PHYTOCHEMICAL ANALYSIS AND *In-vitro* SCREENING OF *Citrus aurantifolia* LEAF EXTRACTS FOR SCHIZONTICIDAL ACTIVITY ON CLINICAL ISOLATES OF *Plasmodium falciparum*

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ABSTRACT
Malaria control efforts are thwarted by the development of resistance to the available drugs by P. falciparum. The search for alternative drugs should be a never ending exercise. This research was aimed at phytochemical screening and testing the inhibitory effect of Citrus aurantifolia (Leaf extracts) on the schizont maturation of Plasmodium falciparum. The inhibitory effect was studied using 96 well micro titer plates with each well loaded with 100µl of warm sterile Rosswell Park Memorial Institute 1640 medium, 50µl of blood medium mixture, 20µl red blood cells and 20µl serum. Twenty microliters (20µl) plant extracts of different solvents (water, ethanol, hexane, petroleum ether and chloroform) were added into 13 wells for each extract. In another 13 wells, 20µl of chloroquine solution was added. In control wells, 20µl of individual solvents (water, ethanol, hexane, petroleum ether and chloroform) were added. The micro titer plate was kept in the desiccator and a candle was lit. When the candle went off, the desiccator was placed in an incubator at 37.5^{0}C for 24 hours. After which the contents were harvested and form thick film smear on pre- labeled slides. In the phytochemical screening, the result revealed the presence of Tannins, Saponin, Alkaloids and Glycosides in the plant extracts of hexane, petroleum ether, chloroform, ethanol and water. Anthraquinone glycosides, cyanogenic glycosides and Saponin glycosides were only present in ethanol and water extracts. None of the flavonoids and cardiac glycoside was detected in any of the solvents extracts. The percentage inhibition of schizont maturation using different solvents of the leaf extract at various concentrations showed inhibition ranges as follows: 10.1%-51.4% for aqueous, 10.9%-56.0%for hexane, 8.1%-72.7% for chloroform, 7.6%-75.3% for petroleum ether and 8.1%-88.4 for ethanol and 6.4%-41.0% for chloroquine.

Keywords: Plasmodium falciparum, Citrus aurantifolia, Inhibition and Phytochemicals.

1. INTRODUCTION

Plasmodium falciparum is the protozoan parasite that causes a long term, severe and persistent malaria in human. (Chris 1999; Stuart and Artur, 2005; Ron and kirk 2009). Annually about 500million people are infected and the disease claims the life of about 1 to 2 million people worldwide (Kellen 2009). Plasmodiumfalciparum possesses some sophisticated mechanism which qualifies it to be a successful parasite in a hostile environment (Sodeinde, 2004). *Plasmodium falciparum* has the ability to escape clearance by the human immune system as a result of continuously altering the surface exposed antigenic proteins that are vulnerable to antibody recognition (Ron and Kirk, 2009). The use of plants and other plants derived products for therapeutic purpose in contemporary medicine is the most ancient method for curing disease. Plants precursors for the synthesis of drugs (Gills 1992; Arote *et al* 2009; Egwaikhide*et al* 2009; Benmehdi*et al* 2012).

Nearly, all cultures and civilizations from ancient times to the present day have depended fully or partially on herbal medicines because of their effectiveness, affordability, availability, low toxicity and acceptability (Benmehdi*et al* 2012). Plant extracts have been used in preventing or eliminating different types of diseases ranging from physical, mental and those of social origin. Nigerian flora contains plant species used for curing various ailments due to their antibacterial, fungicidal and insecticidal properties, among others. Without the knowledge of the active ingredients responsible for the relief desire to know the active ingredients facilitate researchers to work on Phytochemical and pharmacological studies of these plants. *Citrus aurantifolia* has relieving effect on fever, jaundice, and headache (Gills, 1992). In this research, screening of the Phytochemical and testing the inhibitory effect of the *Citrus aurantifolia* leaf extract on schizont maturation of plasmodium falciparum was conducted.

2. METHODOLOGY

2.1 Sample Collection

The plant (*Citrus aurantifolia*) was collected at Farfaru area in Sokoto and was identified at the Taxonomy unit, Botany Department, Usmanu Danfodiyo University, Sokoto. The leaves were air dried under shade and ground into powder. Malaria parasite infected human blood (Group O+) was obtained from blood bank at Usmanu Danfodiyo University Teaching Hospital, Sokoto. The blood was centrifuged at 3000rpm for 5 minutes. The plasma was collected and inactivated at 56^0C for 60 minutes. The cells were re- suspended in RPMI 1640 medium and aspirated.

