Antiplasmodial Properties of Methanol Leaf Extract of *Laggera aurita* Linn in Experimental Animals

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ABSTRACT

Laggera aurita Linn (Asteraceae) is ethnomedicinally used in the treatment of malaria, epilepsy, fever, pain, stomatitis, bronchitis, nasal congestion, asthma and bacterial infections. This research is aimed at evaluating the antiplasmodial activity of the plant in mice. The plant extract was evaluated using three *in vivo* antiplasmodial models in mice; 4-day suppressive, prophylactic and curative tests against chloroquine-sensitive *Plasmodium berghei-berghei*. Effects of the extract on blood glucose level and hematological indices in mice infected with *P. berghei-berghei* were also evaluated. Antipyretic effect of the extract was also studied in rats using Brewer's yeast induced pyrexia. The extract exhibited significant ($p<0.05$) dose-dependent decrease in parasitaemia level in all the three models when compared to the control groups. The extract extended the mean survival of the mice beyond 21 days in a dose dependent manner. Also, the extract significantly ($P<0.05$) lowered blood glucose level with no significant ($p<0.05$) effect on hematological indices in mice infected with the parasite. In Brewer's yeast-induced pyrexia test, the extract exhibited statistical significant ($p<0.05$) reduction of pyrexia in rats. Results of this study suggested that the methanol leaf extract of *L. aurita* possesses antiplasmodial and antipyretic activities. Therefore, the study lends scientific credence to the acclaimed ethnomedicinal use of the plant in the treatment of malaria.

Keywords: *Laggera aurita,* antiplasmodial, chloroquine, *Plasmodium berghei-berghei*

INTRODUCTION

Malaria is a paradigm of diseases that affect productivity in human endeavours (Saxena *et al*. 2003). It is amongst the most prevalent infectious diseases in the World (Amelo *et al*., 2014) primarily caused by Plasmodium parasites which include *Plasmodium falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi* (WHO, 2017). An intense renaissance of malaria prevalence is as a result of increasing resistance of mosquito vectors to the currently available insecticides and the resistance of parasites, mostly *Plasmodium falciparum* to the available chemotherapeutic agents (Koudouvo *et al*., 2011). Complicated malaria is the principal cause of malaria-related deaths especially if left untreated; it presents with hypoglycemia, anemia, convulsion, acute renal failure and acute pulmonary edema amongst other symptoms (WHO, 2000).

Several plants have been used historically for the treatment of malaria such as *Laggera aurita, Cissus rotundifolia, Parkia biglobosa* and *Veroniaam bigua* (Builders *et al*. 2012). The ethnomedicinal use of *Laggera aurita* in paediatric malaria has also been reported (Odugbemi, 2008). *Laggera aurita* Linn (Asteraceae) is native to Sub Saharan Africa and is a herbaceous plant found growing as a weed in Nigeria (Burkill, 1985). Essential oils from the plant of L. aurita are used for the treatment of different diseases, such as cancer, atherosclerosis, and thrombosis (Edris, 2007). Anticonvulsant activity (Malami *et al.*, 2016), anti-inflammatory and analgesic activity (Shehu *et al*., 2016), antiviral, antibacterial and hepatoprotective properties (Egharevba *et al.*, 2010), and antinociceptive properties (Olurishe and Mati, 2014), have been reported by previous studies. Short term toxicity assessment of the plant extract demonstrated its relative safety (Julde *et al*., 2017).

MATERIALS AND METHODS

Animals

Adult Swiss albino mice (18-24g) and Wistar rats (120-150g) of either sex were procured from the animal facility attached to the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria. The animals were kept in cages in a well-ventilated room, fed on standard animal feeds with free access to water and maintained under standard laboratory conditions of temperature and light prior to study.

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Plasmodium parasite
Chloroquine-sensitive *Plasmodium berghei-berghei* was donated by the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria; maintained in mice by continuous intraperitoneal inoculation every four days into anotherfresh mice (Adzu and Salawu, 2009).

