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Research Article
Antiplasmodial, Antioxidant, Hemolytic Activities and Acute Toxicity of *Costus afer* Ker Gawl (Costaceae) Used in Malaria Healing in Benin

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Abstract

Background and Objective: Overcome the resistance of malaria parasites to most orthodox drugs requires the exploration of different drug sources for new compounds or powerful natural substances against the *Plasmodium* parasite. The current study was designed to investigate antiplasmodial, antioxidant properties and toxicological profile of *Costus afer* Ker Gawl, a medicinal plant widely used traditionally in Bénin Republic to treat malaria. **Materials and Methods:** Crude ethanolic and aqueous extracts were tested *in vitro* against both field isolates and chloroquine sensitive 3D7 strains of *P. falciparum* using *Plasmodium* lactate dehydrogenase (pLDH) assay. Hemoglobin release was measured spectrophotometrically to assess hemolytic power. Antioxidant activity of extracts was assessed in vitro by DPPH radical-scavenging, reducing power (FRAP), superoxide radical scavenging and Hydrogen peroxide scavenging assays. Acute oral toxicity of extracts was evaluated according to OECD 423 guideline. **Results:** Ethanolic extract exhibited very good antiplasmodial activity against both strains with IC_{50} values of 3.19±0.13 μg mL^{-1} on field isolates and 13.68±1.41 μg mL^{-1} on 3D7. The aqueous extract showed moderate inhibition (<50%) on both strains as well as the fractions obtained from fractionation of ethanolic extract at single dose of 100 μg mL^{-1}. Both extracts exhibited dose-dependent antioxidant activity. Both extracts presented hemolytic power less than 3% with a LD_{50} greater than 2000 mg kg^{-1} of b.wt. There was no significant change on biochemical and hematological parameters. **Conclusion:** The study demonstrated the strongest antiplasmodial and antioxidant properties of *Costus afer* as well as an absence of acute oral toxicity. It could be used as an alternative in the management of malaria and oxidative diseases through a standard formulation.

Key words: *Costus afer*, antiplasmodial, antioxidant activity, toxicity, phytochemicals

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Historically, medicinal plants constitute an important source of satisfaction for the primary health care needs of populations, particularly in the developing regions of the world. According to the WHO, up to 80% of the population in some developing countries still continues to rely on traditional medicine for treatment of diseases[1]. The increasing use of medicinal plants is supported by broadly accepted beliefs and practices. To this can be added the difficulties related to the low income of these populations and the accessibility of conventional drugs. Furthermore, the discovery of quinine and artemisinin, the most worldwide used antimalarial drugs, is the best proof that medicinal plants represent a potential source of bioactive compounds, thus enhancing the belief of the population. However, it has been reported that traditional medical systems could not guarantee the efficacy of herbal medicines on the basis of traditions or supposed beliefs[2]. A thorough investigation of the pharmacological quality and safety issues guarantees a better knowledge of these plants.

Costus afer (Costaceae) is pantropical species encountered in Africa in the forest belt from Senegal to Ethiopia and in the East to Tanzania, Malawi and Angola, in the South and in West Africa[3]. Called tétégoungoun (fon, goun: Benin Republic) and tétérégoun (yoruba), It's commonly crossed from southern to northern of Benin[4]. According to ethnobotanical surveys, *C. afer* is one of the commonly used medicinal plants in folk medicine to treat malaria in Benin[5–7], in Tanzania[8] and in Nigeria[9]. Moreover, it is also used to treat gastralgia, tuberculosis and azoospermia in Benin[10], cough and rubella in Nigeria[9]. Previous research have reported anti-inflammatory[11], hepato-protector[12,13] and hypoglycaemic effect[12] and antioxidant activity[14–17] of *C. afer*.

Despite several ethnobotanicals reports on considerable use of *C. afer* in treatment of malaria, no scientific data was available to validate its antimalarial properties and safety. The present study was designed to assess phytochemicals, acute toxicity and *in vitro* antiplasmodial and antioxidant activities of this medicinal plant used in Bénin Republic.

MATERIALS AND METHODS

Plant material and extracts preparation: The present study was carried out for 18 months from May, 2015 in the laboratories of University of Abomey-Calavi, Bénin. Aerial parts of *Costus afer* were collected in their natural habitats in south Benin in April, 2015. Identification and authentication were done by National Herbarium, University of Abomey-Calavi, Bénin and voucher specimens (YH 354/HNB) were deposited at the same institute for further references.

