BIOACTIVE COMPOUNDS FROM THE FRUITS OF *ZANTHOXYLUM LEPRIEURII*[†]

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Summary

Four acridone alkaloids, tegerrardin A **1**, 1-hydroxy-3,4-dimethoxy-*N*-methylacridone **2**, arborinine **3** and xanthoxoline **4** and a coumarin, scoparone **5** have been isolated from the fruits of *Zanthoxylum leprieurii*, used as spice in many Cameroonian traditional dishes. Compounds **3** and **4** displayed a good *in vitro* antiplasmodial activity against 3D7 strains of *Plasmodium falciparum* with IC_{50} values of 4.5 ± 1.0 and 4.6 ± 0.6 µg/ml, respectively. Compounds **1** and **3** are good chelating agents with 90% and 61% Radical Scavenging Activity (RSA), respectively. Compound **2** showed good activity in the superoxide anion scavenging assay with 90.3% RSA. The structures of the compounds were elucidated on the basis of their spectroscopic and physical data.

Key words: *Zanthoxylum leprieurii*, acridone alkaloids, xanthoxoline, antiplasmodial, antioxidant.

Introduction

In years past, there has been a growing interest in the study of the bioactivity of phytochemical constituents in food (1). Several studies indicate that a plant-based diet protects against the development of chronic diseases such as atherosclerosis, diabetes, cancer, cirrhosis and ageing-related diseases (2). Dietary plants contain hundreds of antioxidants many of which are believed to act in a synergistic way, protecting the body against oxidative stress (3).

In a program aimed at identifying bioactive substances in diet, the fruits of *Zanthoxylum leprieurii* Guill. & Perr. (Rutaceae) (syn. *Fagara leprieurii*) which are used as spice in many traditional Cameroonian dishes (4), have been studied. *Z. leprieurii* is an under storey forest tree, 15-80 ft. high with stems covered with large broad-based thorns having sharp points (5).

Previous studies on the fruits of this plant included the antibacterial and antifungal analyses of the volatile constituents as well as the phytochemical studies of a sample collected from Nigeria (4,6,7). We report herein the antiplasmodial and antioxidant activities of compounds isolated from the ethyl acetate extract of these fruits.

Methods

Plant materials

The fruits of *Z. leprieurii* Guill. & Perr were purchased from a local market in Yaoundé (Cameroon) and identified by Mr. Nana, a Botanist at the National Herbarium of Yaoundé, Cameroon, where a voucher specimen (N^{o} 10669 / SRFCAM) was deposited.

Extraction and isolation of constituents

The dried fruits (1 kg) of *Z. leprieurii* were chopped in a blender and extracted with ethyl acetate (4 L) for 48 hrs. at room temperature. The filtrate was concentrated to give an orange-brown oil. Twenty grams (20 g) of the concentrated extract were applied to a silica gel Column Chromatography (CC) and eluted with increasing amounts of EtOAc in petroleum ether (pet. ether). Sixty fractions of 250 ml each were collected and pooled according to their TLC profile giving four major fractions (A-D). Fraction B obtained from the main column with pet. ether-EtOAc (80:20–75:35) was further chromatographed over a silica gel CC and eluted with pet. ether-

EtOAc of increasing polarity to give 1-hydroxy-3,4-dimethoxy-*N*-methylacridone **2** (orange crystals, 30 mg) and tegerrardin A **1** (yellow pellets, 100 mg). Fraction C obtained from the main column with pet. ether-EtOAc (70:30) was applied to a silica gel CC, eluted with pet. ether-EtOAC (80:20) to furnish arborinine **3** (yellow pallets, 400 mg) and scoparone **5** (colorless needles, 2 g). On the other hand, fraction D, obtained from the main column with pet. ether-EtOAc (50:50), was passed through a silica gel CC and eluted with pet. ether-EtOAc (50:50) to give xanthoxoline **4** (yellow pallets, 200 mg). The structures of compounds **1**-**5** were elucidated by analysing their ^{1}H and ^{13}C NMR and mass spectra data and by comparison with those already reported (8,9,10,11).

Antioxidant activity

Iron chelating scavenging assay

The Fe^{+2}-chelating ability of samples was determined following the modified method of Decker and Welch (12). The Fe^{+2} ions were monitored by measuring the formation of ferrous ion-ferrozine complex. The pure compound (0.5 mM) was mixed with $FeCl_2$ (0.0625 mM) and ferrozine (0.5 mM). The resulting mixture was then shaken and left at room temperature for 10 min. The absorbance of the resulting mixture was measured at 562 nm. A lower absorbance of reaction mixture indicated a higher Fe^{+2}-chelating ability. Radical scavenging activity of pure compounds was calculated by using the following formula:

% RSA = 100 – {(OD test compound / OD control) x 100}.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The assay was adapted from Shaheen and others (13) methodology. Test samples were allowed to react with DPPH, a stable free radical for half an hour at 37 ºC. The concentration of DPPH was kept as 300 µM. Ten microlitres of test samples (0.5 mM**)** were dissolved in 90 µL DMSO, while DPPH solution was prepared in ethanol. After incubation, decrease in absorption was measured at 515 nm using multiplate reader (Spectra MAX-340, Molecular devices, CA, USA). Percent Radical Scavenging Activity (% RSA) of samples was determined in comparison with a DMSO treated control group. Radical scavenging activity was calculated using the following formula:

% RSA = 100 – {(OD test compound / OD control) x 100}.