2.2 Phytochemical Screening

Two grammes of the powdered plant were dissolved in 20mls each of distilled water, hexane, chloroform, petroleum ether and ethanol. Alkaloids, tannins, saponins, glycosides, flavonoids, flavonoid glycosides, cyanogenic glycoside, cardiac glycoside and anthraquinone glycosides were screened according to the method of El-Olemyl *et al*(1994).

2.3 Slides Preparation and Microscopic Examination

About 50µl of the malaria infected blood sample were used to make thick and thin film smear on pre-labeled slides in accordance with the method described by Maurice (1973). The slides were examined under x100 oil immersion objective. Thin smears were used for identification of malaria parasite species. Slides with mixed infections or parasite other than

P. falciparum were discarded. Thick smears were used for parasite count. The number of asexual forms (mostly young trophozoites which appeared as incomplete rings or spot of blue cytoplasm with a detached red chromatin dot) was counted against 100 leucocytes and then multiplied by 8000 (standard leucocyte count per mm^3 of blood) for slides with low parasitemia. While for slides with high density parasite only 10 fields were examined. Slides with mixed infections or infections other than *P.falciparum*were excluded.

2.4 In-Vitro Schizonticidal Effect of the Plant Extracts

Using a 96 well micro titer plate, each well was loaded with 100µl of warm sterile RPMI- 1640 medium, 50µl of blood medium mixture, 20µl red blood cells and 20µl serum. Twenty microliters of the plant extracts of different solvents (water, ethanol, hexane, petroleum ether and chloroform) were added into 13 wells for each extract. In another 13 wells 20µl of chloroquine solution was added. In the control wells, 20µl of individual solvents (water, ethanol, hexane, petroleum ether and chloroform) were added. The micro titer plate was kept in the desiccator in which a candle has been lit. When the candle went off, the desiccator was placed in an incubator at 37.5^0C for 24 hours. After incubation, the contents of the wells was harvested by removing the supernatant with an eppendorf pipette and the red blood cells deposited on the flat bottom wells. The red blood cells were transferred to clean slides where thick films were made. Parasite count for each blood film was made using an oil immersion. Each film was observed at three different visual fields, the number of schizonts was compared with control wells for the determination of percentage inhibition. The percentage inhibition was calculated as the number of parasite in the test well divided by the number of parasites in the control well multiplied by hundred.

3. RESULTS

Table 1 shows results of phytochemical screening which revealed the presence of Tannins, Saponin, Alkaloids and Glycosides in the C. aurantifolia leaf extracts of hexane, petroleum ether, chloroform, ethanol and water. Bioactive compounds such as antraquinone glycosides, cyanogenic glycosides and saponin glycosides were only present in ethanol and water extracts. While none of the flavonoids and cardiac glycoside was detected in any of the solvents extracts.

Table 1: Phytochemical Screening of *Citrus aurantifolia* Leaf Extracts

Plant	Class of compounds	Hexane	Petroleum ether	Chloroform	Water	Ethanol
Citrus aurantifolia	Tannin	+	+	+	+	+
	Saponin	+	+	+	+	+
	Alkaloids	+	+	+	+	+
	Glycosides	+	+	+	+	+
	Anthraquinone glycoside	–	–	–	+	+
	Cynogenic glycosides	–	–	–	+	+
	Saponin glycoside	–	–	–	+	+
	Flavonoids	–	–	–	–	–
	Cardiac glycoside	–	–	–	–	–

+, indicates presence and – indicates absence

Figure 1 shows the graphical presentation of the percentage inhibition of schizont maturation by different solvent extracts at various concentrations. The result shows inhibition ranges as follows: 10.1%-51.4% for aqueous, 10.9%-56.0%for hexane, 8.1%-72.7% for chloroform, 7.6%-75.3% for petroleum ether, 8.1%-88.4 for ethanol and 6.4%-41.0% for chloroqiun.

