

Research Article



Phytochemical Composition and Biological Activity of *Faidherbia albida* (Mimosaceae) Roots and Leaves

Tchebemou Bakang Bruno¹, Soh Désiré², Nkwengou Tchouboun Zondegoumba Ernestine^{1*}, Nganso Ditchou Yves Oscar³, Emmanuel Mouafo Tekwu⁴, Kerstin Andrea-Marobela⁵, Nyasse Barthélemy^{1*}

¹Department of Organic Chemistry, Faculty of Science, University of Yaoundé I, Box 812 Yaoundé, Cameroon.

² Department of Chemistry, Higher Teacher Training College, University of Bamenda, PO Box 39 Bambili, Cameroon /TWAS Research Unit (TRU) of The University of Bamenda, Bamenda, Cameroon.

³Department of Chemistry, Faculty of Science, University of Maroua, P.O Box 814, Maroua, Cameroon.

⁴Laboratory of Tuberculosis Research and Pharmacology, Biotechnology Centre, Nkolbisson, University of Yaoundé 1, Yaoundé, Cameroon.

⁵Department of Biological Sciences University of Botswana Block 235/Room 251 Private Bag 0022 Gaborone Botswana.

*Corresponding author's E-mail: ernestine.nkweng@gmail.com

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ABSTRACT

Phytochemical composition of roots and leaves of *Faidherbia albida*, led to the isolation and the identification of fourteen (14) compounds namely 3 β -Friedelinol (1), Friedelan-3-one (2), Heptadecanoic acid (3), Ergosterol- β -D-glucoside (4), (3R,4R,5S,6R)-2-(hydroxymethyl)-6-(4,4a,6b,8a,11,11,12b,14a-octamethyl-docosahydricen-3-yloxy)-tetrahydro-2H-pyran-3,4,5-triol (5), Brassicasterol Benzoate (6), Lupeol (7), Betulin (8), Oleanolic acid (9), Maslinic acid (10), Apigenin (11) Kaempferol (12)], Quercetin-3-O- α - rhamnoside (13) and Trans-Tiliroside (14). The structures of all the isolated constituents were determined by a comprehensive use of spectroscopic analysis such as 1D- and 2D-NMR, EI-MS, and ESI-MS coupled with the comparison of data thereof obtained with those of known analogs listed in the literature. These compounds and various extracts, fractions were investigated for their antioxidant potential, and antischistosomal and antifungal effects against *Schistosoma mansoni* and *Candida albicans* respectively, α -Glucosidase and Trypsin activity. The results revealed that all the extracts (roots and leaves), exhibited antifungal, antioxidant and enzyme α -Glucosidase inhibition activities. Moreover, Quercetin-3-O- α - rhamnoside (13) inhibited α -Glucosidase, Trypsin and *Schistosoma mansoni*. The synergic effect of the extract and fraction, and also their triple of Quercetin-3-O- α - rhamnoside (13) could classified *Faidherbia albida* among the potential candidate for the development of multitarget drugs. This could reduce the combination of multiple biological active agent (multitherapy) therapy in favor of multitarget drug strategies.

Keywords: *Faidherbia albida*, Quercetin-3-O- α - rhamnoside, α -Glucosidase inhibition, Trypsin inhibition, Anti-cercaricidal activity, Multitarget drugs.

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INTRODUCTION

This work is a follow up of our previous efforts on the study of medicinal plants of Africa in general and in particular of the chemical constituents of *Dissotis perkinsiae* (Melastomaceae) so as to promote sources of compounds with potential antimicrobial activities¹. These research axe could be explored in the development of new the new therapeutic natural product, which corroborates with the assumption that more than 60% of the African population make use of their traditional medicine with more implication of the medicinal plants². The special interest on *Faidherbia albida*³, also known as *Acacia albida*⁴, a plant of Mimosaceae/Leguminosaceae/Fabaceae family is based on the fact that the species grows in more

