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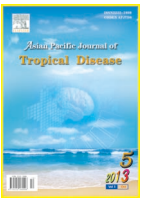

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Isolation and identification of an antiparasitic triterpenoid estersaponin from the stem bark of *Pittosporum mannii* (Pittosporaceae)

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PEER REVIEW

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Comments

The study reported the antiparasitic activities of the crude extracts and Compound 1 from the plant *P. mannii* that was used for treatment of malaria *in vitro*, and the findings showed that Compound 1 appeared active against both malaria and leishmamniasis, proving its potential as lead compound for treatment of parasitic infections. This finding is of great significance for the further screening and development of novel antiparasitic agents.
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ABSTRACT

Objective: To screen for antiparasitic properties of *Pittosporum mannii* Hook (Pittosporaceae) through *in vitro* bioassay tests and to identify the bioactive compound(s).

Methods: The stem bark of *Pittosporum mannii* was harvested in Bali Nyonga in January 2007. The CH_2Cl_2 and MeOH extracts were tested *in vitro* for antiparasitic activity. NF54 (an airport strain of unknown origin and sensitive to all known drugs) and K1 (a clone originating from Thailand and resistant to chloroquine/pyrimethamine) strains were used for the antiplasmodial screening while *Leishmania donovani* MHOM-ET-67/L82 was used for antileishmanial testing. ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-500 spectrometer using $CDCl_3$ as solvent. EIMS were recorded on a double-focusing mass spectrometer (Varian MAT 311A) while HREIMS were recorded on a JEOL HX 110 mass spectrometer.

Results: The MeOH extract was active on both the chloroquine-resistant (K1) strain (IC_{50}=4.3 μg/mL) and on the macrophages of *Leishmania donovani* (IC_{50}=8.6 μg/mL). The CH_2Cl_2 extract was considered inactive on both parasites (IC_{50}>5.0 μg/mL and 21.7 μg/mL respectively). Compound 1, a constituent that precipitated from the MeOH extract, showed pronounced activity on both *Plasmodium falciparum* and *Leishmania donovani* parasites (IC_{50}=1.02 and 1.80 μg/mL respectively) with artemisinin and miltefosine included as reference drugs. Its structure was identified as 1-O-[apha-L-(Rhamnopyranosyl]-23-acetoxyimberbic acid 29-methyl ester, a pentacyclic triterpenoid estersaponin.

Conclusions: The present study constitutes the first report on the antiparasitic activity of this plant and provides some support for the traditional use of the plant in the treatment of malaria. The plant has therefore been identified as a potential source for the discovery of antiparasitic lead compounds.

KEYWORDS
Phytochemical, Pittosporum, Antiplasmodial, Triterpenoid, Saponins

1. Introduction

Parasitic diseases such as malaria and leishmaniasis constitute a major public health threat worldwide, with developing countries the most hit. It accounts for about 10% of total disease burden with some 39.3 million cases reported annually[1]. This increased disease burden is partly due to the high cost of currently available chemotherapy that puts them out of reach for the local poor[2,3].

In Cameroon a greater percentage of rural populations are now using phytomedicines/traditional remedies as alternative means to their primary healthcare[4]. This

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increasing use of phytomedicines/traditional remedies has prompted further interest in research in medicinal plants used traditionally in a view to validating their traditional claims and then developing them into efficacious drugs or purified phytomedicines[5-7].

As part of our ongoing efforts to validate the traditional claims and discover antiparasitic drugs from medicinal plants of Cameroon, we embarked on the bioassay-guided phytochemical screening of the stem bark of *Pittosporum mannii* Hook (Pittosporaceae) (*P. mannii*), a medicinal plant used in traditional medicine by the people of Bali Nyonga, Cameroon for the treatment of malaria and related fevers. It is a highland shrub that grows to a height of 5-10 m. In South Africa and Kenya it is used in traditional medicine for the treatment of fever, malaria, inflammation, stomach ache and as an antidote for insect bites[8,9]. The ethyl acetate extract of this plant has also been shown to possess high radical scavenging activities[10]. Previous phytochemical screening of the stem bark of *P. mannii* showed the presence of flavonoids and saponins[11,12]. The presence of essential oils, sesquiterpenes, triterpenes, flavonoids, carotenoids and saponins were equally shown to be present in the genus *Pittosporum*[13-16]. The aim of the present investigation was to screen for antiparasitic properties through *in vitro* bioassay testing and to determine if its use in traditional medicine for the treatment of malaria and other parasitic diseases could be substantiated and to discover lead compounds that could be developed into efficacious drugs.