The plant material collected was dried for two weeks in laboratory (22°C) and ground to a fine powder using an electric grinder (LONG YUE LY-989, China). The aqueous extract was obtained by boiling of 150 g of powder in water (1.5 L) at 100°C for 30 min. The mixture was filtered with Whatman paper No. 1 and concentrated using rotary evaporator coupled with vacuum pump (BUCHI Rotavapor RII, Switzerland; Vacuubrand PC101NT, Germany) at 60°C. Ethanolic extract was obtained by maceration of 500 g of powder in ethanol 76% (ratio 1:10, powder: ethanol) overnight under circular agitation at 200 rpm with rotary agitator (IKA-Werke KS 260, Germany). After three washing, the filtrate was concentrated by same way as aqueous extract. Dried crude ethanolic extract was subjected to liquid-liquid partition using three solvent systems of varying polarity in sequential order. 2 g of ethanolic extract were dissolved in 100 mL of distilled water/ethanol (80:20, v/v) and extracted successively with hexane, dichloromethane and ethyl acetate using a separating funnel. The hexane (F-Hex), dichloromethane (F-DCM), ethyl acetate (F-AcOEt) and the last remaining aqueous (F-aq) fractions were concentrated using the rotary evaporator. All extracts were stored at 4°C.

Phytochemical screening: The both crude extracts were tested for the presence of alkaloids, flavonoids, triterpenes, tannins, coumarins, anthraquinone, saponins, lignanes, essential oils and anthocyanins by time layer chromatography (TLC) and colorimetric test using respectively a standard protocol of Wagner and Bladt[18] and Shah and Hossain[19].

Estimation of total phenolic content: Total phenolic content (TPC) was estimated by colorimetric method using Folin-ciocalteu reagent[20]. Briefly, 200 µL of sample (100 µg mL^{-1}) were added to 1 mL of Folin-ciocalteu reagent (10%). After 4 min, 800 mL of saturated sodium carbonate (75 g L^{-1}) were added. The mixture was incubated for 2 h at room temperature and the absorbance was measured at 765 nm. Standard curve for estimation of TPC was plotted with gallic acid. The essays were performed in triplicate and the results were expressed as mg of gallic acid equivalents GAE g^{-1} of extract.

Estimation of total flavonoid content: Total flavonoid content was measured by the aluminum chloride colorimetric assay[21]. About 250 µL of extract at 100 µg mL^{-1} were mixed with 750 µL of ethanol (96%), 50 µL of potassium acetate (1M), 50 µL of aluminium chloride (10%) and 1400 µL of distilled water. After 30 min of incubation at room temperature, the absorbance was measured at 450 nm. Quercetin was used to plot standard calibration curve and total flavonoid content was expressed as mg of quercetin equivalent (QE)/g of extract.

Antioxidant assays

DPPH radical scavenging activity assay: DPPH radical (2,2-diphenyl-1 picrylhydrazyl), scavenging activity of extracts was assessed according to the method previously described by Amoussa *et al.*[20] with slight modification. Briefly, 1.5 mL of a freshly prepared methanolic solution of DPPH (0.04%) was mixed with 0.75 mL of extract solution diluted in methanol from 300-2.34 µg mL^{-1}. After 20 min of incubation in dark at room temperature, absorbencies were read at 517 nm. Methanolic DPPH solution constitutes a blank and the assays were replicated three times. The DPPH radical scavenging percentage was determinate according to the formula:

$$\text{Inhibition (\%)} = \frac{\text{Ab - As}}{\text{Ab}} \times 100 \quad (1)$$

where, Ab is absorbance of the blank and As is absorbance of the test sample.

The percentage of inhibition was plotted as a function of extracts concentration and curve fitting was obtained by nonlinear regression analysis. The IC_{50} value was extrapolated as the concentration that induced 50% inhibition of DPPH.

Potassium ferricyanide reducing antioxidant power (FRAP) assay: The ferric reducing capacity of extracts was evaluated following the potassium ferricyanide-ferric chloride method[21]. The reaction mixture was constituted with 2 mL of extract (100 µg mL^{-1}), 2 mL of phosphate buffer (0.2 M, pH 6.6) and 2 mL of potassium ferricyanide (10 mg mL^{-1}). After 20 min of incubation at 50°C, the reaction was stopped with 2 mL of trichloroacetic acid (100 mg L^{-1}). The mixture was then centrifuged at 3000 rpm for 10 min. About 2 mL of the upper layer were mixed with 2 mL of distilled water and 0.4 mL of fresh ferric chloride (0.1%, w/v). After 10 min, the absorbances were read at 700 nm. Ascorbic acid was used to produce the calibration curve ($y = 0.0069x+0.015$, $R^2 = 0.9625$). The iron (III) reducing activity assay was performed in triplicate and expressed in mmol of ascorbic acid equivalent (AAE) g^{-1} of extract.

