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Antiplasmodial Activity of *Phyllanthus amarus* Preserves Renal Function in *Plasmodium berghei* Infected Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Authors I. Onyesom and I. F. Onumaechi designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors J. Ehiwario and R. Dagana managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

The antiplasmodial effect of ethanolic leaf extract of *Phyllanthus amarus* on the biomarkers of renal function was investigated. The phytochemical constituents of the extract were screened. The renal function markers investigated include levels of uric acid, creatinine, urea and electrolytes (HCO_3^-, Na^+, K^+ and Cl^-) in serum of *Plasmodium berghei* infected mice treated with *P. amarus.* Twenty-five (25) adult mice (22-27g bwt) randomly divided into 5 groups (n= 5/grp) were used. Group 1; Normal control (uninfected and untreated), Group 2; malarial control (infected with *P. berghei* and untreated), Group 3; infected and treated with *Pyllanthus amarus* leaf extract (200mg/kg bwt), Group 4; uninfected but treated with same dose of *Phyllanthus amarus* and Group 5; standard control (infected and treated with quinine, 5mg/kg). Each group was so treated for 5 days and on the 6th day the animals were sacrificed under chloroform anaesthesia after an overnight fast. Whole blood samples were obtained by cardiac puncture and then, prepared for biochemical assay using standard methods. Results show that *P. berghei* malaria infection significantly (p<0.05) increases

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serum uric acid (9.68±0.21mg/dl), creatinine (1.60±0.26mg/dl) and urea (47.46±0.24mg/dl), but reduced (p<0.05) electrolytes` (Na^+: 106.40±10.10mEq/L, K^+:2.20±0.42mEq/L, Cl^-: 60.00±14.38mEq/L and HCO_3^-: 10.98±2.64mEq/L) levels when compared with the control mice. However, treatment of the malaria infection with *P. amarus* abated the malaria effects in a manner that compares well with the standard quinine treatment and there were no significant differences compared with the control values. The extract and quinine suppressed *Plasmodium* by 94.5% and 94.7%, respectively in experimental mice. Phytochemicals identified in the leaf extract include alkaloids, flavonoids, tannins, saponins, terpenoids and glycosides. Results indicate that malarial infection induces renal dysfunction. However, this compromise could be mitigated by the ethanolic leaf extract of *P. amarus* probably because of its potent *in vivo* antiplasmodial activity arising from its active phytochemicals. It would therefore be worthwhile to purify and extract the active components of the herb, by a bioassay-guided isolation. With the enriched fractions or the pure compounds, researchers would be able to assess the parasite life phase on which the plant extract is most active.

Keywords: Antiplasmodial; Plasmodium berghei; ethanolic extract, Pyllanthus amarus; phytochemicals; renal function; electrolytes.

1. INTRODUCTION

The World Health Organization has estimated that in 2010, there were 219 million documented cases of malaria. That year, the disease killed between 660,000 and 1.2 million people [1], many of whom were children in Africa. This observation could be due to the growing resistance of the *Plasmodium* parasite to drugs known to be previously active [2]. So, there is need for a vitalized search for new antimalarial drugs and the use of plants is increasing [3].

Herbalism could be traced to early man who probably acquired skills of healing through deliberate or accidental selection of plants and their parts [4]. In the identification of new medicinal plants or focusing on those earlier reported which have bio-active phytochemical constituents, herbalism (ethno-medicinal study) is regarded as the most viable and feasible method [5]. Traditional medicine, a major socio-cultural heritage in Africa for several decades, was once believed to be primitive and challenged by foreign religion and orthodox or conventional medical practitioners [6]. However, as a result of increasing chemotherapeutic failure in recent times, natural herbs are becoming of interest in providing remedy.

