SEARCH FOR ANTIMALARIAL COMPOUNDS FROM *PYCNANTHUS ANGOLENSIS*

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INTRODUCTION

Malaria is one of the most important infectious diseases in underdeveloped countries, particularly in Africa. [1, 2, 3] It affects about 500 million people each year, leading to 1.5 million deaths per year. [2, 3] Multi-resistance to most antimalarials in use is now wide spread, while the cost of effective treatment, through different antimalarial drug combinations, is prohibitive for the majority of the affected populations. [4] Plants used in traditional medicine are one major potential source for new antimalarial compounds. The recognition and validation of traditional medicine practices as well as the search for natural antimalarial compounds could lead to new strategies for malaria control. The species *Pycnanthus angolensis* (Myristicaceae) is described to be used by traditional healers of São Tomé and Príncipe islands for the treatment of malaria and fever. [5] Beside its use in traditional medicine against malaria and fever, it is also used in the cure of oral thrust, fungal skin infections, shingles, chest pain and headaches. [6, 7] The only reported compounds isolated from this species include allantoin, flavonoids, dihydroguaiaretic acid and pycnanthuquinones A, B and C. [6, 7, 8] A previous study demonstrated that the crude ethanolic extract of the bark of *Pycnanthus angolensis* had evident antiplasmodial activity against chloroquine resistant *Plasmodium falciparum.* [5]

In this communication we present a bioguided phytochemical study of this species and the results regarding the antimalarial *in vitro* tests against two strains of *Plasmodium falciparum*, 3D7-chloroquine sensitive and Dd2-chloroquine resistant, for the extracts, fractions and isolated compounds.

Figure 1 – The species *Pycnanthus angolensis*

RESULTS AND DISCUSSION

A new dibenzylbutane lignan was isolated, 4,4'-dihydroxy-3-methoxylignan (**1**) along with other four known lignans: (-)-dihydroguaiaretic acid (**2**), hinokinin (**6**), heliobuphthalmin (**7**) and talaumidin (**9**). Three new lignans were obtained from derivatization of **2**, 4'-hydroxy-3,3',4-trimethoxylignan (**3**), 3,3',4,4'-tetramethoxylignan (**4**) and 4,4'-diacetoxy-3,3'-dimethoxylignan (**5**). (-)-dihydrocubebin (**8**) was got by reduction of **7**. Ozic acid (**10**), was isolated and submitted to a methylation reaction yielding methyl 4*R*,5*S*,9*R*,10*S*-8(17),12,14-labdanetrien-18-oate (**11**). The steroids stigmast-4-en-6β-ol-3-one (**12**), β-sitosterol (**13**) and stigmasterol (**14**) were also isolated. The extracts, the main fractions and the isolated compounds were tested *in vitro* for their antimalarial activity. In contrast with the crude extract and fractions, the compounds have not shown significant antimalarial activity in both strains. Unless the active compounds were lost during fractionation, these results might be explained by synergistic effects between the different components of the complex extracts and could suggest that a standardization of the bark extract might be the best solution to a rational use of this traditional antimalarial plant.

Table 1: Antimalarial *in vitro* activity against 3D7-chloroquine sensitive *Plasmodium falciparum* of the extracts prepared for the preliminary study and the fractions derived from the fractionation of the total extract.

Samples	**IC_{50} (μg/mL)**
Dichlorometane extract	1.6
Methanol extract	6.4
Ethanol 70 % extract	27.0
Fraction A	105.9
Fraction B	54.4
Fraction C	8.4
Fraction D	11,6
Fraction E	3.1

Table 2: Antimalarial *in vitro* activity against $3D_7$-chloroquine sensitive and Dd2-chloroquine resistant *Plasmodium falciparum* of the isolated compounds.

Compound	**3D7 strain**		**Dd2 strain**	
	IC_{50} (μg/mL)	**IC_{50} (μM)**	**IC_{50} (μM)**	**IC_{50} (μM)**
4,4'-dihydroxy-3-methoxylignan	31.0	103	37.6	125
(-)-dihydroguaiaretic acid	78.2	237	42.3	128
4'-hydroxy-3,3',4-trimethoxylignan	49.2	143	50.2	146
3,3',4,4'-tetramethoxylignan	106.2	298	174.2	489
4,4'-diacetyl-3,3'-dimethoxylignan	39.1	101	40.8	115
Talaumidin	36.2	106	20.7	61
Heliobuphthalmin	87.4	211	35.1	85
(-)-dihydrocubebin	78.1	218	82.4	230
Hinokinin	90.7	256	54.4	154
Ozic acid	110.0	364	119.9	397
Methyl 4*R*,5*S*,9*R*,10*S*-8(17),12,14-labdanetrien-18-oate	52.0	165	19.9	63
Stigmast-4-en-6β-ol-3-one	74.8	175	24.5	57
β-sitosterol/Stigmasterol	121.4	-	109.1	-

