Research Article

In Vitro Antimicrobial Properties of Chemical Constituents Isolated from Leaves of *Trichilia emetica* (Meliaceae)

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ABSTRACT

Trichilia emetica a plant native to Africa belonging to the Meliaceae family is used in traditional medicine by indigenes to treat various ailments such as: malaria, cough, gastric ulcer, dysmenorrhea, asthma, intestinal worms, high blood pressure, skin infections and oral infections. The purpose of this study was to evaluate the antimicrobial properties of compounds isolated from the leaves of *Trichilia emetica* (Meliaceae). *In vitro* studies conducted by many researchers have shown that *Trichilia emetica* has antimicrobial properties. The chemical study of the methanol extract of the leaves of *Trichilia emetica* led to the isolation of three compounds: stigmasteryl palmitate (1); 3β-Lup-20(29)-en-3-ol or Lupeol (2); Rohituca-3 (3) isolated for the first time from the leaves of *Trichilia emetica*. Their structures have been elucidated based on spectroscopic analysis and comparison of their spectral data with those reported in the literature. The results of the antimicrobial activity showed that compounds 1, 2 and 3 strongly inhibit *Staphylococcus aureus ATCC BAA 977* and *Streptococcus pneumoniae ATCC49619* and moderately inhibit *Staphylococcus aureus NR46374*. Compounds 2 and 3 showed strong inhibition on *Hemofilus enfluenza ATCC 49247*, with minimal inhibitory concentrations (MICs) less than or equal to 0.5 mg/mL. The results of this study suggests that *Trichilia emetica* represents an untapped source of compounds with potential antimicrobial activity that could be explored in the development of new therapeutic natural product

Keywords: Antimicrobial properties, Limonoids, Meliaceae, Steroids, *Trichilia emetica*, Triterpenes.

INTRODUCTION

Plants play a vital role in the daily life of men. Since time immemorial, they are used as firewood, raw materials in the real estate, decoration and in the treatment of the diseases[1]. Today, these are a hive of drugs because they are fully integrated into African customs and play a pivotal role in traditional pharmacopoeia in the fight against many diseases [1]. In addition to these ethno-pharmacological uses, it is impossible to deny that nature offers to pharmacopoeia the traditional use which gives indications of the expected effect. Thus, several plants have been used as medicine in traditional medicine and are now introduced in modern medicine[1]. Despite the success of modern medicine through certain pharmaceutical and health care products, most African populations still consult traditional healers for their health problems. This is because of the climate of trust between the traditional practitioner and the patient, the high cost of health services and conventional medicines, and the inadequacy of health education campaigns. However, despite the integration of herbal medicines (decoction, infusion, macerate ...) in our customs and their efficiency more or less observed, clarifications remain to be made on the chemical composition of these phyto medications. Above all, there is a question of health and more and more pathogens resist the treatments prescribed for the total recovery of health by the patients. However, researchers continue to develop new drugs that may be more effective. Naturally derived drugs with antibacterial, antifungal, anticoagulant, antiparasitic, immunosuppressive and anticancer activity are able to treat 87 % of the listed human diseases [2]. Of the 520 new drugs approved between 1983 and 1994, 39 % were natural products or derived from natural products and 60 to 80 % of antimicrobials were derived from natural products [3]. These data justify work in the field of natural products, especially given their importance in the search for new drugs against bacterial and fungal infections. With all the above, we are interested in a Cameroonian medicinal plant of the Meliaceae family *Trichilia emetica.*

Trichilia emetica is used in traditional medicine to treat various ailments such as: abdominal pain, malaria, skin infections, oral infections, cough, gastric ulcer, hemorrhoids, jaundice and chest pain [4]. *In vivo* and *in vitro* studies carried out by many researchers have shown that *Trichilia emetica* has anti-inflammatory[5], antioxidant[6,7], antimicrobial[6], antiplasmodic[7,8], antitripanosomal and antimutagenic properties [9]. Previous chemical studies on *Trichilia emetica* have led to the isolation and characterization of a few secondary metabolites, including steroids, terpenoids, limonoids,

flavonoids and coumarins [10]. The limonoids are the family of compounds most represented in the Meliaceae family [10]. Thus, the general objective of this study is to evaluate the antimicrobial properties of secondary metabolites isolated from leaves of *Trichilia emetica* (Meliaceae).

MATERIALS AND METHODS

General experimental technique

After drying, crushing of the leaves of *Trichilia emetica* was carried out using a crushing machine. The maceration of the powder in methanol was done in a tightly sealed 20 L can.

