Research Article

Antiplasmodial and cytotoxic activities of flavonoids and arylbenzofuran derivatives from *Morus mesozygia*

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ABSTRACT

***Morus mesozygia* Stapf (Moraceae) is a plant found in many regions and used in treating many diseases including malaria and fever. Fractionation of the methanolic extract of its stem bark, using various chromatographic methods has led to the isolation and identification of 3 flavonoids: artocarpesin, artochamin C and kushenol E. And 4 arylbenzofuran derivatives: moracin M, moracin C, moracin L and mulberofuran F. The methanolic extract and the seven isolated compounds were tested for antiplasmodial activity against the chloroquine-resistant FcB1 *Plasmodium falciparum* strain and cytotoxicity on MCF-7 human breast cancer cells. Relating to the antiplasmodial activity, it was found that all compounds were active against the FcB1 strain of *Plasmodium* with artocarpesin, koushenol E and mulberrofuran F showing particular potency (with the median inhibitory concentrations IC_{50} = 2.5-2.6 µg/ml). Cytotoxicity tests performed on MCF-7 cells revealed all the IC_{50} varying from <1.0 to 5.0 ± 0.6 µg/mL. A structure – activity relationship is discussed.**

Keywords: *Morus mesozygia;* Antimalarial; Arylbenzofurans; Flavonoids; Antiplasmodial; Cytotoxicity

INTRODUCTION

Malaria remains the one of the most infectious diseases in the world. It constitutes a public health problem in more than 90 countries, inhabited by about 40% of the world's population. The World Health Organization estimates that there are 300—500 million malaria cases annually, causing 2—3 million deaths, mostly in children under five years old (Who, 2002). Africa accounts for over 90% of malaria mortality (Who 2002).
The tide of Malaria has had significant economic impacts in endemic countries, costing Africa $ 12 billion in lost gross domestic product every year and consuming 40% of all public health spending (Sachs et al., 2002).
It is well documented that several plant species have been used in Africa and elsewhere for varieties of ailments including microbial infections of man. (Soforowa,1993; Adjanohoun et al., 1991). Consequently, African plant species appear to be a natural resource of secondary metabolites, which constitute a source of potentially bioactive compounds. (Soh et al., 2007). In Cameroon, the use of plants in traditional medicine has also been documented to some extent. (Soh et al., 2007) Nowadays, the scientific interest on these metabolites has increased, due to the urgency in the availability of new drugs. The great potential of Cameroon in terms of biodiversity, traditional knowledge and practice, has led many researchers to undertake an ethnopharmacological investigation on medicinal plants.(Zelefack et al, 2009; Ngouamegne et al, 2008). Several active secondary metabolites have been isolated from number of these plants, amongst which can be cited flavonoids, alkaloids, coumarins, chromenes, triterpenes and arylbenzofurans. (Baumgartner et al., 1990; Singab et al., 2005). Plants of the family Moraceae (*Morus mongolica, Morus alba, Ficus septica, Ficus formosana…*) are known to be very good sources of such compounds.(Sheu et al., 2005; Kang et al., 2006)

Morus mesozygia Stapf (Moraceae) is a shrub growing in the tropical and subtropical regions of the world. In many of the regions where it is found, the local population use the roots, the stem and the leaves to treat traditionally: syphilis, dermatitis, rheumatism, asthenias, fever and malaria. (Berhaut, 1979, Burkill, 1997) Investigations on plants of the Moraceae family have been of great interest due to its numerous biological compounds. It is reported in the literature that previous studies carried out on *Morus mesozygia* reveled antimicrobial activity of the methanol crude extract and isolated compounds, (Kuete et al 2009) and isolation of prenylated arylbenzofuranes with antioxidant activity. (Kapche et al 2009).

As a part of a large project seeking new anti-malaria lead compounds, we investigated the antiplasmodial and cytotoxic activity of this plant. We were precisely interested on the phytochemical studies, antiplasmodial and cytotoxic activities of the crude methanolic extract and of purified compounds obtained from *Morus mesozygia* Stapf.

