



Comparative *in vivo* antiplasmodial activities of different extracts of *Lawsonia inermis*, *Tithonia diversifolia* and *Nauclea latifolia* against *Plasmodium berghei*

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Abstract

Traditional herbal medicines are potentially rich sources of new drugs against malaria and other infectious diseases. However, phytochemicals extracted from a medicinal plant is strongly dependent on the nature of the extracting solvent. This study therefore aimed to investigate the antimalarial activity of three plants traditionally used to treat malaria using solvents of different polarities as solvents of extraction. The leaves of *Lawsonia inermis*, *Tithonia diversifolia*, and stem barks of *Nauclea latifolia* were extracted with water, n-hexane, ethanol and dichloromethane: methanol (1:1). The extracts were thereafter investigated for their antiplasmodial activity against *Plasmodium berghei* in infected mice using Peter's 4-days suppressive test. In *N. latifolia* stem bark, the aqueous extract showed the highest percentage chemosuppression (79.04%) followed by the dichloromethane: methanol (1:1), ethanolic and hexane extracts with percentage chemosuppressions of 74.66%, 61.47% and 8.81% respectively. The dichloromethane: methanol (1:1) extract of *T. diversifolia* leaves exhibited moderate antiplasmodial activity with percentage chemosuppression of 43.90% while its other extracts showed weak activity. Only dichloromethane: methanol (1:1) extract of *L. inermis* showed good antiplasmodial activity with percentage chemosuppression of 61.57% while the other extracts had between weak to inactivity. The antimalarial activity exhibited by these plants, most especially *N. latifolia* and *L. inermis*, against *P. berghei* suggests that these plants have active principles against malarial as claimed by the folklore, and further work is necessary to isolate, identify and characterize the active components of the plants.

Keywords: *Nauclea latifolia*, *Tithonia diversifolia*, *Lawsonia inermis*, Malaria

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1. Introduction

Malaria is a life threatening disease caused by parasites that are transmitted through the bites of female anopheles mosquito which bites mainly between dusk and dawn. Malaria is caused by Plasmodium parasites of which there are five species causing malaria in humans—*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi*. In 2013, most of the cases of malaria worldwide were reported in the African region followed by the South-East Asia region and the Eastern Mediterranean

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region with 82% of the cases seen in African countries (WHO, 2014). In 2014, 95 countries and territories had ongoing malaria transmission of which about 3.2 billion people estimated to be almost half of the world's population are at risk of malaria (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 followed by South-East Asia region and Eastern Mediterranean region with 7% and 2% cases respectively (WHO, 2016). 214 million malaria cases that led to 438,000 deaths was reported by WHO in 2015 (WHO, 2016), of which about 80% were children under age five.

The high rate of mortality and morbidity associated with this disease is due to rapid growth and multiplication of Plasmodium parasites in the human host blood cells (Cheng et al., 2012). Decades ago, malaria was effectively treated using antimalarial drugs such as chloroquine, Fansidar, Mefloquine and Malarone. However, development of resistant strains of Plasmodium, particularly *P. falciparum* to these drugs has since led to abandonment of use of the drugs (Cheng et al., 2012). In the absence of a credible vaccine and with emergence of resistance to almost all antimalarial drugs, the dream of eradication of malaria appears to be a huge challenge (Kaushik et al., 2015).

Traditional herbal medicines are a potentially rich source of new drugs against malaria and other infectious diseases. Traditional medicines have been used to treat malaria for thousands of years; a remarkable example being artemisinin from *Artemisia annua* and quinine from the bark of Cinchona tree. Quinine has also provided a lead template to chemists who successfully synthesized aminoquinoline-based anti-plasmodial analogs, such as chloroquine, amodiaquine, primaquine and mefloquine, all of which considerably improved the treatment of malaria (Toma et al., 2015). Artemisinin derivatives such as dihydroartemisinin, arthemeter, arteether, artesunate and arteminic acid have also been successfully synthesized.

Despite the recent successes in rational drug design and synthetic chemistry techniques by pharmaceutical companies, natural products and particularly medicinal plants have remained an important source of new drugs (Kaushik et al., 2013). This fact has encouraged the continue search for new natural product-derived antimalarial drugs. Additional research is therefore needed in order to realize the full benefits of natural plants and respond to the health needs of people especially in developing countries like Nigeria.

