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ENZYME INHIBITORY SECONDARY METABOLITES FROM *CANARIUM SCHWEINFURTHII* Engl.

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ABSTRACT

Sixteen secondary metabolites have been obtained from *Canarium schweinfurthii* subsequently characterized with the help of physical and spectral data as amentoflavone (**1**), agastiflavone (**2**), (+)-catechin (**3**), luteolin (**4**), *β*-amyrin (**5**), oleanolic acid (**6**), olean-12-en-3*β*-24-diol (**7**), *β*-amyrenone (**8**), 3*β*- hydroxyolean-9(11),12-dien (**9**), *α*-amyrin (**10**), *α*- amyrenone (**11**), 3β-hydroxytirucalla-8,24-dien-21-oic acid (**12**), 3α-hydroxytirucalla-8,24-dien-21-oic acid (**13**), 3-oxotirucalla-8,24-dièn-21-oic acid (**14**), lupeol (**15**) and *β*-sitosterol glucoside (**16**), respectively. All of these have been subjected to biological screening in order to establish the scientific basis for the medicinal uses of the plant.

Key-words: *Canarium schweinfurthii,* Burseraceae, secondary metabolites, biological screening.

INTRODUCTION

Members of the family Burseraceae are known for their fragrant resins having economic and medicinal values (Ngbede *et al*., 2008). *Canarium* is one of the genera of Burseraceae, native to tropical Africa and southern Asia (Hoang *et al*., 2012). *C. schweinfurthii* is a huge tree abundantly found in forests of Cameroon. The plant is recommended for a variety of ailments such as rheumatism, fevers. sexual transmitted diseases and diarrhea (Dongmo *et al*., 2010). No systematic pharmacochemical studies have so far been carried out on this plant. The ethno-pharmacological importance of the genus *Canarium* motivated us to undertake pharmacochemical studies on aerial parts of *C. schweinfurthii.* As a result of current chemical analysis, the secondary metabolites **1-16** have been isolated and characterized by matching their obtained data with those recorded in literature. All of these were subjected to comprehensive biological screening to ascertain the scientific basis of a variety of medicinal uses of the plant. However, only significant results were obtained in case of inhibitory studies against the enzymes lipoxygenase (LOX), urease and butyrylcholinesterase (BChE), respectively.

MATERIALS AND METHODS

General experimental:

In a glass column, silica gel (Si 60, 70-230 mesh, E. Merck, Darmstadt, Germany) was used to purify phytochemicals. FTIR Spectrometer IR-460 (Shimadzu Corporation, Tokyo, Japan) was used to obtain I.R spectra. Thermo scientific spectrophotometer was used to scan UV spectra. Bruker AM 600 and AM 400 NMR spectrometers (Bruker, Fallendel, Switzerland) were used to scan both one and two dimensional NMR spectra. The chemical shifts (δ) were measured in ppm, while the coupling constant (J) was measured in hertz. The EIMS were taken using a JEOL JMS600H-1 spectrometer. The HREIMS spectra were obtained using a Thermo Finngal MAT-95 XP mass spectrometer. A polatronic D Polarimeter was used to measure optical rotation. TLC cards were used to monitor the purity of the constituents (Si-gel, Merck PF254, 0.25 mm thickness) and a praying reagent, cerric sulphate was used to visualize the spots.

Collection of Plant Material:

The aerial parts of *C. schweinfurthii* Engl. (young plant) were taken from Yaounde, Cameroon and authenticated by Mr. Victor Nana, Taxonomist, the National Herbarium, Cameroon (specimen # HNC 25918).

Extraction and Isolation

Fresh plant material (5.5 kg) was shade dried and extracted thrice with methanol for three days each. The combined methanolic extract was condensed on a rotary evaporator to obtain a crude extract (180 g).

