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In vivo assessment of the antimalarial activity of *Cassia Singueana* and *Cymbopogon Citrutus*

Lydia D. Ior[1], Noel N. Wannang[1], Clement A. Ior[2] and Kennedy I. Amagon[1]

[1]Department of Pharmacology, University of Jos, Nigeria
[2]Department of Medical Microbiology, University of Jos, Nigeria

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ABSTRACT

The problem of increasing drug resistance in malaria therapy has made its treatment a major source of concern globally. This has necessitated the need to explore traditional medicines in the search for new/potential anti-malarials for both prophylaxis and chemotherapy. Cassia singueana and Cymbopogon citrutus are widely spread in northern Nigeria and claimed to possess multiple therapeutic properties, including anti-malarial activity. Cassia singueana bark was extracted with 50% ethanol,while the root and leaf of Cymbopogon citrutus were extracted with cold water. Phytochemical analysis and oral acute toxicity of the extracts, the Suppressive and Prophylactic anti-plasmodial activities against chloroquine sensitive strain of Plasmodium berghei berghei in mice were evaluated. Pathological effects associated with malaria infections; pyrexia and weight loss or poor weight gain were similarly assessed. Results showed the presence of carbohydrates, alkaloids, tanins, flavonoids, cardiac glycosides, saponins and steroids in Cassia singueana, while Cymbopogon citrutus showed the presence of alkaloids, carbohydrates, tannins, flavonoids, steroids and saponins. The oral median lethal dose of both extracts was greater than 5000 mg/kg body weight. Cassia singueana at (400-600 mg/kg) exerted significant ($P < 0.05$) chemosuppressive effects between 72.7% to 90.5% and prophylactic effects between 79% to 83% against the Plasmodium berghei berghei. Similarly, Cymbopogon citrutus (200-800 mg/kg) produced a significant chemosuppressive effects between 20.83 - 80.56% for the leaf extract and 55.38-77.78% for the root extracts. This result showed that the plants have anti-plasmodial property that can be explored for the management of malaria.

Keywords: Malarial activity, *Plasmodium berghei berghei*, Extracts

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INTRODUCTION

Malaria, caused by *Plasmodium specie,* is world's most widespread infectious disease. The parasite is transmitted to human through a bite from infected female anopheles mosquito. Although more than 100 different species of *Plasmodium* exist, only four are recognized to infect human: *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium vivax* and *plasmodium knowlesi* (Daneshvar *et al.*, 2009), with *Plasmodium falciparum* being the deadliest. It is responsible for the high rate of deaths recorded annually from malaria incidence (WHO, 2008). According to the World Malaria Report of 2011, malaria is prevalent in 106 countries of the semitropical and tropical world, with highest burden of cases and deaths recorded in 35 Central African countries. People at risk of *Plasmodium falciparum* malaria worldwide in 2007 was estimated at 2.37 billion, of which 26% were at the WHO AFRO region and 62% in the combined SEARO-WPRO regions (WHO, 2011). Annual estimates of malaria incidence vary widely. The Report estimated malaria episodes in 2010 to be at 216 million, of which

approximately 81% were in the African Region, 13% from South-East Asia and 5% from Eastern Mediterranean Regions (WHO, 2011). In 2007, an estimated 2.37 billion people worldwide was reported as being at risk of *Plasmodium falciparum* malaria (UK Aid, 2010). About 91% of malaria episodes reported in Africa in 2010 were due to *Plasmodium falciparum* (WHO, 2011).

The use of natural products as medicinal agents dates back to prehistory. Plants, which are being used for thousands of years, have formed the basis of Traditional Medicine (TM) systems. Various but specific plants have been used to treat illnesses over the years. They have been relied upon to support, promote, retain and regain human health and are used in every country of the world (Li *et al*., 2008; Shi *et al*., 2009; Sucher, 2008). *Cassia singueana* belongs to the class Leguminosae and family Caesalpinioideae (Ode and Asuzu, 2011). It is a woody annual herb or under shrubs between 1.2 and 1.5m high with small yellow flowers. It is widely spread in India and tropical Africa including northern Nigeria, especially in cultivated or old clearings by the road side and open grassy areas (Dalziel, 1956). In Northern Nigeria *C. singueana* is used for the treatment of acute malaria. It has also been reported to exhibit profound anti-ulcer potential (Ode and Asuzu, 2011). *Cymbopogon citrutus* is a tropical perennial grass rising from swollen bases, cultivated by the natives of Asia, parts of South America and tropical regions of the world. The plant leaves are thin, along with slender bladder that grows to a height of 1.5m. The root spread in to branches of rootlet network that rapidly exhaust the soil, propagated by dividing the root clump. *C. citrutus* is used traditionally in different parts of the world to treat varying degree of illnesses, in Nigeria decoction preparation of the leaves is used as antimalaria, antipyretic, stimulant, cough remedy and antispasmodic (Asaolu *et al*., 2009).