arid zones, which confers its with anti-stress ability and a capacity to fix nitrogen, water and other useful nutrients from deep soils⁵⁻⁸. The bark and leaves extracts are prescribed in traditional medicine to treat respiratory infections, fertility disorders, and digestive ailments, backache, malaria, etc⁹⁻¹². Moreover *in vitro* qualitative phytochemical studies shown this plant possess a great quantity of triterpenes, sterols, glycosides, and tannins¹³⁻¹⁴. Pharmacological studies have indicated antiinflammatory, anti trypanosomal, antidiabetic, antimalarial, antifungal, Neumaticidal activities of their bark crude bark extracts¹⁴⁻²¹, providing scientific resources for their indigenous use. Previous phytochemical studies indicated that the seeds and fruits essential oil of were rich in fatty acids²² and the GC-MS analysis of essential oils from the stem bark revealed the presence of 37 different constituents of which α -pinene was the major one²². In addition, the extraction and characterization of the fruits of the plant revealed the presence non polar constituents such as of stearic acid, oleic acid, linoleic acid, methyl ester and linolenic acid, methyl ester²³. In our knowledge, there is unreported literature on their roots and leaves. The chemotaxonomic and ethnopharmacological significance



of the genus *Faidherbia* prompted us to investigate the secondary metabolites of *Faidherbia albida*. Thus, this work reports on the isolation and identification of 14 constituents isolated from the leaves and roots of *Faidherbia albida* and on the biological activities observed on some extracts and isolated secondary metabolites.

MATERIALS AND METHODS

General experimental procedures

Mass spectroscopic data [Electrospray ionization mass spectrometry (ESI-MS)] were measured on a Waters Synapt HDMS spectrometer. NMR spectra were recorded with a Varian spectrometer at 400 MHz. Chemical shifts (δ) were quoted in parts per million (ppm) from the internal standard tetramethylsilane (TMS). Deuterated solvents dimethyl sulfoxide (DMSO-*d*6), and chloroform (CDCl₃) were used as solvents for the NMR experiments. Column chromatography was performed on silica gel 60 [(0.2-0.5 mm) and (0.2-0.063 mm)] mesh (Sigma-Aldrich, Germany). Pre-coated silica gel 60 F254 thin layer chromatography (TLC) plates (Merck, Germany) were used for monitoring fractions and spots were detected with UV light (254 and 365 nm) and then sprayed with 30% sulphuric acid (H₂SO₄) followed by heating to 110°C.

Collection of Plant and Preparation

Roots and leaves of *Faidherbia albida* were collected in Maroua-Cameroon, (June, 2013) by Dr Frumsia (Plant taxonomist) and identified at the Cameroon National Herbarium (HNC), where a voucher specimens N°58978 /SRF/Cam are deposited.

Extraction and isolation of *Faidherbia albida* roots and leaves

The roots and leaves were dried at room temperature and powdered. 1500g of powdered roots were extracted by maceration in 07 liters (7l) of dichloromethane/methanol (DCM/MeOH, 1:1, v/v) mixture at room temperature for 72 h. The filtrate was concentrated in rotavapor (Büchi R-200) under reduced pressure at 65°C to yield 240g of extract. The crude material was dissolved in water and successively extracted with hexane, dichloromethane, ethyl acetate and n-butanol. All these extracts collected were preserved for chemical analysis.

Hexane fraction (14.3 g) was subjected to vacuum liquid chromatography (VLC) over silica gel grade (GF254) eluted with *n*-hexane/ethyl acetate (AcOEt) mixture with increasing polarity from *n*-hexane to AcOEt. Eight fractions (F1-F8) were obtained after combining subfractions according to their TLC profiles. Fraction F1 was subjected to column chromatography with *n*-hexane/AcOEt gradient mixtures to yield 3 β -friedelinol (9.15mg). Fraction F2 obtained at a polarity of *n*-hexane/AcOEt (85:15), friedelan-3-one (5.05 mg), heptadecanoic acid (4.64 mg). Fraction F8 obtained with *n*-hexane/AcOEt (20:80) was subjected to other column chromatography with *n*-hexane/AcOEt increasing polarity to yield Ergosterol- β -D-glucoside (7.26 mg).

The ethyl acetate fraction was subjected to column chromatography (CC) over silica gel (4x150 cm, 250 g, 70 - 230 mesh) and eluted with *n*-hexane: AcOEt mixture with increasing polarity to afford 96 subfractions combined in five fractions (F1-F5) according to their TLC profiles. Fraction F2 was subjected to other column chromatography to yield (3R,4R,5S,6R)-2-(hydroxymethyl)-6-(4,4a,6b,8a,11,11,12b,14a-octamethyl-docosahydricen-3-yloxy)-tetrahydro-2H-pyran-3,4,5-triol (6.27 mg) and Brassicasterol Benzoate (7.12 mg). Fraction F4 was subjected to column chromatography (CC) and eluted with *n*-hexane/AcOEt mixture with increasing polarity to yield Kaempferol (6.14 mg) and Trans-Tiliroside (9.16 mg) (Table 1).