Nauclea latifolia, which belongs to the family Rubiaceae known commonly as African peach or pin cushion tree, is a straggling shrub with rounded ovate leaves. It is a native of Africa and thus widely distributed throughout the forest and tropical forests of Benin, Burkina Faso, Cameroon, Democratic Republic of Congo, Ghana and Nigeria (Lamidi, 1993). Among the Ibibios of South-South Nigeria, the stem bark either as an infusion or decoction is used as antimalarial, antipyretic and aphrodisiac. The leaf has the potential to relieve dysentery and diarrhoea (Etukudoh, 2003). Different parts of the plant including the inner bark, stem, sap, roots, fruits and bark of root have been used in the treatment of sleeping sickness, cough, febrile conditions, thrush, jaundice, piles, stomach and menstrual disorders as well as sores (Odugbemi, 2008). A previous study confirms the presence of tannins, flavonoids, alkaloids, glycosides and saponins in the root and stem bark of this plant (Egbung et al., 2013). Various works have been done on antimalarial activity of extracts of the plant parts of *N. latifolia*—leaf, stem bark and root. It was reported that *N. latifolia* inhibits *P. falciparum* erythrocytic cycle mainly at the end of schizogony (32nd to 48th hour) (Benoit-Vical et al., 1998; Ogbonna et al., 2008; Abbah et al., 2010; and Etebong et al., 2014).

Tithonia diversifolia (Hemsely) A. Gray belongs to the family Asteraceae. It is commonly known as tree marigold or Mexican sunflower, and is used traditionally for the treatment of malaria. Its anti-inflammatory anti-amoebic or spasmolytic activities have been described (Rungeler et al. 1998). The plant contains tagitinin A-C and F with tagitinin A showing insecticidal property. Goffin et al. (2002) reported that tagitinin C, a sesquiterpene lactone from the plant exhibited antiplasmodial action and possessed cytotoxic property *in vitro*. Phytochemical analysis revealed the presence of saponins, alkaloids and tannins in the aqueous extracts (Nafiu et al., 2014).

Lawsonia inermis Linn. belonging to the Lythraceae family and commonly called henna. It is a biennial dicotyledonous herbaceous shrub. Though the plant is a native of North Africa and South-West Asia, the plant is now widely cultivated throughout the tropics as an ornamental and dye plant (Chaudhary et al., 2010). It is a widespread medicinal plant and natural dye in the world. Henna leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments such as rheumatoid arthritis, headache, ulcers, diarrhoea, leprosy, fever, leucorrhoea, diabetes, cardiac disease and hepatoprotective (Chetty and Andhra, 2008). The biological review of the plant reported activities against diverse health conditions such as antidiabetics

(Arayne et al., 2007), anti-inflammatory (Gupta et al., 1986), antiparasitic (Okpekon et al., 2004), antifungal (Anwar et al., 2007).

The bioactive chemical compounds in plants regarded as phytochemicals are the ones responsible for the treatment of illnesses. They are produced via secondary metabolism in relatively small amounts. Studies on the number of these phytochemicals have increased greatly over the last decades and those of significant health benefits have been grouped into classes which include alkaloids, terpenes, glycosides, flavonoids, phenolics, saponins, tannins, steroids and many more. They exhibit various pharmacological activities such as anti-inflammatory, anti-oxidant, anti-malaria, anti-cancer, anaesthetics, anti-viral, anti-fungal and anti-bacterial activities (Wadood et al., 2013). The phytochemicals extracted from a medicinal plant is strongly dependent on the nature of the extracting solvent and the method because of the presence of different chemical compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent (Sultana et al., 2009; and Jakopic et al., 2009). Previous studies showed that the dichloromethane:methanol (1:1) extracts of *L. inermis*, *Chromolaena odorata*, *T. diversifolia* singly and in combination were active against *Plasmodium* parasite than the aqueous extracts (Afolayan et al., 2014; and Afolayan et al., 2016).

This study therefore aimed at determining the most active extract out of hexane, ethanolic, aqueous and dichloromethane : methanol (1:1) extracts of the leaves of *L. inermis*, *T. diversifolia* and stem bark of *N. latifolia* against *Plasmodium berghei* in mice model for the purpose of further studies.

