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In Vitro Determination of Anti-Plasmodial Activity of *Artemisia Annua* Leaf Extract on *Plasmodium* Parasite

***Asma'u Mahe[1], Mustapha Iliyasu Utai[2], Umar Abdullahi Zakariyya [1], Bashir Ado Ahmad [1], Adam Abdullahi Adam[1]**
[1]Biochemistry Department,
Federal University Dutse

[2]Microbiology and Biotechnology Department,
Federal University Dutse
Email: maheasmau@gmail.com

Abstract
Malaria is a life-threatening disease caused by Plasmodium parasites. Plasmodium falciparum is the most prevalent malaria parasite on the African continent responsible for most malaria-related deaths globally. This research aimed at the determination of in vitro activity of Artemisia annua leaves extract against Plasmodium falciparum. The leaf of the plant was extracted using hexane, phytochemical screening was carried out and secondary metabolites such as Flavonoid, Saponin, Terpenoid, alkaloids, cardiac glycoside, phenols were found to be positive and its activity against plasmodium parasite was found to be positive with percentage elimination obtained as 81.84% and 70.59% for the control and the extract respectively. These observations showed that Artemisia annua plant may contain compounds with potential anti plasmodial properties which can assist clinically in the management of malaria.

Keywords: *Atemisia annua*, anti-plasmodia, phytochemicals, secondary metabolites, plasmodium parasite

INTRODUCTION

Malaria is currently the world's number one infectious disease caused by *Plasmodium* parasites. Female Anopheles mosquitoes are the vectors that transmit the malarial parasites to humans. Two main species poses the greatest threat, these are *Plasmodium falciparum* and *Plasmodium vivax*. *Plasmodium falciparum* is the most predominant malaria parasite on the African continent and is responsible for the majority of deaths associated with malaria. Worldwide, nearly 3.2 billion people are at risk of malaria (WHO 2013; 2015). In 2015, 95 countries and territories had ongoing malaria transmission and still in 2015 alone, there were an estimated 214 million new cases of malaria and 438,000 deaths (WHO, 2015). Sub-Saharan Africa carries a disproportionately high share of the global malaria burden. In 2015, the region was home to 88% of malaria cases and 90% of malaria deaths, globally. Nigeria accounted for up to 25 percent of the global cases and deaths (WHO 2013, 2015). The transmission frequency depends on factors specific to the transmission parasite, the vector, the human host, and the environment (FMoH, 2015). The plant called Sweet Wormwood, Sweet Annie, Sweet

**Author for Correspondence*

Sagewort and Annual Wormwood is scientifically known as *Artemisia annua*. The *Artemisia annua* genus belongs to the *Asteraceae* family. It is a common type of wormwood native to temperate Asia, but it can be found worldwide. The plant has leaves resembling ferns leaves, with bright yellow flowers, and have a fragrance like camphor. The seed is extremely small and the plant is normally grown and transplanted at a seedling stage. On the surfaces of the entire plant (leaves, stems and flowers), can be found glandular structures (trichomes) that contain a wide range of bioactive compounds (mostly terpenoids) (Elfawal, 2012). The phytochemical composition of *Artemisia annua* has been reviewed in great detail by Bhakuni *et al* 2001. The most *A. annua* relevant compounds are sesquiterpenoids (ex. artemisinin), triterpenoids, flavonoids (polymethoxylated flavonoids), chromenes, saponins, cardiac glycosides, alkaloids etc and essential oil components (Ferreira *et al*., 2005). *Artemisia annua*, (sweet wormwood), is the main source of *artemisinin* and its derivatives which are powerful medicines known for their ability to swiftly reduce the number of *Plasmodium* parasites (especially *Plasmodium falciparum*) in the blood of person infected with malaria (WHO, 2015). It's globally known that Malaria infections are treated through the use of antimalarial drugs such as chloroquine, and pyrimethamine but drug resistance is drastically increasing. Hence, the research is aimed at *in vitro* determination of anti-plasmodial activity of *artemisia annua* leaf extract on *plasmodium* parasite which might be used as an alternative way to overcome the challenge.