Superoxide radical scavenging assay: This test consists to measure the capacity of extract to quench superoxide radical generated by alkaline DMSO[22]. Superoxide radical scavenging activity of extract is proportional to nitro blue tetrazolium (NBT) reduction by superoxide into formazan dye at room temperature and can be measured around 630 nm. In microplate 96 wells, the reaction mixture was constituted by 50 µL of extract (100 µg mL^{-1} in DMSO) and 170 µL of alkaline DMSO (1 mL DMSO, 100 µL NaOH 5 mM) and 30 µL of NBT (1 mg mL^{-1} in DMSO). The mixture was incubated for 5 min at room temperature and the absorbance was measured at 630 nm using microplate Reader (Rayto R 6500, China). Quercetin was used as a reference. The percentage of superoxide quenching (PI) of extracts was determinate using the following formula:

$$\text{PI} = \frac{\text{A0 - A1}}{\text{A0}} \times 100 \quad (2)$$

where, A0 is absorbance of the blank constituted by alkaline DMSO with NBT and A1 is absorbance of the tested sample.

Hydrogen peroxide radical scavenging assay: The ability of extract to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.*[23] with slight modification. 0.6 mL of hydrogen peroxide solution in phosphate buffer (0.1 mM, pH 7.4) was mixed with 0.5 mL of extract (100 µg mL^{-1}). The mixture was incubated for 10 min at room temperature. All tests were performed in triplicate. The absorbances were determined spectrophotometrically at 295 nm against a blank containing the phosphate buffer without hydrogen peroxide and control constituted by hydrogen peroxide in phosphate buffer. The percentage of hydrogen peroxide scavenging of extracts and gallic acid (standard) was calculated using the following formula:

$$\text{Scavenging } (H_2O_2\,\%) = \frac{\text{Ac - As}}{\text{Ac}} \times 100 \quad (3)$$

where, Ac is absorbance of control and As is absorbance of sample or standard.

Antiplasmodial activity: The plant crude extracts and fractions were evaluated *in vitro* for their antiplasmodial activity against a field isolates and chloroquine-sensitive (3D7) strains of *Plasmodium falciparum* obtained respectively from Laboratory of Infectious Vector Borne Diseases (LIVBD), IRSP/UAC, Benin and Institut Pluridisciplinaire Hubert Curien, UMR 7178-CNRS/Unistra, France. Parasites were maintained on culture in fresh O^+ human erythrocytes in malaria complete medium (RPMI 1640 with 0.8% AlbumAX II, 25 mM HEPES, 1 mM L-glutamine, 0.4 mM hypoxanthine and 0.05 mg mL^{-1} gentamicin) according to the modified method of Trager and Jensen[24]. The cultures were maintained in a standard gas mixture (1% O_2, 3% CO_2, 96% N_2) at 37°C. Parasitaemia was maintained daily between 1 and 5% using smears colored with Giemsa which are visualized on microscope (Optika Microscopes DM-25, Italy). *Plasmodium falciparum* sensitivity to extracts and fractions was carried out in 96-well plates as described by Desjardins *et al.*[25] with slight modification. Crude extracts and fractions were preliminary tested at single concentration of 100 µg mL^{-1} and only active extracts and fractions were submitted to dose-response assay (concentration ranging from 100-0.78 µg mL^{-1} by two-fold dilution) to determine the 50% inhibitory concentration (IC_{50}). In triplicate by concentration in 96-well plates, 100 µL of parasite suspension (1% parasitaemia, hematocrit, 4%) were mixed with 100 µL of each concentration of extracts and fractions previously dissolved in complete medium and the plate was incubated for 96 h at 37°C in *P. falciparum* culture conditions. Parasites viability was assessed by immune-dosage of *Plasmodium* lactate dehydrogenase (pLDH) using ELISA malaria antibody test kit (ApDia, Belgium) according to manufacturer protocol. The concentrations of pLDH in the tests well were measured at 450 nm with microplate reader (Rayto R 6500, China) against a positive control (parasite suspension only) and negative control (red blood cells only). The positive and negative controls of the kits were also plotted to access efficacy of test following the recommendations of the manufacturer. The percentage of parasite growth inhibition (PI) was extrapolated using the following formula:

$$PI = 100 - \frac{ODs - ODnc}{ODpc} \times 100 \quad (4)$$

where, ODs is the optical density generated by sample, ODnc is the optical density generated by negative control and ODpc is the optical density generated by positive control.

The PI was plotted as a function of extract or fraction concentration and the IC_{50} was estimated by nonlinear regression analysis. Chloroquine diphosphate and artesunate were used as antimalarial drug.

Toxicity assays

Hemolytic power essay: Hemolytic power assay was performed to assess toxicity effects of extracts and fractions on human red blood cells. The test was assessed following the method used by Sarr *et al.*[26] with slight modification. Briefly, 100 µL of extracts or fractions dissolved in RPMI (ranging from 200-1.56 µg mL^{-1} by two-fold dilution) were mixed with 100 µL of non-infected erythrocytes (4% hematocrit) in 96-well plate. The mixture was incubated at 37°C under circular agitation. After 1 h, the plate was centrifuged at 2000 rpm for 5 min and 150 µL of supernatant was transferred into new 96-well plate. Haemoglobin content in the supernatants was determined by absorbance measurements at 450 nm in microplate reader. The 100% hemolysis (positive control) was obtained with 5% sodium dodecyl sulfate (SDS) and non-infected erythrocytes suspension was used as the blank. Hemolysis percentage was expressed according to the formula:

$$\text{Hemolysis (\%)} = \frac{As - Ab}{Ac} \times 100 \quad (5)$$

where, As is absorbance of the sample, Ab is absorbance of the blank and Ac is absorbance of positive control.