The study was aimed at evaluating the ethanol bark extract of *C. singueana* and aqueous leaf and root of extracts of *C. citrutus* for antimalarial activity.

MATERIALS AND METHODS

Plant Collection and Authentication

C. singueana and *C. citrutus* were collected in March, 2012 in Jos, Nigeria. The plants were authenticated by a taxonomist at the Federal College of Forestry; Jos and Herbarium sample specimen was prepared and deposited at the Museum of the College.

Extraction of Plant Material

The fresh bark of *C. singueana* and the leaves and roots *C. citrutus* were air dried at room temperature. The dry plant parts were crushed and grounded into coarse powder in a mortar. 60 g of *C. singueana* was measured and dissolved in sufficient quantity of ethanolfor 48hrs and 60 g each of the leaf and root of the powdered plant of *C. citrutus* were measured separately and dissolved in sufficient quantity of water for 48 hrs, the mixture was filtered with ashless filter paper, the extract was concentrated using rotary evaporator at a temperature of 4 °C. The concentrate was heated over a water bath to obtain a solvent free extract. All the extracts were stored in a refrigerator at 4 °C in well-closed container to protect from light and moisture

Experimental Animals

Both sexes of Swiss albino mice weighing 25-30 g, obtained from Animal House Unit of the University of Jos were used for the study. They were housed in plastic cages with saw dust as beddings at the Animal House Unit of the University. The mice were given standard laboratory diet and water *ad libitum* and maintained under laboratory conditions of temperature (24 ± 1°C), and a non-reversed 12 hr light/12 h dark cycle.

Ethics

The study was carried out in accordance with standard experimental procedures approved by the ethical and research Committee, Department of Pharmacology University of Jos.

2.1 Phytochemical Screening

The extract were screened for phytochemical constituents using standard procedures as described by Trease and Evans (2005) to test the presence/absence of alkaloids, saponin, cardiac glycosides, carbohydrates, flavonoids, tannins, anthraquinones and steroids.

Acute Toxicity Test (LD_{50})
The oral acute toxicity of the ethanol extracts were estimated in albino mice using modified Lorke's method (1983). The study was carried out in two phases. In the first phase, nine mice were randomly divided into three groups of three mice each and were given 10, 100 and 1000 mg extract /kg body weight orally respectively. They were observed for the first 24 hours after dosing for signs of toxicity and mortality. In the second phase of the study three fresh mice, one per group, were each given doses of 1600 mg/kg, 2900 mg/kg and 5000 mg extract/kg body weight respectively based on the results of the first phase. These were also observed for signs of toxicity and mortality for 72 hour. The LD_{50} was calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose, i.e., the geometric mean of the consecutive dose for which 0 and 100% survival rates were recorded in the second phase. The oral median lethal dose was calculated using the formula:

LD50 =$\sqrt{}$*maximum dose for all survival × minimum dose for all death* (Lorke 1983)

Rodent Parasite (*Plasmodium berghei*)
The rodent parasite was sourced from National Institute for Medical Research (NIMR), Lagos, Nigeria and maintained alive in mice by serial intraperitoneal passage of blood from mouse to mouse (Calvalho *et al.*, 1991). The re-infected mice were kept at the Animal House Unit, Department of Pharmacology, University of Jos where the study was carried out. Prior to the commencement of the study, one of the infected mice was kept and observed to reproduce signs of diseases similar to human malarial infection (English *et al*., 1996).