MATERIALS AND METHOD

Plant Sample collection

Fresh sample of the plant *Artemisia annua,* was obtained from the Bioresouces Development Centre (BIODEC) Kano, and was classified and identified by the Ethnobotany Unit of the centre. The identification number of the plant is BDCKN/EB/1896. It was ensured that the sample was taken at a suitable temperature and other environmental conditions. It was then allowed to shade dry in the laboratory and then grinded to powder using mortar and pestle. The powdered form of the sample was then measured using Digital Weighing balance. The mass of the powder was noted and recorded.

Extraction of plant leaves

About 80g of the powder was measured and placed into a conical flask. The powder was macerated using methanol as a solvent and stirred gently and continuously with a stirrer, the extract was evaporated leaving a colorless methanol layer. Hexane was gradually added until the extract is partitioned into polar and non-polar fractions. To the methanol extract (polar fraction), 10ml of distilled water was added. Also 50ml ethyl acetate was gradually added to the mixture until the solution partitioned again. This was done to produce a semi-polar extracts and to elute the anti plasmodial compound. The Overall mixture was shaken thoroughly and the most viscous extract was collected. (It is the one containing the anti-plasmodial compound). The extract was then stored under suitable conditions of temperature and pressure. The target population of this research was randomly selected among patients who reported symptoms of malaria fever at Bayero University Kano Clinic. Blood samples of 8 patients were collected and then screened to detect presence of malaria parasite (i.e *Plasmodium spp*).

Erythrocyte separation

Blood sample with 5% parasitaemia was centrifuged at 2500 rpm for 15 minutes. After centrifugation, the supernatant (plasma) was discarded while the sediments (containing the

erythrocytes) were further centrifuged with normal saline at 2500 rpm for 5 minutes. Then the supernatant was discarded and the erythrocytes were suspended in normal saline.

Test Concentrations Preparation

20 mg of the extract was dissolved in 1ml of DMSO in separate EDTA bottles (stock solution). Using serial doubling dilution, four different concentrations (10mg/ml, 5mg/ml, 2.5mg/ml, and 1.25mg/ml) of the extract were prepared.

Culture Media (RPMI 1640) and Antiplasmodial Assay

The media was prepared by dissolving 10.4g of the powdered material into 1 liter of distilled water and then autoclaved at 121^{o}C for 15 minutes as instructed by the manufacturers.

Equal volume of the extract solution (0.5ml) and the culture media were transferred into fresh flat bottomed test tubes and labeled accordingly (10mg/ml, 5mg/ml, 2.5mg/ml, and 1.25mg/ml). For each concentration of the extract, 0.1ml of the malaria positive erythrocytes was added and shaken gently to ensure even distribution of the erythrocytes. The test tubes were transferred into a bell jar containing burning candle. The cover of the bell jar was then replaced until the flame of the candle stopped burning. This supplied about 95% nitrogen, 2% oxygen, and 3% carbon dioxide as described by (Trager *et al.*, 1976). The whole set up was transferred into an incubator maintained at 37^{o}C for 24 to 48 hours.

A control group containing of culture media plus positive erythrocytes (negative control) and culture media plus positive erythrocytes and a known antimalarial agent Artemether (positive control) were incubated along with the test concentrations. After 24hrs of incubation, a thin smear from bottle was made on clean glass slides and fixed in absolute methanol, then stained with Giemsa's stain. Each smear was observed under microscope using oil immersion to count the number of infected erythrocytes. After 48 hours of incubation, a second extract was taken through the same procedures as done at 24hrs of incubation.

RESULTS

Table 1 shows the physical properties of the extract obtained from *Artemsia annua* leaves which was extracted using organic solvent. The extract weighs 21.70g, appeared brownish green in color, with a gummy texture and a yield of 27.13%.

Table 1: Physical Properties of Leaf Extract from *Artemisia annua*

Property	Amount
Weight of plant powdered sample (g)	80.00
Weight of extract (g)	21.70
Percentage yield (%)	27.13
Color of extract	Brownish green
Texture of extract	Gummy

Table 2 shows the results of phytochemical analysis giving the distribution of the secondary metabolites in the extract. Flavanoids, Alkaloids, Steroids, Tannins, etc were present but Anthroquinones was negative.

