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RESEARCH ARTICLE

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Antimalarial Activity of Yaoundamine a Naphthyl Iso-quinoline Alkaloid, Extracted from Stem of *Ancistrocladus heyneanus*

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ABSTRACT

Naphthyl iso-quinoline alkaloid was extracted from stem of Ancistrocladus heyneanus, in methanol and then separated by TLC. It was further assayed by LC/MS and NMR. The alkaloid identified as Yaoundamine was tested for its anti-malarial activity using in vitro method and two strains of Plasmodium falciparum a Chloroquine-sensitive strain 3D7 and the other Chloroquine-resistant strain K1. Based on the IC_{50} value and SI (Selective Index) of the test samples (Yaoundamine) it was found that the stem extract containing Yaoundamine exhibited promising anti-malarial activity against both 3D7 and K1 strains of Plasmodium falciparum along with good SI. It can be concluded that the extract is active against Chloroquine resistant parasites and efficacy is comparable to drug Arteether

Keywords: Naphthyl iso-quinoline alkaloid, Yaoundamine, *Plasmodium falciparum,* Anti-malarial, *Ancistrocladus heyneanus*

INTRODUCTION

The impetus for this work was continual development of resistance of malarial parasites to the available drugs.

Presence of unique Naphthyl iso-quinoline (NIQ) alkaloids in all the species of Ancistrocladus have been reported in nineteen seeventies [1, 2]. NIQ alkaloid is found in very few plant families viz. Ancistrocladaceae and Dioncophyllaceae. As the name suggests, these alkaloids comprise of two moieties; a Naphthyl group and an iso-quinoline group. These alkaloids are unique due to several reasons e.g. their unusual substitution pattern, including an unprecedented methyl substitution pattern at C-3, a meta-oxygenation pattern at C-6 and C-8 and a stereo chemically interesting biaryl linkage- the linkage connecting the iso-quinoline part to the Naphthyl moiety (Fig 1).This unusual structure arises from acetic acid units and not from aromatic amino acids like most of the alkaloids [3]. Because of such unusual structures, remarkable biological activities have been shown by different genera of Ancistrocladus e.g. anti-malarial activity in extracts of *Ancistrocladus korupensis* (Hallock et al 1997), anti-HIV activity [4] and anti-tumor activity [5].

Youndamines A [6] have been reported to have anti-malarial activity. Hence, for the present work the alkaloid were extracted from the stem of *Ancistrocladus heyneanus;* TLC separation of these alkaloids in exhibited 3 alkaloid bands one of them was analyzed to be Yaoundamine by LC/MS and NMR [7]. Youndamine fraction was used for anti-malarial assay. The work reported in this paper is a preliminary attempt to know whether compound isolated had any anti-malarial activity or not.

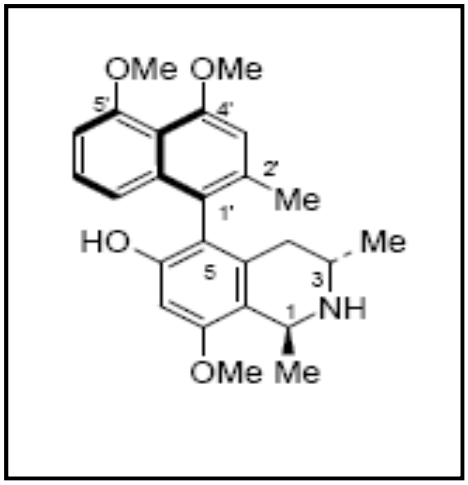

Fig 1: Basic structure of Naphthyl Iso-quinoline Alkaloid

Figure -1: *Ancistrocladus heyneanus* growing at Matheran [Inset- arrows show characteristic hooks of *A. heyneanus*]

Malaria is widespread in tropical and subtropical regions and it is caused by a female mosquito bite. These mosquitoes introduce the Plasmodium through saliva into the blood. Through the blood circulatory system the Plasmodium travels to the liver where it matures and reproduces. It is an infectious disease of humans and other animals. Malaria in severe cases can progress to coma or death. Five species of *Plasmodium* are known to infect transmitted by humans i.e. *P. falciparum* , *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. Effective vaccine for malaria has not been created. However, a variety of anti-malarial medications are available e.g. quinine,

artemisinin derivative artesunate, mefloquine. However, Plasmodium has developed resistance to several anti-malarial drugs.