Plant collection, identification and extraction
Whole plant of *Laggera aurita* was collected from Bauchi Local Government Area in February, 2017. The plant was identified and authenticated by a botanist at the herbarium unit of the Department of Plant Biology, Bayero University Kano. The sample was compared with an already deposited specimen and voucher number (BUKHAN 0138) was given for reference.

Fresh leaf of *Laggera aurita* was shade dried at room temperature to a constant weight. The dried leaf was grounded into fine powder with the aid of pestle and mortar. 297.8g of the powdered leaf was extracted in 4 Litres of 70% v/v methanol using cold maceration method for one week with regular shaking. The resultant extract was concentrated and evaporated to dryness using water bath at temperature of 45^{o}C. The dark-brownish extract was weighed and stored in an air-tight container.

Preliminary Phytochemical Screening
Preliminary phytochemical screening of the extract was carried out as described by Trease and Evans (2002).

Determination of Median Lethal Dose
Median lethal dose (LD_{50}) was determined using Lorke's method (1983).

Antiplasmodial studies
Suppressive test
This was determined using 4-day suppressive test (David *et al*., 2004). Mice were inoculated intraperitoneally with standard inocula containing $1x10^{7}$ *P. berghei-berghei* parasite infected erythrocytes. The infected mice were distributed randomly into five groups (n=6) and treated once daily by oral route as follows: Group I received distilled water, Group II received the standard drug (chloroquine 5mg/kg), Groups III, IV and V received the extract at 600, 300 and 150 mg/kg, respectively. On day 4 (D4) of the experiment, the animals were tail-bled and blood smears were prepared using Giemsa solution. Parasitaemia level was determined by microscopically at 3 different fields.

Percentage suppression of parasite growth was thus calculated as shown in the equation below:

% Suppression of parasite=

$$100 - \left\{\frac{meanparasitaemia\ of\ treated\ animals}{meanparasitaemia\ of control(negetive)}\right\}\ x\ 100$$

Prophylactic test
This was assessed by using the method described by Adzu and Salawu (2009). Thirty mice were randomly distributed into five groups (n=6). Group I received distilled water and served as control group. Group II received the standard drug (Pyrimethamine 1.2mg/kg) while Groups III, IV and V received extract at 600, 300 and 150 mg/kg (*p.o.*) respectively for three consecutive days. On the fourth day (D4), the mice were inoculated with the standard inocula of *P. berghei-berghei*. Seventy-two hours later (D7), thin films were made from the tail blood of each mouse. The films were fixed with methanol, stained with Giemsa solution and parasitaemia level was determined microscopically.

Curative test
This was assessed using the method described by Akuodor et al., (2014). Thirty mice were divided into six groups (n=5) and each mouse was inoculated intraperitoneally with P. berghei-berghei. Seventy-two (72) hours after (D4), thin films were made from the blood of each mouse and baseline parasitaemia level was determined. Group I received distilled water (negative control) while Groups II and III received artesunate (2 mg/kg) and chloroquine (5 mg/kg). Mice in Groups IV, V and VI received 600, 300 and 150 mg/kg (p.o.) of the extract respectively. Extract treatment continued daily until the seventh day (D7) when thin films were made from the blood of each mouse (Chandel and Bagai, 2010). Mice were monitored for 28 days and mean survival period was determined for each group.

Effect of the Methanol Leaf Extract of*Laggeraaurita* on Blood Glucose Level and Hematological Indices in mice infected with *P. berghei-berghei*
Thirty mice were used and baseline blood glucose level of each was determined. They were divided into five groups (I-V) of 6 mice each. Group I served as control and Groups II-V were infected with the parasites. On day 4, groups III, IV and V were treated with the extract at 600, 300 and 150 mg/kg (*p.o.*) respectively. Extract treatment, blood glucose monitoring using glucometer and glucose strips, continued daily until the tenth day of the study. On

day 10, the animals were sacrificed and blood samples were taken and analyzed using hematology analyzer.