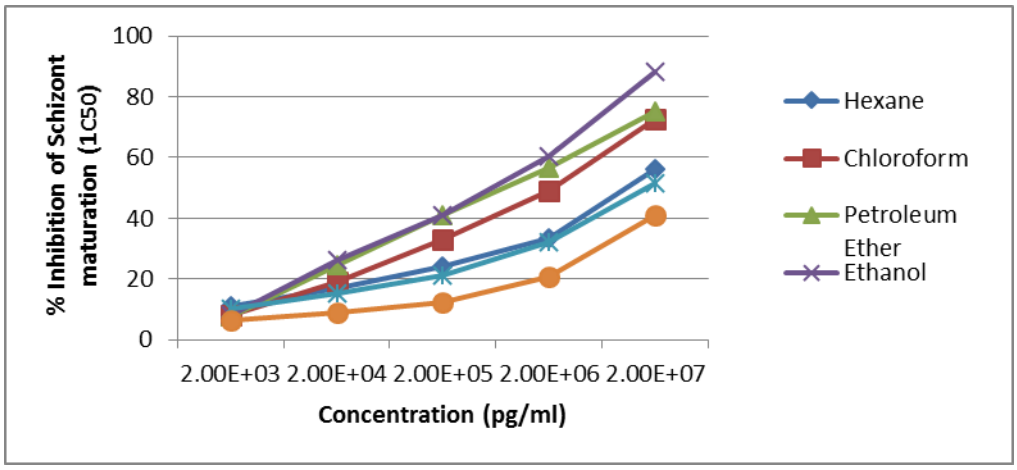

Figure 1: Inhibitory Effect of the Plant Extracts on schizont Maturation at Different Concentrations

4. DISCUSSION

One of the objectives of this research was to find out some of the bioactive components present in the leaves of *C. aurantifolia*. The outcome of the phytochemical screening indicates presence of about seven different secondary metabolites out of nine that were screened. These include Tannins, Saponin, Alkaloids and Glycosides in the plant extracts of hexane, petroleum ether, chloroform, ethanol and water. Anthraquinone glycosides, cyanogenic glycosides and saponin glycosides were only present in ethanol and water extracts. None of the flavonoids and cardiac glycoside was detected in any of the solvents extracts. The extracts of all the solvents used show significant inhibitory effect on the schizont maturation of *P. Falciparum* parasites when compared with the standard Chloroquine.This shows that in the plant there may be polar, semipolar and non-polar compounds with inhibitory effect on schizont maturation. The ethanol extract produced the highest inhibitory action on the schizont maturation at the highest concentration. Extracts of petroleum ether and chloroform nearly show equal inhibition at the highest concentration, although from the lower concentrations the extract of petroleum ether has nearly similar inhibitory action with that of ethanol extract. The outcome of the water and hexane extracts also show nearly equal inhibitory effect on the schizont maturation but that of hexane seems to show slightly higher inhibitory effect. The extracts may contain different bioactive component in combined state that are responsible for more pronounced inhibitory effect than chloroquine. It has been reported by Iwalewa *et al.*, (2008) that essential volatile oils from *Citrus aurantifolia* produce significant in-vitro inhibition on the growth of *Plasmodium falciparum*. This research shows that solvent leaf extracts of *C. aurantifolia* possesses antimalarial property and may be potential antimalarial agents.

5. REFERENCES

Arote, S.R., Dahikar, S.B., and Yeole, P.G., (2009). *Phytochemical screening and antibacterial properties of leaves of Pongamia Pinnata Linn.* From India. Afr.Joun. Of Biotech.Vol 8 (22) pp 6393-6396.

Benmehdi, H., Hasnaous, O., Benali, O., and Salhi, F., (2012). *Phytochemical investigation of leaves and fruits extract of Chamaecrops humilis L. J. mater.* Environ. Sci. 3(2) 237-320.

Chris, I.N., (1999). *Antigenic variation in plasmodium falciparum: mechanism and consequences.* Current opinions in microbiology.Vol 2, pp 420- 425.

Egwaikhide, P.A., Okeniyi, S.O., and Gimba, C.E., (2009). *Screening for antimicrobial activity and phytochemical constituents of some Nigerian medicinal plants.* Journal of medicinal plants research Vol. 3 (12), pp1088-1091.

El. Olemy, M, M; Almuhtadi, F, J and Afifi, A.A (1994), Experimental Phytochemistry; A laboratory manual, King Saudi University Press.

Gill, L.S., (1992). *Ethnomedical uses of plants in Nigeria.* Uniben press Benin City. Pp 1-174.

Kellen, L.O., Joanne, M.M., Daniel, W., James, M.B., Akhil, B.V., Joshua, D.R., and Manuel, L. (2009). *Host parasite interactions revealed by plasmodium falciparum metabolomics.* Cell Host & Microbes Vol 5 pp 191-199.

Ron, D., and Kirk, W.D., (2009). *Genetics of antigenic variation in Plasmodium falciparum.* Curr Genet vol 55 pp 103-110

Sodeinde, O., (2004). *Molecular aspect of Host-parasite interaction in malaria.* Annals of Ibadan post graduate medicine Vol 2 (2) pp12-15.

Stuart, A.R., and Artur, S., (2005). *The epigenetic control of antigenic variation in plasmodium falciparum.* Current opinion in microbiology. Vol 8. Pp 434-440.