MATERIALS AND METHODS

General

NMR spectra were recorded on Bruker DRX 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) and DRX 300 (300 MHz for ^{1}H) instruments. Chemical shifts were reported with TMS as internal standard. Mass spectra (EI and CI) were recorded with a GC/MS Nermag R10-10 mass spectrometer. HRCI/MS were recorded with a Thermo Finnigan Mat 95XL mass spectrometer. TLC was carried out using Merck silica gel Si 60 F254 20 × 20 cm aluminum sheets and RP-18 $F_{254}S$ 20 × 20 cm aluminum sheets. and 50% H_2SO_4 spray reagent Analytical HPLC was carried out on a Thermo Separation Products system equipped with a P-4000 quaternary gradient pump, a UV-6000LP photodiode array detector, using analytical 125-4 mm columns packed with Merck Lichrospher 100 RP-18 (5 μm), and Macherey-Nagel Nucleosil 100-5 C_6H_6 (end capped). HPLC purifications were performed with a gradient solvent system (water-acetonitrile) and a flow rate of 1 mL/min. This yielded products with chemical purity greater than 93%. Medium pressure liquid chromatography was carried out using Merck silica gel 60 (40-63 μm) or Lichroprep 60 RP-18 (40-63 μm) with UV detection at 254 and 366 nm. The structures of the compounds were confirmed by comparison with reference data from available literature.

Plant material

The stem bark of *Morus mesozygia* Stapf was collected in august 2006 in the Centre province of Cameroon. M. Nana Victor, botanist at the National Herbarium of Cameroon (NHC), performed the botanical identification of the plant and a voucher specimen was conserved under the reference number 1391/SRFK.

Preparation of extract and isolation

Air-dried material of the above plant was grounded. 2.0 Kg of the obtained powder was macerated in 10 L of methanol, overnight, at room temperature. The macerate was filtered using filter paper whatman No.1. The filtrate was concentrated under vacuum to a paste, which constituted the crude extract (262 g).

The crude extract (83g) was subjected to silica gel (230-400 mesh) vacuum liquid chromatography (VLC), using hexane, hexane-ethyl acetate and ethyl acetate-methanol of increasing polarity and finally methanol as eluents. Fractions of 500 mL each were collected, concentrated under vacuum and grouped on the basis of TLC analysis, to yield four main fractions A, B, C and D. Fraction B (8.5g) was subjected to medium pressure liquid chromatography (MPLC) over Silica gel, eluting with a gradient of n-hexane-ethyl acetate, of increasing polarity to yield 57 main fractions of 50 mL each, which were combined on the basis of TLC analysis to 8 fractions (F1-F8). Fraction F3 (1.5 g) was submitted to column chromatography over the Sephadex (LH-20) eluted with methanol to give compounds **3** (11.0 mg) and **4** (63.0 mg). Fraction F4 (2.5 g) was treated as fraction F3 to yield **2** (30.0 mg), **5** (25.0 mg), **6** (39.0 mg), and **7** (11.8 mg). Fraction C (12g) was purified on a silica gel column with a continuous gradient of methylene chloride - methanol (95-5) to give compound **1** (50.0 mg).

Antiplasmodial activity testing

Parasites were cultured according to the method described by Trager and Jensen (Trager et al., 1976) with modifications described by Munoz. (Muñoz et al., 1999) Briefly, parasites (FcB1-Columbia strain, considered to be chloroquine-resistant with an IC_{50} of 145 nM for chloroquine), were maintained on human red blood cells in RPMI 1640 medium (Cambrex, Belgium) supplemented with 7.5% human AB+ serum and grown in a 5% CO_2 atmosphere. Cultures were synchronized every 48 h by magnetic concentration of old stages followed by 5% D-sorbitol lysis. (Ribaut et al 2008, Lambroset al.1979). For *in vitro* antiplasmodial activity evaluation, we took the stock solutions of extracts and drugs firstly in DMSO and then in culture medium and added to parasite culture (1% parasitaemia, 2% haematocrit) in 96-well plates. Parasite *in vitro* growth was followed by [^{3}H]-hypoxanthine (Perkin Ellmer, France) incorporation. The positive control was chloroquine (Sigma). Inhibition values were

plotted versus extract concentrations (average of three independent experiments) and the 50% inhibitory concentration (IC_{50}) was graphically determined by interpolation. The [^{3}H]-hypoxanthine incorporation, in the presence of extracts, was compared with that of control cultures without extract.

