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Original article

Evaluation of the antiplasmodial activities of methanol leaf extract of *Andrographis paniculata* (burm. f.): An *in vitro* and *in vivo* study

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ABSTRACT

Background: *Andrographis paniculata (A. paniculata)* has been exploited for its ethnomedicinal properties as an antimicrobial agent across decades. The antiplasmodial efficacy of *Andrographis paniculata* methanol leaf extract was established in this study. **Methods:** Forty-two mice were divided into 7 treatment groups. Groups 1 and 7 served as negative and positive control respectively, while groups 2 – 6 were treated with different concentrations of the extract. The parasites were also cultured in Roswell Park Memorial Institute (RPMI) 1640 medium, and treated with different concentrations of the extract. **Results:** This study showed a significant reduction in the parasitaemia level in the blood of mice treated with the extract. The different concentrations of the extract also had a positive effect on some haematological parameters. The white blood cell counts changed significantly with concentration of 10, 50, 100 and 500mg/ml. Also, there was significant increase in the packed cell volume (31.00±0.00, 36.33±0.58, 38.33±0.58 and 46.67±0.58%) at concentration of 10, 50, 100 and 500mg/ml respectively, while the untreated group remained low. The extract displayed a significant antiplasmodial activity against *Plasmodium berghei (P. berghei)* in vitro at different concentrations of 5, 10, 20, 30, 40 and 50mg/ml with plasmodial load of 1.8%, 1.21%, 0.68%, 0.51%, 0.44% and 0.26% respectively compared with chloroquine and untreated samples that had 0.21% and 4.0% parasitized erythrocytes. **Conclusion:** This study has demonstrated the in vivo antiplasmodial efficacy of *A. paniculata* methanol leaf extract. Hence, the study recommends that the leaf extract of *A. paniculata* should be considered for production of novel antimalarial drugs.

Introduction

As indicated by the World Health Organization (WHO) in 2019, Nigeria represented the most prominent region of malaria incidence in Africa cases (25 %) [1]. Five species of Plasmodium are recognized to cause human infections: *Plasmodium falciparum, P. vivax, P. ovale, P. malariae*, and *P. knowlesi* [2,3]. Most fatalities (about 91 %) happen in Africa, and are a result of infection by *P. falciparum*. Be that as it may, malaria is as yet responsible for half a million deaths yearly and the case casualty of severe malaria stays high. Resistance among the parasites has developed to a few antimalarial medications; for instance, chloroquine-resistant *P. falciparum* has spread to most malarial endemic regions, and artemisinin

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resistance has turned into a major problem in certain parts of Southeast Asia [4,5]. This calls for tried and trusted ethnobotanical solutions through the exploitation of medicinal plants.

Andrographis paniculata (A. paniculata) (Burm. f.) Nees is an important pharmaceutical plant and is commonly used globally. It belongs to the family Acanthaceae. *Andrographis paniculata* is used as a traditional homegrown medicine in most Asian nations [6-8]. Its medicinal properties have been attributed to the presence of different phytochemical constituents, mainly lactones, diterpenoids, diterpene glycosides, flavonoids, and flavonoid glycosides [9]. The plant has been reported to show various degrees of biological activities in *in vivo* as well as *in vitro* assays for its antimalarial potential [10-13]. In this study, we evaluated *in vivo* antiplasmodial activities of *A. paniculata* methanol leaf extract on experimental mice, with a particular focus on the changes in the haematological parameters, and also carried out this study *in vitro* with the use of RPMI 1640 culture medium.

Hence, the evaluation of the biological activities of *A. paniculata* methanol extract on *Plasmodium berghei (P. berghei)* -induced mice was ascertained in this study.

Materials and Methods

Collection of plant material

The leaves of *A. paniculata* were collected at the Obakekere campus of the Federal University of Technology, Akure, (FUTA), Nigeria where they were growing naturally, and then authenticated at the Department of Crop, Soil and Pest Management, FUTA, Nigeria.