Parasite Inoculation
The inoculums consisted of $5x10^{7}$ of *Plasmodium berghei berghei* parasitized erythrocytes per ml. This was carried out by determining the percentage parasitaemia of the donor mouse and diluting the blood with normal saline in proportions indicated by the percentage parasitaemia determination (Odetola and Basir, 1980). Each mouse was inoculated intraperitonealy with 0.2 ml of infected blood containing about $0.1 x10^{7}$ *Plasmodium berghei berghei* parasitized red blood cells.

Activity on Early Infection (Suppressive Test)
The Peter's 4-day suppressive test against chloroquine sensitive *Plasmodium berghei berghei* NK 65 infection in mice was employed (Peters, 1965) On the first day (Day 0), adult Swiss albino mice were inoculated by intraperitoneal (I.P) injection with 0.2ml of standard inoculums containing about $0.1 X 10^{7}$ *Plasmodium berghei berghei* parasitized red blood cell. These mice were randomly divided into 5 groups of 5 mice per group and treated for 4 consecutive days (Day 0-3). Within 3 hours post-inoculation of mice with the parasite (i.e. on Day 0), treatment of the experimental groups was initiated. Group I mice were given 10 ml normal saline/kg body weight orally daily. Groups II, III and IV were given 200, 400 and 600 mg extract/kg body weight orally daily respectively, while group V mice received 5mg chloroquine/kg body weight orally daily. On the 5th day (Day 4), blood from the tail of each mouse was smeared on to a microscope slide to make a thin film (Saidu *et al*., 2000). The blood films were fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 min (English *et al.*, 1996) and parasitaemia examined microscopically (100 x magnification). The parasitaemia level was determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice and the results multiplied by 100.

Percentage Suppression = (A – B/A) × 100
Where;
A= average percentage parasitaemia in the negative control group
B=average parasitaemia in the test/standard group.

The mice were reweighed on Day3, 7, 11, and 15 and the differences between the pre- and post-treatment body weight recorded. The mean survival time (MST) of the mice in each treatment group was determined over a period of 29 days (D_0–D_{28}).

Evaluation of Prophylactic Activity (Repository Test)
The prophylactic activity of the extracts were tested using the residual infection procedure described by Peters (1965). Twenty-five mice of both sexes were weighed and randomized into five groups of five mice each. Group I mice were given 10ml normal saline/kg body weight. Groups II, III and IV were given 200, 400 and 600 mg

extract/kg body weight orally respectively while group V mice received 5 mg chloroquine/kg body weight orally daily for five days (D_0-D_4). On the fifth day (D_4), all the mice were inoculated with 0.2 ml standard inoculum containing 0.1 x 10^7 *P. berghei berghei* infected erythrocytes. Blood smears were made from each mouse 72 hours post treatment (Abatan and Makinde, 1986) and examined microscopically for parasitaemia level. The mean survival time (MST) of the mice in each treatment group was determined over a period of 29 days (D_0–D_{28}).

Statistical Analysis

Graph pad prism version 5.02 was used to analyze data obtained and these were expressed as mean ± standard error of mean. The differences between means were compared using One way analysis of variance (ANOVA) followed by Dunnet's post hoc test. $P \leq 0.05$ were considered significant.

RESULTS

Extract Yield Percentage

The percentage yield of the ethanolic bark extract of *C. singueana* and aqueous root and leaf of *C. citrutus* were calculated to be 35.1%, 39.3%, and 44.5% respectively.

Phytochemical Screening

Results showed the presence of carbohydrates, alkaloids, tanins, flavonoids, cardiac glycosides, saponins and steroids in *Cassia singueana*, while *Cymbopogon citrutus* showed the presence of alkaloids, carbohydrates, tannins, flavonoids, steroids and saponins as shown in Table 1.

Table 1. Phytochemical Screening Ethanolic Bark extract of *C. singueana* and Aqueous Root and Leaf Extracts of *C. citrutus*

Phytochemical Constituents	*C. singueana* Bark	*C. citrutus* Root	*C. citrutus* Leaf
Alkaloid	+++	++	+++
Anthraquinone	-	-	-
Carbohydrate	++	++	+++
Cardiac glycoside	++	-	-
Flavonoid	+++	+++	+
Saponins	+++	++	-
Steroid	+++	++	++
Tannins	+++	+++	+

- Absent; + Present; ++ Moderately present; +++ Abundantly present.