Superoxide radical scavenging assay

The assay protocol was adapted from Pieker and Fridovich (14) methodology. Ten microlitres of sample (0.5 mM) was mixed with 40 µL of β–nicotinamide adenine dinucleotide (NADH-280 µM), 40 µL of nitro blue tetrazolium (NBT-80 µM), 20 µL of phenazine methoxysulphate (PMS-8 µM), 10 µL of sample (1 mM) and 90 µL of phosphate buffer (0.1 M, pH 7.4). The reagents were prepared in buffer, while the sample was prepared in DMSO. The reaction was performed in 96-well microtitre plate at room temperature and absorbance was measured at 560 nm. The formation of superoxide was monitored by measuring the formation of water soluble blue Formazan dye. A lower absorbance of reaction mixture indicated a higher scavenging activity of the sample. Percent Radical Scavenging Activity (% RSA) of the sample was determined in comparison with propyl gallate as control using the following formula: % RSA = 100 – {(OD test compound / OD control) x 100}.

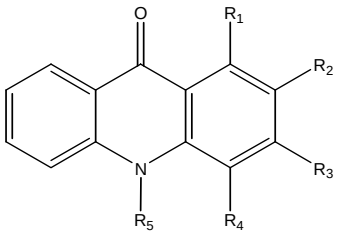
Antiplasmodial activity

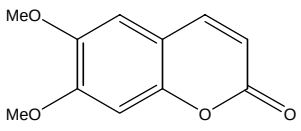
In-vitro antiplasmodial activity was performed on the *Plasmodium falciparum* 3D7 strains using the parasite lactate dehydrogenase assay (pfLDH) as described by Makler and others (15). The 96-well plate formate was employed for the assay. Plant constituents and standard drugs (chloroquine diphosphate) were serially diluted (two fold) with a complete culture medium in 96-well plates. A solution of infected RBC with 2% parasitemia and 1% hematocrit was added to make a total volume of 200 µl in each well. Plates were incubated in a candle jar with 5% CO_2, 5% O_2 and 90% N_2 at 37 ºC for 72 hours. To carry out the Malstat reaction (an immunocapture diagnostic for malaria), 20 µl in 100 µl of Malstat solution from each well of plate was added to respective well of Malstat plate. Plates were placed in an agitating water bath at 37 ºC for 30 minutes. Twenty five microlitres (25 µl) solution (1:1 solution of NBT (2 mg/ml) and PES (0.1 mg/ml) was added in each well and the plates were kept in the dark for a complete reaction and color development (a positive reaction was seen as a dark blue or reaction product. Plates were read at 650 nm and the OD was recorded. IC_{50} value was calculated with the help of an EZfit computer program.

Results and Discussion

Four acridone alkaloids, namely tegerrardin A **1** (10), 1-hydroxy-3,4-dimethoxy-*N*-methylacridone **2** (8), arborinine **3** and xanthoxoline **4** (6,9) and a coumarin, scoparone **5** (11) have been isolated from the fruits of *Z. leprieurii*. Compound **1** has been reported only from *Teclea gerrardi* (10), whereas only one source (8) reports the isolation of compound **2.** Compounds **3**, **4** and **5** have been previously reported from the fruits of *Z. leprieurii* and various Rutaceae species, whereas compounds **1** and **2** are reported herein for the first time from that plant.

All the isolated acridone alkaloids **1**-**4**, scoparone **5** and crude extract of the fruits of *Z. leprieurii* were tested against the 3D7 strains of *Plasmodium falciparum*. After 72 hrs. of incubation, compounds **3** and **4** were found to exhibit a good *in vitro* antiplasmodial activity with IC_{50} values of 4.5 ± 1.0 and 4.6 ± 0.6 µg/ml, respectively whereas compounds **1**, **2** and **5** and the crude EtOAc extract were found to be inactive against the parasites (Table 1). The lack of activity in constituents and the crude extract may be due to the antagonistic effect of the different constituents present in the crude extract. The antiplasmodial effects of compounds **3** and **4** were comparable to those of some natural acridone alkaloids reported earlier (10,16,17,18,19).