Preparation of *A. paniculata* plant extract

The air-dried leaf samples of *A. paniculata* were powdered using a Marlex blender (Electroline model IS 4780, CM/L 7902804) for extraction. The powdered materials were extracted using methanol. Three hundred grams (300 g) of the grinded samples were weighed into three different containers and labeled appropriately after which 2000 ml of the solvent was added, covered and shaken. The mixtures were left for about 72 hours, after which the solvents along with the extracts were drained out with muslin cloth, filtered with Whatman filter paper. The semi-solid extracts were obtained *in vacuo* using a rotary evaporator (RE-52A Union Laboratories, England). One gram of the methanol extract was dissolved in 10ml of 30 % Dimethyl sulfoxide (DMSO).

In vivo antiplasmodial analysis (Curative assay)

Ethical approval was sort and obtained from the Centre for Research and Development (CERAD) at the Federal University of Technology, Akure. The *in vivo* experiment was then carried out following the Animal Research Reporting of *In vivo* Experiments (ARRIVE) guidelines. Forty-two (42) healthy male and female mice (weighing 18-20 g) were obtained from the animal house of the Department of Animal Production and Health, FUTA, Nigeria. The mice were allowed to adapt for 7 days and were subsequently screened for the absence of malarial infection. The animals were nurtured under standard environmental conditions of dark/light cycle (12:12 hours), at room temperature (25 ± 2 °C) and a humidity of 60 ± 5%. The mice received a standard laboratory diet nutritionally adequate for their metabolism. Likewise, *P. berghei*-infected donor mice with rising parasitaemia count were obtained from the Department of Animal Production and Health, FUTA, Nigeria, and blood was obtained into heparin bottles through cardiac puncture using sterile needles and syringes.

Experimental design

The curative test was carried out as described by **Christian et al**. [14]. On the first day of the experiment, 42 mice were administered intraperitoneally with a standard inoculum of 1×10^7 *P. berghei* infected erythrocytes. After 72 hours, the mice were randomly divided into 7 groups of 6 mice per cage.

Group 1: Negative control group

Mice received 0.2 ml of normal saline

Group 2 – 6: Test groups

Mice in these groups received 10, 50, 100, 500 and 1000 mg/kg of the *A. paniculata* methanol leaf extract respectively.

Group 7: Positive control group

Mice in group 7 received 10 mg/kg of chloroquine diphosphate.

All doses were administered orally. Treatment continued every day until the fifth day when thin films were made from the blood of each mouse. At the end of the experiment, the mice were anesthetized with diethyl ether and sacrificed by cervical dislocation and the blood samples were then obtained through cardiac puncture for haematological tests.

Determination of plasmodial load in the blood samples mice

Collection of blood samples, staining and parasite counts were carried out using the method of **Adu-Gyasi et al**. [15]; **Flores-Garcia et al**. [16]. Thick and thin blood films were prepared on microscope slides, stained with 10 % Giemsa (v/v) and examined under the microscope.

Haematological analysis

Packed cell volume (PCV) was determined by micro-hematocrit technique using a capillary tube as described by **Schalm et al**. [17]. Red blood cell (RBC) and white blood cell (WBC) counts were determined as described by **Chan et al**. [18]. Differential leucocytic counts (DLC) and the haemoglobin (Hb) concentration (determined using the cyano-methemoglobin method) were as described by **Angelo et al**. [19]. Haematological indices such as the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from determined values of PCV, RBC and HB as described by **Tayebi et al**. [20].

***In vitro* antiplasmodial analysis**

Plasmodium berghei collected from malarial positive mice were cultured using the method of **Trager and Jensen** [21]; **Wahyuni et al**. [22]. Briefly, the parasites were cultured in 7 ml Roswell Park Memorial Institute (RPMI) 1640 medium with 5% serum, 10% haematocrit, hypoxanthine, and gentamicin (complete medium) in 75 ml flasks. The medium was changed every 48 hours and flasks were incubated at 37 °C with 5% CO_2, 5% O_2, and 90 % N_2 at 0.5 % heamatocrit. This suspension was then added into different flasks labelled A to H.

