



ANTI-PLASMODIUM FALCIPARUM ACTIVITY OF EXTRACTS FROM DENDRANTHEMA INDICUM

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ABSTRACT

Malaria being a major health problem, demands for search for alternative anti-malaria drugs. *Dendranthema indicum*/ *Dunkufea* plant used in the treatment of malaria in the northern part of Nigeria was evaluated for activity against *Plasmodium falciparum*. The crude ethanol extract of the plant, n-hexane, chloroform and ethyl acetate and last residue fractions obtained by maceration of the crude ethanol extract were screened for activity against *Plasmodium falciparum*, the last residue and hexane fraction did not inhibit significantly even at a concentration of 200µg/ml, the ethyl acetate fraction obtained after maceration was the most active fraction. From the result of the photochemical analysis all the common classes of secondary metabolites tested were present in the plant except alkaloid.

Keywords: Anti-malaria, *Plasmodium falciparum*, maceration, *Dendranthema indicum*

INTRODUCTION

Malaria continues to be a major global health problem, with over 40% of the world's populations. More than 2400 million people exposed to varying degrees of malaria risk in some 100 countries (WHO, 2002). *Plasmodium falciparum* causes the most serious form of the disease, and it is common in the tropics. Infections with this parasite can be fatal in the absence of prompt diagnosis of the disease and its complications, and urgent appropriate patient management taken. The situation is complicated by the increasing occurrence of *falciparum* parasites that are resistant to some anti-malaria drugs like chloroquine and sulphadoxine, pyrimethamine (WHO 2002). Due to this resistance, the search for new anti-malaria drugs is now a major research activity throughout the world.

In spite of control programs in many countries there has been very little improvement in the control of malaria and infections can reduce the effectiveness of labour and can lead to both economic and human losses. Control of malaria is complex because of the appearance of drug resistant strains of *Plasmodium* and with the discovering that man may become infested with species of simian (monkey) malaria (Symth, 2011). At the same time the anopheles mosquito have developed resistance to many insecticides (Srisilam and Veersham, 2010)

Thus it is important to search for new anti-malarial compounds, either synthetic or natural compounds that kill either the vector or parasite. The use of plant-derived drugs for the treatment of malaria has a long and successful tradition. For example, quinine isolated from *Cinchona* and quinghaosu from *Artemisia annua* L. illustrates the potential value of investigating traditionally used anti-malarial plants for developing pharmaceutical anti-malarial drugs (Srisilam and Veersham, 2010).

A combination of medicinal plants commonly used by the locals in Nigeria for the treatment of

malaria are *Azadirachta indica*. *Azadirachta indica* is antipyretic, hypoglycaemic, antifungal, spermicidal, antimalarial, antibacterial and diuretic (Biswas *et al.* 2002]. *Cymbopogon citratus* is commonly used in teas, soups and curries. It is antifungal and has been used for the treatment of flu, fever, pneumonia, malaria and type 2 diabetes (Adeneye and Agbaje 2007). *Anacardium occidentale* is antimicrobial (Aderibigbe *et al.*, 1999) and used for the treatment of malaria.

In Ghana, several plant species including *Alstone iboone* De Willd (Apocynaceae), *Azadirachta indica* A. Juss, (Meliaceae), *Cryptolepis anguinolenta* (Lindl.) Schtr. (Asclepidaceae), *Morinda lucida* Benth. (Rubiaceae), *Nauclea latifolia* Sm. (Rubiaceae) and *Ocimum viride* Willd. (Lamiaceae) are used in the treatment of malaria (Ayitey-Smith, 1989; Abbi, 1990; Mshana *et al.*, 2009).

In Africa and elsewhere, plant extracts are still widely used in the treatment of malaria and other ailments, and up to 80% of the African population use traditional medicines for primary health care (WHO, 2002). Since little scientific data exist to validate antimalarial properties of these medicinal plants, it is important that their claimed antimalarial properties are investigated, in order to establish their efficacy and determine their potential as sources of new antimalarial drugs (such as, artemisinin isolated from *Artemisia annua*).