Anti-pyretic Study

Thirty Wistar rats were divided into five groups (n=6) and baseline rectal temperature was recorded. Rats were fevered by injecting 20 mg/kg of 15% suspension of Brewer's yeast subcutaneously (Burne and Alpermann, 1983). After 18 hours, animals that showed an increase of at least 1°C in rectal temperature were selected. Distilled water (control), aspirin (300 mg/kg) and extract at doses of 600, 300 and 150 mg/kg, were administered orally, each respectively. Rectal temperature of each rat was recorded by digital thermometer at 0, 1, 2, 3 and 4 h post treatment.

Statistical analysis

Data were analyzed using SPSS statistical software (V.23). Results were expressed as mean±SEM; presented as tables and charts. Analysis for difference between means was carried out using one way analysis of variance (ANOVA) followed by least significant difference (LSD) post hoc test. P-Values of $p < 0.05$ were considered statistically significant.

RESULTS

Phytochemical screening

Preliminary phytochemical screening of the methanol leaf extract of L. aurita revealed the presence of alkaloids, saponins, flavonoids, cardiac glycosides, carbohydrates, steroids, terpenoids, and tannins.

Acute toxicity study

The intraperitoneal median lethal dose (LD_{50}) values of the extract in both rats and mice were found to be 2,154mg/kg and the oral median lethal dose (LD50) values in both rats and mice were found to be greater than 5,000mg/kg respectively.

Table 1: Suppressive effect of the methanol leaf extract of *L. aurita* in mice infected with *Plasmodium bergheiberghei*

Treatment	Dose (mg/kg)	Mean Parasitemia Density (D4)	%Suppression
Distilled water	10 ml/kg	24.02±3.42	
Chloroquine	(5)	5.80±0.51*	75.9
MLLA	(600)	6.98±1.36*	70.9
MLLA	(300)	8.94±1.17*	62.8
MLLA	(150)	12.68±1.78*	47.2

Values presented as Mean±SEM, and percentage, n=6, * significantly different from control at p<0.05 using 0ne-way ANOVA and LSD Post Hoc Test. MLLA= Methanol Leaf Extract of *Laggera aurita*, D4 indicates day 4.

Table 2: Prophylactic effect of the methanol leaf extract of *L. aurita* in mice infected with *P. bergheiberghei*

Treatment	Dose (mg/kg)	Mean Parasitaemia Density (D7)	% Prophylaxis
Distilled water	10 ml/kg	22.88±3.92	
Pyrimethamine	(1.2)	5.95±0.92*	74.0
MLLA	(600)	9.23±1.23*	59.7
MLLA	(300)	11.50±1.93*	49.7
MLLA	(150)	18.27±2.58	20.2

Values presented as Mean±SEM, and percentage, n=6, * significantly different from control at p<0.05 using 0ne-way ANOVA and LSD Post Hoc Test. MLLA= Methanol Leaf Extract of *Laggera aurita*, D7 indicates day 7.

Table 3: Curative effect of the methanol leaf extract of *L. aurita* in mice infected with *P. bergheiberghei*

Treatment	Dose (mg/kg)	Mean Parasitaemia Density		% Clearance	Mean Survival (Days)
		D4	D7		
Distilled water	10 ml/kg	16.00±5.65	26.17±3.79		14.83±3.61
Artesunate	(2)	17.00±2.41	4.77±1.56*	81.8	27.83±1.67*
Chloroquine	(5)	17.38±1.70	5.10±1.15*	80.5	27.67±0.33*
MLLA	(600)	18.78±2.65	6.06±1.21*	76.8	26.67±1.33*
MLLA	(300)	15.17±2.70	9.95±1.54*	62.0	23.33±3.00*
MLLA	(150)	13.88±2.21	10.68±1.94*	58.2	21.33±3.78

Values presented as Mean±SEM, and percentage, n=6, * significantly different from control at p<0.05 using 0ne-way ANOVA and LSD Post Hoc Test. MLLA= Methanol Leaf Extract of *Laggeraaurita*, D4 and D7 indicate day 4 and day 7 respectively.