Acute oral toxicity test: The acute toxicity of ethanolic extract was carried out using[27] Organization for Economic Cooperation and Development (OECD) guidelines N°423. Healthy, nulliparous and non-pregnant females Albinos Wistar rats aged 10-12 weeks (180-200 g body weight) were used. Animals were kept in animal house and maintained under laboratory conditions (22-25°C, 12 h light/dark cycle) with diets and water available *ad libitum*. A total of 6 rats divided in 2 groups of 3 animals each were ranged in different cage during experiments. The animals were deprived of food over-night before administration of extract. The control group (group 1) has received the vehicle (distilled water) and second group has received plant extract at a single dose of 2000 mg kg^{-1} b.wt. The rats were observed in detail during the first 8 h and daily for a period of 14 days. The monitoring was based on general toxicity signs, behavior changes, body weight evolution (days 0, 7 and 14)

and mortality. The animals were anesthetized with ether on day 15 after an over-night food privation and blood samples were collected into tubes with EDTA and tubes without EDTA respectively for hematological and biochemical analysis.

Hematological analyzes were carried out using an automated hematology analyzer (Sysmex XP-300, Japan). Parameters as hematocrit (HCT), haemoglobin (Hgb), Mean corpuscular haemoglobin concentration (MCHC), red blood count (RBC), leukocytes formula (lymphocytes), mean corpuscular volume differential (MCV), mean corpuscular haemoglobin (MCH), platelet count (PLT), white blood cells count (WBC) were examined. Biochemical parameters including blood glucose, blood urea, creatinine (Crea), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analyzed using clinical chemistry analyzer (CHEM-7 ERBA diagnostics mannheim Gmbh, Germany).

Statistical analysis: All data were presented as Mean±SD. The IC_{50} values were estimated by nonlinear regression analysis using Graphpad prism version 8.0.2 (GraphPad Prism software Inc., San Diego CA).The graphical representations of the data were performed using Microsoft Excel 2010 and Graphpad prism version 8.0.2. The difference was considered statistically significant when $p<0.05$.

RESULTS

Phytochemical constituents: Preliminary phytochemical analysis of extracts of *C. afer* revealed the presence of various phytoconstituents such as flavonoids, anthraquinones, coumarins, triterpenes and tannins. Alkaloids, saponins, anthocyanins, lignans and essential oil were not detected in both extracts as well as tannins in aqueous extract (Table 1). Total phenolic content (TPC) and total flavonoid content (TFC) were presented in Fig. 1. Ethanolic extract showed the highest TPC (10.51±0.16 mg GAE g^{-1} of extract) compared to aqueous extract (8.07±0.07 mg GAE g^{-1} of extract) with significant different ($p<0.05$). However, both extracts presented similar amount of TFC with respective values of 20.17±0.36 and 20.38±2.59 mg QE g^{-1} of extract.

Antioxidant activity: Antioxidant activities of *C. afer* ethanolic and aqueous extracts assessed *in vitro* against DPPH free radical, iron III, superoxide anion and hydrogen peroxide anion are presented in Fig. 2. The results showed

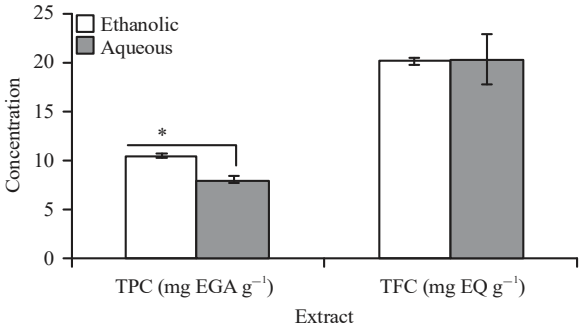

Fig. 1: Total phenolic (TPC) and flavonoids (TFC) contents crude extracts of *C. afer*
*Statistically significant at $p<0.05$, each value represents a Mean±SD (n = 3)

Table 1: Phytochemical contents of *Costus afer* crude extracts

Chemicals components	*Costus afer*	
	Aqueous extract	Ethanolic extract
Alkaloids	-	-
Flavonoids	+	+
Saponins	-	-
Tannins	-	+
Anthraquinones	+	+
Anthocyanins	-	-
Coumarins	+	+
Triterpenes	+	+
Lignans	-	-
Essential oils	-	-