A small amount (170 g) of the extract was used in flash chromatography over silica gel (200-400 mesh; 400 g, 80 x 5 cm), eluting with combinations of *n*-hexane, ethyl acetate and methanol in increasing order of polarity. This gave five fractions A-E. The fraction B which eluted with *n*-hexane/ ethyl acetate (39:1-9:1; 22.4 g), was re-chromatograped (silica gel: 50 g; column: 30 x 3 cm) run with hexane- EtOAc in increasing order of polarity to afford *β*-amyrin (**5**) (22.3 mg), oleanolic acid (**6**) (8.1 mg), olean-12-en-3*β*-24-diol (**7**) (20.3 mg), *β*-amyrenone (**8**) (3.2 mg), 3*β*- hydroxyolean-9(11),12-dien (**9**) (7.9 mg), *α*-amyrin (**10**) (8.4 mg), *α*- amyrenone (**11**) (14.6 mg), 3*β*-hydroxytirucalla-8,24-dien-21-oic acid (**12**) (5.6 mg), 3α-hydroxytirucalla-8, 24-dien-21-oic acid (**13**) (18.2 mg), 3-oxotirucalla-8,24-dièn-21-oic acid (**14**) (9,7 mg) and lupeol (**15**) (4.1 mg). The fraction C eluted with hexane/ethyl acetate (3:2-2:3; 15.9 g) was again loaded on column (silica gel: 50 g; column: 50 x 3 cm), and dichloromethane-methanol was using as a mobile phase. The fraction eluted with dichloromethane-methanol (95:5) was further chromatographed over Sephadex LH-20 to afford amentoflavon (**1**) (9.2 mg), agastiflavon (**2**) (11.5 mg), (+)-catechin (**3**) (5.2 mg) and luteolin (**4**) (8.2 mg). *β*-Sitosterol glucoside (**16**) (280 mg) was obtained from fraction D as methanol insoluble stuff.

Biological study

All of the above isolates were subjected to extensive biological testing, including inhibition studies against different enzymes. However, only LOX, urease, and BChE yielded notable results.

Lipoxygenase inhibition activity:

LOX activity is important to the plant's defense against pathogens. This activity and the resulting fatty acid hydroperoxides get started free radical carbon chains, resulting in protein modifications. The isolated constituents have been tested for LOX using a modified spectrophotometric method developed by Tappel (1962) which showed much potential compared to the other studied enzymes.

Urease inhibition activity:

Urease has capability to hydrolyze urea. As a result, CO_2 and NH_3 are released into the atmosphere. It also promotes *Helicobacter pylori* growth in the stomach by providing an acidic environment. Peptic ulcers, Gastric cancer, and urinary tract infections are all caused by this (Zahid *et al*., 2014). Our extracted natural products were also tested for urease inhibitory potential. Compounds **5**-**6** and **13** produced promising results. Weatherburn's method was used in this study (Weatherburn *et al*., 1967). Thiourea was used as a standard.

Butyrylcholinsterase inhibition activity:

In alzheimer's patient, level of BChE increases progressively. Therefore, for the treatment of alzheimer, BChE inhibitors have been established (Wang, 2013). Same samples were also tested against BChE. None of the sample showed noticeable activity except compounds **1**and **4**.

RESULTS

The methanolic extract of the aerial parts of *C. schweinfurthii* was subjected to a series of chromatographic resolutions, resulting in isolation and characterizations of sixteen secondary metabolites belonging to different classes including four flavonoids namely: amentoflavone (**1**) (Tamboué *et al*., 2000), agastiflavone (**2**) (Tamboué *et al*., 2000), (+)-catechin (**3**) (Mogana *et al*., 2014), luteolin (**4**) (Xiang *et al*., 2010); eleven triterpenes: *β*-amyrin (**5**) (Maffo *et al*., 2015), oleanolic acid (**6**) (Maffo *et al*., 2015), olean-12-en-3*β*-24-diol (**7**) (Kouam *et al*., 2008), *β*-amyrenone (**8**) (Bandaranayake, 1980), 3*β*- hydroxyolean-9(11),12-dien (**9**) (Tanaka *et al*., 1988), *α*-amyrin (**10**) (Bandaranayake, 1980), *α*- amyrenone (**11**) (Bandaranayake, 1980), 3β-hydroxytirucalla-8,24-dien-21-oic acid (**12**) (Takashi *et al*., 2012), 3α-hydroxytirucalla-8,24-dien-21-oic acid (**13**) (Takashi *et al*., 2012), 3-oxotirucalla-8,24-dièn-21-oic acid (**14**) (Keller *et al*., 1996), lupeol (**15**) (Bruneton *et al*., 1995) and *β*-sitosterol glucoside (**16**) (Bouic *et al*., 1996) (Fig.1).