2. Materials and methods

2.1. Plant collection and extraction

Fresh leaves of *T. diversifolia* and *L. inermis* as well as stem bark of *N. latifolia* were collected. The collected plants materials were air-dried at ambient temperature (25 °C) for two weeks. The dried plant samples were blended and each plant was soaked separately in distilled water, ethanol, n-hexane and dichloromethane-methanol (1:1) for 24 h. The aqueous extracts were prepared by heating the soaked sample in a water bath at 72 °C for one and half hours and left to stand for 24 h. For a proper mixing, plant materials and solvent were continuously shaken at regular intervals. The resultant mixture was doubly filtered with muslin cloth and then with Whatman No. 1 filter paper and the filtrate concentrated under pressure using a rotary evaporator. Extracts were stored in a refrigerator at 4 °C until used.

2.2. Determination of percentage yield of extracts

Determination of percentage yield of extract was done using the formula:

$$\text{Percentage Yield} = \frac{\text{Weight of final extract}}{\text{Soaked sample material}} \times 100\%$$

2.3. Animals and parasites

Healthy albino mice 6-8 weeks old weighing 18 ± 2 g were obtained from the animal house of the Department of Physiology, University of Ibadan. Animals were maintained in clean and well ventilated cages in the animal house of the Department of Zoology, University of Ibadan and acclimatized for two weeks preceding the experiments. They were fed with mice feed and water. Rodent parasites used were Chloroquine sensitive *P. berghei* ANKA strain donated by Dr. Falade of the Department of Zoology, University of Ibadan.

2.4. Preparation of inoculum

Parasitized blood from a mouse donor with high parasitaemia was obtained by first anaesthetizing the mouse with 0.1 ml ketamin, and blood collected through cardiac puncture using sterile syringe into sterile bottles. The 1% parasitaemia was determined by counting the number of parasitized red blood cells against the total number of red blood cells. The desired volume of blood then obtained from the donor mouse was suitably diluted with sterile normal saline so that the final inoculums (0.2 ml) for each mouse contained the required number of parasitized red blood cells (1.0×10^7). The 0.2 ml of the final inoculums therefore contained 1×10^7 parasitized red blood cells, the standard inoculums for the infection of a single mouse.

2.5. In vivo determination of antimalarial activity

Antiplasmodial activity of the test extract was performed using Peter's 4-day suppressive standard test (Knight and Peter, 1980). In this study, albino mice were randomly assigned into four treatment groups (ethanolic,

n-hexane, aqueous, dichloromethane-methanol (1:1) extracts) of five mice each for each group separately for each plant. Negative and positive controls with five mice per group were also set. Two hours after infection, 250 mg/kg/day of each extract was orally administered. Chloroquine diphosphate at the dose of 10 mg/kg/day and an equivalent volume of vehicle (10% of Tween 80) was administered to the positive and negative control groups respectively for four consecutive days (D_0 to D_3).

2.6. Determination of parasitaemia

The blood films of the mice were taken at four and six days after infection for these three models. Percentage parasitaemia determined by counting four fields of the blood smear in a view under the microscope was used to calculate percentage reduction in parasitaemia and chemosuppression for the different test extracts, using the formulae below:

$$\text{Percentage parasitaemia} = \frac{\text{Number of parasitized Red Blood Cell}}{\text{Total number of Red Blood Cell}} \times 100$$

$$\text{Percentage chemosuppression } A = \frac{B - C}{B} \times 100$$

where:

A = Percentage chemosuppression.

B = Average percentage parasitaemia in the negative group.

C = Average percentage parasitaemia in the test group.

3. Results

3.1. Extract yield

The percentage yield of extracts ranged from 0.34% to 31.69%. The aqueous extracts had the highest yields than the organic extracts (ethanolic, n-hexane, DCM-Methanol (1:1)) for both *T. diversifolia* (31.69%) and *L. inermis* (20.91%) while the ethanolic extract had the highest yield for *N. latifolia* (6.26%). The n-hexane extract had the lowest yield of all the plants (Table 1).