+: Presence, -: Absence

that DPPH radical scavenging activity of both extracts was dose-dependent (Fig. 2a). Additionally, the both extracts exhibited interesting DPPH radical scavenging activity with respective IC_{50} values ($p>0.05$) of 181.90±26.99 and 159.60±15.62 µg mL^{-1}. The ferric reducing antioxidant capacity of both extract was respectively 1272.88±50.29 and 1294.83±34.26 µM AAE g^{-1} of extract ($p>0.05$) indicating strong reducing capacity of extracts when compared to Ascorbic acid (1004.24±62.03 µM AAE g^{-1}) with significant difference ($p<0.05$) (Fig. 2b). According to superoxide method, it was observed that extracts quench superoxide anion more than 90% at 100 µg mL^{-1} where quercetin exhibited 83.58% (Fig. 2c). Thus, the both extracts possess strong superoxide anion scavenging capacity significantly different from quercetin ($p<0.05$). At the same concentration, both extracts exhibited moderate hydrogen peroxide quenching capacity (inhibition <60%) when compared to gallic acid (inhibition >70%) with significant difference ($p<0.05$) on the one hand between both extracts and on the other hand between extracts and quercetin (Fig. 2d).

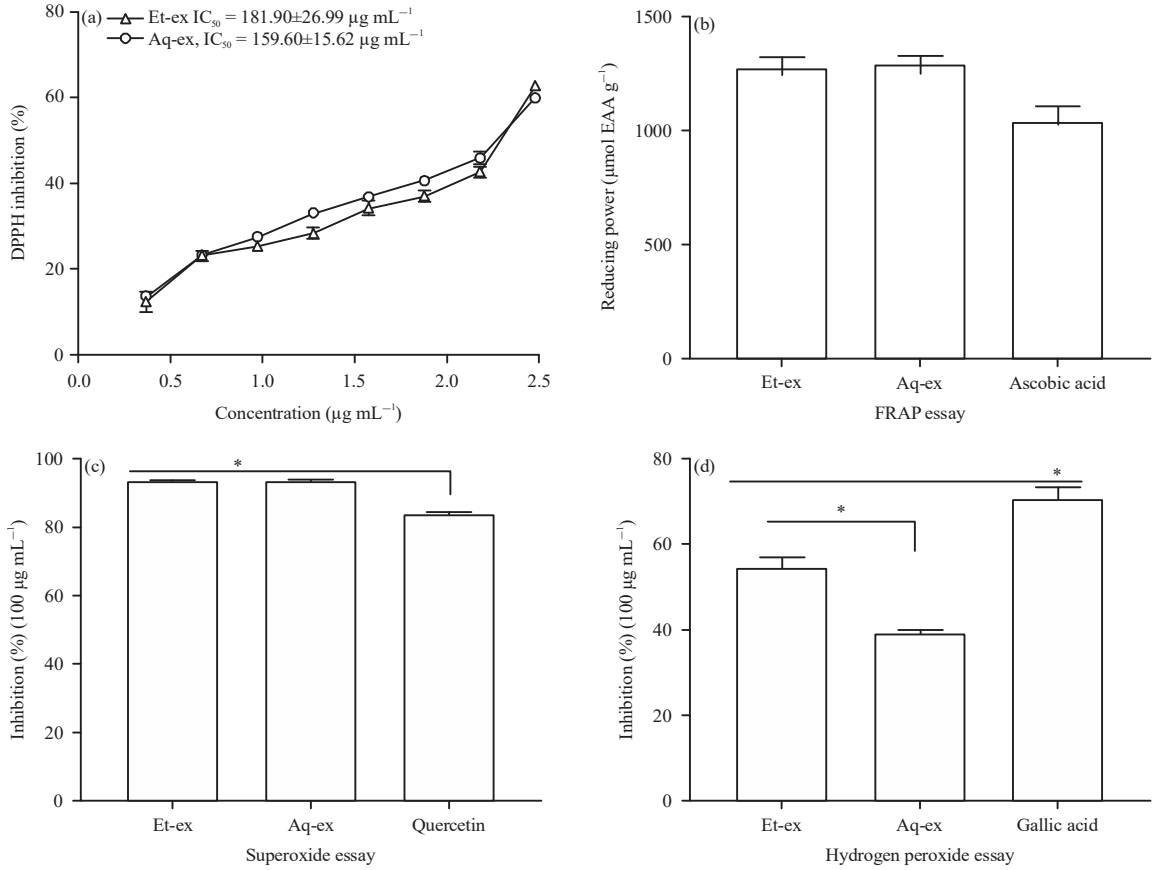

Fig. 2(a-d): Antioxidant activity of crude ethanolic and aqueous extracts of *C. afer*, *in vitro* free radicals scavenging capacity of both extracts obtained against (a) DPPH radical, (b) Iron III, (c) Superoxide anion radical and (d) Hydrogen peroxide
Each value represents mean for 3 experiments±SD (standard deviation), *Statistically significant difference ($p<0.05$), Ascorbic acid, quercetin and gallic acid were used as reference compounds, Et-ex: Ethanolic extract, Aq-ex: Aqueous extract