Acute Oral Toxicity

The acute toxicity study indicated that none of the different doses of all the extracts in all the groups and phases caused mortality of mice. The mice appeared normal and no treatment - related sign of toxicity was observed during and after treatment at doses below 5000 mg/kg. However, behavioral signs of toxicity observed at 5000 mg extract /kg body weight include; paw licking, restlessness, sniffing and reduced activity over time. The oral median lethal dose (LD_{50}) of the extracts in mice were estimated to be greater than 5000 mg /kg body weight.

Table 3: Suppressive Effect of *C. singueana* Bark on Mice Infected by *Plasmodium berghei*

Treatment (mg/kg)	Mean parasite count	% Suppression
Normal saline	66.0 ± 04.0	-
Extract 200	41.3 ± 14.0	37.4
Extract 400	18.0 ± 07.2*	72.7
Extract 600	06.3 ± 00.3 **	90.5
Chloroquine	05.5 ± 00.6**	100

*Values expressed as Mean ± SEM; n= 5; * (P < 0.05); ** (P < 0.01)*

Table 4: Suppressive Effect of *C. citrutus Root* on Mice Infected by *Plasmodium berghei*

Treatment (mg/kg)	Mean parasite count	% Suppression
Normal saline	7.20±0.58	-
Extract 200	4.00±0.55*	50.38
Extract 400	1.60±0.40**	77.78
Extract 800	0.00**	100
Chloroquine 5	0.00**	100

*Values expressed as Mean ± SEM; n= 5; * (P < 0.05); ** (P < 0.01)*

Table 5: Suppressive Effect of *C. citrutus Leaf* on Mice Infected by *Plasmodium berghei*

Treatment (mg/kg)	Mean parasite count	% Suppression
Normal saline 5 ml	7.20±0.58	-
Extract 200	4.80±0.93*	20.83
Extract 400	3.20±0.74**	55.56
Extract 800	1.40±0.25**	80.56
Chloroquine 5	0.00**	100

*Values expressed as Mean ± SEM; n= 5; * (P < 0.05); ** (P < 0.01)*

Table 6: Prophylactic Effect of *C. singueana* Bark on Mice Infected by *Plasmodium berghei*

Treatment mg/kg	Mean parasite count	% inhibition
Normal saline 5 ml	66.0 ±04.0	-
Extract 200	42.0 ± 22.0	30.0
Extract 400	12.5 ± 5.3**	79.0
Extract 600	10.0 ± 4.5**	83.3
Chloroquine 5	6.00± 2.34**	90.0

*Values expressed as Mean ± SEM; n= 5; * (P < 0.05); ** (P < 0.01)*

DISCUSSION

The menace of multi-drugs resistant malaria parasite and the absence of a functional safe and widely available malaria vaccine have necessitates research in the direction of development of new antimalarial drugs. It is of importance that chemical components derived from plants used in traditional medicine for treatment of malaria be investigated. In this study, the *in vivo* antimalarial activities of the ethanolic bark extract of *C.singueana and* aqueous root and leaf of *C. citrutus* plant used in traditional medicine in Nigeria, on *Plasmodium berghei berghei* infected mice both in a 4 day suppressive and a prophylactic test models are reported.

The choice of young mice for the study was to avoid the effect of anemia in old mice and the possible physiological changes associated with ageing which may be induced on the treatment outcome. (Pierrot *et al.*, 2003). The *in vivo* model was employed for this study because it takes into account possible pro-drug effect and possible involvement of immune system in eradication of infection (Waako *et al.*, 2005). The rodent model of malaria has been employed in this study for prediction of efficacy of antimalaria effect of the plant extracts. *Plasmodium berghei berghei* parasite is used in predicting treatment outcomes of any suspected antimalaria agent due to its high sensitivity to chloroquine making it an appropriate parasite for this study. Moreover, several studies (Calvalho *et al*., 1991; Agbedahunsi *et al.*, 1998; Adzu and Salawu, 2009) have employed *Plasmodium berghei berghei* in predicting treatment outcome of suspected antimalarial agents. Where substances that reduce parasite multiplication (anti plasmodial effect) in the host were considered to possess antimalarial activity (Ryley and Peters, 1970).