An electronic scale of the HANGING SCALE Electronic type was used to measure the powdered mass of the crushed leaves;

An electronic scale of the LUTRON GM-300P type was used to weigh the mass of the raw extract of the sub-fractions and the products obtained;

A Buchi brand Heidolph WB 200 rotary evaporator was used for the evaporation and the condensation of the crude extract and the various fractions obtained;

The flash chromatography was carried out using a VELP Scientifica vacuum cleaner, a micropore Buchner and a vacuum flask;

The column chromatography was carried out in a column 2 cm in diameter and 40 cm in length and another 3 cm in diameter and 23 cm in length. KIESELGEL silica type 60 (0.04-0.063 mm) was used as stationary phase;

Thin-layer analytical and preparative chromatographies were carried out on aluminum support plastic plates of dimension 20 x 20 cm^2, 0.2 mm thick and covered with a 60F254 silica layer Merck;

The purity of the compounds was revealed by heating TLC using a PHILIPS brand hairdryer after spraying each plate with sulfuric acid diluted to 10 %;

The ^1H NMR and ^{13}C NMR spectrum was obtained through a Delta JEOL ECA500II spectrometer with TMS as reference at a frequency of 600 MHz for the proton and 125 MHz for the carbon. This was done at the Pharmacognosy Division of the Institute of Natural Medicine, Toyoma University, Japan.

Plant Material

The leaves of *Trichilia emetica* were collected in Mokolo in the Far North Region of Cameroon in November 2017 by Dr. Froumsia Moskia of the Department of Biological Sciences of the Faculty of Sciences of the University of Maroua. This plant was identified in comparison with the sample collected by Leeuwenberg A.J.M. 7651 of the specimen collection of the National Herbarium of Cameroon (Yaounde) under the identification number 40986/HNC.

Extraction and Isolation

After cutting, drying and grinding the leaves of *Trichilia emetica*, 1060 g of powder was obtained and macerated twice at room temperature with pure methanol for 48 hours. The filtrate obtained was evaporated using a rotary evaporator under atmospheric pressure and gave a mass of 153 g of crude extract. 80 g of crude extract was hot-fixed on 80 g of silica gel (0.063-0.200 mm) and the Büchner was filled with 100 g of silica gel as a stationary phase and then subjected to flash chromatography. The elution with a solvent system (hexane/ethyl acetate) by increasing polarity gradient made it possible to obtain eighty-five (85) vials grouped based on the analytical TLC in four (04) major fractions. Indexed from A to D.

Purification of the Different Fractions

Chromatography of fraction A

Fraction **A (0.889 g)** was fixed on 3 g of hot silica gel and then introduced into a chromatographic column containing 30 g of silica gel (0.063-0.200 mm) as a stationary phase. Elution of this fraction was done in the hexane/ethyl acetate gradient system by increasing polarity. Twenty-seven (27) vials were collected grouped into five (05) fractions ranging from A_1 to A_5 based on analytical TLC. The sub fractions A_3 and A_5 crystallized in pure methanol and, after washing (decantation-filtration), two compounds were obtained: **stigmasteryl palmitate (1)** and **(3β)-lup-20(29)-en-3-ol** or **Lupeol (2)**. **(1)** was obtained in the form of milky white crystals and is soluble in the hexane/ethyl acetate system (7:3), **(2)** was obtained in the form of white crystals soluble in the hot hexane/ethyl acetate system (6:4).

Chromatography of fraction B

Fraction **B (2.06 g)** was hot-fixed on 4 g of silica gel and then introduced into a chromatographic column containing 40 g of silica gel (0.063-0.200 mm) as a stationary phase. Elution of this fraction was done in the hexane/ethyl acetate system by gradient of increasing polarity. This made it possible to collect one hundred and thirty-three (133) vials grouped into eight (08) sub-fractions ranging from B_1 to B_8 based on analytical TLCs. At the end of the column chromatography on the major fraction B, **Rohituca-3 (3)** was obtained from leaves of *Trichilia emetica* in the form of white crystals in hexane/ethyl acetate (3:7). It melts at 174-176 °C and gives a positive reaction to the Erhlich test.

Physical and spectral data of compounds (1, 2 and 3)

Stigmasterol palmitate (1): Calculated mass of $C_{45}H_{78}O_2$, m/z = 651 g/mol

^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 11.1 (2H, m); 1.53 (2H, m); 3.53 (1H, m); 2.32 (2H, d); 5.39 (1H, m); 2.38 (1H, m); 1.53 (2H, m); 2.02 (2H, m); 0.84 (2H, m); 1.09 (1H, m); 1.12 (1H, m); 0.68 (3H, s); 1.01 (3H, s); 0.96 (3H, d); 5.18 (1H, m); 5.06 (1H, m); 0.95 (1H, m); 1.70 (1H, m); 0.88

(3H, m);0.86 (3H, m); 0.84 (3H, m); 0.86 (3H, m); 2.30 (2H, t); 1.19 (2H, m); 1.25 – 1.29 (20H, m); 0.92 (3H, t).