1240g of powdered leaves were extracted by maceration in seven liters (5l) of dichloromethane/methanol (DCM/methanol (MeOH), 1:1, v/v) mixture at room temperature for 72 h. The filtrate was concentrated in rotavapor (Büchi R-200) under reduce pressure at 65°C to yield 143g of extract. Dried extract was dissolved in water and successively extracted with hexane, dichloromethane, ethyl acetate and n-butanol. All these extracts collected were preserved for chemical analysis.

Hexane fraction (21.6 g) was subjected to vacuum liquid chromatography (VLC) over silica gel (GF254) and eluted with *n*-hexane/AcOEt mixture with increasing polarity from *n*-hexane to AcOEt. Four fractions (F1-F4) were obtained after combining subfractions according to their TLC profiles. Fraction F1 was subjected to column chromatography with *n*-hexane/AcOEt gradient mixtures to yield Lupeol (4.16 mg), Betulin (3.25 mg). Fraction F2 obtained at a polarity of *n*-hexane/AcOEt (85:15), yield Maslinic acid (7.15 mg) and Oleanolic acid (5.20 mg). Fraction F4 obtained with *n*-hexane/AcOEt (20:80) was subjected to other column chromatography with *n*-hexane/AcOEt increasing polarity to yield Ergosterol- β -D-glucoside (3.26 mg). The ethyl acetate soluble fraction was subjected to column chromatography (CC) over silica gel (4x150 cm, 250 g, 70 - 230 mesh) and eluted with *n*-hexane: AcOEt mixture with increasing polarity to afford 66 subfractions combined in six fractions (F1-F6) according to their TLC profiles. Fraction F2 was subjected to other column chromatography to yield Kaempferol (4.02 mg), Quercetin-3-O- α - rhamnoside (3.17 mg), Apigenin (2.10 mg). Fraction F3 was subjected to column chromatography (CC) and eluted with *n*-hexane/AcOEt mixture with increasing polarity to yield Trans-Tiliroside (5.13 mg).

Preparation of stock solution of plants crude extracts, fractions and compounds

The different stock solution of plant, fractions and compounds were prepared by dissolving 2 mg in 1 ml of DMSO 10 % for a final concentration of 2 mg/ml. Reference antibiotics were prepared in the same condition by dissolving 512 mg of Fluconazole (Sigma Aldrich) and 2mg of Chloramphenicol and Ampicillin (Sigma Aldrich) in 1 ml of DMSO 10 % to yield 512 μ g/ml and 2 mg/ml respectively. After preparation, the different stock solutions were



sterilized with 0.20 µM Syringe Filter and store at -20°C before use.

Antioxidant assay

A sample of 20 ml of potassium persulphate (50 mg/ml) was added to the 1 ml of azino-bisethylbenzothiazoline-sulphonic acid diammonium salt (ABTS, Fluka, Switzerland). The mixture was incubated for 1 h at room temperature until persulphate had oxidized ABTS to a green-black coloured product. The oxidized ABTS stock solution was then diluted 1:10 in distilled water and 200ml of diluted working solution was pipetted into each well of a 96well-microtiter plate. A solution of 60% ethanol in water was used as a negative control and ascorbic acid (1 mM) was used as appositive control. An amount of 10 ml of controls or compounds was added to duplicate wells of the plate and stirred gently using the pipette tip. Antioxidant activity was recorded for compounds which reduced ABTS from green to colourless.

α-Glucosidase and Trypsin Inhibition & Activity Assay Starch

Agar plates were prepared by boiling agar (10 g/IHi Media) with potato starch (15 g/l) in distilled water. The mixture was poured into petri dishes (Falcon) to solidify. α-Glucosidase was Isolated from 4-day old pea shoots by grinding fresh shoots with phosphate buffered saline (oxid). An amount of 10 ml of each compound was spotted onto the agar along the edge of the plate. In the center of the plate, 10 ml of 60% ethanol (negative control) and 10ml of a carbose (4 mg/ml, SIGMA) were spotted side by side. Onto each spot, 10 ml of pea shoot extract (α-Glucosidase) was added and left to digest for 10 min; 1 ml of iodine solution (UniLAB/Saarch, South Africa) was then poured over the surface of the plate and the excess discarded. Areas where the starch was digested by α-Glucosidase appeared clear in colour, indicating no inhibition of enzymatic activity. Spots which appeared blue due to iodine dyeing the undigested starch indicated the inhibition of α-Glucosidase.