Although rodent models do not exactly produce the same observed signs and symptoms in the human plasmodial infection but, they have been reported (Pedroni *et al.*, 2006; Pierrot *et al.*, 2003) to produce disease features similar to those of plasmodial infection in human, when infected with *Plasmodium berghei* (Thomas *et al.*, 1998). Several conventional anti-malaria agents such as chloroquine, mefloquine, halofantrine and more recently artemisinin derivatives have been identified using rodent malaria model (David *et al*., 2004). The 4-day suppressive test is a standard test commonly used for antimalarial screening and the determination of percent inhibition of parasitaemia has been the most reliable parameter. A mean group parasitaemia level of less than or equal to 90% of the normal

saline-treated control animals usually indicate that the test material is active in standard screening studies (Peter and Anatoli., 1998).

The results obtained from this study showed that the ethanolic bark extract of *C. singueana* and aqueous root and leaf of *C. citrutus* possess significant suppressive effect against early infection in parasitaemia of *Plasmodium berghei berghei* infected mice as compared to the standard antimalarial drug. The ethanolic bark extract of *C. singueana* also showed prophylactic effect against residual infection of the parasite at doses used. In the prophylactic study, it was shown that the extract produced a dose dependent reduction in parasitaemia levels similar to that of the chloroquine treated group (positive control). The average percentage prophylactic suppression of parasitaemia of the extract treated group was significant at 400 and 600 mg/kg body weight of the mice with 79% and 83% respectively. Chloroquine at 5 mg/kg/day exerted a 61.54% decrease in parasite count.

The anti-malarial activity of the extract observed in the established may be due to the inhibitory effect of the extract on generation of free radicals and haemolytic principles such as free fatty acids resulting from high parasitaemia level (Calvalho *et al*., 1991). It may also be due to the direct plasmocidal effect of the extract as shown by the decrease in parasite count produced by the extract. In addition to these effects the plant extract may possess other pharmacological benefits to the hosts, such as acting as analgesics, antipyretics or as immune stimulators (Dahanukar *et al*., 2000). Though these tests were not assessed in this study.

Although, the exact mechanism of action of these extract has not been explicated, antiplasmodal effects of natural plant products have been shown to depend on their constituent active phytochemicals (Ayoola *et al*., 2008). The presence of pharmacologically active phytochemicals such as saponins, tannins, flavonoids and alkaloids, which were identified present in these plant extracts, have been suggested to act as primary antioxidant or free radicals scavengers that can counteract the oxidative damage induced by the malaria parasite (David *et al*., 2004, Okokon *et al*., 2008). Furthermore, studies have shown that antiplasmodal effect of natural products of plant origin may be mediated via inhibition of protein synthesis (Peter and Anatoli, 1998).The antiplasmodal effect demonstrated by these extracts may be due to the presence of saponins, tannins and the alkaloidal constituent acting through either of the two mechanisms mentioned above or synergistically through a combination of the mechanisms suggested or another possibly unknown mechanism. Furthermore this phytochemicals may be acting independently or in synergy with one another to produce the observed anti-malarial activity in this study.

CONCLUSION

The present work has demonstrated the efficacy of the ethanolic bark extract of *C. singueana* and the aqueous root and leaf extracts of *C. citrutus* traditionally used in chemotherapy of malaria infection. These observations provide the basis for the traditional use of this herb in treatments of malaria.