A= untreated parasitized,
B= treated with 5 mg/ml of extract,
C= treated with 10 mg/ml of extract,
D= treated with 20 mg/ml of extract,
E = treated with 30 mg/ml of extract,
F= treated with 40 mg/ml of extract,
G= treated with 50 mg/ml of extract,
H= treated with 2.5 mg/ml of chloroquine

The flasks were incubated at 37^{o}C with 5% CO_2, 5% O_2, and 90% N_2 at 0.5% heamatocrit, after 72 hours, the smear was prepared and stained with Giemsa stain for determination of percentage parasitemia and viewed under the microscope.

Analysis of data

Data obtained were subjected to one-way analysis of variance while the means were compared by Duncan's New Multiple Range Test at 95 % confidence interval using Statistical Package for Social Sciences (SPSS) version 23.0. Differences were considered significant at $p<0.05$.

Results

Effect of treatment on the parasitaemial count of infected mice

Table 1 shows the antiplasmodial activity of the *A. paniculata* methanol leaf extract. There was reduction in the percentage parasitaemial level of mice treated with methanol extract of *A. paniculata.* The parasitaemia level of the infected mice reduced significantly ($p<0.05$) with days of administration and is dose-dependent. Also, the extract at 500 and 1000 mg/ml competed favourably with chloroquine by reducing the parasites count to zero at day 1.

Effect of treatment on the haematological parameters of *P. berghei*-infected mice

Haematological parameters of *P. berghei* infected mice treated with varying concentrations of *A. paniculata leaf extract* are shown in **table (2)** at treatment concentrations of 50, 100, 500 and 1000 mg/ml. The results revealed that the extract maintained the PCV of the infected mice at all concentrations compared with the untreated group which has a reduction in PCV. Also, the value of WBC increases as the concentrations of extract increased except at high dose (1000 mg/kg). Differential WBC revealed high neutrophil in the treated groups.

***In-vitro* antiplasmodial activity of *A. paniculata* leaf methanol extract**

In-vitro antiplasmodial activity of *A. paniculata* extract is shown in **figure (1)**. The extract displayed a good antiplasmodial activity against *P. falciparum* at 5, 10, 20, 30, 40 and 50 mg/ml with plasmodial load of 1.8%, 1.21%, 0.68%, 0.51%, 0.44% and 0.26% respectively compared with chloroquine (2.5 mg/ml) and untreated samples that have 4.00% and 0.21% parasitized erythrocytes. Likewise, an increase in chemosuppression was observed with an increase in the concentration of the extract. At the lowest concentration of 5 mg/l, the % chemosuppression was at 55 % while at a concentration of 50 mg/ml, the % chemosuppression was 93.5%. The highest concentration of the extract (50 mg/ml) competed favourably with 2.5 mg/ml of

chloroquine which was used as the drug control and had a % chemosuppression of 94.75%.

Table 1. Antiplasmodial activity of the *Andrographis paniculata* methanol leaf extract.

Drug/extracts	First day	Second day	Third day	Fourth day	Fifth day
Distil water	5.53±0.06^{e}	6.00±0.10^{e}	6.73±0.06^{e}	7.40±0.10^{e}	9.60±.10^{e}
10mg/ml	3.90±0.00^{d}	2.73±0.06^{d}	2.20±0.10^{d}	1.87±0.6^{d}	1.63±0.06^{d}
50mg/ml	2.13±0.06^{c}	1.80±0.00^{c}	1.50±0.00^{c}	1.30±0.00^{c}	1.10±0.00^{c}
100mg/ml	1.17±0.06^{b}	0.80±0.00^{b}	0.53±0.06^{b}	0.33±0.58^{b}	0.13±0.05^{b}
500mg/ml	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
1000mg/ml	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Chloroquine	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