In vitro studies with *S. mansoni* stock and working plant extract solutions

In vitro schistosomicidal evaluation was conducted on cercariae evolutionary form. Stock solutions of extract, fractions and isolated compounds were prepared according to the procedure described previously²⁴.

Preparation of Cercariae Suspension

Schistosome cercariae's were obtained from experimentally infected *B. pfeifferis* nails as previously described²⁴.

In Vitro Cercaricidal Activity Test

The effects of crude extract (extract code5), fractions (extracts codes 6, 7, 8) (FAF2, FAF4, FAF5) and isolated compounds FAF6 - FAF11 (extracts codes 9, 10 and 11) on *Schistosoma* infectious stage (cercariae) were assessed as previously²⁴.

Antimicrobial assay

Microorganisms and growth conditions

C. albicans (Donated by Mr. M. Morobe, Department of Biological Sciences of the University of Botswana) were used as test organisms²⁵:

C. albicans Microorganisms was grown in nutrient Sabouraud Dextrose Broth (HiMedia, South Africa) at 32°C for 48 h. Microorganisms were maintained on agar plates at 4°C or stored as glycerol stocks at 80°C.

Antimicrobial assays

Sterile molten agar medium (400 mL) was pipetted into each well of a sterile 24-well culture plate (Corning) and left to solidify.

Yeast (*C. albicans*) were grown overnight in the irrespective media to a density of 1-106 cfu/ml. 10 ml of culture were pipettes onto each well of the agar plates. EtOH (60% aqueous solution, B&M, Scientific, Cape Town, South Africa) was the negative control across all antimicrobial assays. The positive controls were and penicillin/Streptomycin/fungizone (PSF, 100x; Highveld Biological, South Africa). 10 ml of EtOH, antibiotic, or compound were pipetted onto the surface of the agar in duplicate wells. The plates were later inverted and incubated in a moist chamber. Inhibition was indicated by a clear area on the agar surface, while visible growth of colonies indicated inhibition, poor/very poor/no inhibition.

RESULTS AND DISCUSSION

Fractionation and Isolation of Compounds Using Column Chromatography

In this study, the phytochemical composition of crude *Faidherbia albida* (Mimosaceae) roots and leaves extracts and fractions from methylene chloride/methanol (1v, 1v), n-hexane, and ethyl acetate solvents, afforded 14 compounds for the first time by silica gel column chromatography and identified as 3β-Friedelinol²⁶ (1), Friedelan-3-one²⁷(2), Heptadecanoic acid²⁸ (3), Ergosterol-β-D-glucoside²⁹ (4), (3R,4R,5S,6R)-2-(hydroxymethyl)-6-(4,4a,6b,8a,11,11,12b,14a-octamethyl-docosahydricen-3-yloxy)-tetrahydro-2H-pyran-3,4,5-triol³⁰ (5), Brassicasterol Benzoate³¹(6), Kaempferol³² (7), Trans-Tiliroside³³ (8), Lupeol³³⁻³⁴ (9), Betulin²⁷ (10), Maslinic acid²⁷ (11), Oleanolic acid³⁵ (12), Quercetin-3-O-α-rhamnoside³⁰ (13) and Apigenin³⁰ (14). The structures of the compounds were determined by analysis of their NMR data and comparison with those reported in the literature (Figure 1).