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 11.88; 12.00; 14.14; 18.9; 19.05; 19.1; 19.42; 19.85; 21.10; 21.2; 22.72; 23.08; 24.32; 24.74; 25.5; 26.08; 28.27; 29.09; 29.16; 29.27-29.73; 31.65; 31.8; 31.92; 31.92; 33.83; 33.85; 33.96; 33.96; 36.17; 36.52; 37.27; 39.79; 39.9; 40.52; 42.29; 42.34; 45.85; 50.15; 51.26; 55.97; 56.07; 56.78; 57.0; 71.85; 121.76; 129.29; 138.34; 140.76; 178.38.

(3β)-lup-20(29)-en-3-ol or **Lupeol (2):** Calculated mass of $C_{30}H_{50}O$; m/z = 426 g/mol

m/z: 426.3862 (100.0 %), 427.3895 (32.4 %), 428.3929 (5.1 %)

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 0.76; 0.79; 0.83; 0.94; 0.97; 1.03; 1.68; 3.18; 4.56; 4.685.

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 14.5; 16.2; 16.5; 17.2; 18.7; 18.7; 19.6; 21.3; 25.5; 27.7; 27.8; 28.3; 29.8; 34.6; 36.0; 37.5; 38.5; 39.1; 39.2; 40.4; 41.4; 43.0; 43.2; 48.4; 48.7; 50.8; 55.7; 79.4; 109.7; 151.0.

Rohituca-3 (3): Calculated mass of $C_{32}H_{40}O_{11}$; m/z = 600 g/mol, m/z: 600.2571 (100.0 %).

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 0.79 (3H, s); 0.83 (3H, t, 7.5); 0.93 (3H, d, 6.5); 1.17 (1H, m); 1.25 (3H, s); 1.33 (1H, m); 1.64 (3H, s); 1.66 (1H, m); 2.40 (1H, m); 2.44 (1H, t; 2.0); 2.72 (1H, m); 2.79 (1H, m); 2.80 (1H, m); 2.81 (1H, m); 2.98 (1H, t; 9.0); 3.22 (1H, d; 10.0); 3.78 (1H, d, 3.5); 3.99 (1H, d, 11.0); 3.99 (1H, t; 3.0); 3.01 (1H, m); 4.22 (1H, t; 10.0); 4.24 (1H, d, 11.0); 5.35 (1H, s); 5.42 (1H, s); 5.56 (1H, d; 10.0); 6.10 (1H, s); 7.07 (1H, s); 7.32 (1H, s).

^{13}C NMR (150 MHz,$CDCl_3$) δ (ppm): 12.1 (q); 13.1 (q); 15.1(q); 16.9 (q); 24.2 (t); 24.2 (t); 26.9 (q), 31.4 (t); 31.4 (t); 35.1 (d); 37.3 (t); 37.3 (t); 39.4 (d); 42.1 (t); 42.1 (t); 43.9 (d); 48.2 (s); 51.8 (s); 54.8 (d); 72.0 (t); 72.0 (t); 74.5 (d); 75.2 (d); 78.7 (d); 79.3 (s); 79.7(s); 79.8 (d); 110.6 (d); 121.5 (s); 122.7 (d); 122.7 (d); 138.9 (s); 140.0 (d); 143.3 (d); 167.7(s); 171.3 (s); 175.4 (s); 209.1 (s).

ANTIMICROBIAL ACTIVITIES

Microorganisms

The antimicrobial activities of the crude methanol extract and compounds isolated from the leaves of *Trichilia emetica* were determined using certain microbe pathogens according to established standard procedures and conditions. The microorganisms used consist of strains and isolates of bacteria and yeasts. Thus, strains of bacteria are: *Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 43300, Klepsiella pneumoniae ATCC 700603, Staphylococcus aureus BAA 977, Streptococcus pneumoniae ATCC 41619, Hemofilus influenza ATCC 49247* from American type culture collection, *Staphylococcus aureus NR46374, Shigella NR518 flexineri, Salmonella enterica NR13555* from EIB resources and *Salmonella typhi CPC* isolate from CHU (University Hospital Center).

Study of the antimicrobial activity of the crude extract with methanol and compounds (1,2 and 3).

The study of the antimicrobial activity of the crude methanol extract and the compounds isolated from the leaves of *Trichilia emetica* consisted in determining parameters of antimicrobial activity (CMI and CMB/CMF) of the extract and compounds isolated from *Trichilia emetica*. All manipulations were performed under sterile conditions under a microbiological hood.

Preparation of extract solutions, compounds and reference antibiotics

500 mg of the various extracts and compounds were weighed and dissolved in 1 mL of 100 % DMSO to obtain a stock solution of 500 mg/mL extract concentration. Antibiotic solutions (ciprofloxacin and fluconazole) were prepared under the same conditions by dissolving respectively in 2 mg in 1 mL of distilled water.