Antiplasmodial activity: Crude ethanolic and aqueous extracts of aerial part of *C. afer* and fractions obtained by bioassay-guided fractionation were tested *in vitro* on asexual stage of both field isolates and chloroquine sensitive (3D7) strains of *P. falciparum* using pLDH assay (Fig. 3). At single dose of 100 µg mL^{-1}, the crude ethanolic extract (Et-ex) showed the highest activity with a parasites growth inhibition more than 60% on 3D7 strain and 80% against field isolates. The aqueous extract exhibited moderate inhibition (<50%) on both strains (Fig. 3a). Additionally, the Et-ex showed interesting dose-response-inhibition effect on both strains and mainly on field isolates with IC_{50} values of 3.19±0.13 µg mL^{-1} and 13.68±1.41 for 3D7 strain (Fig. 3b). However, among the fractions obtained from partition of Et-ex, ethyl acetate fraction (F-AcOEt) exhibited moderate activity against both strains (40%<inhibition<50%) while the others inhibited at less than 40% at the single dose (Fig. 3c). The chloroquine and artesunate used as control drugs are very active on chloroquine sensitive (3D7) and field isolates strains with respective IC_{50} values of 1.87±0.32 and 2.57±0.38 µg mL^{-1}. However, the field isolates is less sensitive to chloroquine with an IC_{50} value of 19.85±2.71 µg mL^{-1} (Fig. 3b).

Toxicity effect

Hemolytic power: The cytotoxic effect of *C. afer* crude extracts assessed on human red blood cells is shown in Fig. 4. It has been observed that both extracts possess very low hemolytic power less than 3%. This indicates non cytotoxic effect of both extract against human red blood cells.

Acute oral toxicity: The acute oral toxicity effect of *C. afer* ethanolic extract assessed at single dose of

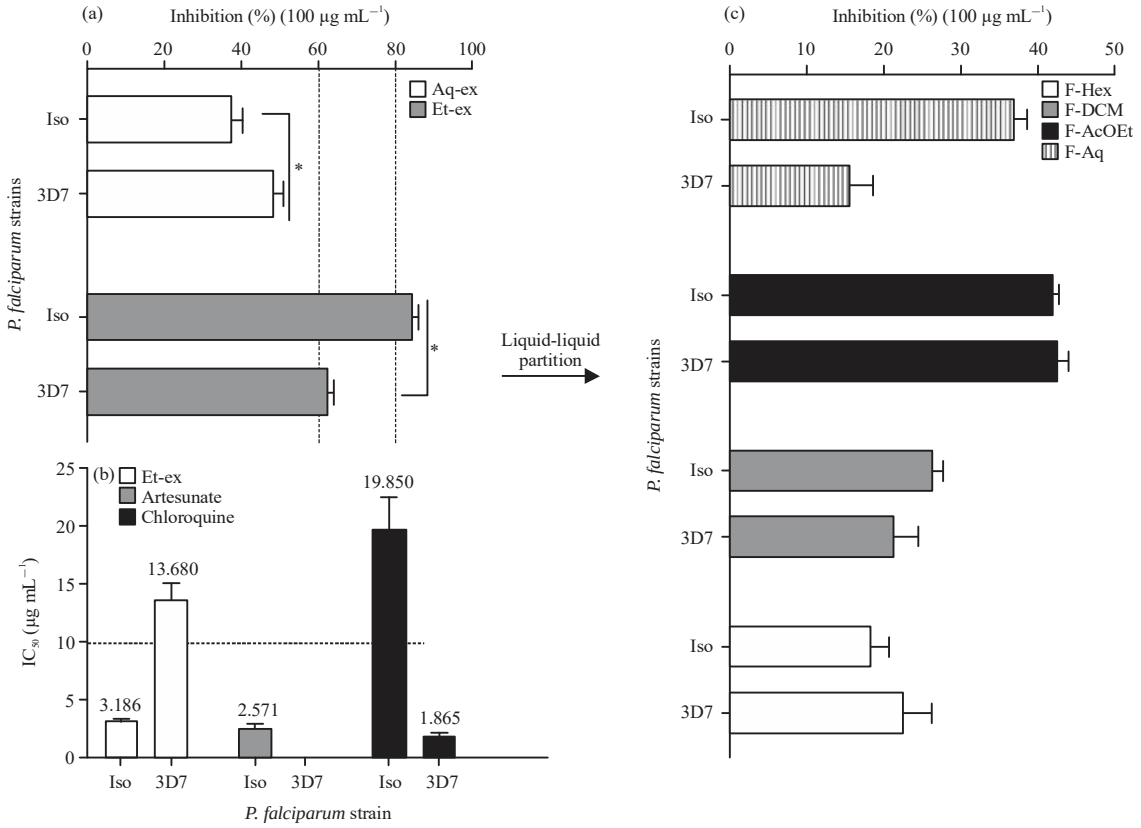

Fig. 3(a-c): Antiplasmodial activity of *C. afer* against chloroquine sensitive (3D7) and field isolates strains (Iso) of *P. falciparum*, (a) *In vitro* inhibition of parasites by the crude extracts at single dose, (b) IC_{50} values of crude extracts and control drugs (artesunate and chloroquine) and (c) *In vitro* inhibition of parasites by the fractions at single dose