Extract type	<i>N. latifolia</i>	<i>T. diversifolia</i>	<i>L. inermis</i>
Aqueous	5.23	31.69	20.91
Ethanol	6.26	4.29	17.56
DCM-MeOH	3.70	5.83	16.90
n-hexane	0.34	1.19	2.05

3.2. Outcome of *In vivo* antiplasmodial bioassay

3.2.1. Antimalarial activity on day 4 post-infection

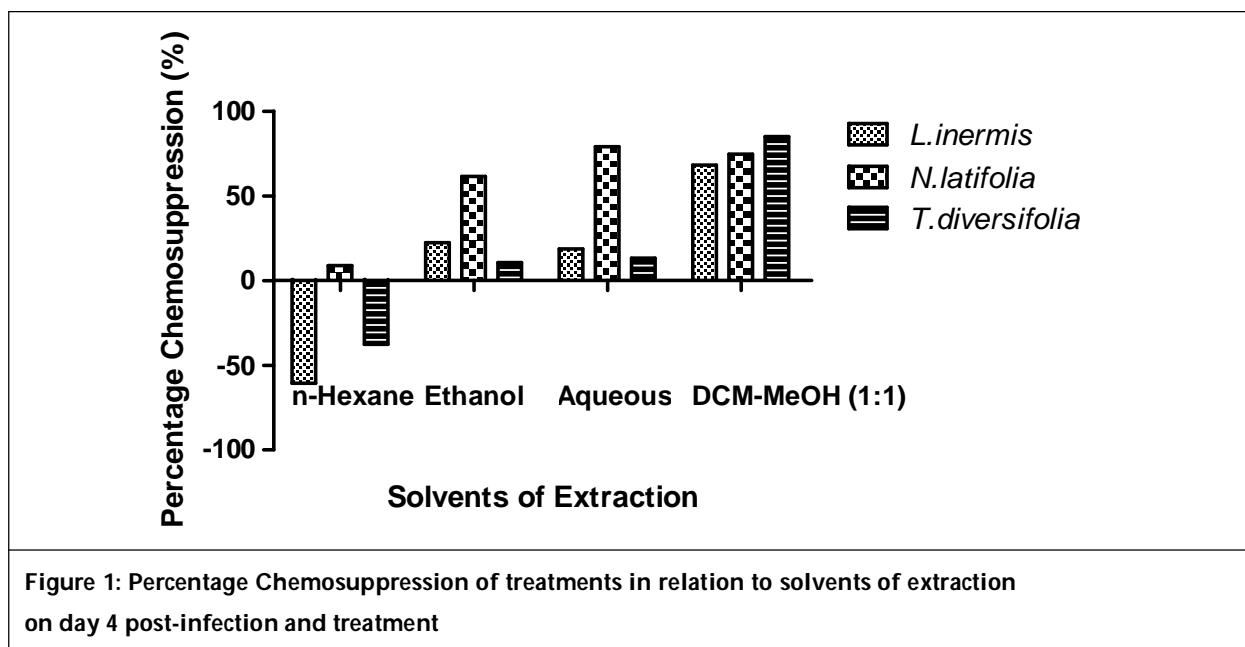
All the extracts exhibited varying degrees of chemosuppression. A therapeutic dose lowering parasitaemia by >60% was considered high chemosuppression. The aqueous stem bark extract of *N. latifolia* induced chemosuppression that was not significantly different (79.04%) from the effect of Chloroquine (82.44%) in positive control (Table 2). Other extracts of *N. latifolia*, except hexane extract, had high percentage chemosuppression with DCM-MeOH (1:1) having a chemosuppression of 74.66% and 3.90 ± 2.38 percentage parasitaemia, followed by ethanolic extract with percentage chemosuppression of 61.47% and 5.93 ± 4.04 parasite density. Furthermore, *L. inermis* was observed to show highest activity in DCM, MeOH (1:1) extract treated group with chemosuppression of 68.2%. Other extracts of *L. inermis* however showed weak to inactivity.

Similarly, *T. diversifolia* had its highest percentage chemosuppression of 43.9% in DCM, MeOH (1:1) extract treated group. While other groups displayed weak to inactivity. On day 4-post infection and treatment, the lowest chemosuppression was observed in the group treated with the hexane extracts of all the plants (Figure 1).