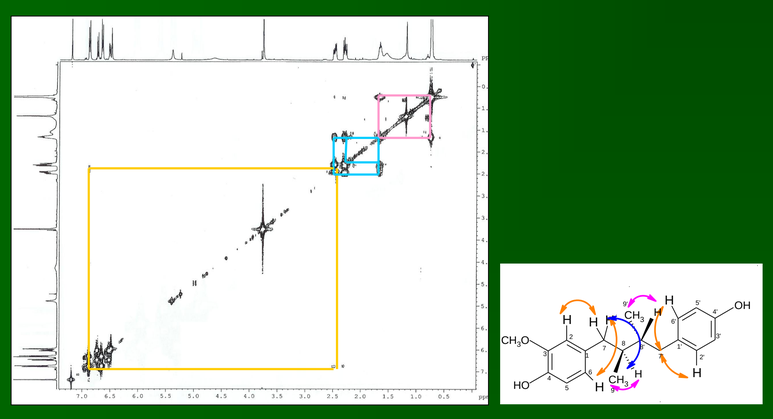

Figure 2 – ^1H-^1H COSY correlations of **1**

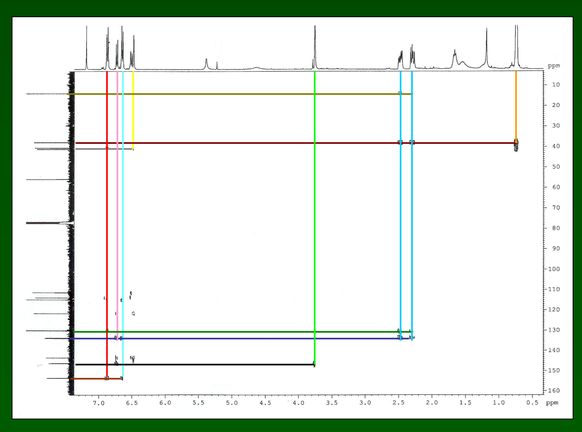

Figure 3 – HMBC correlations of **1**

MATERIALS AND METHODS

Extraction and fractionation: The powdered stem bark of *Pycnanthus angolensis* was extracted at room temperature with dichloromethane (4 x 10L). Fractionation and purification were performed by classic chromatographic techniques. Methylation of **2** and **10** was done with diazomethane. [9] Acetylation of **2** was achieved with acetic anhydride and pyridine. **7** was reduced with $LiAlH_4$. [10] Identification of all compounds was achieved by physical and spectroscopic methods (IR, EIMS, ^1H NMR, ^{13}C NMR, DEPT and 2D experiments – ^1H-^1H COSY, HMQC, and HMBC) and data obtained was in agreement with data reported in the literature.

Antimalarial activity assays: Extracts, fractions and isolated compounds were tested by the susceptibility microassay technique. [11] Two strains of *Plasmodium falciparum*, 3D7-chloroquine sensitive and Dd2-chloroquine resistant, were continuously maintained in culture [12] and used in these assays.

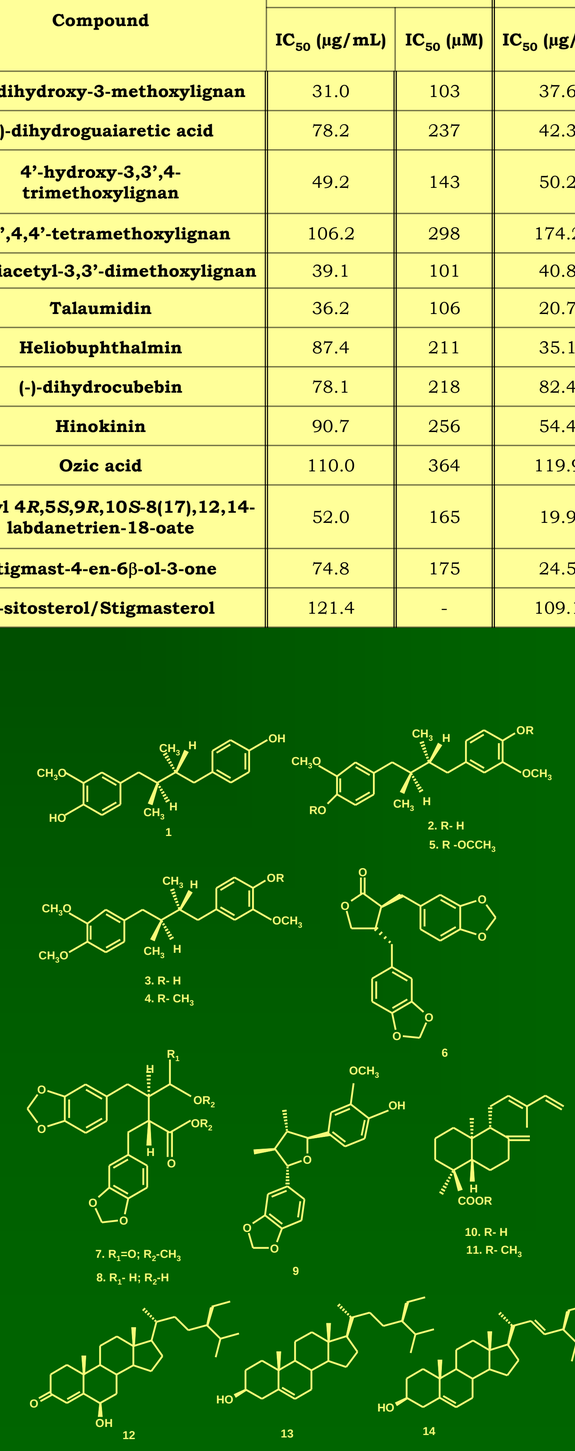

Figure 4 – Chemical structures of the compounds obtained from *Pycnanthus angolensis*

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