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In-Vitro Cultivation of *Plasmodium Falciparum* in *Jatropha Curcas* Plant Medium to Determine Anti-Plasmodia Activities.

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Abstract
Most studies for in-vitro cultivation of *Plasmodium falciparum* were based on RPMI 1640 medium which was found expensive and cumbersome most especially for Africa settings where the disease is endemic with high poverty rate. This present study involved the usage of *Jatropha curcas* plant medium for cultivating malaria parasite in laboratory environment. The cultivation of the parasites were achieved by using liver extract and 10% human serum and the sensitivity test was carried out using ethanolic extracts of *Azadracta indica.* During the cultivation at 72 hours, medium with liver extract only has an increased parasitaemia of 2 to 5.9% and with addition of human serum increased from 3.1 to 7.4% which was discontinued at 72 hours due to complete digestion of red blood cells next day. Also, the experiment was continued with another set of *Jatropha curcas* plant medium and when 0.2% uninfected blood was added at interval for 120 hours, the growth yielded to 17.5%. Also, when the sensitivity test were carried out using ethanolic extracts of *Azadirachta indica* at 500µg/ml and 31.3µg/ml concentration, the growth rate increased to 334 (30.1%) and 449 (6.1%) for the parasitized cells respectively.
Keywords: In-vitro cultivation, *Azadracta indica , Plasmodium falciparium, Jatropha curcas*

INTRODUCTION

Malaria is the most important parasitic disease in humans, affecting 40% of the world's population with an estimated toll of 1.5–2.7 million deaths and 300–500 million clinical cases per year (Good, 2001). Plasmodium is the causative agent of malaria, one of the most prevalent and severe human infectious diseases, more than 90% in children under 5 years of age in Africa (Good, 2001). The methods for cultivation of the erythrocytic stages of *P.falciparum* reported by Trager and Jensen (1976) have been usefully applied in nearly all aspects of research on malaria: chemotherapy, drug resistance, immunology and vaccine development, pathogenesis, gametocytogenesis and mosquito transmission, genetics, the basis for resistance of certain red cells, cellular and molecular biology and biochemistry of the parasites and their relationship with their host erythrocytes (Ringwald *et al*.,1999). Therefore, life cycles of species that infect humans have been established invitro, of these four *Plasmodium* species, *P. falciparum* remains the only species for which all stages have been cultured invitro ; different degrees of success have been achieved with the other human *Plasmodium* spp. The commercially prepared culture RPMI 1640 medium to which serum and erythrocytes are added for cultivation of *Plasmodium* species were found to be less commonly used in this environment probably because of its cost implication and also storage problem as a result of instability in electricity for proper refrigeration .Studies have shown relative lower inhibition by some African indigenous plants such as *Azandrachta indica* and ***Jatropha curcas*** because the plants is usually boiled with a mixture of certain other plants for prophylaxis , traditional chemotherapy, antipyretic as well as schizonticidal agent malaria therapy (Igbinosa *et al*,2009) It is therefore necessary to seek for an alternative mode of cultivation to by- pass the aforementioned problem of RPMI as well as serving the same purpose. The current study therefore aimed at using indigenous *Jatropha curcas* plant medium to cultivate *Plasmodium falciparum* in-vitro as well as using indigenous *Azadirachta indica* to test for sensitivity.

MATERIALS AND METHODS

The preparation of *Jatropha curcas* plant medium involves; 0.2g/ml of glucose preparation, 0.13g/ml of saline preparation, 0.126g/ml of (Phosphate buffer) PBS whereby 1x PBS was prepared from the solution, 0.25g/ml in 1X PBS of liver extract was centrifuge for 20 mins then debris was removed and stored at 4^{o}C, 0.05mg/ml of hypoxanthine solution and 1ml of *Jatropha curcas* to 99ml of distilled water was prepared.

PREPPARATION OF COMBINED *JATROPHA CURCAS* PLANT MEDIUM;

10ml *Jatropha curcas*, 20ml water, 2ml glucose solution, 1ml saline solution, 1ml PBS 1X, 12ml liver extract, 5ml 10% human serum and 0.2ml uninfected human red blood cell and 15µl Gentamycin. The pH of the new media is about 6.6 but usually adjusted to 7.4-7.6 using $NAHCO_3$ solution (0.002g/ml). The complete new medium was stored at -21^{0}C.

The Preparation of *Azadirachta indica* extracts

1mg/ml extract of *Azadirachta indica* was prepared and the parasites were exposed to a range of different concentrations of the extract in culture tubes at 4% cultured parasitaemia to *Jatropha curcas* plant medium. Each

extract concentration has a replicate and serial dilutions of 1:2 (500µg/ml), 1:4 (250µg/ml), 1:8 (125µg/ml), 1:16 (62.5µg/ml) and 1:32 (31.25µg/ml). The numbers of parasitized cells were counted by fluorescent microscopy after 48 hours in culture incubated at 37^{o} C.