Pyllanthus amarus belongs to the family *Euphorbiaceae*. It is a small, erect, perennial herb having large number of phytochemicals that are attributed to its leaves, stem and roots. The herb is a distinguished plant worldwide which has been used over the years because it is non-toxic to the liver and kidney and contains rich medicinal components [7]. *Pyllanthus amarus* is an important plant of Indian Ayurvedic system of medicine which is used in the problems of stomach, genitourinary system, liver, kidney and spleen; precisely for the treatment of gonorrhea, menorrhagia, jaundice, diarrhoea, dysentery, gastropathy, ophthalmopathy, scabies, fever, uro-genital diseases, ulcer, sore and wounds [8]. Juice from the roots and leaves are taken internally to stimulate the kidney. The aqueous extract of aerial parts is used to break and expel kidney stones in India, Brazil and other Amazonian regions [9], hence called “stone breaker” [10]. However, studies on *P. amarus* toxicity are scarce but available evidence shows no significant toxic effect for the dried water extract; LD_{50} > 5000mg/kg. bwt. [7]. The antiplasmodial effect of *P. amarus* has been reported [10], but the associated changes in renal function markers are yet to be fully documented.

The incidence of renal dysfunction is on the increase; malaria and other infectious diseases may be contributing factors. It is therefore worthwhile to know the changes inrenal function markers (uric acid, creatinine, urea and electrolytes) in *Plasmodium berghei* infected mice treated with *Pyllanthus amarus* leaf extract purported to have antiplasmodial property.

2. MATERIALS AND METHODS

2.1 Harvesting and Preparation of Leaf Extract

Fresh leaves of *Pyllanthus amarus* were collected from natural habitat in Ozoro, Isoko North LGA of Delta State, Nigeria. The plant was identified at the Nigeria Institute of Forestry

Research, Ibadan, where a voucher specimen (No: FHI109728) has been deposited.

The leaves were washed, air-dried and ground to produce a fine powder which was extracted using a soxhlet apparatus sequentially with ethanol. The extract was evaporated to dryness using rotary evaporator (Buchi R-210) under reduced pressure. The percentage yield was 3.6%. The dried extract obtained was dissolved in distilled water. The volume (i.e. equivalent dose used) was then calculated thus:

$$V\ (ml) = D\ (g/kg) \times P\ (kg)/C\ (g/ml)$$

D= Dose used (g/kg b.wt), P= Body weight (kg), C= Concentration of the extract (g/ml), V= Volume of extract (ml) administered.

2.2 Phytochemical Screening

The phytochemical screening of *Pyllanthus amarus* leaf extract was carried out using standard procedures to determine the presence of the following compounds; alkaloid, flavonoids, tannins, saponins, glycosides, steroids, terpenes and carbohydrates [11].

2.3 Experimental Animals

Twenty-five (25) Swiss Albino mice of mixed sexes weighing between 0.022kg (22g) to 0.027kg (27g) were obtained from the Laboratory Animal House, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria and used. Three (3) *Plasmodium berghei* infected(donor)mice were obtained from Nigeria Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria, and were used to inoculate some other mice used for this investigation.

2.4 Animal Care and Handling/Ethical Approval

The mice were fed on growers mash obtained from Top-Feeds, Sapele, Delta State, Nigeria, and given clean drinking water *ad libitum*. The animals were kept in plastic cages, under controlled condition of 12hr light/12hr dark cycle. The animals were maintained in accordance with the guidelines approved by the Animal Ethical Committee, Delta State University, Abraka.

2.5 Animal Grouping, Inoculation and Extract Administration

The animals were caged in groups of five (5) to allow free and easy movement and to avoid crowdedness. Group 1 was uninfected and untreated (normal control), Group 2 was infected with *Plasmodium berghei* and untreated (malaria control), Group 3 was parasitized (infected) and treated with 200mg/kg b.wt of *Pyllanthus amarus* leaf extract, Group 4 was uninfected but treated with 200mg/kg b.wt of *Pyllanthus amarus* leaf extract and Group 5 was infected and treated with quinine (5mg/kg b.wt).