Table 2: Phytochemical Constituents of *Artemisia annua*

S/N	PHYTOCHEMICAL	INFERENCE
1	Fehlings test	+
2	Tannins	+
3	Steroids	+
4	Anthroquinones	_
5	Terpenoids	+
6	Saponins	+
7	Flavonoids	+
8	Alkanoids	+
9	Phenols	+
10	Cardiac glycoside	+

Key:- (+) =present, (-) =absent

Table 3 shows the antimalarial sensitivity of *Artemisia annua* leave extract against the infected erythrocytes at different concentrations. The percentage elimination obtained was 81.84% and 70.59% for the control and the extract respectively, as shown in the table.

Table 3: Antimalarial Sensitivity of *Artemisia annua* Extract Against the Infected Erythrocytes (RBC) at Different Concentrations (mg/ml) and Standard Control Used.

Sample	Average Parasitaemia initial count	Concentrations of extract used (mg/ml)	Average Final parasitaemia count after incubations	Percentage elimination at the end of incubation
Extract	13.6	10, 5, 2.5, 1.25	9.6	$\frac{9.6 \times 100}{13.6}$ = 70.59%
Control	13.6	10, 5, 2.5, 1.25	11.13	$\frac{11.13 \times 100}{13.6}$ = 81.84%

DISCUSSION

The phytochemical screening of the leaf extract *Artemisia annua* (Table 1) showed the presence of different secondary metabolites in the extract. Phytochemicals are compounds that occur naturally in plants, they have biological significance but are not established as essential nutrients. The leaf extract indicated the presence of almost all the tested Secondary metabolites with the exception of anthroquinones. This could be attributed to the polarity index of the solvents. Solvents are classified into polar and non-polar. Generally, the dielectric constant of a solvent provides a rough measure of its polarity. Polarity of a solvent is attributed to its attraction power to different compounds in a solution (Imam *et al.,* 2016). The substances in a solution interact with each other at a molecular level. Polar solvents, like water attract polar compounds like salts and sugars while non-polar solvents, attract non-polar compounds. Methanol with polarity index of 5.1 is known to extract both polar and non-polar compounds. Basically, the choice of solvent relies on the polarity of the compounds of interest present in the plant material. Phytochemical compounds such as alkaloids are commonly implicated in the anti-plasmodial activity of many plants (Okokon *et al.,* 2006). Some alkaloids targets the plastid-like organelles of the parasite called apicoplast, while some inhibit parasitic protein synthesis. Terpenes arrest parasite development and inhibit biosynthesis of isoprenoids in *Plasmodium falciparum* (Al-Adhroey *et al.,* 2010).

Cardiac glycosides can inhibit the membrane sodium-potassium (Na^+-K^+) pump, raising intracellular Na^+. Owing to this property, cardiac glycosides prevent the transport across the malarial parasite cell membrane and hence, the parasite will become inactive (Al-Adhroey *et al*., 2010).

Flavonoids revealed significant anti-parasitic activities against different parasite strains of malaria, trypanosome and leishmania (Al-Adhroey *et al*., 2010). The exact mechanism of anti-malarial action of flavonoids is unclear but some are shown to inhibit influx of L-glutamine and myoinositol in infected erythrocytes. The bioactive compounds which were found in this extract may be acting singly or in synergy with one another to exert the anti-plasmodial activity of the leaves of *Artemisia annua*. The method of maceration was used in the extraction of the fresh leaves of *Artemisia annua*. The present study provides evidence that the extract of *Artemisia annua* leaves exhibited the anti-plasmodial activity. However, it possesses a moderate anti-malarial activity when compared with the standard antimalarial drug artemether as they showed a percentage elimination of 70.59% and 81.84% respectively. The percentage eliminations obtained in this study is in conformity with findings of (Imam *et al*., 2016). The result therefore justifies the traditional use of the whole plant in the treatment of malaria. Nevertheless, further work is necessary to ascertain the *in vivo* toxicity of the plant, identify its active principles and optimum dosage in order to provide effective and low cost intervention drug malaria infection.

CONCLUSION

In conclusion, the leaves extract of *Artemisia annua* was obtained with a yield of 21.7%.The qualitative phytochemicals in the extract were determined and the extract relatively showed antiplasmodial activity *in vitro*.

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