RESULTS

During the cultivation of *Plasmodium falciparum* 0 hour was used as the control sample in which the infected red blood cell was adjusted to 4% parasitaemia. Thick film was made and the number of parasite/ µl of blood was determined in relation to the standard number of white blood cell/µl. Standard number of white blood cell/µl: 8000 (WHO, 2010). All data were entered and analysed with SPSS for window I5.0. Using liver extract supplement, the growth rate of 0- 72 hours yielded 5.9% parasite density and with addition of 10% human serum at the same interval the parasite growth recorded was 7.4% (fig 1). Also, between interval of 0-120 hours when liver extract and human serum were added as showed in fig 2 the growth rate was 17.5%.

Considering the sensitivity test using ***Azadirachta indica*** at 500µg/ml and 31.3µg/ml concentration (table 1), the growth rate increased to 334 (30.1%) and 449 (6.1%) for the parasitized cells respectively.

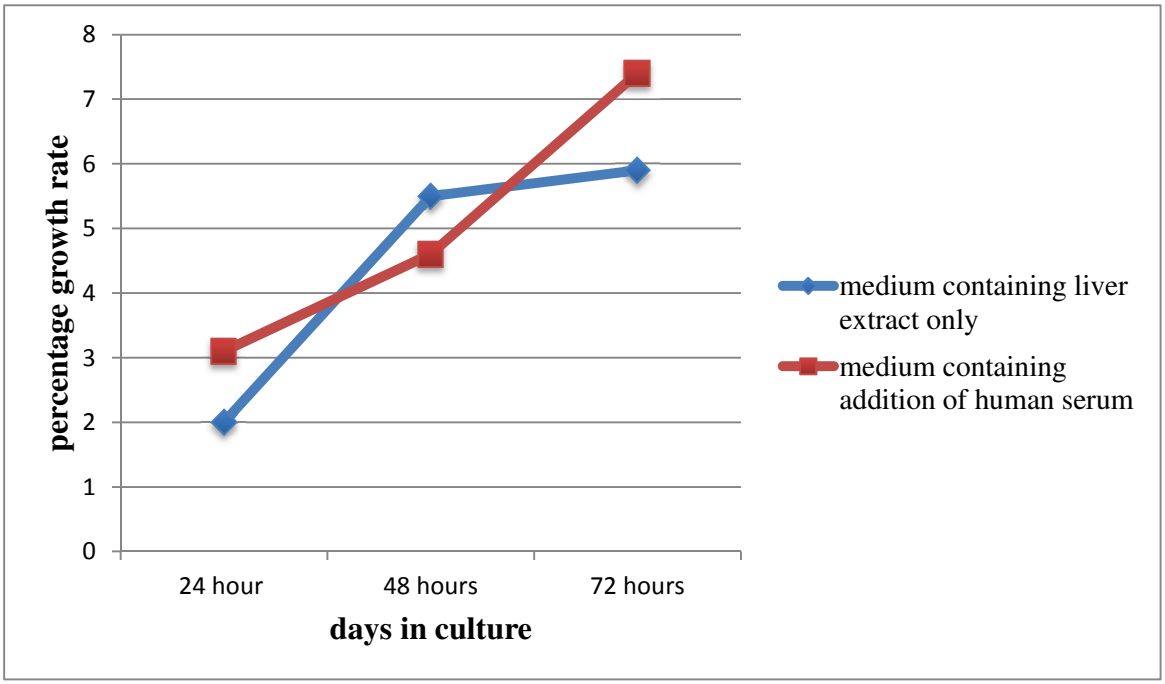

Fig 1; showing the percentage growth rate of the parasite with liver extract.