The mice were infected by obtaining parasitized blood (3-4 drops) from the cut tail tip of an infected (donor) mouse. Then, 0.1ml of infected blood was diluted in 0.9ml of phosphate buffer, pH 7.2 and the mice were inoculated with 0.1ml of the parasitized suspension which contained about twelve thousand (12,000) parasites. Parasitaemia was done using thin blood films from cut tip of the infected mice`s tail and stained with Giemsa stain then viewed with the microscope (model TH-9845,Serico, China) at x100 magnification. The ethanolic *P. amarus* extract and quinine doses were administered once a day, only in the morning, as designed using intragastric cannula for a period of five (5) days.

2.6 Animal Sacrifice and Collection of Specimen

On the 6th day, the experimental animals were fasted overnight and sacrificed under chloroform anaesthetic. Whole blood was collected by heart puncture and centrifuged to obtain serum which was used for the biochemical analysis to determine changes in the renal function markers (uric acid, creatinine, urea and electrolytes - HCO_3^-, Na^+, K^+ and Cl^-).

2.7 Analysis of Specimen

The biochemical investigation was carried out to determine the degree of renal dysfunctions by ascertaining the levels of renal biomarkers in serum of *P. berghei* infected mice treated with *P. amarus.*

Uric acid was estimated using the colorimetric enzymatic method [12], creatinine was determined using the Jaffe`s Alkaline Picrate Method [13], Urea was estimated using Urease-Berthelot colorimetric method [14], Bicarbonate (HCO_3^-) was analyzed using the enzymatic method [15], sodium ion(Na^+) was analyzed using the colorimetric method [16], potassium ion(K^+) was assayed using the colorimetric method [17] and chloride ion (Cl^-) was estimated

using the colorimetric method [18]. All the chemicals used were of Analar grades, supplied by Poole Chemicals, England.

3. STATISTICAL ANALYSIS

Experimental data were expressed as Mean ±SD (Standard Deviation). Results were subjected to statistical analysis using one way analysis of variance (ANOVA) to determine the differences between groups. Differences between means at 5% level (p<0.05) were considered significant.

4. RESULTS

The results obtained from the investigation into the antiplasmodial potential of *Pyllanthus amarus* and associated preservation of renal function in *Plamodium berghei* infected mice are shown in Tables 1-4.

Plasmodium berghei malarial infection significantly increased serum uric acid and urea levels in experimental mice (Table 1). *Pyllanthus amarus* leaf extract appears to ameliorate the malaria induced values in a trend that compares well with the control (Group 1) and standard (Group 5) values (Table 1). Administration of the *P. amarus* leaf extract to uninfected mice (Group 4) reduced serum uric acid level when compared with both control and standard values, but the creatinine and urea estimates were similar (Table 1).

From Table 2, it can be observed that in the infected and untreated mice (Group 2) there is significant reduction (P<0.05) in the electrolytes concentrations when compared with the other remaining groups whose values were not statistically different (P>0.05).

P. amarus extract and standard quinine treatments inhibited parasitaemia alike (Table 3), indicating that the extract has medicinal chemicals that can suppress parasitaemia.

The results obtained from the investigation into the phytochemical contents of *P. amarus* leaf extract are shown in Table 4. Saponins, tannins, flavonoids, glycosides, terpenoids and alkaloids were identified and quantified.