Determination of the minimum inhibitory concentration (MIC) of the extracts and compounds by the liquid microdilution method

Minimal Inhibitory Concentration (MIC) is the lowest antimicrobial concentration that can inhibit any visible growth after incubation[11]. For this work, the minimum inhibitory concentration (MIC) was determined using the M27-A3 microdilution method of the Clinical Laboratory Standards Institute [12] using microtiter plates (12x8 wells).

Principle

This method is based on the assessment of the ability of microorganisms to multiply and survive in a medium containing extracts and compounds isolated from *Trichilia emetica* at different concentrations. The viability of bacteria and yeasts was thus determined by observing the change in colour from blue to pink, thus reflecting the reduction of resazurin (blue) to resorufin (pink) by the dehydrogenases of living cells [11].

Method

The tests were carried out in 96-well microplates. In fact, 100 µL of RPMI 1640 medium were introduced into all the wells of the plate; then 100 µL of a stock solution of an extract were taken and added to the 100 µL of the first wells of row 1 except for column C and F. A series of 10 dilutions of geometric reason 2 was made from line 1 to line 11; and finally, 100 µL of a suspension of a single microorganism prepared under the title 2×10^4 Cells/mL for yeasts and 5×10^4 Cells/mL. For the bacteria, they were sown in the wells of the plates, except those of the columns C and F which constituted the white; the wells on line 12 were the negative control. Extract and control positive concentrations (ciprofloxacin/fluconazole) in the wells of one column ranged from 20 to 0.39 mg/mL and from 32 µg/mL to 0.0625 µg/mL respectively. Plates were sealed and incubated at 37 °C for 24 hours for bacteria and 48 hours for yeasts. At the end of the incubation time, the lowest concentration at which no visible growth

is observed, marked by the reduction of resazurin from blue to pink, corresponds to the MIC of the extract [11].

Determination of minimal fungicide concentrations (CMF) by subculture

The determination of the CMB/CMF of the extracts was made by a subculture, taking 25 µL of the mixture from each of the 4 wells (the cup following the MIC, the cup of the MIC and the two cups preceding the MIC and seeding after homogenization). 10 mL of sterile solid medium (SDA) contained in previously labeled silted petri dishes, which allows four wells to be inoculated per extract. The dishes thus prepared were incubated at 37 °C. For 24 hours for the bacteria and 48 hours for yeasts and growth was then observed. The smaller concentrations showing no growth were noted as representing CMB/CMF.

RESULTS AND DISCUSSION

Elucidation of structures

The leaves of *Trichilia emetica* collected in Mokolo in the Extreme-North Region Cameroon in November 2017, underwent several operations, starting from cutting, to chromatographic techniques, which made it possible to isolate three compounds **(1), (2)** and **(3).**

Compound **(1)** was obtained in the form of a white powder soluble in ethyl acetate. The analysis of its ^{1}H NMR spectrum shows in the weak fields a broad triplet integrating 1 H at 5.35 ppm, two doublets of low intensity, one at 5.18 and the other at 5.06 ppm integrating for 1H. each and a multiplet integrating 1H at 3.53 ppm. In strong fields, there is a large and intense peak between 1.25 and 1.29 ppm, integrating 20H and a peak characteristic of a terminal methyl at 0.91 ppm integrating 3H. On its ^{13}C NMR spectrum, olefinic carbons are observed at δ_C 140.76; 121.76; 138.34 and 129.29 ppm, an oxygenated carbon at δ_C 71.85 ppm, a 178.38 ppm signal characterizing a carbonyl group, a broad and intense peak between 29.27 and 29.73 ppm and a signal, 14.14 ppm. The peaks observed at δ_H 5.18 (1H, m) and at δ_H 5.06 (1H, m) are attributable respectively to the signals of the H-22 and H-23 protons of stigmasterol and to a triplet at δ_H 5.35 integrating 1H. Characteristic of the vinylic proton H-6 of phytosterols, in this case stigmasterol. This is confirmed on the HSQC spectrum by signals at δ_C 121.76 (C-6) and 140.76 (C-5) ppm. Similarly, on the ^{1}H NMR spectrum, the multiplet appearing at δ_H 3.53 (1H) corresponding to a proton carried by an oxygenated carbon (C-3), characteristic of the H-3 proton steroid derivatives. In addition, the peak observed at δ_C 178.38 ppm is attributable to the C-1' carbon which characterizes the carbonyl group bonded to the oxygen at position 3 of the sterol. The presence of a linear chain is explained by the presence of a peak set between 29.27 and 29.73 ppm attributable to C-4' to C-13' carbons and a terminal methyl group [13]. The analysis of its HSQC spectrum allows for the attribution of some carbon protons. Thus, in strong fields, between δ_H 0.67 and δ_H 1.02 ppm, there are several methyl groups that can be observed at δ_H 0.68 (3H, s, Me-18); 0.84 (3H, s, Me-29); 0.88 (3H, d, Me-26); 0.86 (3H, s, Me-27); 1.01 (3H, s, Me-19); 0.96 (3H, s, Me-21). On its HMBC spectrum, the correlation between the proton H-6 and the carbons C-4, and C-10 and C-7, also the correlations between the proton H-4 and the carbons C-1', C-5, C-2, C-3 and C-6 is observed. We also note the correlations of proton H-18 and carbons C-12, C-13 and C-17. Moreover, correlations between the H-25 proton and the C-24 carbon are also observed. Based on these data, and in comparison, with the data of the literature, compound **(1)** has been identified as **stigmasteryl palmitate** of empirical formula $C_{45}H_{78}O_2$ [13].

