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ANTIPLASMODIAL ACTIVITY OF EXTRACTS OF *KHAYA SENEGALENSIS* (DERS.) A. JUS (MELIACEAE) AND *MELIA AZEDARACH* L., PLANTS OF SENEGALESE TRADITIONAL MEDICINE

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ABSTRACT: Two medicinal plants (*Khaya senegalensis* and *Melia azedarach*), frequently used by a large part of the Senegalese population and in particular that of the natural region of Casamance in the traditional treatment of malaria, were selected to study phytochemistry and to compare the antimalarial activity of the different parts used (leaves, bark, and seeds). The extraction of these drugs was carried out successively following a gradient of increasing polarity with cyclohexane, ethyl acetate, dichloromethane, and methanol. *In-vitro* antiplasmodial screening of the different fractions was performed on chloroquine-sensitive and chloroquine - resistant strains of *Plasmodium falciparum* (3D7 strain and W2 strain, respectively). The MDEK fraction is the most active on 3D7 strain with an IC₅₀ = 1.81 ± 0.53 µg / ml (Selectivity index > 55.25). *In-vitro* cytotoxicity assays on human umbilical vein endothelial cells (HUVEC cells) were performed and the selectivity index was calculated. These tests reveal the non-toxicity of the fractions tested with high CC₅₀ and very often greater than 100 µg / ml.

INTRODUCTION: Medicinal plants are part of the history of every continent. In some traditional societies, the medical management of so-called chronic diseases is largely ensured by the use of medicinal and food plants¹. In fact, medicinal plants produce much diversified natural substances. They accumulate secondary metabolites which represent an important source of molecules usable by the man in particular in the pharmacological field².

Thus, research on medicinal plants has shown that they are sources of active principles that can treat various conditions^{3, 4} or are precursors in the synthesis of useful drugs. These plants are often used in whole or in part (leaves, bark stems, roots, and fruits) in galenic preparations.

According to Farnthworth⁵, more than 70% of the African population has had to use medicinal plants in the treatment of various conditions. Similarly, WHO reports that more than 80% of the African population uses medicine and traditional pharmacopoeia⁶. Today, in order to contribute to the protection of the environment and in particular medicinal plants, research should be supported to search for the different families of chemical compounds produced by these plants in order to isolate the active principles or molecules that can

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serve as models for a synthesis of new molecules that mainly respond to the safety and efficacy of conventional drugs. The formulation of measured principles and / or synthetic molecules could not only contribute to the conservation of species in their biotope and thus to the protection of the environment, but also to find solutions to public health.

It is in this sense that we have proposed to work on a research project that is part of a desire to promote and promote medicinal plants through the discovery of principles or new molecules treating or relieving various conditions such as malaria like the discovery of quinine and artemisinin.

Thus, *Khaya senegalensis* and *Melia azedarach* frequently used in the traditional treatment of malaria were selected for a phytochemical and comparative study of the activity of different parts (leaves, barks and seeds).

MATERIALS AND METHODS:

Plant Material: The leaves of *Melia azedarach* and the seeds, barks and leaves of *Khaya senegalensis* were harvested in April 2016 in Ziguinchor (Senegal). The plants have been authenticated by Prof. E. Bassene, department of pharmacognosy and botany, Cheikh Anta Diop University, Dakar, Senegal. Reference specimens have been deposited in the herbarium of the pharmacognosy and botany laboratory under the respective numbers 2016/018 and 2016/019.

Extraction and Fractionation: The dried drugs (27 °C) in the laboratory were crushed using a grinder (Bradender OHG Duisburg type). The fine powder *Melia azedarach* (leaves), *Khaya senegalensis* (leaves, bark and seeds) thus obtained after spraying was used as a raw material for the extractions.

Successive depletion of the powder by maceration was carried out by solvents of increasing polarities (cyclohexane, ethyl acetate, dichloromethane, and methanol). Indeed, 100 g of fine powder of the leaves of *Melia azedarach* L. were introduced into a 2 L flask containing 1 L of cyclohexane and macerated for 24 h. The residue obtained after evaporation of the extract is placed in a watch glass and placed under the hood for complete evaporation of the extraction solvent.

The product from the cyclohexane extract is taken up successively under the same conditions as above with 1 L of ethyl acetate, dichloromethane and methanol. The same procedure was carried out with 100 g of fine *Khaya senegalensis* powder (leaves, barks and seeds) **Table 1**.

Phytochemical Methods: Phytochemical screening of secondary constituents present in the plant extracts was carried out using methods adopted in similar surveys ⁷. This quantitative and phytochemical analysis of these plants was determined as follows: Sterols and terpenoids (Lieberman's reaction); alkaloids (Bouchardat's / Valser - Mayer's / Dragendorff's reagents); flavonoids (concentrated HCl + magnesium ribbon), tannins (Stiasny's reagent, FeCl₃ test); saponins (foaming test); free or combined quinone substances (Borntraegen's reagent).