Table 1. Uric acid, creatinine and urea levels in serum of *P. berghei* infected and uninfected mice treated with *Pyllanthus amarus* leaf extract

Groups	Uric acid (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
1	3.34±1.25^a	0.90±0.14^a	29.24±0.24^a
2	9.68±0.21^b	1.60±0.26^a	47.46±0.24^b
3	1.16±0.42^c	0.72±0.19^a	26.44±9.05^a
4	0.98±0.65^c	0.96±0.15^a	29.89±9.84^a
5	3.90±1.27^a	0.74±0.23^a	26.68±5.54^a

Values are expressed as Means±SD for n=5 mice per group
Values that bear another superscript on a column differ significantly (p<0.05)
1= Normal control (uninfected and untreated mice)
2= Malaria control (mice infected with Plasmodium berghei and untreated)
3=Parasitized (infected) mice treated with 200mg/kg b.wt of Pyllanthus amarus leaf extract
4= Uninfected mice but treated with 200mg/kg b.wt of Pyllanthus amarus leaf extract
5= Standard control (infected mice treated with quinine 5mg/kg)

Table 2. Electrolytes` concentrations in serum of *P. berghei* infected and uninfected mice treated with *Phyllanthus amarus* leaf extract

Groups	Na^+(mEq/L)	K^+(mEq/L)	Cl^-(mEq/L)	HCO_3^-(mEq/L)
1	142.80±6.91^a	4.25±0.84^a	101.80±3.19^a	28.94±4.05^a
2	106.40±10.10^b	2.20±0.42^b	60.00±14.38^b	10.98±2.64^b
3	144.00±8.48^a	4.56±2.12^a	102.00±5.65^a	30.80±6.50^a
4	140.20±5.30^a	4.04±0.69^a	99.20±1.17^a	27.00±4.28^a
5	143.60±6.65^a	4.26±0.57^a	101.40±2.60^a	27.76±3.79^a

Values are expressed as Means±SD for n=5 mice per group
Values that bear another superscript on a column differ significantly (p<0.05)
1= Normal control (uninfected and untreated mice)
2= Malaria control (mice infected with Plasmodium berghei and untreated)
3= Parasitized/infected mice treated with 200mg/kg b.wt of Phyllanthus amarus leaf extract
4= Uninfected mice but treated with 200mg/kg b.wt of Phyllanthus amarus leaf extract
5= Standard (infected mice treated with quinine, 5mg/kg)

Table 3. *Pyllanthus amarus* and quinine *in vivo* antiplasmodial activities

No.	*P. berghei* infection	*P. amarus* treated	Quinine treated
1	10700	600 (93.9)	500 (95.3)
2	12800	600 (95.3)	550 (95.7)
3	10600	700 (93.4)	700 (93.4)
4	10800	500 (95.3)	520 (95.2)
5	10900	580 (94.7)	525 (95.2)
Mean±SD	11160±923.58	606±75.37(94.52±0.9)	559±80.81 (94.7±0.9)

Values are parasite count for n=5mice per group.
Values in parenthesis are percentage in vivo parasite inhibition.
Values ≥ 50% are significant.
Malaria parasite count: about 12,000 parasites per 0.1ml of parasitized suspension

Table 4. Chemical constituents of the ethanolic leaf extract of *Pyllanthus amarus*

Phytochemicals	Amount (mg/g)
Saponin	0.25
Tannin	0.03
Flavonoid	0.22
Glycosides	0.08
Terpenoids	0.04
Alkaloid	0.14

5. DISCUSSION

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans (a type of unicellular microorganism) of the genus *Plasmodium*. Commonly, the disease is transmitted via a bite from an infected female *Anopheles* mosquito. The management of malaria is complicated because the parasites are resistant to most of the conventional drugs available, hence, most individuals in developing countries use herbs in treating malaria. One of such herbs is *Pyllanthus amarus*, which has been claimed to be potent against malaria infection.