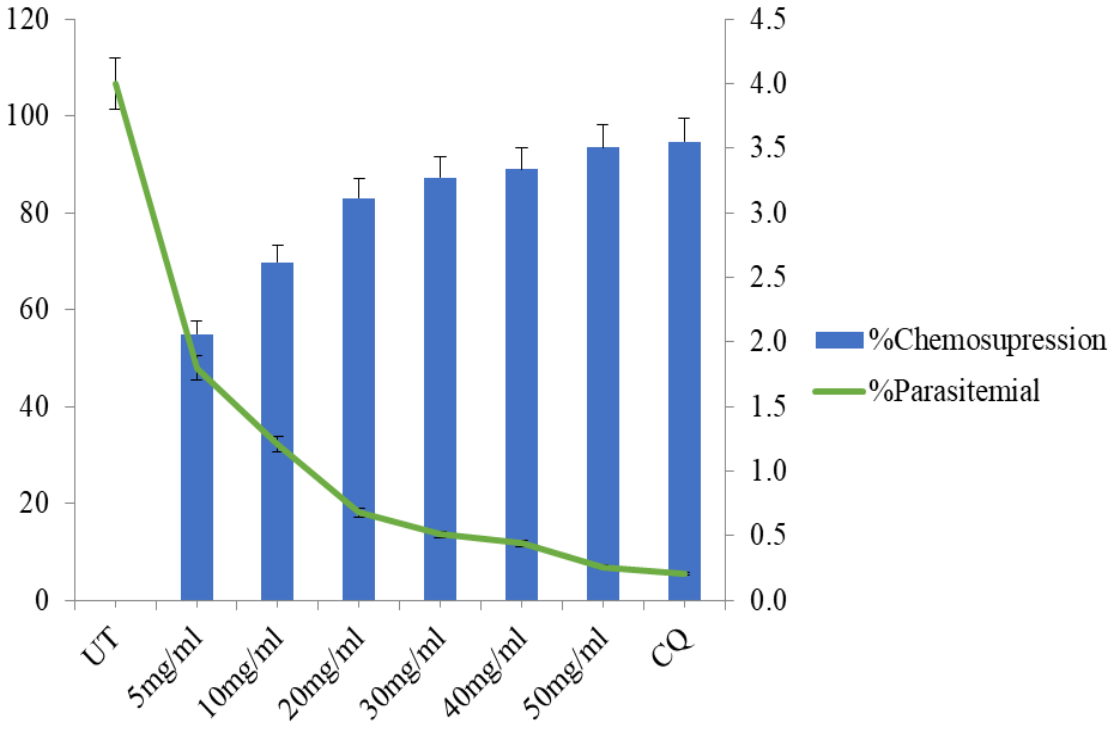
Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same row are not significantly different (p<0.05).

Table 2. Haematological parameters of *P. berghei*-infected mice treated with different concentrations of *Andrographis paniculata* methanol leaf extract.