Compound **(2)** was obtained as white crystals in hexane/ethyl acetate 6 %, was soluble in hot hexane/ethyl acetate 40 % and crystallized in methanol. On its ^{1}H NMR spectrum, six (06) singlets integrating three protons each (δ_H=0.76 ppm, 0.79 ppm, 0.83 ppm, 0.94 ppm; 97 ppm, 1.03 ppm) corresponding to six methyl groups. These results suggest the structure of a triterpene [14]. We also note the presence of a singlet integrating for three protons (δ_H=1.68 ppm) attributable to a methyl group carried by an ethylenic carbon and two signals integrating for a proton each. In addition, we note the presence of a multiplet at δ_H=4.56 ppm and a doublet at δ_H=4.69 ppm (J=2.2 Hz) attributable to the two germinal ethylenic protons. On the other hand, the observation of a double doublet integrating for a proton [δ_H=3.18 (J=5.3 and 10.8 Hz)] proves the presence of a hydroxyl group in compound [15]. The decoupled ^{13}C NMR spectrum reveals the presence of thirty (30) carbon signals, including two ethylenic carbons at δ_C=109.47 ppm and δ_C=151.12 ppm attributable respectively to the C_{29} and C_{20} carbons. Moreover, a signal at δ_C=79.14 ppm attributable to the carbon C_3 characteristic of a hydroxylated carbon [16] is observed. These physical and spectroscopic data compared to those described in the literature made it possible to identify the compound **(2)** as **(3β)-lup-20(29)-en-3-ol** or **lupeol** with empirical formula $C_{30}H_{50}O$, having six degrees of unsaturation, isolated for the first time leaves of *Trichilia emetica* [15].

Compound **(3)** was obtained as white crystals in hexane/ethyl acetate (3:7). It melts between 174-176 °C and gives a positive reaction to the Erhlich test thus suggesting its limonoid nature. Its ESI-TOF ionization mass spectrum shows the peak of the molecular ion at m/z=600.6, whose high-resolution analysis m/z=600.6659 gives it the raw formula $C_{32}H_{40}O_{11}$ (calc. 600,6678) containing thirteen (13) degrees of unsaturation. Its Fourier transform IR spectrum shows carbonyl absorption bands at (υ_{max} 1744 cm^{-1}, 1710 cm^{-1}) and olefins at υ_{max} 1636 cm^{-1}. Its proton decoupled ^{13}C NMR broadband spectrum shows 32 signals corresponding to the 32 carbon atoms present in the molecule. The analysis of these signals by DEPT technique and by interpretation of the HSQC spectrum reveals the presence of: Five methyl groups whose resonance signals appear at δ_c 12.1; 13.1; 15.1; 16.9 and 26.9. Six methylenes including a sp^2 hybrid