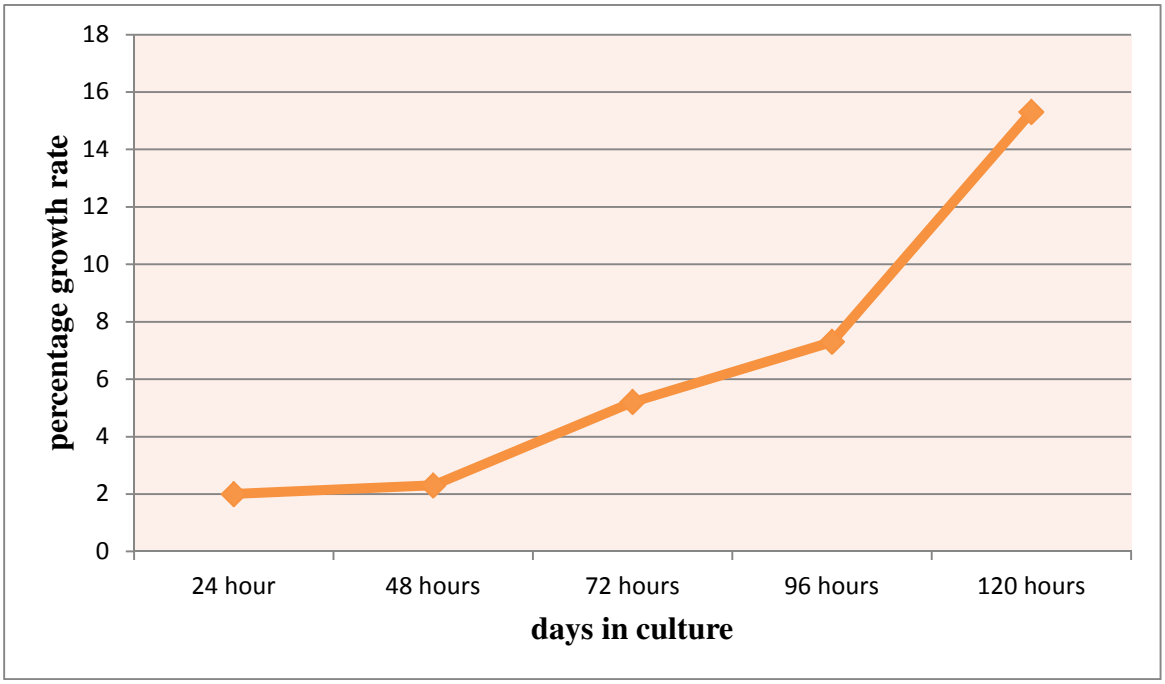

Fig 2; shows the parasite growth rate at interval of 0 - 120 hours (liver extract+ human serum).

Table 1; showing the antiplasmodial effect of *Enantia chlorantha* and *Azadirachta indica* on cultured malaria parasite.

Extract conc. (µg/ml)	Parasitized cells/500 cells	Parasitaemia (%)
Enantia chlorantha		
500	334	30.1
250	340	28.9
125	362	24.3
62.5	445	6.9
31.3	449	6.1
Azadirachta indica		
500	186	61.1
250	206	56.9
125	218	54.4
62.5	251	47.5
31.3	292	38.9

DISCUSSION

The present study focused on the cost effectiveness of RPMI 1640 medium which is not readily available in Africa settings with the indigenous *Jatropha curcas* plant medium (liver extract and human serum as part of the essential supplement) for in-vitro cultivation of *plasmodium falciparum*. So, also the anti-plasmodia effects of *Azadracta indica* were determined on in-vitro cultured malaria parasite. Since Cultivation of *Plasmodium falciparum* has been an important tool for the understanding of parasite biology, molecular biology, immunology, and pharmacology in which its application will contribute immensely for anti-malaria drugs, monitoring of drug sensitivity, and the detection of cross resistance patterns against the *Plasmodium falciparum* as reported by Trager and Jensen, 1976 ; Jensen *et al.,*1983 ; Trager and Jensen,1997; Oduola *et al.,* 1992 .This present study therefore tried to access the in-vitro cultivation of *Plasmodium falciparium* using indigenous plant as both the medium for cultivation and medium for sensitivity. In the present study using liver extract, the parasitized cells increases from 2% to 5.9% at day 3 using 0.05µg/ml of hypoxanthine solution and when human serum was added to the medium, the parasitaemia increases from 3.1% to 7.4% on day 3 with same concentration. The reason for this increment maybe due to the fact human serum constitute more nutritive value than the liver extract which aids parasite growth in this medium. However, at day 3 the resulted growth was discontinued, it may also be due to the digestion of red blood cell in the medium. 17.5% yielded parasitaemia cells recorded in this study is to prolong the growth rate with 0.2ml uninfected washed red blood and the growth rate recorded was in consonance with the study carried out by Aderounmu, 2003. When 5 different

concentrations of *Azadirachta indica* extracts were tested for 48 hours. The effect of *Azadirachta indica* at 500µg/ml and 31.3µg/ml concentration showed growth rate increase to 334 (30.1%) and 449 (6.1%) for the parasitized cells respectively . The general observations indicated no chemical injury for both media using the two indigenous plants from the environment and this complement the previous study on the chemical constituent of the plants which includes alkaloids, phenols, saponins, triterpenoils, proteins and tannins which might be responsible for the increase in their anti- plasmodia activitie (Biswas *et al.,*2002 ; Odugbemi *et al.,* 2007 ; Igbinosa *et al*., 2009 ; Ikpeme *et al.,* 2011)

CONCLUSION

The cultivation of *Plasmodium falciparum* using plant based medium provide a short term growth of the parasite. Therefore, these attempts can be used to investigate other related aspects of plasmodium studies such as different developmental stages of the parasite invitro as this may probably lead to development of malaria vaccine and drug resistant activities.

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