Cytotoxicity evaluation

For the most active extracts (on *P. falciparum* culture), cytotoxicity was estimated on human breast cancer cells (MCF7, ATCC n°: HTB-22). The cells were cultured in the same conditions as those used for *P. falciparum*, except the replacement of human serum by 5% foetal calf serum (Cambrex). Cells were distributed in 96-well plates at 2 x 10^4 cells/well in 100 µL of culture medium added to 100 µL of the same medium containing the extracts or drugs at various concentrations. Positive control was doxorubicin (Sigma). Cell growth was estimated by [^{3}H]-hypoxanthine incorporation after a 48h incubation. The [^{3}H]-hypoxanthine incorporation, in the presence of extracts, was compared with that of control cultures without extract. (Roumy et al., 2007)

RESULTS AND DISCUSSION

The structural identification of the isolated compounds was established by comparing the ^{1}H NMR and ^{13}C NMR spectral data with reference data from available literature. These compounds were found to be flavonoids and arylbenzofurans (Fig. 1). Flavonoids **1**-**3** were identified as Artocarpesin (Kijjoa et al., 1996), Artochamin C (Wang et al., 2004) and Kushenol E **(**Mizuno et al., 1990) respectively; compounds **4**-**7,** all arylbenzofurans, were respectively found to be Moracin C, Moracin M, Moracin L, and Mulberrofuran F **(**Mizuno et al., 1990; Takasugi et al. 1979; McAllister et al., 1998; Singab et al., 2005; Kang et al., 2006).

Due to its wide use in folk medicine in the treatment of Malaria and other microbial infections (Berhaut et al., 1976), the methanolic crude extract of *M. mesozygia* as well as its compounds Artocarpesin, Artocamin, Kushenol E, Moracin C, Moracin M, Moracin L and Mulberrofuran F were tested for antiplasmodial activity against the FcB1-Columbia strain of *P. falciparum* and cytotoxicity on MCF-7 human breast cancer cells. From the results of the antiplasmodial activity and cytotoxicity assays presented on Table 1, it can be seen that the MeOH extract of *Morus mesozygia* had a lower antiplasmodial activity (IC_{50} >10 µg/ml). Its cytotoxic activity indicated a less toxic drug (IC_{50} =26 µg/ml) compared to pure isolated compounds. Artocarpesin (**1**), Kushenol E (**3**), two flavonoids and mulberrofuran F (**7**) have exhibited the highest activity with an identical IC_{50} of 2.5 ± 0.4 µg/mL. Apart from Moracin C (**4**) with an IC_{50} = 7.5 µg/mL, the two other arylbenzofurans (**5** and **6**) were potentially inactive (IC_{50}>10 µg/mL). According to Kuypers *et al.* (Kuypers et al., 2006), a compound is classified as an active potential anti malaria drug when its IC_{50} is less than 8 µg/mL. From these results, we could argue that compounds **1**, **3**, **4** and **7** might be interesting sources of potential antimalaria drugs. But these conclusions should be modulated by the cytotoxic effect exhibited by all the compounds against MCF-7 cells. Table 1 showed that all the isolated compounds were more toxic than the methanolic crude extract. Their IC_{50} varying from <1.0 to 5.0 ± 0.6 µg/mL; with regards to the threshold of toxic compounds stipulated by Kuypers *et al.,* "for a compound to be considered cytotoxic, its IC_{50} should be less than 32 µg/mL"

On the subject of the structure activity relationships, it can be found that, in artocarpesin (**1)** and artochamin C (**2)**, the presence of the second prenyl group at position 8 reduced considerably the cytoxic and antiplasmodial activities. Meanwhile, the prenylation of the arylbenzofuran ring at position 4' contributed to an increase in the cytotoxicity with the reduction of antiplasmodial activity when regarding moracin M (**4)** and moracin C (**5)**. The presence of the other cycles on the benzofuran ring increases the cytotoxic activity (compounds **6** and **7**). As it concerns the other compounds, it seemed that the cytotoxic and antiplasmodial activities cannot be correlated with the presence or absence of a specific functional group, and it is probably influenced by a combination of factors.