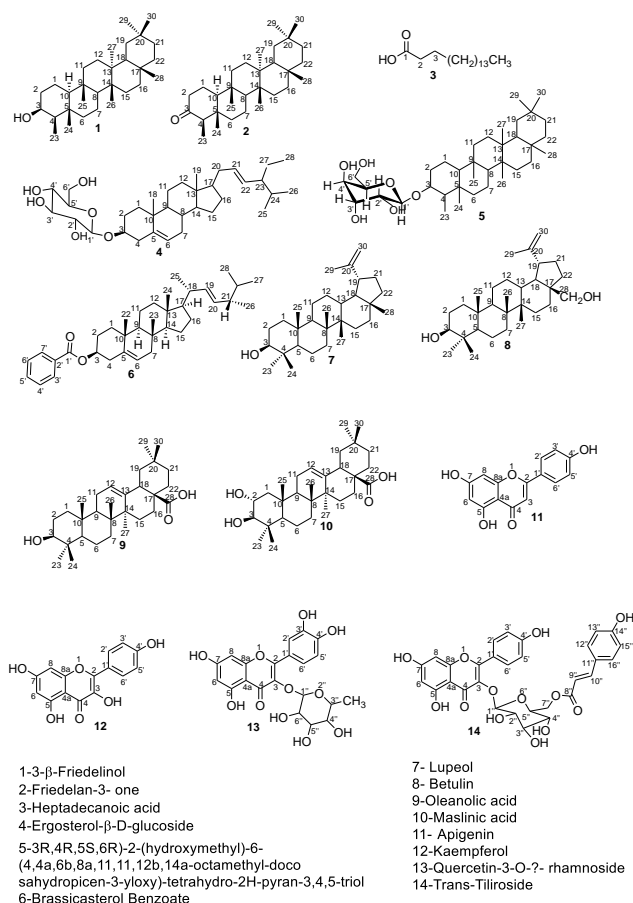


Figure 1: Structures of compounds isolated from *Faidherbia albida* (Mimosaceae)

Among these phytochemicals, Trans-Tiliroside (14) and 3β-Friedelinol (1) were found to be the majority compounds.

The first, a Triterpenes type is found in both ethyl acetate fractions, but approximately two times more present in roots than in leaves; whereas, the second, Flavonoids were found only in n-hexane fraction of roots. The result corresponds to the report in the literature that different solvent extraction and geographical area may result in obtaining different phytochemical constituents³⁶.

In an attempt to characterize their bioactivities, isolated compounds and various extracts, fractions were investigated for their antioxidant potential, anthelmintic and antifungal effects against *Schistosoma mansoni* and *Candida albicans* respectively, α-Glucosidase and Trypsin activity.

Cercaricidal activity of crude roots and Leaves extracts, fractions and the compounds (13)

Schistosomiasis is a chronic and debilitating disease caused by a trematode of the genus *Schistosoma* and affects over 207 million people³⁶. Chemotherapy is the only immediate recourse for minimizing the prevalence of this disease and involves predominately the administration of a single drug, praziquantel (PZQ). Although PZQ has proven efficacy, there is a recognized need to develop new drugs as schistosomicidal since studies have shown that repeated use of this drug in areas of endemicity may cause a temporary reduction in susceptibility in isolates of *Schistosoma mansoni*³⁶.

The kinetics of mortality of the cercariae of *S. mansoni* after exposure to the crude extract, fractions and the compounds from *Faidherbia albida* are presented in figure 2: A-F.