Table 2: *In vivo* suppressive antimalarial screening of *Tithonia diversifolia*, *Lawsonia inermis* and *Nauclea latifolia* at 250 mg/kg against *Plasmodium berghei* infected mice at day 4 post-infection (%)

Extracts	<i>N. latifolia</i>		<i>L. inermis</i>		<i>T. diversifolia</i>	
	Parasitaemia	Chemo-suppression	Parasitaemia	Chemo-suppression	Parasitaemia	Chemo-suppression
Hexane	13.97 ± 0.95	8.81	13.97 ± 0.95	-60.54	21.19 ± 1.13	-37.64
Ethanol	6.75 ± 1.25*	61.47	6.75 ± 1.25	22.30	13.77 ± 1.03	10.56
Aqueous	7.08 ± 1.00*	79.04	7.08 ± 1.00	18.68	13.35 ± 1.54	13.30
DCM/MeOH (1:1)	2.77 ± 0.77*	74.66	2.77 ± 0.77*	68.20	8.64 ± 1.71*	43.90
Chloroquine	2.31 ± 0.35*	85.03	2.31 ± 0.35*	75.25	3.02 ± 0.49*	85.03
Negative control	8.70 ± 2.50		8.70 ± 2.50		15.39 ± 1.54	

Note: * Significantly different from negative control.



3.2.2. Day 6 post-infection

Decrease in percentage chemosuppression was generally observed on day 6 post-infection in the different extracts of *L. inermis* and *N.latifolia* when compared to chemosuppression of day 4 post-infection. However, increase in chemosuppressive activity was noticed in *T. latifolia* extracts' treated groups (Tables 2 and 3). The DCM-MeOH (1:1) extract of both *T. diversifolia* and *L. inermis* induced the highest chemosuppression with percentage chemosuppression of 50.27% and 31.33% respectively. The aqueous extract of *N. latifolia* showed the highest percentage chemosuppression among other extracts with percentage chemosuppression of 65.55%. In all plant groups, hexane extract showed the lowest chemosuppression with no activity in *L. inermis* groups (Figure 2). It was further observed that DCM, MeOH (1:1) solvent extracts of the various plants exhibited the

Table 3: *In vivo* suppressive antimalarial screening of *Tithonia diversifolia*, *Lawsonia inermis* and *Nauclea latifolia* at 250 mg/kg against *Plasmodium berghei* infected mice at day 6 post-infection (%)

Extracts	<i>N. latifolia</i>		<i>L. inermis</i>		<i>T. diversifolia</i>	
	Parasitaemia	Chemo-suppression	Parasitaemia	Chemo-suppression	Parasitaemia	Chemo-suppression
Hexane	38.44 ± 9.44	28.80	44.13 ± 20.50	-45.51	39.48 ± 21.81	26.87
Ethanol	34.25 ± 12.73*	36.55	24.70 ± 13.11	18.55	35.03 ± 12.90*	35.03
Aqueous	18.60 ± 9.91*	65.55	26.70 ± 10.90	11.96	35.67 ± 20.94*	33.92
DCM/MeOH (1:1)	29.52 ± 19.36*	45.31	20.83 ± 8.51*	31.33	26.85 ± 7.02*	50.27
Chloroquine	1.65 ± 0.76*	96.95	1.60 ± 0.71	94.72	1.65 ± 0.76	96.95
Negative control	53.98 ± 19.68		30.33±17.52		53.98 ± 19.68	

Note: * Significantly different from negative control.

