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IN VITRO ANTIMALARIAL ACTIVITY OF LEAVE PETROLEUM ETHER EXTRACT OF FICUS SYCOMORUS PLANT

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ABSTRACT

In vitro anti-malarial activity of petroleum ether extracts of Ficus sycomorus leaves was investigated using Candle Jar method. The extractions were carried out through activity guided fractionation. Petroleum ether extract has the highest activity, at the concentration of 1mg and 0.5mg with percentage growth inhibition of (33.12 and 31.9%) respectively. These results suggest that the leaves of Ficus sycomorus plant have significant antimalarial activity and that the antiplasmodial agents might be as a result of the extract fractionated using the petroleum.

1. INTRODUCTION

Malaria is a mosquito-borne infections disease caused by a eukaryotic protest of the genus plasmodium. It is wide spread in tropical and subtropical regions, including parts of the American, Asia and Africa. Each year there are approximately 350-500 million cases of malaria, killing between one and three million people, the majority of whom are young children in sub-Saharan Africa (Snow *et al.*, 2005). Ninety percent of malaria related deaths occur in sub-Saharan Africa. Malaria is commonly associated with poverty, but it also a cause of poverty (Malaria: Disease impacts and long-run difference, 2008) and a major hindrance to economic development.

Five species of the plasmodium parasites can infect humans; the most serious forms of the disease are caused by *Plasmodium falciparum*. Malaria cause by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* causes milder disease in humans that is not generally fatal. A fifth species, *Plasmodium knowlesi*, is zoonoses that causes malaria in macaque monkeys but can also infect humans (Fong *et al*., 1971) and (Singh *et al*., 2004).

Malaria is naturally transmitted by the bites of a female Anopheles mosquito. When a mosquito bites an infected person, a small amount of blood is taken, which contains malaria parasites. These develop within the mosquito and about one week later, when the mosquito takes the next blood meal, the parasites are injected with the mosquito's saliva in to the person being bitten. After a period of between 2 weeks and several months (occasionally years) spent in the liver, the malaria parasites start to multiply within red blood cells, causing symptoms that include fever and headache. In severe cases, the disease worsens, leading to coma and death (Snow *et al*., 2005).

Malaria transmission can be reduced by preventing mosquito bites with mosquito nets and insect repellants or by mosquito control measures such as spraying insecticides inside houses and draining standing water where mosquitoes lay their eggs. Although many countries are under developed, the challenge of producing a widely available vaccine that provides a high level of protection for a sustained period is still to be met.

2. MATERIALS AND METHOD

2.1. MATERIALS

Table 2.1: List of reagents

Name	Grade	Manufacturer
Roswell part memorial institute (RPMI 1640)	Sigma reagent	Himedia Laboratories PVT Ltd. Latd.
Petroleum ether	Analar	BDH Chemical Ltd. Poole England
Chloroform	Analar	BDH Chemical Ltd. Poole England
Hexane	Analar	BDH Chemical Ltd. Poole England
Giemsa stain	Laboratory reagent	BDH Chemical Ltd. Poole England
Sodum Dihydrogen Orthophosphate (N-a$H_2PO_4 2H_2O$)	Laboratory reagent	BDH Chemical Ltd. Poole England
Sodium bicarbonate ($NaHCO_3$)	Laboratory reagent	BDH Chemical Ltd. Poole England
Distilled water		Chemistry Department UDUS
Gentamycin	Injection	Ranbaxy, India
Artessunate	Tablet	Pfizer

2.2. BLOOD SAMPLE COLLECTION

The infected blood sample used for the research was obtained at city campus, Pathology Department Usmanu Danfodio University Teaching Hospital, Sokoto from patient suffering from malaria. Then the sample was passed through malaria parasite (MP) test, which was found to be positive (+ve).

The uninfected blood sample was obtained from one of my research colleague. The blood sample were collected in Ethylene Diamine Tetra acetic acid (EDTA) bottles and kept in refrigerator at 4^0C for further analysis.

2.3. PREPARATION OF INFECTED AND UNINFECTED RED BLOOD CELLS

The infected blood sample was spinned for 5 minutes at 2500g to separate the red blood cells from white blood cells and serum components. The uninfected blood sample was spinned for 5 minutes at 2500g and supernatant was decanted into another tube. The serum was inactivated by heating.

2.4. PLANT MATERIALS

The plant used for the research was obtained from Kebbi State and the plant was taken to Herbarium section of Biological Science Department of Usmanu Danfodiyo Univesity Sokoto for identification. A voucher number UDUH/ANS/0103 was obtained. The voucher number was deposited at the herbarium unit. The leaves of *Ficus sycomorus* plant was used for the research.

2.5. PREPARATION OF THE PLANT MATERIAL

The plant material was air dried for one week and then pulverized in to powdered form. The powder was then sieved to get fine powder.

60g of powdered leaves of *Ficus sycomorus* were measured and soaked in 900mls of distilled water for 24hours at room temperature respectively. The soaked plant material were filtered and the residues were discarded. The filtrates were then passed through activity guided fractionation.

2.6. ACTIVITY GUIDED FRACTIONATION

In this process, an equal proportion of the aqueous extract (filtrate) and organic solvent were used.