Data represent means for three experiments±SD (standard deviation), Artesunate and chloroquine diphosphate are positive drug control, *Statistically significant difference ($p<0.05$), Et-ex: Ethanolic extract, Aq-ex: Aqueous extract, F-Hex: Fraction hexane, F-DCM: Fraction dichloromethane, F-AcOEt: Fraction ethyl acetate, F-Aq: Aqueous residual fraction

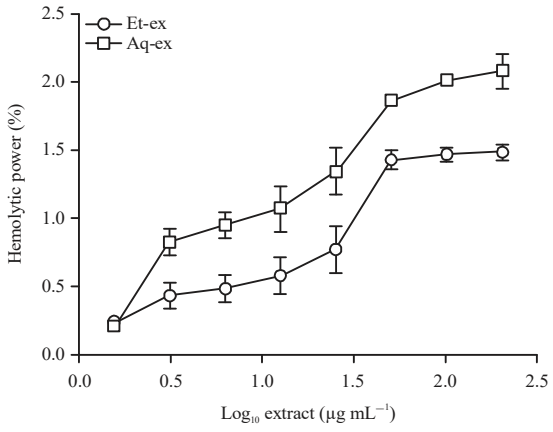

Fig. 4: Hemolytic activity of crude ethanolic and aqueous extracts of *C. afer*

Data represent means for 3 experiments±SD (standard deviation), Et-ex: Ethanolic extract, Aq-ex: Aqueous extract

2000 mg kg^{-1} b.wt., caused no death in rats. The rigorous monitoring of behavior (respiration, food consumption, contortion and coma) of the rats showed no toxicity signs during the experiment period. The body weight of treated animals in comparison to control showed progressive increasing without significant change ($p>0.05$) (Fig. 5).

Among the hematological parameters analyzed, only platelet count of treated group decrease significantly ($p<0.05$) when compared to control group. No significant difference was observed between the others hematological parameters of treated and control groups (Table 2). Similarly, *C. afer* ethanolic extract not induces significant changes in biochemical markers of liver and renal functions as well as serum glucose of tested compared to control rats (Table 2).

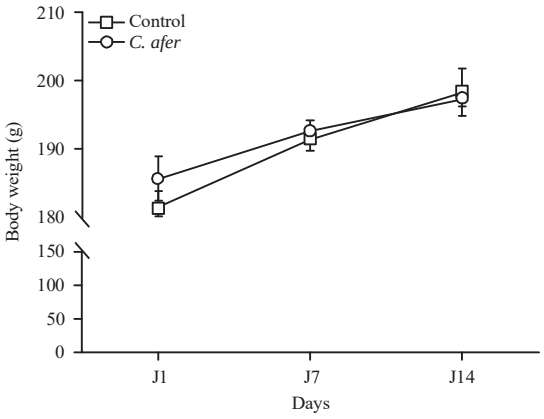

Fig. 5: Effect of *C. afer* crude ethanolic extract on body weight of Wistar rats

Data represent means for 3 experiments±SD (standard deviation), p<0.05 indicates statistically significant difference, J1: Day 1, J7: Day 7, J14: Day 14

Table 2: Effect of the extract on biochemical and hematological parameters

Parameters	Control	EE-CA	p-value
Biochemical parameter			
Glucose (g L^{-1})	1.00±0.30	1.46±0.12	0.12
Creatinine (mg L^{-1})	6.40±0.98	6.31±1.04	0.46
Urea (g L^{-1})	0.53±0.15	0.64±0.13	0.07
ASAT (IU L^{-1})	116.67±8.85	157.93±16.01	0.05
ALAT (IU L^{-1})	43.02±5.40	47.73±9.19	0.32
Hematological parameter			
WBC (×10^3 µL^{-1})	14.30±2.05	14.13±0.070	0.75
Red blood count (×10^6 µL^{-1})	7.98±0.24	6.97±0.88	0.70
Haemoglobin (g dL^{-1})	15.67±0.40	15.10±0.96	0.14
Hematocrit (%)	47.60±1.49	40.90±4.63	0.27
MCV (fL)	59.80±1.31	59.80±0.36	1.00
MCH (pg)	19.70±0.12	19.97±0.61	0.56
MCHC (g dL^{-1})	32.93±0.58	33.40±0.26	0.27
Platelet (×10^3 µL^{-1})	1229.67±126.06	853.67±25.97	0.01*
Lymphocytes (×10^3 µL^{-1})	9.17±2.12	10.60±0.95	0.33