Antiplasmodial Assay: The antimalarial activity of extracts/compounds was evaluated against *P. falciparum* 3D7 and *P. falciparum* W2 strains, using the fluorescence-based SYBR Green I assay approach in 96-well microplates as described by Smilkstein *et al.*, ⁸ with some modifications. Positive control wells for each assay contained no inhibitor while negative controls contained chloroquine (CQ). The CQ molecule was provided from Worldwide antimalarial resistance network (WWARN network). Experiments were run in duplicate with both test and control drugs employed at varying concentrations. Stock solutions (extracts) were prepared in dimethylsulfoxide (DMSO) and diluted with culture medium to give a maximum DMSO concentration of 0.5 % in a final well volume of 200 µL containing 1 % parasitemia and 2.5 % haematocrit.

Extracts and negative control [Chloroquine (CQ)] were prepared by two-fold dilution, in a dose-titration range of 0.098-100 µg / mL, to obtain 11 concentrations each, in duplicate. The concentrations used for CQ were between 0.5 and 1000 nM. After 48 h incubation, the plates were subjected to 3 freeze thaw cycles to achieve complete hemolysis. The parasite lysis suspension was diluted 1:5 in SYBR Green I lysis buffer (10 mM NaCl, 1 mM Tris HCl pH 8, 2.5 mM EDTA pH 8, 0.05 % SDS, 0.01 mg/mL proteinase K and 10X SYBR Green I). Incorporation of SYBR Green

I in parasite DNA amplification was measured using the Master epRealplex cycler® (Eppendorf, France) according the following program to increase the SYBR green incorporation: 90 °C for 1 min, decrease in temperature from 90 °C to 10 °C for 5 min with reading the fluorescence 10 °C for 1 min and a new reading at 10 °C for 2 min. The IC₅₀ was calculated by nonlinear regression using icestimator website 1.2 version:

<http://www.antimalarialicestimator.net/methodintro.htm>.

Cytotoxicity on HUVEC: HUVEC cells were cultured in Gibco™ RPMI 1640 medium (Life technologies, France) complemented with 10 % fetal bovine serum and 1 mM L-glutamine (Sigma-Aldrich, France) and incubated in 5 % CO₂ at 37°C. The cytotoxicity of extracts was evaluated using the SYBR Green I assay as previously described. HUVEC were seeded in a 96-well plate at 100,000 cells/well and incubated for 24 h to adhere. After discarding the old medium, the cells were incubated in the medium containing eight concentrations (0.78 - 100 µg/mL) of each extract in duplicate. After 48 h incubation, cells were visualized using an inverted microscope to check their morphology or the cell viability. The medium was subsequently removed and replaced by lysis buffer without SYBR Green I and the plates were subjected to 3 freeze-thaw cycles. The cell lysis suspension was diluted 1:2 in SYBR Green I lysis

buffer. The incorporation of SYBR Green I in cell DNA and the IC₅₀ analysis were obtained as previously.

RESULTS AND DISCUSSION: This study describes the extraction of the leaves *Melia azedarach* and *Khaya senegalensis* (leaves, barks, and seeds) **Table 1** and the examination of their antiplasmodial activities **Table 2, 3** and **4**. We noticed in this table that the leaves and barks of *Khaya senegalensis*, and the leaves of *Melia azedarach* are very rich in polar compounds, and in *Khaya senegalensis* seeds, the cyclohexane fraction is richer in fatty acid⁹.

Phytochemical screening carried out on the *Khaya senegalensis* barks has detected the presence of alkaloids, saponins, tannins and flavonoids¹⁰, and as for the extracts of *Melia azedarach* we have detected in MMFM (methanol extract from *Melia azedarach* leaves) polyphenol, flavonoids, tannins alkaloids and saponins, MAFM (ethyl acetate extract from *Melia azedarach* leaves) sterols and polyterpenes, MDFM (dichloromethane extract from *Melia azedarach* leaves) alkaloids, and MCFM (cyclohexane extract from *Melia azedarach* leaves) sterols, polyterpenes, and alkaloids. Phytochemical studies on *Khaya senegalensis* leaves, bark and seeds have isolated limonoid, triterpenoid, α-tocopherol, khayanolide and khayanonone -type molecules^{11, 12, 13, 14, 15, 16, 17, 18, 19}.