Suppressive test

The extract exhibited significant (p<0.05) dose dependent reduction in the mean parasitemia density at the doses (600, 300 and 150mg/kg) employed in the study. The extract at 600mg/kg caused 70.9% suppression as against 62.8% and 47.2% suppression caused by 300mg/kg and 150mg/kg respectively. However, the standard drug (chloroquine 5mg/kg) caused a suppression of 75.9% (Table 1).

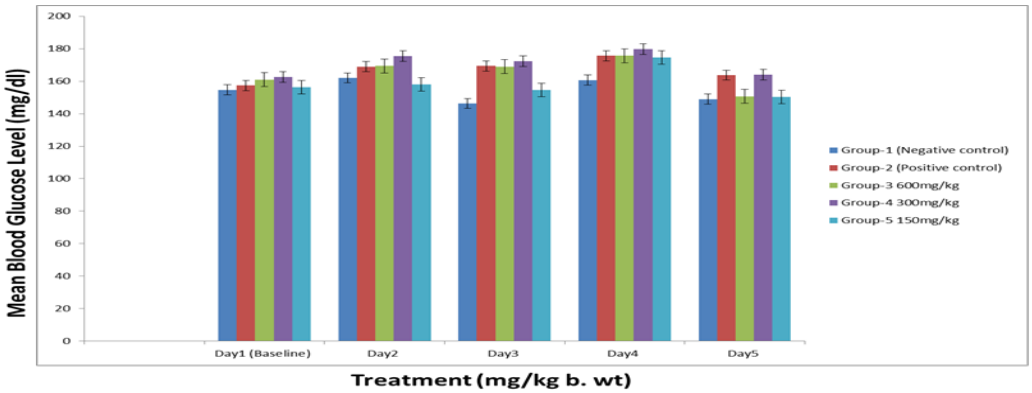

Figure 1a: Effect of the Methanol Leaf Extract of *L. aurita* on Mean Blood Glucose Level in Mice Infected with *P. berghei-berghei* parasite.
Values presented as Mean±SEM, n=6, * significantly different from control at p<0.05 using One-way ANOVA and LSD Post Hoc test for comparison among groups using SPSS software (V. 23). *= comparing with positive control, *=comparing with negative control, b. wt = body weight.