Mice infected with *Plasmodium berghei* and treated with *P. amarus* leaf extract were studied in order to determine serum uric acid, creatinine, urea and electrolytes levels which are markers of renal function tests [19]. Results show increased level of serum uric acid, creatinine and urea with concomitant decrease in electrolytes concentrations in parasitized untreated mice (Tables 1 and 2). These data suggest compromise in the normal functioning of the kidneys by the *P. berghei* malarial infection. This observation with experimental mice agrees with earlier findings in human patients infected with *P. falciparum* [20]. Acute renal failure has been associated with severe *Plasmodium falciparum* malaria but *P. vivax* and *P. malariae* can occasionally contribute to renal impairment [21]. Two mechanisms have been reported to be involved in the pathogenesis of acute renal failure in severe malaria. The first mechanism is the impairment of microcirculation by parasitized erythrocytes and the second is the non-specific effects of the malarial infection, which include hypovolaemia, intravascular haemolysis, intravascular coagulation, endotoxaemia and cholestatic jaundice [22].

The results obtained for the levels of serum uric acid, creatinine and urea (Table 1) show significant increase for the *P. berghei* malarial infected mice without treatment (Group 2) when compared with the other groups. This indicates that kidney function has been affected by malaria parasite, because under normal working condition, the kidney excretes these substances and maintains their levels in the body.

The changes in electrolytes (HCO_3^-, Na^+, K^+ and Cl^-) concentrations were also reported (Table 2). Correction of fluid volume and electrolyte deficits has been the standard of care for any critically ill patients including those with severe malaria infection. This is because acidaemia, hypokalaemia, hypocalcaemia and hyponatraemia exacerbate myocardial dysfunction and increase the risk of arrhythmias [23,24]. The electrolytes levels decreased significantly in *P. berghei* infected mice with no treatment (Group 2) when compared with the control mice (Group 1). This also indicates that malarial infection affects renal secretion or reabsorption of electrolytes. The decrease in concentration of sodium (Table 2) may be due to losses in urine, because losses in urine are compensated by increase in urea concentrations (Table 1). This observation has been found to be common in *falciparum* malaria infection, and occurs in order to maintain constant body osmolality [25]. Impaired glomerular filtration amidst hyponatraemia reduces the amount of sodium ions available in the renal tubule for

potassium exchange and therefore contributes to the elevation of serum potassium concentration. Erythrocytes infected with *falciparum* malaria are associated with decreased cytosolic concentration of potassium (hypokalemia) [26], and this present study with mice agrees with this findings. Another report presented by [27], shows low level of serum chloride in malarial infection which is in accordance with the present study. Serious cases of renal problem associated with malaria take the form of nephritic syndrome which gradually progresses to renal failure characterized by metabolic acidosis arising from low level of serum bicarbonate [28].

In this study also, the phytochemical contents of the ethanolic leaf extract of *Pyllanthus amarus* and associated degree of plasmodial suppression in mice was determined. The results obtained from the suppressive activity of the extract are shown in Table 3. The extract at 200mg/kg yielded 94.5% inhibition as against 94.7% for quinine. The plant *Pyllanthus amarus* was observed to show significant *in vivo* antimalarial activity as judged in its percentage chemo-suppression which compared well with that of quinine, the standard drug. The *in vivo* antiplasmodial activity of *P. amarus* has been demonstrated earlier [10]. Also, the lethal rodent malaria parasite, *Plasmodium berghei,* can also be treated with the medicinal plant *Thlaspi arvense* [29].

Phytochemical analysis of the ethanolic leaf extract of *Pyllanthus amarus* as shown in Table 4 revealed the presence of saponins, tannins, flavonoids, glycosides, terpenoids and alkaloids. This is similar to research findings by [7]. Of all the metabolites, saponin was the most frequent followed by flavonoids, alkaloids and glycosides. Terpenoids and tannins were the least frequent. Alkaloids, saponins, and flavonoids are suggested as being responsible for the anti-malarial activities of the plant [30]. These secondary metabolites could have elicited the observed antiplasmodial activity either singly or in synergy with each other. Alkaloids have been implicated in antimalarial activity of many plants [31]. It has been shown to mediate its anti-malarial properties by blocking protein synthesis in *Plasmodium falciparum* [32]. Triterpenoids, steroids and saponins have been found to be detrimental to several infectious protozoans such as *Plasmodium falciparum* [33]. Flavonoids are known to chelate nucleic acid base pairing of malaria parasite [34]. Flavonoids and tannins are phenolic compounds, and plant phenolics are major group compounds that act as primary antioxidants or free radicals scavengers [35]. So, these phenolics could contribute to the protection of the kidneys from oxidative damage arising from malaria parasite infection.