2. Materials and methods

2.1. Instrumentation

^{1}H and ^{13}C spectra were recorded on a Bruker AMX-500 spectrometer using $CDCl_3$ as solvent. Coupling constants (J) were measured in Hz. The electron impact mass spectrum were recorded on a double-focusing mass spectrometer (Varian MAT 311A). HREIMS were recorded on a JEOL HX 110 mass spectrometer. Precoated silica gel thin-layer chromatography plates (E. Merck, 254) were used to check the purity of the compound and iodine vapour was used for the visualization of spots on the TLC plates.

2.2. Plant materials

Samples of the stem bark of *P. mannii* were harvested in Bali Nyonga, a village in the North West Region of Cameroon in January 2007 and identified in collaboration with botanists at the National Herbarium, Yaoundé where a voucher specimen (Ref. No. 32235/HNC) has been deposited.

2.3. Extraction and isolation

The chopped, air-dried stem-bark of *P. mannii* (4.2 kg) was pulverized to give 800 g of dry powdered material. It was macerated sequentially in CH_2Cl_2 and MeOH for 2 days×3 with repeated stirring. The extracts were concentrated under reduced pressure to a minimum volume and allowed to stand resulting in the precipitation of Compound 1 as brownish-white solids from the MeOH extract. This afforded CH_2Cl_2 (2.0 g) and MeOH (3.0 g) crude extracts. Compound 1 was purified by washing several times with acetone to give 2.0 g of the compound.

2.4. Sources and culture of parasites

NF54 (an airport strain of unknown origin and sensitive to all known drugs) and K1 (a clone originating from Thailand and resistant to chloroquine/pyrimethamine) strains were used for the antiplasmodial testing while *L. donovani* MHOM-ET-67/L82 (obtained from the spleen of an infected hamster and grown in axenic cultures) was used for antileishmanial testing. Cytotoxicity test was done using HT-29 (human bladder carcinoma), with podophylotoxin included as reference drug at a concentration of 0.1 μg/mL.

All cultures and assays were conducted at 37 °C under an atmosphere of 4 % CO_2, 3% O_2 and 93% N_2. Cultures were kept in incubation chambers filled with the gas mixture. Subcultures were diluted to a parasitaemia of between 0.1% and 0.5 % and the medium was changed daily.

2.5. Antiparasitic screening

The crude MeOH and CH_2Cl_2 extracts of *P. mannii* and Compound 1 were tested *in vitro* at the Swiss Tropical Institute (STI) in Basel, Switzerland and the London School of Hygiene and Tropical Medicine according to WHO/TDR Standard Operating Procedures (SOPs)[17]. Antiplasmodial screening was based upon the ^{3}H-hypoxanthine incorporation assay with its modifications[18-19], in which the test substances were subjected to primary and secondary screens. In the primary screen, K1 strain was used and the test substances were tested at 7 concentrations (0.078-5.000 μg/mL). If the IC_{50}>5 μg/mL, the sample was classified as inactive. For IC_{50} between 0.5 and 5.0 μg/mL, the sample was classified as moderately active. IC_{50}<0.5 μg/mL, the sample was classified as active and was further evaluated on 2 strains, K1 and NF54. Artemisinin was included as a reference drug, with IC_{50} values in the range of 0.8-2.4 μg/mL for NF54 and 0.4-1.5 μg/mL for K1.

For antileishmanial screening, the samples were tested in duplicate at 7 concentrations (0.123-90.000 μg/mL). If the IC_{50} >10 μg/mL, the test substance was classified as inactive. For IC_{50} between 2.0 and 10.0 μg/mL, the test substance was moderately active. IC_{50}<2.0 μg/mL, the sample was classified as active. Miltefosine was used as the reference drug and showed an IC_{50} value in the range of 0.19-0.49 μg/mL.

3. Results

3.1. Identification

Compound 1 showed a positive Liebermann-Burchard test for triterpenes and a positive test for saponins when agitated in warm water. This is in line with other findings that the plant contains saponins[19].

The HR-MS of Compound 1 indicated a $[M+NH_3]$+ion peak at 708.468 19 (calculation 708.468 12) which suggested a molecular formula of $C_{39}H_{66}O_{10}$ having seven degrees of unsaturation. Comparison of the experimental data (^1H, ^{13}C NMR and HR-MS) with the literature values revealed a match with 1-O-[apha-L-(Rhamnopyranosyl]-23-acetoxyimberbic acid 29-methyl ester (Figure 1), a derivative of imberbic acid, previously isolated from the leaves and flowers of *Combretum sundaicum* and *Lantana camara*[20,21].