Traditional remedies have always been sources of important anti-malaria drugs. The population of developing countries world-wide continues to rely heavily on the use of traditional medicines as their primary source of health care. Ethno botanical studies carried out throughout Africa confirms that indigenous plants are the main constituents of traditional African medicines (Adjanohoun *et al.* 1991; Kokwaro, 1976; Mann, 2009; Oliver Bever, 1986).

Various drugs have been developed for treatment of malaria over the years with a guide from knowledge of malaria commonly used traditional herbs. Extracts for example artemisinin (a sesquiterpene lactone end peroxide), which is now the WHO number one drug required for malarial treatment (WHO 2002) is an extract from the Chinese herb *Artemisia annua* (ginghaosu) which have been used over 1000 years for the treatment of fever. *Dendrothema indicum* has been of use traditionally among the Hausa people of northern Nigeria to treat fever of unknown aetiology. Bioactive component of such extracts were not scientifically determined. This work investigated the chemotherapeutic activity of extracts from *Dendrothema indicum* against *Plasmodium falciparum*, with the ultimate goal of identifying potent preparations for the treatment of malaria.

MATERIALS AND METHODS

Plant material

Dendrothema indicum which is also known as 'dunkufe' or 'riyarkasa' among the Hausas (Kano), is a faintly aromatic branching annual herb which belongs to the family of Compositae (Dalziel, 1976). The plant is commonly found in grasslands on mountains slopes, wet places by rivers, fields, roadsides and by sea shores. The plant is used as antiphlogistic, blood tonic, depurative and febrifuge.

The flowers are used in the treatment of furuncle, scrofula, deep rooted boils, inflammation of the throat, eyes and cervix.

Collection and Identification of Plant Materials

The plants were collected in fresh condition at Kano and taxonomical identification was done at Biological Department Bayero University Kano and at herbarium unit of Biological Science Department Ahmadu Bello University Zaria and the voucher number of the plant is 676. The plant was air dried, pulverised and stored until needed for use.

Extraction of Plant Materials

The sieved powder (1.5kg) of *Dendrothema indicum* was percolated with 95% ethanol (6 litres) for two weeks. The extract was decanted, filtered and labeled. The process was repeated three times for exhaustive extraction. The three sets of extracts were combined on confirmation by TLC. All the combined extracts were macerated using n-hexane, chloroform and ethylacetate.

Phytochemical Analysis of Extracts

The crude ethanol extract of the plant was phytochemically screened using standard techniques for the detection of carbohydrates, saponins, tannins, terpenoids, glycosides and alkaloids (Harbone 1976)

Test for carbohydrate

- (a) **Molisch test:** A few drops of molisch reagent was added to test extract (2ml) in a test tube and of concentrated H_2SO_4 (1ml) was allowed to flow down the side of the inclined test tube so that the acid forms a layer beneath the aqueous solution without

mixing with it. A reddish brown solution indicates a positive test.

- (b) **Fehling's test:** standard test for reducing sugar. Equal volumes (5ml) of Fehling's solution A and B was added to the test extracts (2ml) in a test tube. The resultant mixture was boiled for a minute. A brick red precipitate of copper (I) oxide indicates a positive test.

Test for alkaloids

One percent (1%) HCl (1ml) was added to the test fraction (3ml) in a test tube. These solutions were treated with a few drops of Mayer, Wagner and Dragendoff reagents respectively. A creamy white (Mayer), reddish brown (Wagner) and an orange brown (Dragendoff) precipitates observed indicates a positive test.

Test for tannins

Five percent (5%) $FeCl_3$ (2 drops) was added to the test extract (1ml). A dirty green precipitate is an indication of the presence of tannins.

Test for glycosides

Fifty percent (50%) H_2SO_4 (10ml) was added to the test extract (1ml) in test tube. The mixture was heated in boiling water for 15 minutes. Fehling's solution (10ml) was then added and the mixture was boiled. A brick red precipitate indicates a positive test.

Test for Saponins

(a) Frothing test

The test extract (2ml) in a test tube was vigorously shaken for 2 minutes. Frothing in the test extract indicate the presence of saponins.