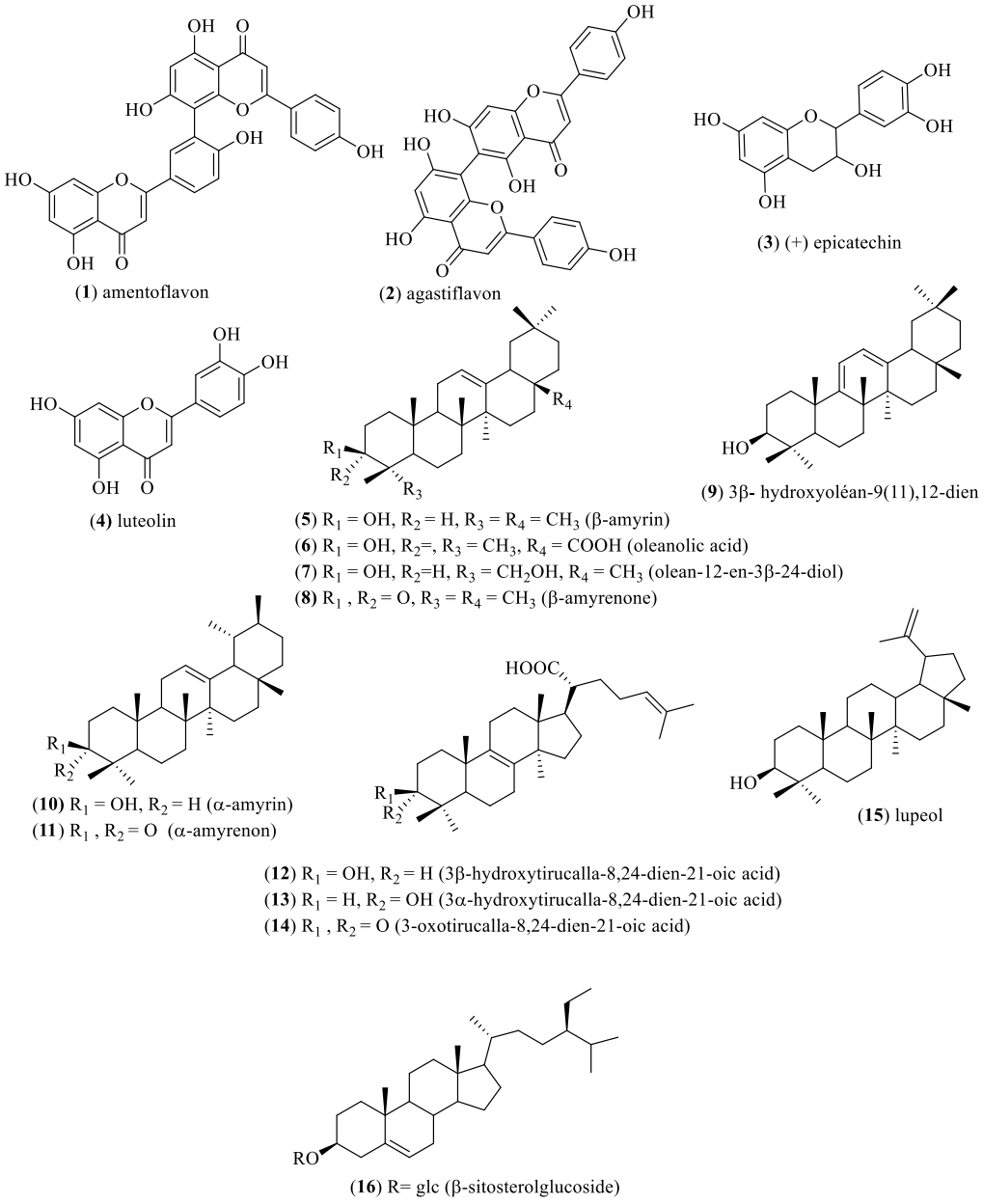

Fig.1. Structures of isolates from *Canarium schweinfurthii*.

Bio-screening of pure isolates:

All the isolates were subjected to detailed bio-screening but only showed significant results against the enzymes urease, BChE and LOX, respectively. The compound **13** showed significant urease inhibitory potential with the IC_{50} value of 13.4 ± 0.11 compared to that of thiourea (IC_{50} 21.7 ± 0.32). It can itself or its derivatives may be taken up by the pharmaceutical industries for preparation of drugs for the treatment of ulcer and urinary infections. In addition, compounds **5** and **6** also showed significant urease inhibition with IC_{50} values of 37,5 ± 0,34 and 23.7 ± 0.31, respectively. The compounds **1** and **4** revealed significant BChE inhibitory tendancy with the IC_{50} values of 17.5 ± 0.90 and 23,1 ± 0,19, respectively, compared to that of eserine used as standard (IC_{50} value of 7.8 ± 0.27). On the other hand, much more significant LOX inhibitory results were exhibited by the compounds **1-2, 4, 9** and **13-14** showing IC_{50} values of 29.8 ± 0.11,

33.6 ± 0.32, 28.7 ± 0.17, 35.6 ± 0.18, 33.6± 0.11 and 32.5 ± 0.66 (Table 1); being compared with that of baicalein used as standard (IC_{50} value of 22.4 ± 0.24).