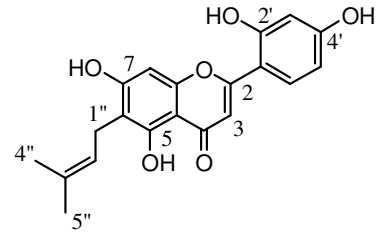
CONCLUSION

Three flavonoids and four arylbenzofufuran derivatives were isolated from the stem bark of *Morus mesozygia* and characterized. From the results presented above it appears that flavonoids: artocarpesin (**1**) and koushenol E (**3**) are potentially good antiplasmodial and antimicrobial agents; they presented the best IC_{50} indicating that they could be possible sources of drugs. Their relative low cytotoxicity compared to other molecules, was an indicator of their therapeutic potential. Mulberrofuran F (**7**) developed an antiplasmodial activity, but its exploitation could be limited due to its high cytotoxicty. The MeOH extract of *M. mesozygia* had a lower antiplasmodial activity (IC_{50} >10 µg/ml). Its cytotoxic activity indicated a less toxic drug (IC_{50} =26 µg/ml) compared to pure isolated compounds. These results could be a preliminary explanation concerning the traditional use of this plant as antimalarial drug in the African traditional folk medicine. Nevertheless, these data need to be complemented by additional experiments in particular to evaluate the cytotoxic effect on other cell lines of usual preparations.

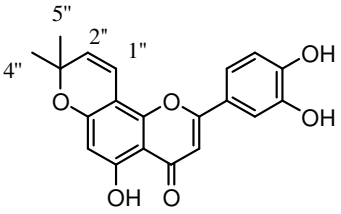
Table1. Antiplasmodial and Cytotoxicity Assays of compounds and MeOH extract of *Morus mesozygia*

	Antiplasmodial activity IC_{50} (µg/mL)	Cytotoxic activity IC_{50} (µg/mL)
Artocarpesin (**1**)	2.5 ± 0.4	3.8 ± 0.6
Artocamin (**2**)	8.6 ± 0.1	5.0 ± 0.9
Koushenol E (**3**)	2.6 ± 0.4	2.5 ± 0.9
Moracin C (**4**)	7.5 ± 0.1	3.3 ± 0.5
Moracin M (**5**)	>10	2.7 ± 0.3
Moracin L (**6**)	>10	<1
Mulberrofuran F (**7**)	2.6 ± 0.1	1.4 ± 0.2
MeOH extract	>10	26
Chloroquinine [a]	0.19	> 100
Doxorubicin [a]	ND	4.5

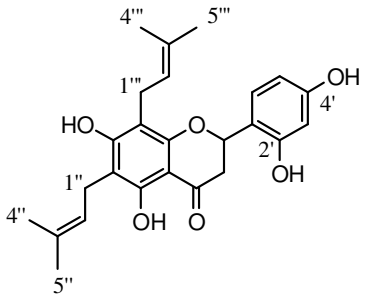
Results are means ± SD deviations of triplicates, ND not determined, [a] Positive controls



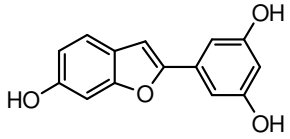
1, Artocarpesin



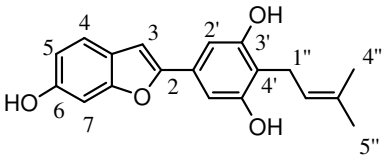
2, Artochamin C



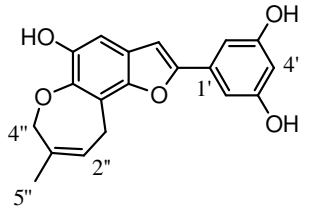
3, Kushenol E



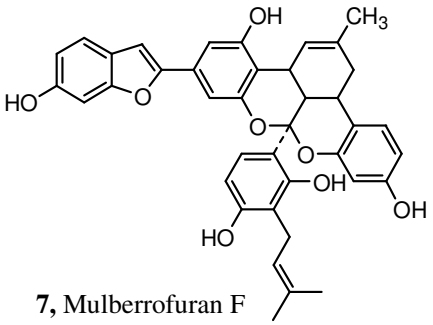
4, Moracin M



5, Moracin C



6, Moracin L



7, Mulberrofuran F

Fig.1. Structures of the compounds isolated from *Morus mesozygia*

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