6. CONCLUSION

On the whole, *P. amarus* was observed to restore the renal dysfunction associated with *P. berghei* malaria infection in experimental mice (Tables 1 and 2). This ability may be due to the bioactivities of identified phytochemicals (Table 4) which displayed significant *in vivo* plasmodial inhibition (Table 3). The mechanism of *P. amarus* antimalarial potency needs to be understood. Therefore, the phytochemicals should be purified and used to assess the parasite`s life stage in which the plants active ingredient(s) is/are most active.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nayyar GML, Breman JG, Newton PN, Herrington J. Poor-quality antimalarial drugs in Southeast Asia and sub-Saharan Africa. Lancet Infect Dis. 2012;12(6):488–496.
2. Nchinda TC. Malaria: A reemerging disease in Africa. Emerge Infect Dis. 2007;4(3):398-403.
3. Krettli AU. Antimalarial drug discovery: screening of Brazilian medicinal plants and purified compounds. Expert Opin Drug Discov. 2009;4(2):95-108.
4. Sofowora A. Medical plants and traditional medicine in Africa. John Wiley and Sons Ltd., New York; 1982.
5. Adjanahoun E, Ahyi MRA, Ake-Assi L, Elewude JA, Dramane K, Fudoju SD, Gbile ZO, Goudole E, Johnson CLA, Keita A, Morakinyo O, Ojewole JAO, Olatunji AO, Sofowora EA. Traditional Medicine and Pharmacopoeia Contribution to Ethno botanical floristic Studies in Western Nigeria. Organization of African Unity, Scientific Technical and Research Commission, Lagos, Nigeria; 1991.
6. Elujoba AA. Medicinal properties of plants with oral health implications. Proceedings of the 2nd Dr. David Barnes' Memorial

