wjpmr, 2021,7(9), 4 – 7.

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

ANTIPLASMODIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *MONODORA TENUIFOLIA* AND *OXYANTHUS UNILOCULARIS* TWO TRADITIONAL PLANTS.

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Article Received on 04/06/2021 **Article Revised on 24/06/2021** **Article Accepted on 15/07/2021**

ABSTRACT

Medicinal plants are currently being evaluated as source of promising antimalarial agents. Plant extracts contain phytochemical constituents for miscellaneous medicinal activities which are bioactive in nature. There is growing interest in the use of plants for the treatment and prevention of malaria. In this study, we have evaluated the claimed antimalarial properties of four extracts from two plants utilized in traditional malaria remedies and we have investigated phytochemical screening. Schizontocidal activity was measured using a standard *in vitro* assay, with clinical *Plasmodium falciparum* isolates. All the 4 extracts showed a good activity on clinical isolate. All extracts contained alkaloids, flavonoids, polyphenols, polyterpenes and sterols.

KEYWORDS: Traditional medicine; Medicinal plants; Malaria; Antimalarial; *Plasmodium falciparum.*

INTRODUCTION

Malaria is currently the most deadly parasitic disease in the world, especially in developing countries (Kofi. A, *et al.*, 2012). This disease is transmitted by the female of an anophele mosquito and caused by a protozoan of genus *Plasmodium*.

Of the five plasmodial species, only *P. falciparum*, the most widespread in Côte d'Ivoire, is responsible for the deaths of thousands of people, mostly children (Schlitzer. M, 2008).

The emergence and extension of *P. falciparum* resistant strains to the currently available antimalarials such as chloroquine, an antimalarial very reference for its cost and frequency of prescription, worsen the prognosis of this pathology (Guédé-Guina *et al.*, 1995).

The impact and severity of this parasitosis on public health requires the discovery of new molecules effective on resistant strains.

In this study, we proposed to evaluate the antiplasmodial action of extracts of *Monodora tenuifolia* and *Oxyanthus unilocularis* used traditionally against malaria access.

MATERIAL AND METHODS

Material

The plant material used consists of ethanolic and aqueous extracts of *Monodora tenuifolia* and *Oxyanthus unilocularis*, two plants traditionally used as a decoction in the treatment of malaria in Côte d'Ivoire.

In addition to these extracts we have also used chloroquine (CQ) for control tests.

As for the biological material, it consists of human blood parasites.

The culture medium used for the *in vitro* tests was RPMI 1640 (Roswell Park Memorial Institute 1640) supplemented with 25 mM HEPES, a solution of 5% bicarbonate of sodium and 10% human serum (O^+).

METHODS

Vegetable material and preparation of extracts

Vegetable material consisted of stem back of *Monodora tenuifolia* and *Oxyanthus unilocularis*. The plants were collected from Agboville department and were identified by Floristic Center of Félix Houphouet-Boigny University. The plant samples were then dried in shade left over for 20 days and powdered with the help of grinder. Powder was extracted according to Zihiri and

Kra (Zirihi & Kra, 2003) as follows: One hundred grams of powder were macerated in distilled water during 48 hours. The obtained homogenate was filtered successively on cotton then on Whatman paper 3 mm. The filtrate is first reduced using a rotary evaporator BÜCHI type at 60 ° C, then collected brown paste is lyophilized. We obtained the total aqueous extract (Eaq). This method was used with Ethanol to obtain ethanolic extract (Eeth).

In vitro antiplasmodial assay on P. falciparum

For *in vitro* culture of *P. falciparum*, we used the isotopic alternative of the microphone-test (plate of 96 wells) of Reichmann adopted by WHO (Rieckmann *et al.*, 1978). This technique measure and quantify the capacity of drug to inhibit the growth of *P falciparum* at the trophozoïtes stage.

In this technique, the strains are incubated at 37°C in an impoverished in oxygen and enriched with carbon dioxide with 95% of humidity. After 24 h, plates were removed and added tritiated hypoxanthine (0.5 µCi by well). The plates were again returned to incubator for 24 h. After the incubation, the plates were frozen and thawed. Freezing and thawing of plates free plasmodial DNA radiolabeled by hypoxanthine. The DNA is recovered after washing on a filter paper in a rectangular fiberglass tape with a cell collector. Once the collection is complete, the paper was removed and dried. The radioactivity was measured using a Wallac MicroBeta counter. All results were expressed on a listing.

Phytochemical screening

The phytochemical screening was done using the standard protocols (Uddin *et al.*, 2012).

Test for Alkaloids: 5 mL of extract was concentrated to yield a residue. Residue was dissolved in 3 mL of 2% (v/v) HCl, few drops of Mayer's reagent was added. Appearance of the dull white precipitate indicated the presence of basic alkaloids.

Test for Saponins: 2 mL extract was shaken vigorously for 30 s in a test tube. Persistence of thick forth even after 30 mins indicated the presence of saponins.

Test for Polyphenols: In 2 mL of vegetable extract is added a drop of alcoholic solution of 2% ferric chloride. The appearance of a darker or darker blue or green color indicates the presence of polyphenolic derivatives**.**

Test for Polyterpenes and sterols: 5 mL of plant extract are evaporated to dryness. The residue is dissolved hot in 1 mL of acetic anhydride and collected in a test tube. Along the tube, 0.5 mL of concentrated sulfuric acid is added. The appearance at the interphase of a purple or purple ring, turning blue and then green, indicates a positive reaction.

Test for Quinone: 1 mL of extract was taken.1 mL of conc. H_2SO_4 was added. Formation of red color indicated the presence of quinone.