Parameter	Not Infected	Infected, treated with chloroquine	Infected not treated	10 mg/ml	50 mg/ml	100 mg/ml	500 mg/ml	1000 mg/ml
PCV (%)	40.33±0.58^{d}	37.33±0.58^{c}	21.00±0.00^{a}	31.00±0.00^{b}	36.33±0.58^{b}	38.33±0.58^{c}	46.67±0.58^{d}	40.33±0.58^{c}
Hb (g/L)	13.34±0.15^{d}	12.37±0.06^{c}	7.04±0.01^{a}	10.33±0.06^{b}	12.33±0.58^{b}	12.33±0.07^{b}	15.40±0.10^{d}	13.31±0.01^{c}
WBC (10^{9}/L)	5.37±0.15^{b}	3.27±0.06^{a}	5.30±0.10^{b}	3.27±0.12^{a}	7.40±0.00^{b}	8.37±0.12^{c}	6.43±0.06^{c}	5.23±0.06^{b}
RBC (10^{12}/L)	5.44±0.05^{d}	4.85±0.15^{c}	3.12±0.01^{a}	3.40±0.00^{b}	4.00±0.00^{b}	4.14±0.05^{b}	5.13±0.06^{d}	5.41±0.01^{b}
MCV (fL)	73.68±0.50^{b}	76.48±0.50^{c}	67.40±0.10^{a}	91.18±0.02^{d}	90.33±0.58^{d}	92.47±0.03^{d}	90.19±0.01^{d}	73.04±0.01^{b}
MCH (pg)	24.70±0.26^{b}	25.46±0.04^{c}	22.56±0.01^{a}	30.30±0.01^{d}	30.33±0.58^{d}	31.93±0.06^{d}	30.33±0.58^{d}	24.61±0.01^{b}
MCHC (g/dl)	33.32±0.07^{a}	33.36±0.13^{a}	33.53±0.10^{a}	33.24±0.04^{b}	33.45±0.21^{a}	33.68±0.04^{c}	33.26±0.01^{a}	33.27±0.00^{a}
N (%)	52.00±0.00^{c}	50.33±0.58^{b}	44.33±0.58^{a}	50.33±0.58^{b}	60.33±0.58^{d}	50.00±0.00^{b}	58.67±0.58^{d}	58.33±0.58^{d}
L (%)	45.33±0.58^{a}	47.00±0.00^{b}	54.33±0.58^{d}	48.33±0.58^{c}	38.67±0.58^{a}	38.33±0.58^{a}	40.33±0.58^{a}	38.33±0.58^{a}
E (%)	2.33±0.58^{b}	2.33±0.58^{b}	2.33±0.58^{b}	1.00±0.00^{a}	2.33±0.58^{a}	2.33±0.58^{a}	1.00±0.00^{a}	2.00±0.00^{a}
M (%)	1.00±0.00^{b}	1.33±0.58^{b}	0.00±0.00^{a}	1.33±0.58^{b}	0.00±0.00^{a}	3.00±0.00^{c}	1.33±0.58^{a}	2.33±0.58^{a}
B (%)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same row are not significantly different (p<0.05).
Key: PCV= packed cell volume, Hb= haemoglobin, WBC= white blood cell, RBC= red blood cell count, MCV= mean corpuscular volume, MCH= mean corpuscular haemogobin, MCHC= mean corpuscular haemoglobin concentration, N= neutrophil, L= lymphocyte, E= eosinophil, M= monocyte, B= basophil.

Figure 1: *In vitro* antiplasmodial activity of *A. paniculata methanol* extract.



Key: UT= untreated, CQ= Chloroquine (2.5 mg/ml)

Discussion

The results obtained indicate that methanol leaf extract of *A. paniculata* is an effective antiplasmodial agent against *Plasmodium* sp, and it correlates with the submission of **Chander et al**. [23]; **Obonti et al**. [24] who concluded that *A. paniculata* considerably repressed the proliferation of *P. berghei* by reactivating the key antioxidant enzyme superoxide dismutase. Methanol leaf extract from *A. paniculata* has also shown substantial inhibitory activity against *Plasmodium* and the concentration needed for 50% inhibition (IC_{50}) value was recorded at 7.2 mg/ml [25]. The reduction in the percentage parasitaemia level of mice treated with methanol leaf extract of *A. paniculata* was observed and this justifies their use as an antimalarial agent in folklore medicine. This result agrees with the findings of **Omoya and Momoh** [26] who submitted that there was a reduction in the parasitaemia level in rats treated with ethanol extract of *A. paniculata* and *C. odorata*.

When compared with the negative control, there was significant difference in the level of haematocrit/=PCV, Hb, WBC, platelets, lymphocytes, neutrophils and monocyte. The increase in the PCV of those treated with *A. paniculata* points to the efficacy of the plant as a good blood supplement. These are in agreement with the findings of **Odeghe et al**. [27]; **Suriyo et al**. [28] who reported in their review that it has been shown that ingestion of medicinal compounds or drugs can alter the normal range of haematological parameters. The increase in the haemoglobin (Hb) level of infected mice treated with *A. paniculata* correlates with reports of **Dhenge et al**. [29]; **Kusmardi et al**. [30] who both reported the significant increase in the hemoglobin level in broilers having been fed with *A. paniculata* leaves powder.