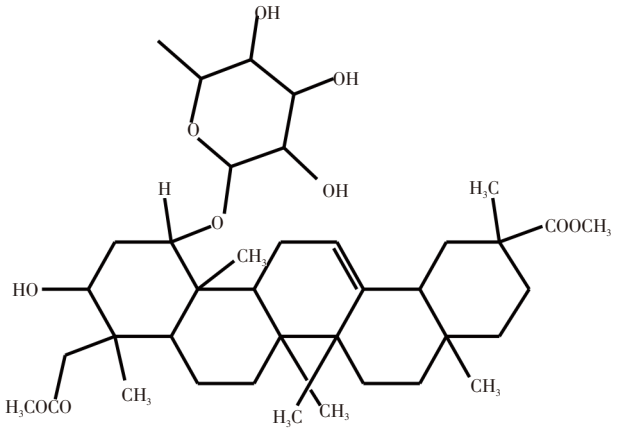

Figure 1. Compound 1.

3.2 Biological testing

In vitro antiparasitic screening of both the CH_2Cl_2 and MeOH extracts and Compound 1 (Table 1) showed that the MeOH extract was moderately active on both the chloroquine-resistant (K1) Thailand strain of *P. falciparum* (IC_{50}=4.3 μg/mL) and on the macrophages of *L. donovani* parasites (IC_{50}=8.6 μg/mL). The SI were 20.93 for *P. falciparum* and 10.47 for *L. donovani*. The CH_2Cl_2 extract was considered inactive for both parasites (IC_{50}>5.0 and 21.7 μg/mL respectively), with low SI of 3.38 for *P. falciparum* and 0.78 for *L. donovani*. Compound 1 showed a pronounced activity for both *P. falciparum* and *L. donovani* parasites (IC_{50}=1.02 and 1.80 μg/mL respectively). Compound 1 was less toxic to mammalian cells with SI 15.59 for *P. falciparum* and 8.83 for *L. donovani* parasites, thus revealing some degree of selectivity in its antiparasitic action.

Table 1

Antiparasitic activity of the CH_2Cl_2 and MeOH extracts and Compound 1 of *P. mannii* (IC_{50}, μg/mL).

Extracts	*P. falciparum* (K1)	SI	*L. donovani* (Macrophages)	SI	Cytotoxicity (Podophylotoxin)
CH_2Cl_2	>5.00	3.38	21.70	0.78	16.9
MeOH	4.30	20.93	8.60	10.47	>90.0
Compound 1	1.02	15.59	1.80	8.83	15.9
Artemisinin	0.80	–	–	–	–
Miltefosine	–	–	0.16	–	–

4. Discussion

As the criteria for activity of a sample is dependent to each research group, we agree that our criteria for activity maybe quite severe and therefore some of the extracts we consider as inactive may actually possess some level of activity. These results which to the best of our knowledge constitute the first report on the antiparasitic activity of this plant provide some support for its traditional use in the treatment of malaria, and open a window for an in depth antiparasitic study of the plant and other related species of the genus *Pittosporum*.

P. mannii Hook (Pittosporaceae) that is used traditionally in Cameroon for the treatment of malaria was investigated to validate this claim. Compound 1 that precipitated from the active MeOH extract of the stem bark showed pronounced *in vitro* activity on both the chloroquine-resistant (K1) Thailand strain of *P. falciparum* and on the macrophages of *L. donovani*, parasites responsible for malaria and leishmaniasis respectively. The structure of Compound 1 was identified as a derivative of imberbic acid, a triterpenoid estersaponin that had previously been isolated from the leaves and flowers of *Combretum sundaicum* and *Lantana camara*. These results provide some support for the traditional use of the plant and identify the plant as a potential source of antiparasitic lead compounds.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Parasitic diseases like malaria and leishmaniasis are still public health concerns worldwide. Currently, control of the disease still relies on drugs. However, resistance of currently available drugs in both parasites has been reported. Screening and development of novel antiparasitic agents is urgently needed.

Research frontiers

This study tested the antiparasitic activities of the compounds isolated from the plant *P. mannii in vitro*, and identified Compound 1 as a potential for an antiparasitic lead compound.

Related reports

The crude MeOH and CH_2Cl_2 extracts of the plant *P. mannii* and Compound 1 were used to test the activity against malaria and leishmania parasites using the test recommended by the WHO/TDR. The methods seem sound and the results are confidential.

Innovations & breakthroughs

The study demonstrated the effective component of the plant *P. mannii* that was used for treatment of malaria, and identified Compound 1, as a novel potential lead compound for treatment of parasitic infections.

Applications

This is the first study to report the antiparasitic activity of Compound 1 extracted from the plant *P. mannii*. Based on the present findings, further studies to investigate the *in-vivo* antiparasitic efficacy and the underlying mechanisms seem justified.

Peer review

The study reported the antiparasitic activities of the crude extracts and Compound 1 from the plant *P. mannii* which was used for treatment of malaria *in vitro*, and the findings showed that Compound 1 appeared active against both malaria and leishmamniasis proving its potential as lead compound for treatment of parasitic infections. This finding is of great significance for the further screening and development of novel antiparasitic agents.

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