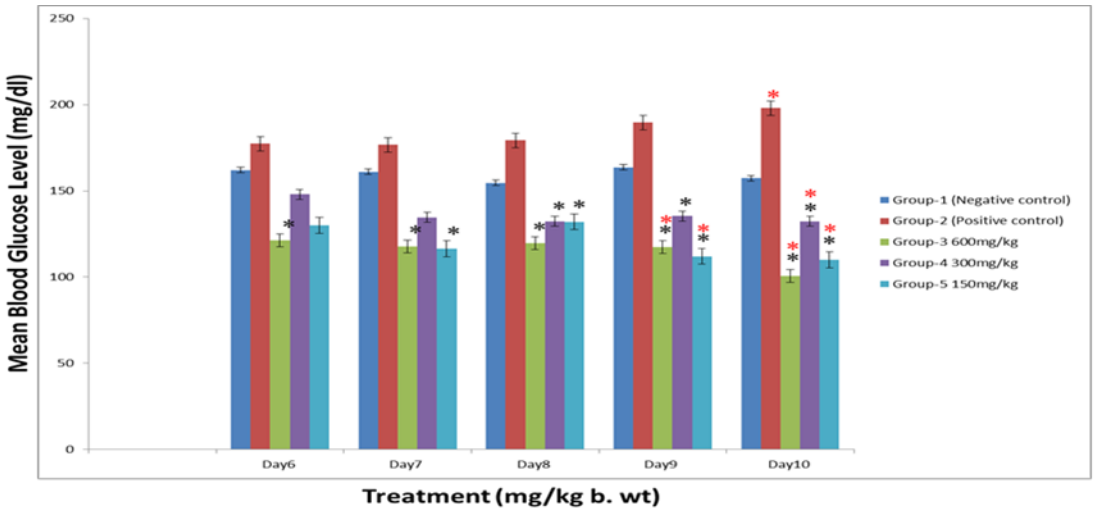

Figure 1b: Effect of the Methanol Leaf Extract of *L. aurita* on Mean Blood Glucose Level in Mice Infected with *P. berghei-berghei* parasite.
Values presented as Mean±SEM, n=6, * significantly different from control at p<0.05 using One-way ANOVA and LSD Post Hoc test for comparison among groups using SPSS software (V. 23). *= comparing with positive control, *=comparing with negative control, b. wt = body weight.

Prophylactic test

The extract (600 & 300mg/kg) and standard drug (Pyrimethamine 1.2mg/kg) caused a statistically significant (p<0.05) decrease in the mean parasitaemia density when compared with the distilled water control. The extract at 600mg/kg caused a prophylaxis of 59.7% as against 49.7% and 20.2% caused by 300mg/kg and 150mg/kg respectively. However, the standard drug (Pyrimethamine 1.2mg/kg) produced a prophylaxis of 74.0% (Table 2).

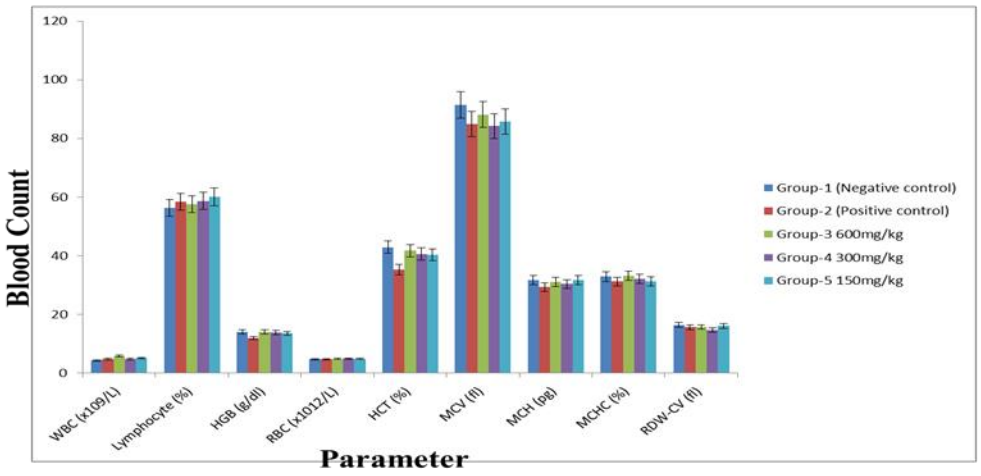

Figure 2: Effect of the Methanol Leaf Extract of *L. aurita* on Hematological Indices in Mice Infected with *P. berghei-berghei* parasites.

Values presented as Mean±SEM, n=6, * significantly different from control at p<0.05 using 0ne-way ANOVA and LSD Post Hoc Tests.

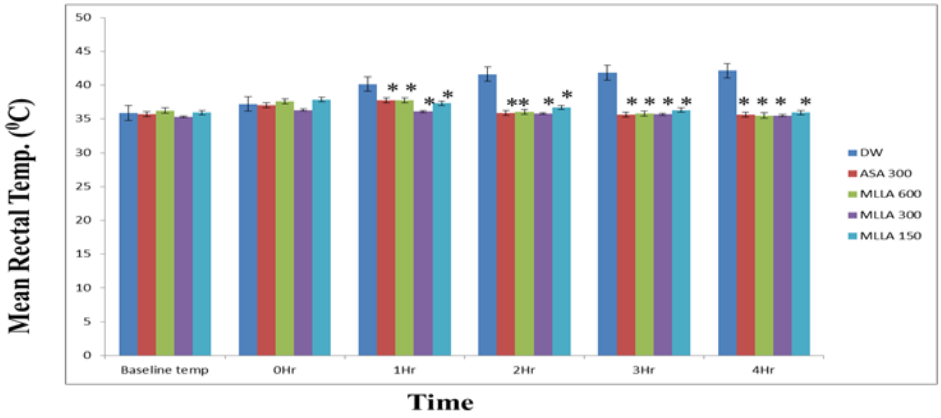

Figure 3: Antipyretic Effect of The Methanol Leaf Extract of *L. aurita* in Adult Rats