REFERENCES

[1] Abatan, M.O. and Makinde, M.J. (**1986**). *J. Ethnopharmacol*., 17: 85-93.
[2] Adzu, B., Salawu O.A. (**2009**). *Int. J. Biol. Chem. Sci*., 3(2): 271-276.
[3] Agbedahunsi, J.M. Elujoba, A.A. Makinde, J.M. Oduda, A.M.J. (**1998**). *Journal of Ethnopharmacology* 67, 321–325.
[4] Asaolu, M.F. Oyeyemi, O.A. Olanlokun, J.O. (**2009**). *Asian Network for Scientific Information Journal*. 234-239
[5] Ayoola, G.A. Coker, H.A.B. Adesegun, S. A, Adepoju-Bello, A. A. Obaweya, K. Ezennia, E.C. Atangbayila, T.O. (**2008**). *Trop J Pharm. Res*. 7(3) 1019-1024.
[6] Calvalho, L.H. Brandao M.G.L. Santos-Filho D, Lopes J.L.C. Krettli A.U. (**1991**). *Br. J. Med. Biol. Res.*, 24: 1113-1123.
[7] Chapman, K.R. & Chomchalow, N. (**2004**). Production of medicinal plants in Asia: 33-42. *In:* Batugal, P. A., Kanniah, J., Lee S. Y. & Oliver, J. T. (eds.) *Medicinal Plants Research in Asia, Volume 1: The Framework and Project Workplans.* International Plant Genetic Resources Institute – Regional Office for Asia, the Pacific and Oceania (IPGRI-APO), Serdang, Selangor DE, Malaysia.
[8] Daneshvar. C, Davis, T M. E, Cox-Singh J, Rafa'ee M. Z, Zakaria S. K, Divis P. C. S, and Singh.B, (**2009**) *Clinical Infectious Diseases* 49:852–60 Infectious Diseases Society of America. 1058-4838.
[9] Dahanukar, S.A. Kulkarni, F.A. Rege, N.N. (**2000**). *Indian Journal of Pharmacology* 32, 81–118.
[10] David, A.F. Philip, J.R. Simon, L.C. Reto, B. Solomon, N. (**2004**). *Nature Reviews*, 3: 509-520.

[11] Dow G.S. Reynoldson J.A. Thompson R.C. (**1998**). *Exp. Parasitol*. 88: 154-156.
[12] English, M.C. Waruiri, C. Lightowler, C. Murphy, S.A. Kirigha, G. Marsh, K. (**1996**). *Arch. Dis. Childhood*, 74: 201-205.
[13] Irvine, F.R. (1961). Woody Plants of Ghana with special reference to their uses. Oxford University Press, London, United Kingdom. 868 pp.
[14] Jutamaad, N.S. Aimmon, S, Yodhtai, T. (**1998**). *Thai J. Phytopharmacy*. 5(20): 14-27.
[15] Kumer, S. Baker, K. Seger, D. (**2002**). *Clinical Toxicology.* 2002, 40:599-673
[16] Lorke, D. (**1983**). *Arch Toxicol*; 54:275-289.
[17] Odetola, A. and Bassir, O. (**1980**). Evaluation of Antimalarial properties of some Nigerian Medicinal Plants. In: Sofowora, A.(editor) *Proceeding of African Bioscience Network,* Fed. Min. of Science and Tech., Nigeria Soc. Of Pharmacognosy and Drug Res. And Production Unit, University of Ife organized Workshop, Ife; 295-298
[18] Okokon, J.E. Ita, B.N. Udokpoh, A.E. (**2006**). *Annals of Tropical Medicine and Parasitology* 100, 585–590.
[19] Pedroni, H.C. Bettoni, C.C Spalding, S.M. Costa, T.D. (**2006**). *Exp. Parasitol*., 113: 193-196.
[20] Peter, I.T., Anatoli, V.K. (**1998**). The current global malaria situation. Malaria parasite biology, pathogenesis, and protection. *ASM press. WDC*. pp. 11-22
[21] Peters W. (**1965**). *Exp. Parasitol*., 17: 80-89.
[22] Pierrot, C. Adam, E. Laffite, S. Godin, C. Dive, D. Capron, M. Khalife, J. (**2003**). *Exp. Parasitol*., 104: 81-85.
[23] Ryley, J.F. Peters, W. (**1970**). *Ann. Trop. Med. Parasitol*., 64: 209-222.
[24] Saidu, K. Onah, J. Orisadipe, A. Olusola, A. Wambebe, C. and Gamaniel, K. (**2000**). *J. Ethnopharmacol*., 71: 275-280.
[25] Sucher, N.J. Carles, M.C. (**2008**). *Planta Medica*, 2008, 74(6):603–623.
[26] Thomas, A.M., Van Der Wel, A.M. Thomas, A.W. Janse, C.J. Waters, A.P. (**1998**). *Parasitol. Today*, 14: 248-249.
[27] Trease, and Evans, W.C. (**2005**). Trease and Evans Pharmacognosy, (14th ed), WB Saunders Company Limited, London, pp. 357-358.
[28] Waako, P.J. Gumede, B. Smith, P. Folb, P.I. (**2005**). *J. Ethnopharmacol.* 99:137-143.