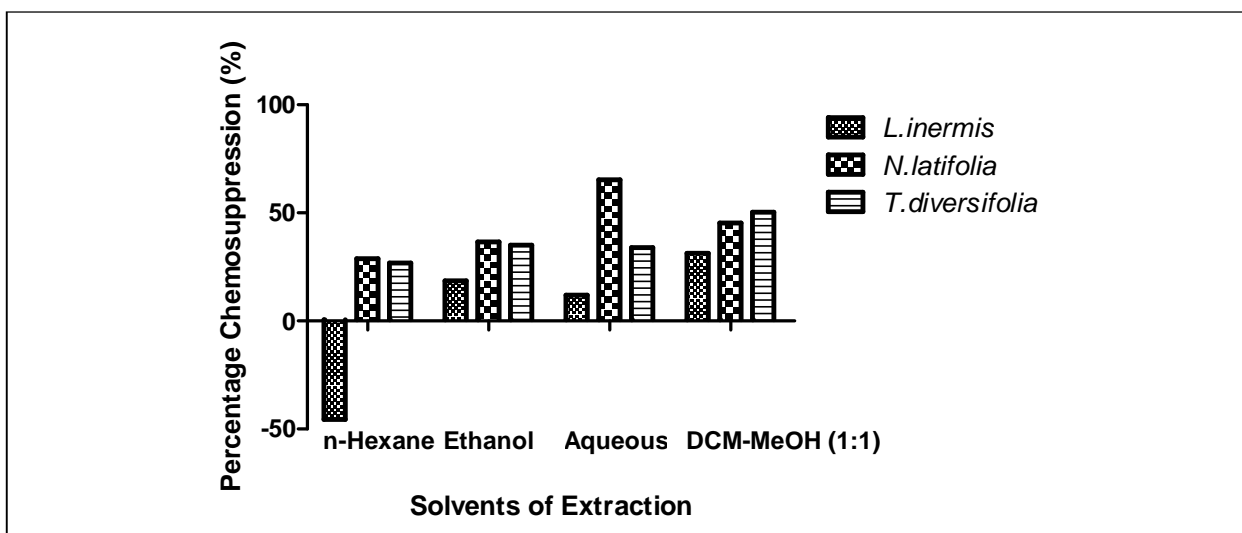


Figure 2: Percentage Chemosuppression of treatments in relation to solvents of extraction on day 6 post-infection and treatment

best antiplasmodial effects on the *P. berghei* infected mice when compared to other solvents' extracts on days 4 and 6 post-infection (Figures 1 and 2).

4. Discussion

Indigenous healthcare systems have always played a vital role in the management of community health and discovery of novel chemotherapeutic agents. Medicinal plants could be attractive start materials, for the discovery of novel drugs, as they are widespread. Also a large population relies on them for their curative effects. In the present study, three medicinal plants, *N. latifolia*, *T. diversifolia*, and *L. inermis*, known for their traditional medicinal usage and pharmacological activities were evaluated for the antiplasmodial activity of their different extracts against chloroquine-sensitive *P. berghei* ANKA strain. The determination of the best solvent of extraction is crucial because the preferred traditional solvent of preparation of these medicinal plants is water.

The dichloromethane-methanol (DCM-MeOH (1:1)) extracts of all the plants which showed the relatively highest chemosuppressive activity could have done so because of abundant phytochemicals present in them.

Afolayan *et al.* (2014) reported that more phytochemicals were present in DCM-MeOH (1:1) extracts of *T. diversifolia* and *L. inermis* than the aqueous extracts. The phytochemicals which include flavonoids, alkaloids, phenols, terpenoids could have been responsible for the antiplasmodial effects (Essiet and Uriah, 2013; Vijayaraghavan *et al.*, 2013; Afolayan *et al.*, 2014; and Singh and Luqman, 2014). Only the aqueous extract of *N. latifolia* had high activity on day 4 post-infection, although the percentage chemosuppression of its DCM-MeOH (1:1) extract was considerably high too as it follows that of the aqueous extract. Although the preferred traditional solvent of extraction of most medicinal plants through decoction is water, this study however revealed that the aqueous extracts of *L. inermis* and *T. diversifolia* had lower antimalarial effects when compared to organic extracts. This trend was observed in previous studies reported by Clarkson *et al.* (2004); Afolayan *et al.* (2016). The high activity of DCM-MeOH extracts can be attributed to the presence of both polar and non-polar bioactive compounds in the extracts. The high antimalarial activity recorded in the dichloromethane-methanol extracts over extracts from other solvents was also reported by Clarkson *et al.* (2004); Melariri *et al.* (2012). It was observed that *T. diversifolia* extracts which exhibited the lowest activity among the extracts on day 4 post-infection later became more active on day 6 post-infection. This could be because the plant was slow acting and needed time to perform.

5. Conclusion

The result of the activities of the different extracts agrees with earlier studies that phytochemicals extracted in plant materials is dependent on the solvent used in the extraction process (Tijani *et al.*, 2007). This study has shown that the aqueous extract of *N. latifolia* stem bark and DCM-MeOH (1:1) extract of the leaves of *T. diversifolia* and *L. inermis* are the most active extract out of the other extracts tested. When a crude extract exhibit high chemosuppressive activity at 250 mg/kg, such an extract deserves further investigation (Rasoanaivo *et al.*, 2004). Therefore, this study paves the way for standardized plant extracts based therapy against malaria, and antiplasmodial activity guided isolation of the extracts and their subsequent development towards phytochemical based novel drugs against malaria.

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