Table 1. Lipoxygenase Inhibition Activity of Isolates **1-16**.

Compound No.	Compound	IC_{50} (µM)
1	Amentoflavone	**29.8 ± 0.11**
2	Agastiflavone	**33.6 ± 0.32**
3	(+)-Catechin	97.4 ± 0.14
4	Luteolin	**28.7 ± 0.17**
5	*β*-Amyrin	87.9 ± 0.22
6	Oleanolic acid	40.3 ± 0.17
7	Olean-12-en-3*β*-24-diol	67.5 ± 0.26
8	*β*-Amyrenone	77.6 ± 0.05
9	3*β*- Hydroxyolean-9(11),12-dien	**35.6 ± 0.18**
10	*α*-Amyrin	87.6 ± 0.83
11	*α*- Amyrenone	67.5± 0.05
12	3β-Hydroxytirucalla-8,24-dien-21-oic acid	48.7± 0.33
13	3α-Hydroxytirucalla-8,24-dien-21-oic acid	**33.6 ± 0.11**
14	3-Oxotirucalla-8,24-dièn-21-oic acid	**32.5 ± 0.66**
15	Lupeol	55.6 ± 0.06
16	*β*-Sitosterol glucoside	55.6 ± 0.26
Standard	Baicalein	22.4 ± 0.24

DISCUSSION

A number of secondary metabolites have been isolated and characterized, followed by their detailed bio-screening to establish scientific basis of the medicinal uses of *C. schweinfurthii* (Dongmo *et al*., 2010; Ngbede *et al*., 2008; Ngbolua *et al*., 2015). Nevertheless, the isolates only displayed notable inhibitory potential in case of enzymes LOX, urease and BChE. It therefore appears that the medicinal uses of the plant may be due to the combined effects of these enzymes.

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