leur importance. *International Journal of Biological and Chemical Sciences*, 8(3):1065-1074.

[3] Lee MW, Kim JH, Jeong DW, Ahn KH, Toh SH, Surh YJ (2000). *Biol. Pharm. Bull*, 23: 517 – 518.

[4] Madani M, Sirigne OS, Issoufou AM, Dackouo B (2016). Antioxidant activity and phytochemical study of leaf extract of *Prosopis africana* (Guill & Perr Taub) an anti-tumor plant used traditionally. *Journal of Chemical and Pharmaceutical Research*, 8(6):521-525.

[5] Kim HJ, Yeom SH, Kim MK, Shim JG, Paek IN, Lee MW (2005). *Arch. Pharm. Res*, 28: 177 – 179.

[6] Lee CJ, Lee SS, Chen SC, Ho FM, W. Lin WB (2005). *J. Pharmacol*, 146: 378 – 388.

[7] Kim JH, Lee KW, Lee MW, Lee HJ, Kim SH, Surh YJ (2006). *FEBS Lett*, 580: 385 - 392.

[8] Lydia OA, Abdullahi HY, Olajumoke MA (2010). Analgesic and anti-inflammatory effects of the methanol stem bark extract of *Prosopis Africana*. *Pharmaceutical Biology*, 48(3): 296–299.

[9] Buniatian ND, Chikitkina VV, Iakovleva LV. Eksp (1998). *Klin. Farmakol*, 61: 53 – 55.

[10] Santhaseelan H, Prabha S, Rathinam AJ, Yi-Hong T, Rahul N, Yang-Chang W, Hans-Uwe D, Fang R C (2017). Biopharmaceutical potentials of *Prosopis spp.(*Mimosaceae, Leguminosa*). journal of food and drug analysis*, 25: 187-196.

[11] Osha JAB, Suzu IUA (2015). Comparative studies of the pharmacological activities of *Prosopis Africana* fruits and its fraction. *Medicinal and Aromatic Plant Research Journal,* 3(1): 1-8.

[12] Ruhnke M, Schmidt WA, Engelmann E, Trautmann M (1996). Comparative evaluation of three antifungal susceptibility test methods for *Candida albicans* isolates and correlation with response to fluconazole therapy. *Journal of Clinical Microbiology,* 34(12): 3208–3211.

[13] Wright L, Scott E, Gorman S (1983). The sensitivity of mycelium, arthrospores and microconidia of *Trichophyton mentagrophytes* to imidazoles determined by *in-vitro* tests. *Journal of Antimicrobial. Chemotheraphy*, 12: 317-327.

[14] Arancibia L, Naspi C, Pucci, G, Arce M (2010). Aromatic plants from Patagonia: Chemical composition and antimicrobial activity of the essential oil of *Senecio mustersii* and *S. subpanduratus. Boletín Latino americano y del Caribe de Plantas Medicinale sy Aromáticas*, 9(2):123-126.

[15] Hattori M, Miyachi K, Hada S, Kakiuchi N, Kiuchi F, TSuda Y, Namba T (1987). Effects of long-chain fatty acids and fatty alcohols on the growth of *Streptococcus mutans*. *Chemical and Pharmaceutical Bulletin*, 35(8): 3507-3510.

[16] Faizi S, Siddiqi H, Bano S, Naz A, Mazhar K, Nasim S, Riaz T, S, Kamal S, Ahmad A, Khan A (2008). Antibacterial and antifungal activities of different parts of *Tagete spatula*: preparation of patuletin derivatives. *Pharmaceutical Biology*, 46(5): 309–320.

[17] Sannah P, NKAMI M (2009). Antituberculosis activity of flavonoids from *Galenia africana* L. var. africana BY A Submitted in partial fulfilment of the requirements for the degree DOCTOR OF PHILOSOPHIAE: PLANT SCIENCE in the Faculty of Natural & Agricultural Science University of Pretoria Pretoria. 55-60.

[18] Reina M, González-Coloma A, Cabrera R, Gutierrez C, Rodriguez M, Fajardo V, Villarroel L (2001). Defensive Chemistry of *Senecio miser*. *Journal Natural Products*, 64: 6-11.

[19] Gonçalves JDS, Vieira IJC, Raimundo BF, Branco A (2015). Chemicals from Agave *sisalana* Biomass: Isolation and Identification. *International Journal of Molecular Sciences*, 16:8761-8771.