Values presented as Mean±SEM, n=6, * significantly different from control at p<0.05 using 0ne-way ANOVA and LSD Post Hoc test for comparison among groups using SPSS software (V. 23). MLLA = Methanol leaf extract of *L. aurita*, ASA = Acetyl Salicylic Acid, DW = Distilled water, Hr = Hour.

Curative test

There was a statistically significant (p<0.05) reduction in the mean parasitaemia density of the treated groups when compared with the distilled water control group. The extract at 600mg/kg produced a clearance of 76.8% as against 62.0% and 58.2% produced by 300mg/kg and 150mg/kg respectively. However, the standard drugs (Artesunate 2mg/kg and Chloroquine 5mg/kg) produced comparable clearance of 81.8% and 80.5% respectively. The extract at 600mg/kg, 300mg/kg and 150mg/kg extended the mean survival of the animals to 27, 23 and 21 days respectively. While, the standard drugs (Artesunate 2mg/kg and Chloroquine 5mg/kg) treated groups survived the 28-day period of the study (Table 3).

Effect of the extract on mean blood glucose level in mice infected with *P. berghei-berghei* parasite.

There was no statistically significant (p<0.05) increase in the mean blood glucose level of the study groups from day-1 to day-5 compared to the negative control group (Figure 1a). Upon initiating treatment on day-4, there was a statistically significant (p<0.05)

decrease in the mean blood glucose level of the extract treated groups from day-6 to day-10 compared to the positive control group (Figure 1b). There was also a statistically significant ($p<0.05$) decrease in the mean blood glucose level of the extract treated groups from day-9 to day-10 compared to the negative control group (Figure 1b). A statistically significant ($p<0.05$) increase in the mean blood glucose level of the positive control group was also noticed on day-10 of the study compared to the negative control group (Figure 1b).

Effect of the extract on hematological indices in mice infected with *P. berghei-berghei* parasite.

Generally, there was a decrease in HGB (g/dl), RBC (x1012/L), HCT (%), MCV (fl), MCH (pg), MCHC (%) & RDW-CV values in the positive control group that are not significantly ($p<0.05$) different from the negative control and extract treated groups. There was increase in the WBC (x109/L) and Lymphocytes (%) of the positive control which are not significantly ($p<0.05$) different from the negative control group (Figure 2).

Antipyretic effect of the methanol leaf extract of *L. aurita* in adult rats

The effect of the methanol leaf extract of *Laggera aurita* plant on yeast-induced pyrexia in adult albino rats is as shown in figure 3. Treatment with the extract at doses of 600mg/kg, 300mg/kg & 150mg/kg body weight and aspirin (ASA) at 300mg/kg body weight significantly ($p<0.05$) decreased the mean rectal temperature of yeast-induced pyrexia in rats. The results obtained from both standard and extract treated groups were compared with the negative control group. A significant ($p<0.05$) reduction in the yeast-induced elevated mean rectal temperature was observed in the standard drug (Figure 3).

DISCUSSION

Preliminary phytochemical screening of the methanol leaf extract of *L. aurita* revealed the presence of alkaloids, saponins, flavonoids, cardiac glycosides, steroids, terpenoids and tannins. These phytochemicals are responsible for various biological activities of plants (Shabbir *et al*., 2013).

The intraperitoneal median lethal dose (LD_{50}) values of the extract in both rats and mice were found to be 2,154 mg/kg while the oral median lethal dose (LD_{50}) values in both rats and mice were found to be greater than 5,000 mg/kg respectively.