(b) Emulsion Test

Olive oil (5drops) was added to the test extract (3ml) in a test tube and the mixture was vigorously shaken. A stable emulsion formed indicates the presence of saponin.

Test for Sterols : Salkowski Test

Concentrated H_2SO_4 (1ml) was added to the test extract (1ml). A red colour indicates the presence of a steroid.

Test for Flavanoids: Shinoda Test

A little amount of magnesium powder and a few drops of concentrated HCl were added to the test extract (3ml). An intense red coloration indicates the presence of flavonoid.

Test for Resins

Copper acetate solution (5ml) was added to the test extract (5ml). The resulting solution was shaken vigorously and allowed to separate. A green coloured solution is an evidence of the presence of resin.

Sample collection/preparation of plasmodia culture

With prior consent of the patients and laboratory authority of Murtala Muhammad Specialist Hospital Kano State, some blood samples positive to *Plasmodium falciparum* were collected and their paracetemia values were determined.

The injected erythrocytes were mixed with red blood cell (RBC) diluents (10ml) and then centrifuged at 20000 rpm for five minutes to separate the plasma as well as the white blood cells (which are detrimental to parasite growth) from the erythrocyte.

Preparation of Alsever's Solution

This is the growth medium for *Plasmodium falciparum* in vitro (Dacie and Lewis, 1998). Glucose (24.6g), trisodium citrate dehydrate (9.6g) and sodium chloride (5.04g) were dissolved in distilled deionised water (1200ml). The PH (which was initially 7.84) was adjusted to 6.1 with citric acid on the solution was sterilized by autoclaving for 30 minutes. The solution was stored at 4°C until needed.

Preparation of RBC Diluents

Trisodium citrate (31.3g) was dissolved in distilled water (1 litre), this makes the RBC to retain its disc like form and not to agglutinate. This helps in preserving the cells for several hours (Dacie and Lewis, 1998)

Inoculation and Incubation of the Parasite in the Media

The diluted erythrocytes (0.2ml) were removed using sterile disposable syringes (HSW luer plastic) without the needle. This was suspended in the Alsever's solution (0.2ml) in a test vial and then the solution (200, 100 and 50 µg/ml) of the test plant extract (0.1ml) was added and then few drops of rabbit serum to enrich the medium. The mixture was maintained at 37°C in a water bath for few hours then it was placed in a bell jar with a burning candle. The whole set up was transferred into an incubator set at 37°C immediately the flame of the candle went off in other to create an anaerobic environment. The control experiment was set up in a similar way except that no test plant extract solution was added.

Estimation of the Anti-Plasmodia Activity of the Plant Extraction

After thorough mixing, thin smear of the content of each vial was made on a clean glass slide, stained and air-dried. This was then observed under microscope to detect the relative killing or elimination of the parasites by taking the difference between the parasitaemia value before and after incubation.

RESULTS AND DISCUSSION

Several authors have pursued the search for natural products with antimalarial effect in plants in the past and in recent years (Rafatroet *et al.* 2005). The rationale for such studies is based on the resistance of the parasites to conventional treatment as observed in the case of malaria (Fidock *et al.*, 2008). This resistance began with *P. falciparum* near the '60s and today is extremely common, especially in Africa, Central and South America (Bloland 2001). This resistance has also been documented for *P. vivax* (Anstey *et al.* 2009) the most common species found outside Africa (Wells *et al.* 2010) and in Costa Rica, were reports account for 95-98% of the cases (Vargas, 2001). Malaria parasites also exhibit resistance to Fansidar, a treatment involving Sulfadoxine and Pyrimethamine,

that act inhibiting the formation of nucleic acids (Bloland 2001).

Based on the foregoing, we started the search for antimalarial components in the plants *Dendrothema indicum* used in the treatment of fever in northern Nigeria. To the best of our knowledge no work has been done on the antimalarial activity of this plant before now, however some works have been found on other species belonging to the same family (composite). In Africa and Asia there has been an intense and enthusiastic search for active compounds against *P. falciparum* especially (Mariath *et al.* 2009, Titanjiet *et al.* 2008) Antimalarial components have been found in *S. andina*, *S. pauciflora*, *S. tonduziana* and *S. aspera*.