resonant δ_c 122.7 and the other five sp^3 hybridized appearing at δ_c 72.0; 42.1; 37.3; 31.4 and 24.2. Eleven methines including 3 sp^2 hybridized at δ_c 143.2; 140.6 and 110.6 and eight other sp^3 hybridized at 79.8; 78.7; 75.2; 74.5; 54.8; 43.9; and 39.4. This makes it possible to deduce the presence of ten quaternary carbons within this compound, among which four carbonyls, including three esters with δ_c 175.4; 171.4 and 167.7 and one of ketone at 209.1; two sp^2 hybridized carbons at δ_c 138.9 and 121.9 and four sp^3 hybridized carbons at δ_c 79.7; 76.8; 51.9 and 48.2. The ^{1}H NMR spectrum analysis which is completed by both the COZY and HSQC spectra, makes it possible to highlight the presence of: Five methyls, three of which give singlet signals, each integrating for three protons at δ_H 1.64 (3H, s), δ_H 1.25 (3H, s) and δ_H 0.79 (3H, s) attributable to the angular methyl a triterpene-type backbone, the two signals at δ_H 0.83 (3H, d, 7.5)/δ_c 12.1 and at δ_H 0.93 (3H, t, 7.0)/δ_c 14.9, being assigned to the two methyl signals of a 2-hydroxy-3-methylvalerate unit, in which appears correlations between the proton H-2'at δ_H 3.78 (1H, d, 3.5) with the proton H-3 'at δ_H 1.66 (1H, m), which in turn correlates with the protons of methyl in position 3'- δ_H 0.93 (3H, d, 7.0) on the one hand and with the protons at position 4' at δ_H 1.33 (1H, m) and at δ_H 1.17 (1H, m) on the other hand. It is also observed on this spectrum: Three broad singlets of a proton each at δ_H 7.07 (1H, s), δ_H 7.32 (1H, s) and at δ_H 6.10 (1H, s) characteristic of a β-substituted furan ring in position 17α and whose presence is confirmed by signals at δ_c 143.2; 140.6 and 110.6 observed on its ^{13}C NMR spectrum. Two broad singlets of a proton each at δ_H 5,35 (1H, s)/δ_c 122,8 and δ_H 5,42 (1H, s)/δ_c 122,8 attributable to the protons of a terminal methylene at position 8 and characteristic of a limonoidic B-type skeleton [17]. A signal at δ_H 2.98 (1H, t, 9.0) typical of the proton in the 17β position, and which has correlation spots on the COZY spectrum with the 2 methylenic and diastereotopic protons at position 16. The fact that the only correlations of the H-16 protons are those with the H-17 proton, lead us to position the resonant ketone carbonyl at δ_C 209.1 at position 15. This was confirmed by the HMBC correlations observed between the H-16 proton and carbon C-15 (δ_C 209.1). Moreover, the correlations observed between the olefinic protons H-30 with the quaternary carbon C-14 resonating at δ_C 79 makes it possible to place the OH group on this carbon. All of this is in accordance with the literature data, according to which the prieurianine derivatives have within their structure either a 14-hydroxy-15-keto system or a 14-15 epoxide ring [17]. Three multiplets of one proton each at δ_H 3.99 (1H, t, 3.0)/78.7; 3.01 (1H, m)/37.3 and 2.80 (1H, m)/37.3 respectively attributable to protons H-1 and H-2 of a lactonized A ring, present in the compounds of the class of prieurianine. Two AB systems of two methylenic diasteriotopic protons, one of which resonates in weak fields at δ_H 4.24; 3.99 (1H, d, 11.0)/δ_C 72.8 and the other in strong fields at 2.79; 2.72 (1H, m)/δ_C 31.4 both show spots of HMBC correlations with C-7 carbonyl at δc 171.3 of a pyronne-type ring. All these spectral data are compatible with the presence of a prieurianine-type *seco*-limonoid A, B skeleton in the structure of the compound **(3)** [17]. It only remains to determine the position of the substituents on the limonoidal skeleton. This was made possible by correlations J^2 and J^3 observed on the HMBC spectrum of this compound. Indeed, the 2-hydroxy-3-methylvalerate group was localized in position C-12 thanks to the HMBC correlations observed between proton H-12 at δ_H 5.87 (1H, d, 10) and carbon C-1' at δ_C 175.4. The ether junction between C-1 and C-11 carbons was established based on biogenetic considerations and literature data [17]**.** As for the relative stereochemistry around carbons, C-1, C-11 and C-12, they were established on the basis of the J^3 coupling constants, observed between the protons carried by these different carbons and confirmed by the data of the literature. Indeed, the coupling constant $J^3_{H9\text{-}H11}$=10 Hz and $J^3_{H11\text{-}H12}$=10 Hz suggests that the protons H-9 and H-11 on the one hand, and the protons H-11 and H-12, on the other hand have a relative trans stereochemistry. On the basis of all these physical and spectroscopic data and in comparison with those of the literature, compound **(3)** was identified with **rohituka-3** isolated for the first time from leaves of *Trichilia emetica*, Gunatilaka et al. [18].