Test for Tannins: About 0.5 g of the dried powdered samples was boiled in 20 mL of water in a test tube and then filtered.

Test for Flavonoids: A portion of the powdered plant sample was heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute Ammonia solution. A yellow coloration was observed indicating a positive test for flavonoids.

RESULTS

Antiplasmodial activity

The results of the *in vitro* antiplasmodial activity of extracts are presented in table I.

The antimalarial activity of extracts was defined according to the IC_{50} values obtained. An extract showing an IC_{50} value ≤ 5 µg/mL was classified as highly activity. Extracts with IC_{50} values ≥ 5 µg/mL and ≤ 15 µg/mL were considered as promising activity. Extracts with IC_{50} values ≥ 15 µg/mL and ≤ 50 µg/mL were considered as moderately activity and those with IC_{50} values > 50 µg/mL inactive (Bero *et al.*, 2009 ; Usman *et al.*, 2012 ; Kipré *et al.*, 2017).

Phytochemical screening

Phytochemical screening of 4 extracts showed the presence of several secondary metabolites which are summarized in Table 2. In the phytochemical screening, Aqueous extracts and ethanolic extracts of *Monodora tenuifolia* and *Oxyanthus unilocularis* were shown to have same compositions except tanins and saponosides. Similarly ethanolic extracts and aqueous extracts of *Monodora tenuifolia* and *Oxyanthus unilocularis* had same compositions.

Table 1: *Monodora tenuifolia* and *Oxyanthus unilocularis* antiplasmodial activity.

				Clinical isolates /IC50 (µg/mL)			
Plantes	**Extracts**	**W6331**	**W6401**	**W6424**	**W6870**	**W6879**	**Average of IC50**
Monodora tenuifolia	aqueous	2,10	2,17	2,83	3,51	2,56	2,63
	ethanolic	1,73	1,86	1,45	2,88	1,17	1,81
Oxyanthus unilocularis	aqueous	2,82	3,09	3,18	4,39	3,77	3,45
	ethanolic	2,05	2,88	2,03	2,85	2,65	2,49
Artesunate nM		0,03	0,04	0,07	0,85	0,85	0,36
Chloroquine nM		11,47	11,27	11,33	11,56	11,56	11,43

Table 2: Phytochemical screening of aqueous extracts and ethanolic extracts of *Monodora tenuifolia* and *Oxyanthus unilocularis.*

Natural	**substances**					**Chemical**	**groups**		
plants	**Extracts**	**Alkaloïds**	**Flavonoids**	**Saponàsides**	**Cat Tannins**	**Gal Tannins**	**polyPhénols**	**Polyterpenes and Sterols**	**Quinonnes**
Monodora tenuifolia	Aqueous	+	+	+	+	-	+	+	-
	Ethanolic	+	+	-	-	-	+	+	-
Oxyanthus unilocularis	Aqueous	+	+	+	+	-	+	+	-
	Ethanolic	+	+	-	-	-	+	+	-

+ : presence - : absence

DISCUSSION

Antiplasmodial activity

The main goal of this work was to investigate the potential antimalarial properties of two plants used in traditional medicine, against malaria and/or fever, and providing scientific validation for their use. Therefore, selection of plants was carried out based mainly on an ethnobotanical approach (Uddin *et al.*, 2012).

Aqueous extracts and ethanolic extracts of *Monodora tenuifolia* and *Oxyanthus unilocularis* have high activity on clinical isolates of *Plasmodium falciparum*. These results justify the use of this plant in the treatment of malaria in traditional medicine.

However, it is important to note that this plant is frequently used to treat fever, generally associated to malaria. Therefore, an explanation for their lack of *in vitro* antimalarial inactivity could be that these plants may act as antipyretics or may enhance the immune system, rather than having direct antiparasitic activity (Bero & Quetin-Leclercq, 2011). Another explanation is that this plant could contain prodrugs inactive by themselves. In this case, these precursors of the active compounds have to be metabolized *in vivo* into active antimalarials, a major limitation in this study.

Phytochemical screening

Regarding the phytochemical sorting, the results obtained with the extracts of *M. tenuifolia* and *O. unilocularis* vary from one extract to another and from one plant to another. These two plants do not contain quinones and gall tannins, but do contain flavonoids, alkaloids, saponosides, polyphenols, sterols and polyterpenes.

In general, these plants are rich in secondary metabolites. The antimalarial properties of these plants could be due to the presence of alkaloids when we know that the majority of antimalarial molecules are from this chemical family. Tannins and terpenoids have been attributed analgesic and anti-inflammatory activities (Okwu and Josiah, 2006).

CONCLUSION

This study is part of the research program of our laboratory whose objective is to identify traditional Ivorian antimalarial plants. The main objective is to search for new molecules from plant extracts that could be used as new drug leads.

At the end of this study, it appears that the plants of the traditional pharmacopoeia can bring an important contribution to the discovery of new effective and

accessible antimalarial drugs. The results of the study give credibility to the use of many active species in the traditional treatment of malaria. Thus, *Monodora tenuifolia* and *Oxyanthus unilocularis* showed very interesting antiplasmodial activities with IC_{50} values below 5 µg/mL.

The results of the phytochemical screening would explain in part, the infatuation of traditional therapists for these plants as antimalarials. The therapeutic effects are justified by the presence of various chemical compounds (alkaloid, flavonoids, polyterpenes, saponosides and sterols) that allow IC_{50} values below 5 µg/mL.

However, this work must continue in order to produce an Improved Traditional Medicine (ITM).

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