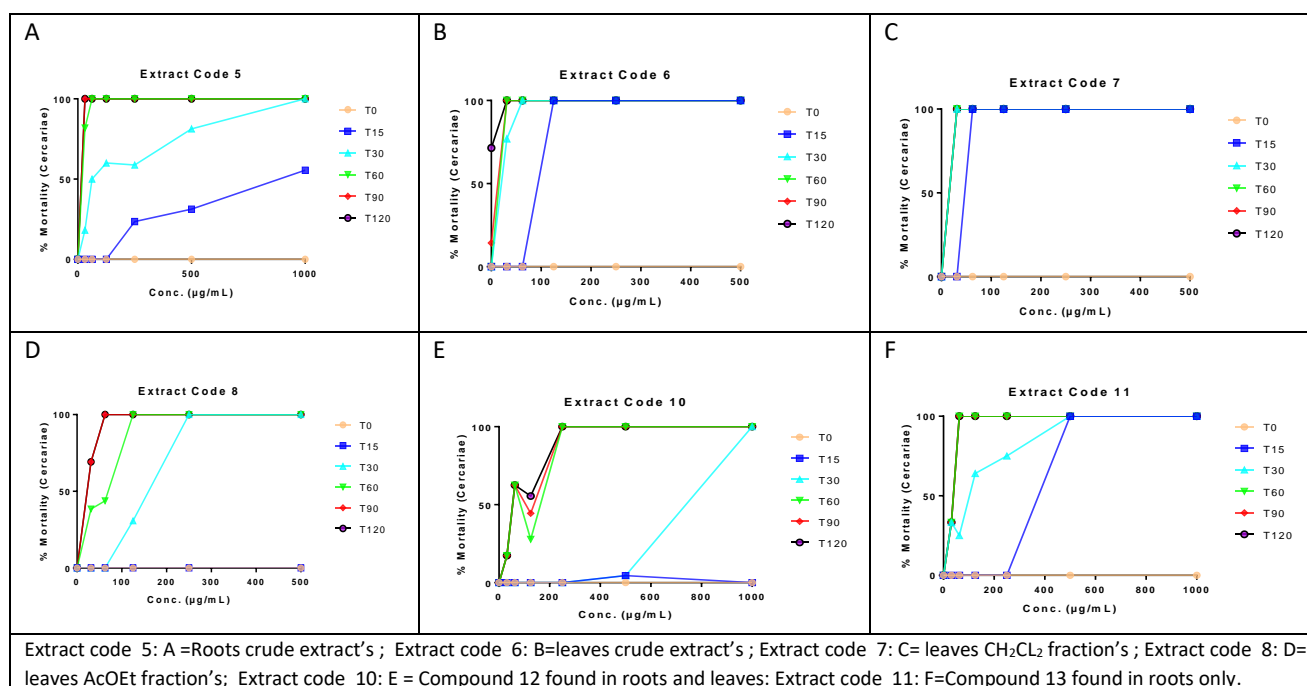


Figure 2: Kinetics of mortality of the cercariae of *S. mansoni* after exposure to the crude extracts, fractions and the compounds from *Faidherbia albida*

The results indicate that cercariae death occur in a dose-dependent manner for all extracts. The beginning of death begins after 15 min of exposition of the cercariae (Figure 2: A, D, E, F). With the concentration of 31.25 µg/ml of the extracts codes 5, 6 and 7, all the cercariae dead after 30, 60 and 90 min for extracts codes C, B and A respectively (Figure 2: A, B, C). On the other hand, one notes a death rate of 100% after 30 min when cercariae are exposed to each sample at 1000 µg/ml. In low concentrations (31.25, 62.5, and 125 µg/ml) some cercariae survived after respective time's intervals of 90 and 120 min, respectively (Figures 2: D, E, F). An increase in the mortality rate of the cercariae for 15, 30, 60, 90, 120 and 150 min (Figures 2: A, B, C, D, F) were observed.

Roots crude extract's caused 100% cercariae death after 90 min at low concentration 31.25 µg/ml, less than 50 µg/ml, while leaves EtOAc fraction's, although was more active than leaves crude extract's, however none of them caused 100 % death. Comparing the effect of different concentrations of the crude extracts, fractions and isolated compounds on the *S. mansoni* cercariae mortality clearly shows that leaves CH₂CL₂ fraction's is the most active, seconded by the isolated compound 13 from roots. This might strongly indicate and justify synergistic effects of the chemical constituent's presents in these samples.

Table 1: Antioxidant, α-Glucosidase and Trypsin activities/inhibition of compounds, fractions and extracts from roots and Leaves of *Faidherbia albida*

Sample	<i>Candida albicans</i>	Glucosidase activity	Glucosidase inhibition	Trypsin activity	Trypsin inhibition	Antioxidant
Crude roots extract	1	3	0	0	0	3
Crude leaves extract	1	3	0	0	0	3
EA roots fraction	2	3	1	0	0	3
EA leaves fraction	2	0	2	0	0	3
Compound 13	0	1	3	0	3	0

3=active, 2=weak activity, 1=very weak activity, 0=not active, NE=not enough material for test, EA=ethyl acetate

In this table 1, all the extracts and ethyl acetate fractions are active on both free radical scavenging by DPPH assay and enzyme α-Glucosidase inhibition. Whereas the ethyl acetate fraction of the leaf is active only on free radical scavenging by DPPH assay. The compound 13 on is active at the same time on α-Glucosidase inhibition, Trypsin inhibition.