Plasmodium berghei-berghei is used in evaluating potential antiplasmodial agents in rodents (Pedroniet al. 2004) and the species induce infection comparable to that of human plasmodial infection (Kumar et al. 2006). The antiplasmodial effects of the methanol leaf extract of *L. aurita* showed significant ($p<0.05$) dose-dependent prophylactic, suppressive and curative activities in mice infected with P. berghei-berghei. Efficacy of the plant extract to suppress the development of early plasmodial infection may be due to the effectiveness of the extract to act on the primary tissue forms of the parasite (tissue schizonticidal) and thus could contain potential agents for casual prophylaxis (Peter, 1980). This further demonstrated the sensitivity of the parasite to the extract and possible large volume of distribution of the extract (Henry and Sanjeev, 2011). Additionally, the increased survival time observed in the extract treated groups might have resulted from clearance of the parasite (Ryler and Peters, 1970). There was a statistically significant ($p<0.05$) decrease in the mean blood glucose level of the extract treated groups from day 6-10 compared to the positive control group (parasites infected group). There was also a statistically significant ($p<0.05$) decrease in the mean blood glucose level of the extract treated groups from days 9-10 compared to the negative control group. The decrease in the blood glucose level observed in the extract treated groups after initiating treatment could either be due to eradication of the *P. berghei-berghei* or/and the extract has potential hypoglycemic activity.

According to World Health Organization, anaemia is established when haemoglobin level or haematocrit and other red blood cell indices such as average red blood cell size (MCV), haemoglobin amount per red blood cell (MCH) and haemoglobin concentration per red blood cell (MCHC) are low (Roger and Cate, 2012). There was no statistical significant ($p<0.05$) difference in the haematological indices of the extract treated groups as compared with the negative and positive control groups.

Pyrexia is induced by inflammatory mediators such as prostaglandins E2 (Ashok et al. 2010). Therefore, drugs acting as antipyretic agents block the synthesis or actions of these inflammatory mediators. The significant ($p<0.05$) antipyretic activity exhibited by the extract against yeast-induced elevation of body temperature could be attributed to the flavonoids content of the plant which target prostaglandin involved in pyrexia (Rajnarayana et al., 2006).

CONCLUSION

The methanol leaf extract of *Laggera aurita* possesses antiplasmodial and antipyretic activities. Therefore, this study lends scientific credence for the acclaimed ethnomedicinal use of the plant in the treatment of malaria.

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REFERENCE

Adzu B, Salawu OA (2009). Screening Diospyrosmespiliformisextract for antimalarial potency. Int J BiolChemSci, 3(2):271-276.

Amelo W, Nagpal P, Makonnen E (2014). Antiplasmodial activity of solvent fractions of methanolic root extract of Dodonaeaangustifolia in *Plasmodium berghei* infected mice. Complementary and Alternative Medicine, 14:462

Akuodor GC, Ajoku GA, Ezeunala MN, Chilaka KC, Asika EC (2014). Antimalarial potential of the ethanolic leaf extract of *Pseudocedralakotschyi*. *Journal of Acute Disease*, 9(14)60077-9.

Ashok BK, Ravishankar B, Prajapati PK, Bhat SD (2010). Antipyretic Activity of *Guduchi Ghrita* Formulations in Albino Rats. *An International Quarterly Journal of Research in Ayurveda*, 31(3): 367–370.

Builders MI, Isichie CO, Aguyi JC (2012). Toxicity Studies of the Extract of Parkiabiglobosain rats. *Br J Pharm Res*, 2(1):1-16

Burkill HM (1985). The useful plants of West/Tropical Africa.Royal Botanic Gardens Kew, surrey. 1:452-453

Burne K,Alpermann H (1983). Non-acidic pyrazoles: Inhibition of prostaglandin production, carrageenino edema and yeast fever. *Agent Action*, 13:360–3

Chandel S, Bagai U (2010). Antiplasmodial activity of Ajugabracteosa against *Plasmodium berghei* infected BALB/c mice. *Indian J Med Res*, 131:440-444

Edris AE (2007). Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. *Phytotheraphy Research*, 21(4): 308–23.