TABLE 1: YIELD OF KHAYA SENEGALENSIS (LEAVES, BARK, SEEDS) AND MAELLA AZADIRACHA (LEAVES)

Vegetable powder	Extract	Extract code	Masse (g)	Yield (%)
Leaves Khaya (100g)	Cyclohexane	MCFK	0.812	0.990
	Ethylacetate	MAFK	1.341	1.635
	Dichlorométhane	MDFK	1.326	1.617
	Méthanol	MMFK	13.816	16.851
Ecorces Khaya (100g)	Cyclohexane	MCEK	0.315	0.426
	Ethylacetate	MAEK	1.318	1.782
	Dichlorométhane	MDEK	0.125	0.169
	Methanol	MMEK	21.13	28.572
Graines Khaya (150g)	Cyclohexane	MCGK	30.648	70.413
	Ethylacetate	MAGK	12.258	28.16
	Dichloromethane	MDGK	1.076	2.472
	Methanol	MMGK	3.157	7.253
Feuilles Maella (100g)	Cyclohexane	MCFM	4.366	4.488
	Ethylacetate	MAFM	1.825	2.195
	Dichloromethane	MDFM	1.142	1.374
	Méthanol	MMFM	7.825	9.413

Extracts cyclohexane (MCFK), ethyl acetate (MAFK), dichloromethane (MDFK) and methanol (MMFK) from *Khaya senegalensis* leaves.

Extracts cyclohexane (MCEK), ethyl acetate (MAEK), dichloromethane (MDEK) and methanol (MMEK) from *Khaya senegalensis* barks.

Extracts cyclohexane (MCGK), ethyl acetate (MAGK), dichloromethane (MDGK) and methanol (MMGK) from *Khaya senegalensis* seeks. **Table 2** lists the results of the antiplasmodial activity of the extracts of *Melia azedarach* and *Khaya*

senegalensis, as regards **Table 3** and **4**, those of the cytotoxicity and the selectivity index (SI) of the extracts respectively for the strain 3D7 and W2 of *Plasmodium falciparum*.

TABLE 2: RESULTS OF ANTIPLASMODIAL ACTIVITY OF EXTRACTS ON PLASMODIUM FALCIPARUM STRAINS 3D7 AND W2

Code	<i>Plasmodium falciparum</i> 3D7 strain	<i>Plasmodium falciparum</i> W2 strain
	IC ₅₀ µg/mL ± SD	IC ₅₀ µg/mL ± SD
MMFM	18.7 ± 1.44	6.16 ± 0.25
MDFM	11.15 ± 2.52	28.65 ± 3.93
MAFM	25.76 ± 4.42	26.28 ± 2.45
MCFM	30.68 ± 2.46	30.03 ± 0.45
MMGK	24.87 ± 4.64	16.67 ± 0.56
MDGK	39.75 ± 10.6	25.57 ± 0.58
MAGK	>100	>100
MCGK	>100	>100
MMEK	>100	>100
MDEK	1.81 ± 0.53	17.59 ± 3.77
MAEK	26.16 ± 2.32	27.02 ± 2.79
MCEK	26.08 ± 4.57	87.43 ± 8.20
MMFK	36.34 ± 6.1	>100
MDFK	32.11 ± 4.88	>100
MAFK	9.93 ± 2.14	22.63 ± 1.42
MCFK	>100	31.45 ± 4.00
CQ	18.29 ± 4.71 nM (9.43 ± 2.43 µg/mL)	>100 nM (51.59 µg/mL)

CQ = Chloroquine

TABLE 3: RESULTS OF THE CYTOTOXICITY OF THE EXTRACTS AND THE SELECTIVITY INDEXES (IS) ON THE STRAIN 3D7

Code	<i>Plasmodium falciparum</i> 3D7 strain	HUVEC cells	Selectivity Index
	IC ₅₀ µg/mL ± SD	CC ₅₀ µg/mL ± SD	=CC ₅₀ /IC ₅₀
MMFM	18.7 ± 1.44	19.51 ± 4.72	1.043315508
MDFM	11.15 ± 2.52	42.92 ± 5.59	3.849327354
MAFM	25.76 ± 4.42	>100	>3.88
MCFM	30.68 ± 2.46	26.44 ± 1.79	0.861799218
MMGK	24.87 ± 4.64	>100	>4.02
MDGK	39.75 ± 10.6	>100	>2.51
MAGK	>100	>100	>1
MCGK	>100	>100	>1
MMEK	>100	>100	>1
MDEK	1.81 ± 0.53	>100	>55.25
MAEK	26.16 ± 2.32	24.28 ± 0.47	0.928134557
MCEK	26.08 ± 4.57	>100	>3.82
MMFK	36.34 ± 6.1	>100	>2.75
MDFK	32.11 ± 4.88	>100	>3.11
MAFK	9.93 ± 2.14	>100	>10.07
MCFK	>100	24.94 ± 2.3	0.2494
CQ	18.29 ± 4.71 nM	34.14 ± 0.42 µM	>314.2

The *P. falciparum* (chloroquine sensitive) 3D7 strain and the W2 (chloroquine-resistant) strain were given with chloroquine, IC₅₀ of 18.29 ± 4.71 nM and > 100 nM respectively. According to WHO recommendations and previous work^{20, 21, 22, 23}, the antiplasmodial activities of plant extracts were classified as follows: highly activity extracts with

IC₅₀<5 µg/mL, promising activity 5 - 15 µg/mL, activity moderate 15 - 50 µg/mL and inactivity > 50 µg/mL.