The use of antioxidants against schistosomiasis have been reviewed³⁸. Works done by Gharib B. et al. in 1999³⁹ showed that oxidative processes triggered by liberation of reactive oxygen species (ROS) resulting from the immunological response and disturbance in cellular antioxidant homeostasis of affected organs during schistosomiasis; this could be due to the physiological properties of antioxidants, which are considered pharmacologically safe agents with minimal side effects¹⁶⁻¹⁷. Here, we showed all the extracts are active on free radical scavenging by DPPH Assay and the cercariae of *S. mansoni*, which reduce the combination of

Previous works done by Gouveia in 2018 showed that Flavonoids were able to form complexes with cholesterol and decreased its level in plasma and increased cholinesterase activity or may be decreased the frequency of cardiac contraction and additionally, they are thus regarded as privileged structures used for a broad spectrum of activities and are potential candidates for sources of new drug prototypes³⁷. Here, the Flavonoid compound (13) is the most active component, making *Faidherbia albida* a potential source of drugs for the fight against schistosomiasis. The exposure of the *S. mansoni* cercariae to crude extract, fractions and compounds showed that the plant have larvicidal activities against *S. mansoni* larvae.

Antioxidant, α-glucosidase and trypsin activities, α-glucosidase and trypsin inhibition and antifungal activities against *Candida albicans* strain of crude roots and leaves extracts, fractions and the compounds (13)

The results of antioxidant, α-Glucosidase and trypsin activities, antifungal activities against *Candida albicans* strain, as well as of α-Glucosidase and Trypsin inhibition of compounds, extracts and fractions of roots and leaves of *Faidherbia albida*, are reported in Table 1.

multiple biological active agent (multitherapy) therapy in favor of multitarget drug strategies.

The rationale for the evaluation of anti-oxidant and α-Glucosidase inhibitory properties is explained by the works done by Nkengfack et al. in 2012⁴⁰, revealing that antioxidants play a vital role in the immune system by reducing oxidative stress. Oxidative stress is, for example, induced by excess production of reactive oxygen species (ROS) due to HIV infection, while those realized by Sandstrom et al. in 1998⁴¹ were showed that an imbalance of cellular oxidant/antioxidant status may, vice versa, stimulate HIV replication. α-Glucosidase inhibitors have attracted interest as potential therapeutic agents against diabetes type 2⁴² and as suppressors of the HIV replication cycle acting at the entry stage⁴³. In contrast, the isolated compounds 13 didn't exhibit anti-oxidant activities. This absence of activity is due to the sugar moiety carried by the compound. In fact, Isoflavone without sugar moiety compounds are known to be among the best natural antioxidant, with the instar of

Seputheisoiflavone (EC₅₀= 0.195 mg/ml) isolated from *Ptycholobium contortum* (N.E.Br.) Brummitt (Leguminosae) by Fotso et al. in 2015²⁵, in comparison, ascorbic acid, which was used as reference drug. However, All the extracts (roots and leaves), as well as the ethyl acetate fraction of the roots were shown exhibited both anti-oxidant and α-Glucosidase activities respectively, while the ethyl acetate leave fraction exhibited only anti-oxidant activity. Interestingly, the compound Quercetin-3-O-α- rhamnoside (13) is active at the same time on α-Glucosidase inhibition, Trypsin inhibition and cercariae of *Schistosoma mansoni*, clarifying a possible candidate for multitarget drugs, although his antischistosomal against adult's worm were revealed by Xiao et al. in 2014⁴⁴.

Susceptibilities of *Candida albicans* choose amongst *Mycobacterium sp.*, which are the two major opportunistic pathogens occurring in an HIV/AIDS context, against extracts fractions and compounds 13 were also determined these samples were investigated. Up to 90% of HIV-infected individuals suffer from at least one episode of candidiasis, which is commonly characterized by oral thrush⁴⁵. Although we could show that 2 mediates fungicidal activity of both fractions, their values are rather high for both extract, none of isolated compounds were active against yeasts. Therefore, the antifungal activity can be considered as weak.

CONCLUSION

Faidherbia albida is a Cameroonian medicinal plants used for the treatment of skin diseases, wounds, fever, rheumatism, malaria and/or infectious diseases. This plant species is rich sources of compounds that can play an important role in the human health, in the broader context, in the treatment of various diseases, such as helminthes, diabetes type 2, schistosomiasis and antioxidant-related ailments and The synergic effect of the extract and fraction, and also their triple of Quercetin-3-O-α- rhamnoside (13) could classified *Faidherbia albida* among the potential candidate for the development of multitarget drugs. This could reduce the combination of multiple biological active agent (multitherapy) therapy in favor of multitarget drug strategies.

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