Egharevba OH, Oladosu P, Okhale ES, Ibrahim I, Folashade KO, Okwute KS (2010). Preliminary anti-tuberculosis screening of two Nigerian Laggera species (*Laggera pterodonta* and *Laggera aurita*. *Journal of Medicinal Plant Research*, 4(12): 1235–1237.

Henry MS, Sanjeev KL (2011). Treatment and prevention of malaria: antimalarial drug chemistry, action and use: Springer Verlag.Pp.45. ISBN9183034604796

Julde S, Kyari H, Malami S (2017). Assessment of Serum Parameters after 28-day Oral Daily Administration of Methanol Leaf Extract of Laggeraaurita (Linn) in Wistar Rats. *Trop J Nat Pro Res*, 1(6):251-254

Koudouvo K, Karou DS, Kokou K (2011). "An ethnobotanical study of antimalarial plants in Togo Maritime Region," *Journal of Ethnopharmacology*, 134(1):183–190

Kumar KA, Singn S, Babu PP (2006). Studies on the glycoprotein modification in erythrocyte membrane during experimental cerebral malaria. *Exp Parasitol*, 114: 173-179

Lorke D (1983). A new approach to practical acute toxicity testing. *Archives of toxicology*, 54:275-287.

Malami S, Kyari H, Danjuma NM, Ya'u J, Hussaini IM (2016). Anticonvulsant properties of methanol leaf extract of *Laggera aurita* Linn (Asteraceae) in laboratory animals. *Journal of Ethnopharmacology*, 191:301-6

Odugbemi T (2008). Medicinal Plants from Nigeria.1. Lagos Nigeria: University of Lagos press; p. 581.

Olurishe TO, Mati, FG (2014). Anti hyperalgesic potentials of Laggera aurita in Swiss Albino mice. *Pakistan Journal of Pharmaceutical Sciences*, 27(1): 169–72.

Pedroni HC, Bettoni CC, Spalding SM, Costa TD (2006). *Plasmodium berghei* development of an irreversible experimental malaria model in wistar rats. *Experimental Parasitology*, 113:193-196

Peter W (1980). Antimalarial Drug Resistance. *British Medical Bulletin*, 32:187-192

Rajnarayana K, Reddy MS, Chaluvadi MR (2006). Bioflavonoids classification, Pharmacological,

Biochemical Effects and Therapeutic Potential, *Indian J. of Pharmaceutical Sciences*, 68(3):380-384

Roger W, Cate W (2012). Clinical Pharmacy and Therapeutics. Churchill Livingstone Elsevier, London, Pp. 769

Ryler JF, Peters W (1970). The Antimalarial Activity of Some Quinolone Esters. *American Tropical Journal of Medicine and Parasitology*, 84:209-22

Saxena S, Pant N, Jain DC, Bhakuni RS (2003), "Antimalarial agents from plant sources," *Current Science*, 85(9):1314–1329

Shabbir M, Muhammad RK,Naima S (2013). Assessment of Phytochemicals, antioxidant, anti-lipid peroxidation and antihemolytic activity of extract and 81 various fractions of Mytenusroyleanus. *BMC Complementary and Alternative Medicine*, 13:143-145

Shehu A, Olurishe TO, Zezi AU, Ahmed A (2016). Acute toxicological, analgesic and anti-inflammatory effects of Laggera aurita Linn (compositae) in mice and rats. *African Journal of Pharmacology and Therapeutics*, 5(2): 65-73.

Trease GE, Evans MC, (2002). Phytochemistry In: Textbook of Pharmacognosy. Fourth edition. WB Sanders Company Ltd. London, UK, Pp 224-343.

World Health Organization (2017). Malaria: Available at: http://www.who.int/mediacentre/factsheets/fs094/en/ Accessed on 22nd August, 2017.

WHO: Severe falciparum malaria (2000). World Health Organization, Communicable Diseases Cluster. *Trans R Soc Trop Med Hyg*, 94(Suppl 1): S1-S90