In this study, the in vitro antimalarial activity of ethanol, hexane, chloroform and ethylacetate extracts of *Dendrothema indicum* a plant used in traditional medicine in Nigeria is reported.. The ethyl acetate fraction after fractionation showed the highest activity of 68% parasite elimination at the end of the incubation as compared to chloroform extract which had antimalarial activity of 61%. The in vivo antimalarial activity of the plant *Vernonia amygdalin* was reported by Abosi and Raseroka (2003), In their findings, leaf extract produced 67% suppression of parasitaemia in a four day test. The result of this work is in agreement with their result, though experimental methods differ. This work also agrees with Misael Chinchilla *et al.* (2012) who reported the *In vitro* antimalarial activity of extracts of some plants from a biological reserve in Costa Rica, In their work, they assessed the antimalarial activity of several plant extracts: using different parts of the plants as well as fresh and dried extracts. The fresh extracts showed stronger activity than the dry ones.

This study validates the traditional use of *Dendrothema indicum* in the treatment of malaria in Nigeria.

Traditional uses of *Dendrothema indicum* extracts particularly in the treatment of fever, eye ailments, gonorrhoea, deep rooted boils, inflammation of the throat eyes and cervix, eczema itchininess of the skin are common (Carvalho, 2005). These can be justified by the presence of various secondary metabolites revealed by the phytochemical analysis

The results of the phytochemical analysis (Table 1) prominently indicate the presence of carbohydrate, glycosides, saponins, tannins, flavanoids, resins and sterols.

The search of new compounds from plants is of course an important area of research for exploring new potential drugs for malaria. However, reliable data on the clinical pharmacology, efficacy and safety of such formulae are extremely scarce, preventing a responsible consideration of their potential benefits (Rath *et al.*, 2004) Further, lack of awareness among people may worsen the situation particularly when the toxicity aspect of herbal drugs is overlooked.

Table 1: Phytochemical screening the crude ethanol extract of *Dendranthema indicum*

Component test type	Remark
Carbohydrate	+
Carbohydrate	+
Alkaloids	-
Tannins	+
Glycosides	+
Saponins	+
Sterols	+
Flavaniods	+
Resin	+

Key: +=Present, -=Absent

Table 2: Anti-malaria test results

S/N	Fraction	Conc. µg/ml	Average no of parasite per field before incubation	Average no of parasite after incubation			Overall average no of parasites after 72 hrs of incubation	Percentage elimination at the end of incubation
				Day 1 st	Day 2 nd	Day 3 rd		
1.	Ethanol	200	21	7	6	5	6	76
2.	Ethanol	100	21	8	8	7	7.66	63
3.	Ethanol	50	21	14	13	12	13	38
4.	Control		21	21	21	21	21	
5.	Hexane	200	21	14	13	12	12	38
6.	Hexane	100	21	16	16	15	15.66	25
7.	Hexane	50	21	19	18	17	18	14
8.	Control		21	21	21	21	21	
9.	Chloroform	200	24	11	9	8	9.33	61
10.	Chloroform	100	24	12	11	11	11.33	52
11.	Chloroform	50	24	20	18	18	18.66	22
12.	Control		24	24	24	24	24	
13.	Ethylacetate	200	21	7	7	6	6.66	68
14.	Ethylacetate	100	21	8	7	7	7.33	65
15.	Ethylacetate	50	21	15	15	14	14.66	30
16.	Control		21	21	21	21	21	
17.	Residue	200	24	20	16	15	17	29
18.	Residue	100	24	18	15	14	15.66	34
19.	Residue	50	24	22	21	20	21	12
20.	Control		24	24	24	24	24	

CONCLUSION AND RECOMMENDATION

In this study, the *in vitro* antimalarial activity of ethanol, hexane, chloroform and ethylacetate extracts of *Dendranthema indicum*, a plant used in traditional medicine in Nigeria was reported.. Still there is a need for continued efforts to discover new antimalaria template molecules from herbal sources.

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