Ancistrocladus heyneanus the Indian species have also shown presence of many NIQ alkaloids e.g. Ancistrocladine, Ancistrocladinine [8], Ancistroheynine [9].

Presence of iso-quinoline moiety in the alkaloid has attracted the attention of pharmacists to study the potential of this plant as anti-malarial agent [10]. It has been shown that Youndamines found in *A. korupensis* exhibits antimalarial activity [6].

Since we have isolated Youndamine from *Ancistrocladus heyneanus* found in Western Ghats of Maharashtra; the present work is an attempt to test the anti-malarial activity of Youndamine isolated from stem extracts of *Ancistrocladus heyneanus*, the only species of Ancistrocladus found in India.

MATERIALS AND METHODS

Stems of *Ancistrocladus heyneanus* were collected from Khandala hills of Maharashtra and sun dried. For extraction of NIQ alkaloids, stem was dried in shade and grinded to fine powder. To the stem powder Methanol was added and left overnight for alkaloid extraction. Next day, it was filtered through Whatman filter paper no. 12. Filtrate was concentrated and separated by TLC using Ethyl acetate: Methanol: 17% Ammonia as mobile phase. TLC exhibited 3 bands. Each band was eluted and analyzed by LC/MS and NMR. The third alkaloid band was identified as Yaoundamine A [7] and used to test its activity against two strains of *Plasmodium falciparum.*

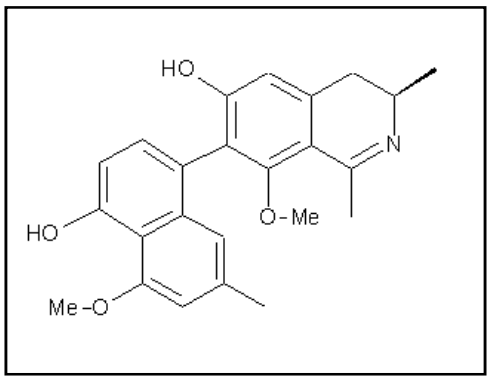

Fig. 2: Structure of Yaoundamine A ($C_{24}H_{25}NO_4$) Mol. Wt. 391.46 g/mol

Anti-malarial assay of alkaloid (Yaoundamine) for in vitro screening using Plasmodium falciparum: Anti-malarial activity of Yaoundamine from stem extracts of *Ancistrocladus heyneanus* was done at CSIR-Central Drug Research Institute, Lucknow.

The Test Model: used for anti-malarial assay were two strains of *Plasmodium falciparum* i.e. 3D 7 a Chloroquine-sensitive strain and K1 Chloroquine- resistant strains.

Alkaloid tested: Only band 3 taken from TLC plate that was identified by GC/MS & NMR as Yaoundamine A was tested for antimalarial assay.

Assay Protocol: *In vitro* anti malarial assay was carried out in 96 well µl plates according to the standard micro assay protocol of [11] with certain modifications. The *in vitro* cultures of both Chloroquine-sensitive (3D7) and resistant (K1) strains of *P. falciparum* 3D7 strain were routinely maintained in RPNI medium supplemented with 25mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% fetal bovine serum [12]. The asynchronous parasites of *P. falciparum* were synchronized after 5 % D-sorbitol treatment to obtain the ring stage parasitized cells only [13].

1 mg/mL stock solution of stem extract was prepared in DMSO and required dilutions were made in test culture medium (RPMI-1640 with 10% FBS). For evaluation of 50% inhibitory concentration (IC_{50}) of the extracts, Malaria SYBR Green I-based fluorescence (MSF) assay was carried out.

Two-fold serial dilutions of the test samples were prepared in 96 well plates and incubated with 1% parasitized cell suspension containing 0.8% parasitaemia (Asynchronous cultures with more than 80% ring stages). The plates were incubated at 37^0C in CO_2 incubator in an atmosphere of 5% CO_2 and air mixtures. 72 hours later 100 µL of lysis buffer containing 2X concentration of SYBR Green –I (Invitrogen) was added to each well and incubated for one hour at 37^0C. The plates were examined at 485 ± 20 nm of excitation and 530 ± 20 nm of emission for relative fluorescence units (RFUs) per well using fluorescence plate reader (FLX BIOTEK). Data was transferred into a graphic program (EXCEL) and IC_{50} values were obtained by Logistic regression analysis. Chloroquine and Arteether were used as the standard reference drugs