Public Health Symposium, 25th March 2003, Organized by Regional Centre for Oral Health Research and Training for Africa, Jos, in collaboration with WHO Regional Office, Brazzaville; 2005.
7. Oyewole OI, Oyedara OO, Olabiyi BF, Fasanya T.S. Phytochemical, antimicrobial and toxicity studies of *Pyllanthus amarus* whole plant extract. Intl J Bioassays. 2013;2(3):519-523.
8. Patel JR, Tripathi P, Sharma V, Chauhan NS, Dixit VK. *Pyllanthus amarus*: ethnomedicinal uses, phytochemistry and pharmacology: a review. J Ethnopharmacol. 2011;138(2):286-313.
9. Obidike I, Okhale S, Aboh MI, Salawu OA. Isolation, fractionation and evaluation of the antiplasmodial properties of *Phyllanthus niruri* resident in its chloroform fraction. Asian Pacific J Trop Med. 2013;169-175.
10. Tolulope OA, Cecilia II, Ibrahim AO, Oluwatoyin AO. The antiplasmodial effect of the extracts and formulated capsules of *Pyllanthus amarus* on *Plasmodium yoelii* infection in mice. Asian Pacific J Trop Med. 2011;283-287.
11. Ekaete DU, Ukana DA, Etoro EU. Phytochemical screening and nutrient analysis of *Pyllanthus amarus*. Asian J Plant Sci Res. 2013;3(4):116-122.
12. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst. 1972;97:142–145.
13. Blass KG, Thibert RJ, Lam LK. Study of mechanism of JAFE reactions. Clin Chem Clin Biochem. 1974;12:336–343.
14. Weatherburn MW. Phenol-hypochlorite reaction for determination of ammonia. Anal Chem. 1967;39:971-974.
15. Forrester RL, Wataji LJ, Silverman DA, Pierre KJ. Enzymatic method for the determination of CO_2 in serum. Clin Chem. 1976;22:243-245.
16. Maruna RFL. Simple methods for measuring serum electrolytes. Clin Chem Acta. 1958;2:581-582.
17. Sesin PG, Terri AE. Potassium reagent tests. Am J Clin Path. 1958;29:86-87.
18. Hochstrasser H, Skeggs LT. Thiocyanate (colorimetric) method of chloride estimation. Clin Chem. 1964;10:918–919.
19. Shivaraj G, Prakash BD, Shruthi SK, Vinayak VH, Avinash AKM, Sonal NV. Markers of renal function tests. N Am J Med Sci. 2010;2(4):170-173.
20. Ekeanyanwu RC, Ogu GI. Assessment of renal function of Nigerian children infected with *Plasmodium falciparum*. Int J Med Med Sci. 2010;2(9):251-255.
21. Das BS. Renal failure in malaria. J Vector Borne Dis. 2008;45:83-97.
22. Prakash J, Agraval DK, Agarval KN, Kulati AK. Etiologic diagnosis of pneumonia in Under-Five children. India Pediatr. 1996;33:329-331.
23. Khilnami P. Electrolyte abnormalities in critically ill children. Crit Care Med. 1992;20:241-250.
24. Kumar A, Haery C, Parrillo JE. Myocardial dysfunction in septic shock: part 1 Clinical manifestation of cardiovascular dysfunction. J Cardiothorac Vasc Anesth. 2001;15:364-376.
25. Mayne PD. Sodium, potassium and water metabolism In: Clinical Chemistry in Diagnosis and Treatment. 6th (ed.), Hodder Arnold, London. 1994;25-104.
26. Matthys B, Sherkanov T, Karimov SS, Khabirov Z, Mostowlansky T, Utzinger J, Wyss K. History of malaria control in Tajikistan and rapid malaria appraisal in an agro-ecological setting. Malaria J. 2008;7:217-221.
27. Akpan EJ, Etim OE, Ekaidem S, Usoh FI, Akpan HD. Changes in electrolytes levels in uncomplicated *Plasmodium falciparum*. Continental J Pharmacol Toxicol Res. 2011;4(1):5-10.
28. Marshal WJ, Bangert SK. Clinical chemistry. 6th (ed.), Harcourt Publishers, London; 2008.
29. Walter NS, Bagai U. Antiplasmodial Potential of Traditional Medicinal Plant *Thlaspi arvense*. Europ J Med Plant. 2014;4(12):1378-1387.
30. Shigemori H, Kagata T, Ishiyama H. New monoterpene alkaloids from *Nauclea latifolia.* Chem and Pharmaceut Bull. 2003;51(1):58-61.
31. Christenzen SB, Kharazmi A. Antimalaria natural products Isolation, characterization and biological properties. In. Bioactive Compounds from Natural Sources: Isolation, Characterization and Biological Properties. Ed. Tringali, C. London, Taylor & Francis; 2001.
32. Abdulelah HAA, Zainal-Abidin BAH. *In vivo* Antimalaria tests of Nigella sativa (Black Seed) different extracts. Am J Pharmacol Toxicol. 2007;2(2):46–50.
33. Delmas F, Di Giorgio CR, Elias. Antileishmanial activity of three saponins

isolated from ivy, α- hederin, β-hederin and hederacolchiside A1, as compared to their action on mammalian cells cultured *in vitro*, Planta Medica. 2000;66(4):343–347.
34. Agrawal P. Carbon-13 NMR of flavonoids. Elsevier, Amsterdam; 1989.
35. Ettebong E, Nwafor P, Okokon J. *In vivo* antiplasmodial activities of ethanolic extract and fractions of *Eluecine indica*, Asian Pacif. J Trop Med. 2012;5(9):673-676.

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