5. Scoparone

1. $R_1 = OCH_3$, $R_2 = R_4 = H$, $R_3 = OH$, $R_5 = CH_3$
Tegerrardin A
2. $R_1 = OH$, $R_2 = H$, $R_3 = R_4 = OCH_3$, $R_5 = CH_3$
1-Hydroxy-3,4-dimethoxy-*N*-methylacridone
3. $R_1 = OH$, $R_2 = R_3 = OCH_3$, $R_4 = H$, $R_5 = CH_3$
Arborinine
4. $R_1 = OH$, $R_2 = R_3 = OCH_3$, $R_4 = H$, $R_5 = H$
Xanthoxoline

Table 1: Antiplasmodial activity of compounds **1**-**5** and crude of extract of *Z. leprieurii* against *Plasmodium falciparum* (3D7 strain), after 72 hrs. of incubation at 37 °C

Compound	**IC_{50} (μg/ml)**
Tegerrardin A (**1**)	17.3 ± 3.0
1-Hydroxy-3,4-dimethoxy-N-methylacridone (**2**)	-
Arborinine (**3**)	4.5 ± 1.0
Xanthoxoline (**4**)	4.6 ± 0.6
Scoparone (**5**)	> 25
Crude	> 25
Chloroquine diphosphate (standard)	0.0250 ± 0.01

Compounds **1**-**5** were screened for their antioxidant activities (Table 2) employing the superoxide, iron chelating, and DDPH scavenging assays. Compound **2** showed the highest activity (RSA 75%) in the superoxide anion scavenging assay, comparable to that of n-propyl gallate used as standard (90%). Compounds **1**, and **3** - **5** exhibited low activity. On metal-chelating assay, arborinine **3** showed the highest iron-chelating ability, followed by tegerrardin A **1** (90 and 61% RSA respectively) in comparison with the standard EDTA (98% RSA). Results suggest therefore that arborinine **3** and tegerrardin A **1** can be considered as strong and moderate chelating agents, respectively. In addition, compound **3** exhibited both iron binding and antiplasmodial activity which may be strongly interrelated. Iron is known to play a critical role in host-parasite interaction (20,21), and is essential for life, because it is required for oxygen transport, respiration, and for activity of many enzymes. Hence it is important for the growth of bacteria, fungi, protozoan parasites and mammalian cells. Iron deprivation can therefore be detrimental to *Plasmodium falciparum* and may be a useful approach for the treatment of malaria. Ironically, iron supplementation in anaemic children and women areas where malaria is endemic may encourage malaria infection (21). The mechanism of action of iron chelators has however not been clearly elucidated. Several hypotheses have been suggested: iron-binding, inhibition of iron-dependent enzymes (ribonucleases, phosphoenolpyruvate carboxykinase, superoxide dismutase, etc) and the formation of free

radicals. According to El Nehir and Karakya (22), iron is capable of generating free radicals from peroxides by Fenton reactions, and the minimisation of the Fe^{2+} ions in the Fenton reaction affords protection against oxidative damage. The present result indicates a positive correlation between the *in vitro* antiplasmodial activities of the iron chelating agents and their iron binding potentials (23). More studies need to be carried out on arborinine **3** for its possible use as an efficient and non-toxic iron chelating agent. The present findings suggest the potential of arborinine **3**, 1-hydroxy-3,4-dimethoxy-*N*-methylacridone **2** and xanthoxoline **4** in malarial treatment. The synergy of these compounds should be considered because when compounds **3** and **4** exhibit antiplasmodial effect, compound **2** and **3** due to their antioxidant activities are expected to boost the patient immune system (24). To the best of our knowledge, this is the first report of the iron chelating activity of acridone alkaloids.

All tested compounds were found to be inactive in the DPPH radical scavenging assay.

Table 2: Antioxidant activities of compounds **1**-**5**.

Compound	**Conc. (µM)**	**Superoxide anion scavenging assay % RSA**	**Metal chelating assay % RSA**	**DPPH radical scavenging assay % RSA**
1	0.5	25	61	5.5
2	0.5	75	-	
3	0.5	33	90	2
4	0.5	18	3	10
5	0.5	24	24	0.5
n-Propyl gallate (standard)	0.5	91	-	- 90
EDTA (standard)	1 mM	-	98	
Ascorbic acid	0.5			92

The antioxidant activities of acridone alkaloids appear to have not been extensively studied. However, Wansi and others (25) reported the moderate free radical scavenging of some prenylated acridone alkaloids isolated from *Oriciopsis glaberrima* (Rutaceae).

In conclusion, the fruits of *Z. leprieurii* are found to possess antioxidant and antiplasmodial potentials. 1-Hydroxy-3,4-dimethoxy-*N*-methylacridone, arborinine, and xanthoxoline isolated in the course of this work may be a potential antimalarial therapy due to their possible synergistic effects. The consumption of these fruits may therefore be beneficial as source of antioxidants and prevention against malaria in endemic areas like Cameroon.

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