The assay was carried out with an initial (ring stage) 43 parasitaemia of 1.0% at 3% haematocrit in a total volume of 200µl of test medium RPMI-1640 with 10% FBS. The provided samples were tested at 1/10, 1/100 and 1/1000 dilutions prepared in test medium. 20 µl of the respective dilutions of test samples were added to the test wells, in duplicate, containing 180 µl parasitized cell suspension. The culture plates were incubated at 37^0C in a 5% CO_2 incubator. After approximately 40h incubation, thin blood smears from each well were prepared on clean, grease free glass slides (Hi Media) and stained with Giemsa's stain. The slides were microscopically examined to record maturation of ring stage parasites into trophozoites and Schizonts in presence of different dilutions of the test agent. At least 300 parasites were counted in each smear.

The test concentration which inhibited the maturation of more than 90% of rings into Schizonts was recorded as the **minimum inhibitory concentration (MIC).** Chloroquine was used as the standard reference drug.

Cytotoxicity Assay: of the extracts was carried out using Vero Cell line (C1008, Monkey Kidney fibroblast) using the method described earlier [14]. The cells were incubated with test sample dilutions for 72 hours and MTT was used as reagent for the detection of cytotoxicity. 50% cytotoxic concentration (CC_{50}) was determined using non-linear aggression analysis. Selectivity index (SI) was calculated as:

$$SI = CC_{50} / IC_{50}$$

RESULTS AND DISCUSSIONS

The IC_{50} value and SI (Selective Index) of the test samples (stem extracts) are depicted in following table 7.1. From the table it is evident that stem extract (alkaloid from band -3 i.e. Yaoundamine A exhibited promising anti-malarial activity against *both* 3D7 and K1 strains along with good SI. It can be concluded that the extract is active against Chloroquine resistant parasites and efficacy is comparable to drug Arteether.

Table – 2: Anti-malarial activity, Cytotoxicity and Selective Index of Youndamine from Stem extracts of *Ancistrocladus heyneanus*

Test Sample	Mean IC_{50} ng/ml		Mean CC_{50} µg/ml of Vero Cells	SI CC_{50} / IC_{50}	
	***3D7*- (Chloroquine Sensitive Strain)**	***K1*- Strain (Chloroquine Resistant Strain)**		***3D7***	***K1***
Youndamine From Stem Extracts	0.79	0.88	61.4	77704	69509
Chloroquine	3.0	150.0	75.0	35627	500
Arteether**	0.43	0.17	42.5	98837	250000

*** Arteether is used for Chloroquine resistant Plasmodium falciparum malaria*

IC_{50} = 50% Inhibitory concentration (of Plasmodium); CC_{50} = 50% Cytotoxic Concentration (of Vero cells); SI = Selective Index (of drug)

Traditional use of three different species of Ancistrocladus; namely *A. abbreviates*, *A. barteri and A. heyneanus* for the treatment for fevers and other diseases has been in record since 1975 [15], but not specifically for treating malaria. Our interest was in confirming whether *A. heyneanus* growing in our area in Maharashtra has the anti-malarial property or not, because there has not been any record of use of this plant for treating malaria by locals.

The first report of anti-malarial activity of extracts of NIQ alkaloids from *Ancistrocladus abbreviatus* and *A. barteri*; tested against *Plasmodium Falciparum* in vitro [10].

Activity of extracts from *Ancistrocladus korupensis* containing Korupensamine A - D, has been tested against *Plasmodium falciparum in vitro* [6, 16] also; later it was shown that another compound Korupensamine-E a Monomer and Yaoundamine-A present in *Ancistrocladus korupensis* as the other Antimalarial compounds.

Two alkaloids from *Ancistrocladus heyneanus* have displayed pronounced in vitro activity against *Plasmodium falciparum*, the malaria parasite i.e. Ancistroheynine A [17, 18] and Jozimine B, which was constitutionally unsymmetrical, anti-plasmodial dimer of the NIQ alkaloid Ancistrocladine [9]. Though as compared with ancistrocladine, it is weakly anti-plasmodial but Jozimine B exhibited a distinctly enhanced anti-malarial activity.
There is ample scope to assess various NIQ alkaloids from different species of Ancistrocladus for its anti-malarial property.

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