There was also an increase in the WBC also known as leucocytes of infected mice treated with *A. paniculata*. This was similar to the report of **Mathivanan and Edwin** [31], who observed that the total leukocyte count was significantly ($p < 0.05$) higher in T5 compared to the control group with no difference between the levels of *A. paniculata* [31]. Similar reports were also made by **Dhenge et al**. [29]; **Kusmardi et al**. [30] who have reported that feeding of *A. paniculata* leaves powder significantly increased the total leukocyte count as compared with control in broilers.

The result obtained in the *in vitro* analysis revealed that the antiplasmodial efficacy of the

extract is dose-dependent, as evidently seen in the percentage reduction of the plasmodial load from 1.8% at a concentration of 5 mg/ml to 0.26% at a concentration of 50 mg/ml. The antiplasmodial activity recorded in this study is similar to the findings of **Bagavan et al**. [32]; **Chauhan et al.** [33] who both tested the antiplasmodial activities of 25 extracts, out of which 6 showed good antiplasmodial activity, 15 were moderate while 4 displayed mild antiplasmodial activity. Similarly, extracts of *Alstonia congensis* root bark have been reported to demonstrate antiplasmodial activities, with the methanol extract producing 75% chemosuppression against *P. berghei* [34].

Conclusion

From the results obtained, the total plasmodial count in the blood of infected mice reduced drastically with an increase in concentration, indicating the efficiency of the extract in the potential treatment of malarial infections. This investigation has been able to identify and prove the *in vivo* and *in vitro* antiplasmodial efficacy of *A. paniculata* methanol leaf extract, hence suggesting that the leaves extracts of *A. paniculata* possess potential properties in the search for novel antimalarial drugs. Tissue culture techniques might be a good alternative to make *A. paniculata* available for further research through pharmacological and phytochemical study to find new bioactive ingredients as well as conservation of this plant.

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Author's contributions

Authors Omoya, F. o. And Ogundare, A. O. designed the study. Authors Isunu, L. E and Akharaiyi, F. C. developed the methodology and acquired the data. Author Ajayi, K. O. analysed and interpreted the data. Author Babatunde, O. J. wrote the first draft of the manuscript. All authors read and approved the final draft of the manuscript.

Conflict of intrest

The authors have declared no conflict of interest.

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References

1-**World Health Organization WHO.** World malaria report 2019. Available at : https://www.who.int/news-room/feature-stories/detail/world-malaria-report-2019

2-**Kantele A, Jokiranta TS.** Review of Cases with the Emerging Fifth Human Malaria Parasite, *Plasmodium knowlesi.* Clinical Infectious Diseases 2019; 52(11), 1356–1362.

3-**Kaur C, Pramanik A, Kumari K, Mandage R, Dinda AK, Sankar J, et al.** Renal detection of *Plasmodium falciparum*, *Plasmodium vivax* and *Plasmodium knowlesi* in malaria associated acute kidney injury: a retrospective case–control study. BMC research notes 2020; 13(1), 1-7.

4-**Caraballo H, King K.** Emergency department management of mosquito-borne illness: malaria, dengue, and West Nile virus. Emergency medicine practice 2014; 16(5), 1-23.

5-**Conrad MD, Rosenthal PJ.** Antimalarial drug resistance in Africa: the calm before the storm? The Lancet Infectious Diseases 2019; 19(10), e338-e351.

6-**Kabir MH, Hasan N, Rahman MM, Rahman MA, Khan JA, Hoque NT, et al.** A survey of medicinal plants used by the Deb barma clan of the Tripura tribe of Moulvibazar district, Bangladesh. Journal of Ethnobiology and Ethnomedicine 2014; 10(1).