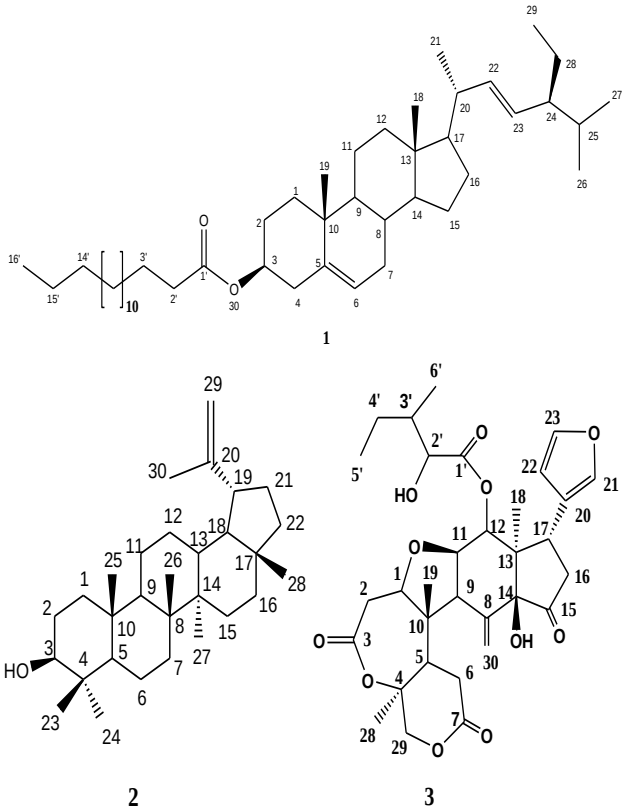

Figure 1: Structure of compounds 1 to 3 isolated from *Trichilia emetica*

Antimicrobial Results

Previous pharmacological studies on *Trichilia emetica* have shown several activities including amongst others: antimicrobial and antifungal. It is in this context that antimicrobial tests on the crude methanol extract and compounds isolated from the leaves of *Trichilia emetica*

were carried out. This is to confirm their use in traditional medicine. The active extracts and/or the isolated products could thus justify the use of this plant in traditional medicine and also permit starting from the present work, to open other avenues of research.

Antimicrobial activities of methanol extract and compounds (1), (2) and (3)

The antimicrobial tests carried out show that the target targets for these different antimicrobial tests are: bacteria, which contain a class of living organisms called microbes. Therefore, it is necessary to carry out the antimicrobial tests of the methanolic extract and compounds isolated from *Trichilia emetica*. According to Aligiannis et al**.** [19]**,** the classification of plant material extracts according to MIC (Minimum Inhibitory Concentration) indicates that:

-For strong inhibition: MIC less than 500 µg/mL;

-For moderate inhibition: MIC varies between 500 µg/mL and 1500 µg/mL;

-For weak inhibition: MIC greater than 1500 µg/mL.

Antibiotics are chemical substances produced by microorganisms or by chemical synthesis of molecules derived from natural compounds. They prevent the growth of other microorganisms and in some cases, can destroy them [20]**.** Bacteria multiply under controlled conditions on a nutrient medium. If this nutrient medium contains an antibacterial substance, two effects must be distinguished:

-Bactericidal effect: the bacteria are killed

-Bacteriostatic effect: the bacteria survive but do not multiply anymore.

If the bacterial multiplication remains intact under the action of an antibacterial substance, we are dealing with a phenomenon of bacterial resistance [20]. The Minimal Inhibitory Concentration (MIC) of an antibiotic is defined as the lowest concentration of antibiotic inhibiting between 18 to 24 hours the multiplication of bacteria (bacteriostasis). This value makes it possible to classify a bacterial strain in the sensitive, resistance or intermediate categories to the action of an antimicrobial agent [21]. Minimal Bactericidal Concentration: (CMB) is the lowest concentration of agent capable of killing at least 99.99 % of bacteria in an inoculum (<0.01 % of survivors). Bactericidal Indicator Value determined by enumeration of bacteria on agar medium. The results of the antibacterial activity of the Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (CMB) of the crude methanol extract and compounds isolated from the leaves of *Trichilia emetica* are contained in Tables 1 and 2 below.

According to Tables 1 and 2, it is observed that the crude extract and the compounds have an antibacterial activity with MICs which vary from 0.25 to 10 mg/mL, not only according to the crude extract and compounds but also depending on the bacterial strains tested. The least sensitive strains are *Staphylococcus aureus ATCC 43300 and Salmonella enterica NR13555* (MIC >10 mg/mL) and the most sensitive is *Staphylococcus aureus BAA 977* (MIC 1.25 mg/mL).

Table 1: Minimum inhibitory concentration (MIC) of the crude methanol extract and compounds (1), (2), (3).

Strains	Crude extract and compounds (1), (2), (3) (mg/mL)				Reference (µg/mL)
	TEEB	(1)	(2)	(3)	CP
Salmonella typhi CHU	5.0±0.00	10±0.00	5±0.00	5±0.00	0.25±0.00
Streptococcus pneumoniae ATCC49619	5±0.00	5±0.00	5±0.00	5±0.00	0.5±0.00
E. coli ATCC 25922	>10	10±0.00	10±0.00	10±0.00	0.125+0.0625/2
Staphylococcus aureus ATCC 43300	>10	>10	>10	10±0.00	0.0625±0.00
Klepsiella pneumoniae ATCC 700603	10±0.00	10±0.00	10±0,00	10±0.00	0.125±0.0625/2
Hemofilus enfluenza ATCC 49247	10±0.00	10±0.00	5±0.00	5±0.00	0.5
Staphylococcus aureus BAA 977	2.5±0.00	2.5±0.00	2.5±0.00	1.25±0.00	0.5
Shigella flexineri NR518	>10	10±0.00	10±0.00	5±0.00	0.125±0.0625/2
Staphylococcus aureus NR46374	10±0.00	10±0.00	10±0.00	5±0.00	1±0.5/2
Salmonella enterica NR13555	>10	>10	10±0.00	10±0.00	0.0625±0.00

CHU: *University Hospital Center;* ***TEEB:*** *Crude methanol extract of Trichilia emetica;* ***(1):*** *Stigmasteryl palmitate;* ***(2):*** *Lupeol;* ***(3):*** *Rohituka-3;* ***CP:*** *ciprofloxacin.*

Table 2: Minimum Bactericidal concentration CMB of the crude methanol extract and compounds (1), (2), (3).