Aqueous extract of plant (100mls)

⇓

Aqueous extract of
Plant + 100mls of petroleum ether

⇓

Petroleum ether extract (evaporate 40-50^{0}C)

On addition of organic solvent to aqueous extract of plant, there was thorough shaking and the separating funnel was left for about 5 minutes for the immiscible layers to be separated. First, 100mls of aqueous extract was placed inside the separating funnel followed by the addition of 100mls of organic solvent (petroleum ether) according to their polarity. These immiscible layers were separated gently in to the weighted dried plates for drying in the drying cabinet for two days. The extracts obtained after drying was weighted on the weighting balance and kept inside the refrigerator for further use. The percentage yield was calculated using this formula;

$$\% \text{ yield} = \frac{\text{Weight of extract (g)}}{\text{Weight of plant material used (g)}} \times 100$$

2.7. METHOD

The method used is the cabled jar method of in vitro test for anti-malarial activity (Trager and Jensen 1976).

Principle

The principle of the "candle jar" method is to maintain the infected erythrocytes in a relatively simple culture medium, with the addition of human serum in an atmosphere of relatively high CO_2 and low O_2 contents. Fresh red blood cells can be added for the continuation of the growth, division and multiplication of plasmodia (Trager and Jensen 1976).

2.8. PREPARATION OF THE MALARIA PARASITE CULTURE MEDIUM

Firstly, all the apparatus were disinfected in an autoclave. The medium was prepared by dissolving 9.8g of RPMI 1640 powder in 900mls of redistilled water and stirred until it dissolved. Seven points eight one gram (7.81g) of sodium dihydrogen orthophosphate was added as buffer and 2.0g of sodium bicarbonate was added. The solution was stirred together, filtered through sterilized Whattman number 1 filter paper and stored at 4^0C.

The blood medium mixture (BMM) was prepared by mixing 0.9ml of RPMI with 100µ1 of malaria parasite infected blood sample. 0.01mg/mol of gentamicin was added in to the RPMI 1640 to inhibit the bacteria contamination of the parasite culture.

One and zero point five milligram (1mg and 0.5mg) concentration of petroleum ether extract was put into different culture plates. As the negative control plate, the parasites were cultured without any extract or drug (100% growth) and the positive control plate contained 20µ1 of artesunate (100% inhibit). The mixture was mixed in order to ensure maximum mixing; the mixing was carried out near flame to minimize bacterial contamination.

The culture plates were covered with a lid and incubated at 37^0C for 48hours.

2.9. PREPARATION OF FILM

The blood films were prepared on the microscope slides. The films were air dried and the films were fixed with methanol which was poured off after 30seconds. The freshly prepared Giemsa stain was used to stain slide films for 20-30 minutes. The slide films were flushed with tap water and stand to dry.

2.10. MICROSCOPIC EXAMINATION OF BLOOD FILMS AND PARASITES COUNT

The dried slide films were examined under 100x magnification (oil immersion) for the presence of schizonts. An area of the film where the total number of erythrocytes is approximately 120 per field was viewed. The number of parasitized erythrocytes in 10 fields were counted and recorded.

2.11. DETERMINATION OF PERCENTAGE GROWTH INHIBITION

The percentage growth inhibition was calculated using the formula;

$$\text{Percentage growth inhibition} = \frac{\text{Control parasitemia} - \text{Extract parasitemia}}{\text{Control parasitemia}} \times 100$$

Parasitemia is the number of schizonts obtained after counting.

3. RESULTS

Table 3.1: Shows the yield and percentage yield of plant material after extraction.

Plant material used	Solvent	Yield (g)	% yield (g)
	Petroleum ether	0.36	0.60

The results of extraction of leaves of *Ficus Sycomorus* obtained through activity guided fractionation are shown above (Table 3.1) with yield and percentage yield of plant material of petroleum ether.

Table 3.2: Number of infected cells for each 10 fields counted on the light microscope of different extracts concentration and percentage growth inhibition.

Plant material	Extract	No. of infected cells	% Growth inhibition		
Concentration		1mg/ml	0.5mg/ml	1mg/ml	0.5mg/ml
	Petroleum ether	745	758	33.12	31.9
Control				43.0	40.0

The result of in vitro anti-malarial activity of leaves of *Ficus sycomorus* is shown in (Table 3.2).

From The results above, the lower the number of infected cells the higher the inhibition and the higher the number of infected cells, the lower the inhibition.

The results obtained shows that, petroleum ether, which has 33.12% at concentration of 1mg/ml and 31.9% at concentration of 0.5mg/ml, however, the extract shows anti-malarial activity.

4. DISCUSSIONS

In sub-saharan African were malaria is endemic and in other parts of the world, plants are extensively used for treating periodic malaria disease. The spread of multi drug-resistance *P. falciparum* has highlighted the urgent need to develop new antimalarial drugs preferably inexpensive drugs that are affordable for developing countries, where malaria is prevalent.

In this study four organic extract were obtained from a plant (*Ficus sycomorus leaves*) that are used for the treatment of fever and malaria were tested *In vitro* against *P. falciparum* using Candle Jar Method.

Table 3.1 shows the percentage yield of petroleum ether extract as against the gram of plant sample used which is 60g. Table 3.2 shows the percentage growth inhibition at different concentration of the extract. Petroleum ether extract exhibit highest growth inhibition of 33.12 and 31.9 at concentration of 1mg/ml and 0.5mg/ml respectively. This indicates that, the anti-plasmodic agent in petroleum ether fraction may be more than the other fractions of hexane, chloroform and water extract.

5. CONCLUSION

The current work confirms the traditional used of *Ficus sycomorus* as an effective antimalarial preparation. The research also indicated that, the active antiplasmodic agent of *F. sycomorus* may be fractionated in the petroleum ether fraction. Also, petroleum ether had shown to have the highest percentage inhibition when compared to the other solvents.

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CONFLICT OF INTEREST

The author have declared that no competing interests exist.

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