7-**Akbar S.** *Andrographis paniculata:* a review of pharmacological activities and clinical effects. Alternative Medicine Review 2011; 16(1), 66-77

8-**Hossain S, Urbi Z, Karuniawati H, Mohiuddin RB, Moh Qrimida A, Allzrag AMM, et al.** *Andrographis paniculata* (Burm. f.) Wall. ex Nees: An Updated Review of

Phytochemistry, Antimicrobial Pharmacology, and Clinical Safety and Efficacy. Life 2021; 11(4), 348.

9-**Isunu LE, Omoya FO, Ogundare AO, Babatunde OJ, Bayode MT, Ajayi KO.** Antibacterial activity of *Andrographis paniculata* (burm. F.) Methanol leaf extract on bacteria consortia isolated from blood of diabetic patients. Bacterial Empire 2022; 5(1), e381.

10-**Siti Najila MJ, Noor RA, Mohamad Kamel AG, Syed Zahir SI, Khozirah S, Lokman HS, et al.** The screening of extracts from *Goniothalamus scortechinii*, *Aralidium pinnatifidum* and *Andrographis paniculata* for anti-malarial activity using the lactate dehydrogenase assay. Journal of Ethnopharmacology 2002; 82(2-3), 239–242.

11-**Dua VK, Ojha VP, Roy R, Joshi BC, Valecha N, Devi CU, et al.** Anti-malarial activity of some xanthones isolated from the roots of *Andrographis paniculata*. Journal of Ethnopharmacology 2004; 95(2-3), 247–251.

12-**Arsenault P, Wobbe K, Weathers P.** Recent Advances in Artemisinin Production Through Heterologous Expression. Current Medicinal Chemistry 2008; 15(27), 2886–2896.

13-**Dwivedi MK, Sonter S, Mishra S, Singh P, Singh PK.** Secondary metabolite profiling and characterization of diterpenes and flavones from the methanolic extract of *Andrographis paniculata* using HPLC-LC-MS/MS. Future Journal of Pharmaceutical Sciences 2021; 7(1), 1-28.

14-**Christian AG, Ahunna AG, Nwakaego EM, Chimsorom CK, Chile AE.** Antimalarial potential of the ethanolic leaf extract of *Pseudocedrala kotschyi*. Journal of Acute Disease 2015; 4(1), 23–27.

15-**Adu-Gyasi D, Adams M, Amoako S, Mahama E, Nsoh M, Amenga-Etego S, et al.** Estimating malaria parasite density: assumed white blood cell count of 10,000/µl of blood is appropriate measure in Central Ghana. Malaria Journal 2012; 11(1).

16-**Flores-Garcia Y, Herrera SM, Jhun H, Pérez-Ramos DW, King CR, Locke E, et al.** Optimization of an in vivo model to study immunity to *Plasmodium falciparum* pre-erythrocytic stages. Malaria journal 2019; 18(1), 1-9.

17-**Schalm SW, Terpstra JL, Achterberg JR, Hegt VN, Haverkate F, Popescu DT, et al.** Orthotopic liver transplantation: an experimental study on mechanisms of hemorrhagic diathesis and thrombosis. Surgery 1975, 78(4), 499-507.

18-**Chan ZH, Su ELM, Lim YM, Randin EL, Yeong CF, Razak MA, et al.** Cellco: portable device for automated blood cell count and abnormal cell detection. Journal of Telecommunication, Electronic and Computer Engineering (JTEC) 2017; 9(3-9), 119-122.

19-**Angelo A, Derbie G, Demtse A, Tsegaye A.** Umbilical cord blood hematological parameters reference interval for newborns from Addis Ababa, Ethiopia. BMC Pediatrics 2021; 21(1).

20-**Tayebi SM, Hanachi P, Niaki AG, Ali PN, Ghaziani FG.** Ramadan Fasting and Weight-Lifting Training on Vascular Volumes and Hematological Profiles in Young Male Weight-Lifters. Global Journal of Health Science 2010; 2(1).