Strains	Crude extract and compounds (1), (2), (3) (mg/mL)				References (µg/mL)
	TEEB	(1)	(2)	(3)	CP
Salmonella typhi CHU	>10	>10	5±0,00	5±0.00	0.25±0.00
Streptococcus pneumoniae ATCC49619	>10	10±0.00	10±0.00	10±0.00	0.5±0.00
Escherichia coli ATCC 25922	>10	10±0.00	10±0.00	10±0.00	0.25
Staphylococcus aureus ATCC 43300	>10	>10	>10	10±0.00	0.5
Klepsiella pneumoniae ATCC 700603	>10	>10	>10	>10	0.5
Hemofilus enfluenza ATCC 49247	>10	>10	>10	10±0.00	0.5
Staphylococcus aureus BAA 977	10±0.00	10±0.00	10±0.00	5±0.00	0.5
Shigella flexineri NR518	>10	>10	>10	10±0.00	0.125
Staphylococcus aureus NR46374	>10	>10	>10	5±0.00	1
Salmonella enterica NR13555	>10	>10	>10	>10	0.125±0.00

CHU: *University Hospital Center:* ***TEEB:*** *Crude methanol extract of Trichilia emetica;* ***(1):*** *Stigmasteryl palmitate;* ***(2):*** *Lupeol;* ***(3):*** *Rohituka-3;* ***CP:*** *ciprofloxacin*

DISCUSSION

Antibiotics are chemical substances produced by microorganisms or by chemical synthesis of molecules derived from natural compounds. They prevent the growth of other microorganisms and in some cases, can destroy them [20]. Bacteria multiply under controlled conditions on a nutrient medium [20]. According to the Ponce's classification stated in this work, the results of antibacterial tests revealed that compounds isolated from leaves of *Trichilia emetica* have more or less important activities for the gram-negative and gram-positive bacterial species tested. These antibacterial activities observed could be due to the presence of different secondary metabolites in the methanol crude extract. Indeed, Marjorie [22] reported that phenolic compounds, alkaloids and saponins inhibited the growth of microorganisms. It also emerges from our results that the isolated compounds have a superior antibacterial capacity to the methanolic extract of the leaves on most of the strains tested. This difference is due to the fact that the sensitivity of a microorganism to a plant extract depends not only on the functional groups, but also on the microorganism itself [23]. The non-effectiveness of the compounds vis-a-vis other bacterial strains can be explained either by the absence of compounds likely to inhibit their growth, or by their low concentration in the methanol crude extract. According to the Aligiannis classification [19] stated in our results, the compounds **(2)** and **(3)** tested showed a strong inhibition on *Staphylococcus aureus ATCC BAA 977*, a moderate inhibition on *Staphylococcus aureus NR46374*, a weak inhibition on *Aerococcus viridans ATCC 11563*, moderate inhibition on *Hemofilus enfluenza ATCC 49247* for compound **(2)** and low for compound **(3)**, finally a low inhibition on *Salmonella enterica NR13555* for compounds **(2)** and **(3)** compared to ciprofloxacin which has a strong inhibition on this strain. The results obtained corroborate with the work of Germano et al. [24] which reported that there are compounds in *Trichilia emetica* that are responsible for antibacterial activities against many microbial strains.

CONCLUSION

The objective of this work was to evaluate the antimicrobial properties of the compounds isolated from the leaves of *Trichilia emetica*, a plant of the Meliaceae family. Found in a large part of Africa, this plant is of great importance both in terms of medicine and socio-economically for indigenous peoples. The chemical study of these leaves led to the isolation of three compounds, stigmasteryl palmitate **(1)**, (3β)-lup-20(29)-en-3-ol) or lupeol **(2)** and Rohituka-3' **(3)**. Antimicrobial tests of compounds isolated from *Trichilia emetica* are strongly related to microorganisms and the composition of extracts. Indeed, the wide range of antimicrobial properties can be explained by the presence of various groups of potentially active secondary metabolites. The existence of these antimicrobial substances was confirmed by phytochemical screening which revealed the presence of certain classes of compounds that had already been demonstrated in their antimicrobial activities. The results obtained support their traditional use by local communities as therapeutic agents for the treatment of gastric ulcer, intestinal worms, malaria and other infectious diseases. Overall, this study showed that compounds obtained from the leaves of *Trichilia emetica* could be a potential source of antimicrobial agents.

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