On the basis of the WHO recommendations, we can say that the results of the extracts are in the range of very high to moderate activities.

TABLE 4: RESULTS OF THE CYTOTOXICITY OF THE EXTRACTS AND THE SELECTIVITY INDEXES (IS) ON THE STRAIN W2

Code	<i>Plasmodium falciparum</i> W2 strain	HUVEC cells	Selectivity Index
	IC ₅₀ µg/mL ± SD	CC ₅₀ µg/mL ± SD	=CC ₅₀ /IC ₅₀
MMFM	6.16 ± 0.25	19.51 ± 4.72	>3.16
MDFM	28.65 ± 3.93	42.92 ± 5.59	>1.49
MAFM	26.28 ± 2.45	>100	>3.80
MCFM	30.03 ± 0.45	26.44 ± 1.79	0.8804528805
MMGK	16.67 ± 0.56	>100	>5.99
MDGK	25.57 ± 0.58	>100	>3.91
MAGK	>100	>100	>1
MCGK	>100	>100	>1
MMEK	>100	>100	>1
MDEK	17.59 ± 3.77	>100	>5.68
MAEK	27.02 ± 2.79	24.28 ± 0.47	0.8985936343
MCEK	87.43 ± 8.20	>100	>1.14
MMFK	>100	>100	>1
MDFK	>100	>100	>1
MAFK	22.63 ± 1.42	>100	>4.41
MCFK	31.45 ± 4.00	24.94 ± 2.3	0.7930047695
CQ	>100 nM	34.14 ± 0.42 µM	0.3414

Most of the extracts show a moderate activity on strain 3D7 with IC₅₀ values between 15 and 50 (15 < IC₅₀ < 50). On the other hand, three of the extracts (MDFM, MMFM and MAFK) were active (5 < IC₅₀ < 15) with IC₅₀ of 11.15 µg/mL; 6.16 µg/mL and 9.93 µg/mL respectively. It should also be noted that for the same 3D7 strain of *P. falciparum*, the MDEK extract is the most active with an IC₅₀ = 1.81 ± 0.53 µg/mL (IC₅₀ < 5) and a better selectivity index (> 55.25).

In the case of W2 (resistant chloroquine) strain, the tests showed as for 3D7 strain a moderate activity for most extracts unlike the reference molecule chloroquine (CQ) which showed no activity on this W2 strain whose IC₅₀ > 100 nM. MMFM represents the most active extract on the *P. falciparum* W2 strain (IC₅₀ = 6.16 ± 0.25). The MMFM IC₅₀ on the *P. falciparum* W2 strain (6.16 ± 0.25 µg/mL) is lower than that of *P. falciparum* 3D7 strain (18.7 ± 0.55). This shows that there is no correlation between the IC₅₀ value obtained with the MMFM and the chloroquine sensitivity of the strain tested. This phenomenon has been described by several authors testing the anti-plasmodial activity of natural products^{24, 25, 26}.

Phytochemically, a chemical characterization of *Khaya senegalensis* showed the presence of alkaloids, limonoids and terpenes and *Melia azedarach* polyphenol, flavonoids, tannins, alkaloids, sterols and polyterpenes and saponins. Several studies have described the antiplasmodial

effect of its secondary metabolites^{27, 28, 29, 30, 31, 32, 33} which could explain the observed results on the activity of extracts of *Khaya senegalensis* and *Melia azedarach*.

The cytotoxicity tests carried out on the 16 extracts show no toxicity on human umbilical vein endothelial cells (HUVEC cells) used in the case of the two strains (3D7 and W2); which will explain their traditional use without any noticeable side effects.

CONCLUSION: This article reports a bioactive study of the antiplasmodial activity of *Khaya senegalensis* and *Melia azedarach*. The antiplasmodial activity of the extracts showed promising results in the field of malaria research. The results of this study showed that *Melia azedarach* leaves extract (MMFM) and *Khaya senegalensis* bark (MDEK) have potent antiplasmodial activity and can therefore serve as potential sources of effective and affordable antimalarial agents. Therefore, this study provides a molecular basis to justify the use of these plants in Senegalese traditional medicine.

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CONFLICT OF INTEREST: Nil**REFERENCES:**

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