21-**Trager W, Jensen JB.** Human malaria parasites in continuous culture. Journal of Paraasitology 2005; 91(3), 484-486.

22-**Wahyuni DK, Rahayu S, Zaidan AH, Ekasari W, Prasongsuk S, Purnobasuki H.** Growth, secondary metabolite production, and

in vitro antiplasmodial activity of *Sonchus arvensis* L. callus under dolomite [$CaMg(CO_3)_2$] treatment. Plos one 2021; 16(8), e0254804.

23-**Chander R, Srivastava V, Tandon JS, Kapoor NK.** Antihepatotoxic Activity of Diterpenes of *Andrographis paniculata* (Kal-Megh) Against *Plasmodium Berghei*-Induced Hepatic Damage in *Mastomys natalensis*. International Journal of Pharmacognosy 1995; 33(2), 135–138.

24-**Obonti AT, Alam S, Kamal TB, Zaman A, Hasnat H, Saha T, et al.** Prospective Plants with Corroborated Antimalarial Actions: A Review. Bangladesh Pharmaceutical Journal 2021; 24(2), 180-193.

25-**Mishra K, Dash AP, Swain BK, Dey N.** Anti-malarial activities of *Andrographis paniculata* and *Hedyotis corymbosa* extracts and their combination with curcumin. Malaria Journal 2009; 8(1).

26-**Omoya FO, Momoh AO.** The Efficacy of *Andrographis paniculata* and *Chromolaena odorata* Plant Extract against Malaria Parasite 2014; repository.elizadeuniversity.edu.ng

27-**Odeghe OB, Uwakwe AA, Monago CC.** Some biochemical and haematological studies on the methanolic extract of *Anthocleista grandiflora* stem bark. Int J Appl Sci Technol 2012; 2(5), 58-65.

28-**Suriyo T, Pholphana N, Ungtrakul T, Rangkadilok N, Panomvana D, Thiantanawat A, et al.** Clinical parameters following multiple oral dose administration of a standardized *Andrographis paniculata* capsule in healthy Thai subjects. Planta medica 2017; 83(09), 778-789.

29-**Dhenge SA, Shirbhate RN, Bahiram KB, Wankar AK, Khandait VN, Patankar RB.** Haematobiochemical profile of broilers supplemented with *Withania somnifera* (Ashwagandha) and *Andrographis paniculata* (Bhuineem). The Indian Journal of Field Veterinarians 2009; 5(1), 5-8.

30-**Kusmardi K, Elya B, Wahyuni T, Paramita RI.** Combination of sambiloto (*Andrographis paniculata* (Burm. F.) nees) extract and spirulina (*Arthrospira platensis* gomont) to prevent anemia in mice infected with Plasmodium berghei anka. Asian Journal of Pharmaceutical and Clinical Research 2017.

31-**Mathivanan R, Edwin SC.** Hematological and serum biochemical parameters of broilers fed with *Andrographis paniculata* as an alternative to antibiotic growth promoter. Journal of Medicinal Plants Research 2012; 6(44), 5647-5650.

32-**Bagavan A, Rahuman AA, Kamaraj C, Kaushik NK, Mohanakrishnan D, Sahal D.** Antiplasmodial activity of botanical extracts against *Plasmodium falciparum*. Parasitology Research 2011; 108(5), 1099-1109.

33-**Chauhan ES, Sharma K, Bist R.** Andrographis paniculata: A review of its phytochemistry and pharmacological activities. Research Journal of Pharmacy and Technology 2019; 12(2), 891-900.

34-**Cimanga RK, Nsaka SL, Tshodi ME, Mbamu BM, Kikweta CM, Makila FBM, et al.** In vitro and in vivo antiplasmodial activity of extracts and isolated constituents of *Alstonia congensis* root bark. Journal of ethnopharmacology 2019; 242, 111736.