EE-CA: Ethanolic extract of *Costus afer*, WBC: White blood cells, ALAT: Alanine aminotransferase, ASAT: Aspartate transaminase, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular hemoglobin concentration, *p<0.05 indicate statistically significant difference when compared to control, each value represents mean for 3 experiments±SD (n = 3)

DISCUSSION

The use of medicinal plants in the treatment of malaria is a very old practice which for decades has served as a track record for the search for new anti-malarial drugs. In this study, *in vitro* antiplasmodial potential of *C. afer* against field isolates and chloroquine sensitive 3D7 strains of *P. falciparum* have been investigated. According to the appreciation grid of natural substances[28,29], *C. afer* ethanolic extract is highly active on field isolates strain and active on 3D7 strain while aqueous extract is moderately active against both strains. The difference in activity could be explained by the variability of their secondary metabolites which depends on the extraction solvent. Otherwise, the fractions obtained from the liquid-liquid partition of the ethanolic extract are weakly active on both strains. This result indicated that the strong antiplasmodial activity demonstrated by the ethanolic extract could result from the synergistic action of secondary metabolites. The qualitative analysis of secondary metabolites of both extracts revealed the presence of diverse phytoconstituent groups that are known to have antiplasmodial activity including flavonoids[30], tannins, coumarins[31], triterpenes[32] and anthraquinones[33]. These groups of secondary metabolites are also known to act either alone or in synergy against *P. falciparum*[30]. The interesting antiplasmodial activity observed with ethanolic extract in comparison to the aqueous extract could be attributed to the presence of tannins that are absent in aqueous extract. The same extract presented the high amount of phenolic compounds which is also associated with the antiplasmodial activity of extract[34]. Although no antiplasmodial activity report of *C. afer* exists in the literature, interesting antimalarial activity has been reported with related species, *Costus Lucanusianus* extract[35].

Malaria infection has been associated with the production of reactive oxygen and nitrogen species, responsible for the oxidative stress observed in the pathophysiology of the disease[36]. Antioxidants, mainly from natural sources, are important for minimizing or reversing the oxidative damage that may occur during malaria. Moreover, antioxidants can block the conversion of free heme to hemozoin, thus making the host environment toxic to the survival of the parasite[37]. For this purpose, the antioxidant potential was evaluated from both crude extracts of *C. afer* using different *in vitro* antioxidant models. The results showed that both extracts have interesting antioxidant potential and can act as scavengers, electron or hydrogen donors, or as reducing agents[38]. These antioxidant capacities could be attributed to phenolic compounds, mainly the flavonoids present in these extracts[39]. *Costus afer,* through his antioxidant activity, could be useful in the management of malaria mainly oxidative damages and red blood cell lysis induced by the infection. These findings are supported by reports of several studies[15-17,40].

Red blood cells membrane is a delicate structure that can be easily altered by plant extract[41] resulting in erythrocyte rupture. Thus, hemolytic power of extracts was assessed to know whether they have cytotoxic effect on red blood cells. The results showed that *C. afer* ethanolic and aqueous extracts do not have hemolytic effect on human red blood

cells. These results confirmed that the antiplasmodial activity observed with the extracts is induced by the direct effect of secondary metabolites on *P. falciparum*.

In the acute oral toxicity assessed with ethanolic extract (most active extract against strains of *P. falciparum*), LD_{50} is above 2000 mg kg^{-1} b.wt., as indicated in the OECD Guidelines. The absence of changes in behavior, of an overt sign of distress, of death and the normal increase of body weight in the test animals during the experimentation period is good evidence for lack of acute toxicity as set by Institute for Laboratory Animal Research (ILAR)[42]. Biological parameters, mainly serum biomarkers that provide information on the health status of vital organs including the liver (ALT and AST)[43] and kidneys (creatinine and urea)[44] and serum glucose were also investigated in this study. The results revealed no statistically significant change in these biological parameters indicating that *C. afer* ethanolic extract does not induces alteration of renal and liver functions as well as glycaemia. Hypoglycaemic and organ protective properties of *C. afer* have been also reported[12,13]. Moreover, no statistically significant variation between hematological parameters of group treated with *C. afer* ethanolic extract and control group (Table 2) indicated normal physiological condition of treated animals.

CONCLUSION

The present study demonstrated the greatest antiplasmodial activity of ethanolic extract of *Costus afer* against field isolates and chloroquine-sensitive (3D7) strains of *P. falciparum* with moderate activity exhibited by aqueous extract as well as fractions from the ethanolic extract. This suggested a probable synergetic action provided by phytoconstituents observed in ethanolic extract. Strong antioxidant activity as well as the lack of toxicity was found with this extract. However, further *in vivo* antiplasmodial investigations in order to better interpret some physiological factors and immune responses in the use of *Costus afer* need to be carried out.

SIGNIFICANCE STATEMENT

This study discovers the possible synergistic effect of secondary metabolites of *Costus afer* ethanolic extract, a combination that can be beneficial for the management of malaria as well as oxidative damages resulting from the pathophysiology of the disease. This study will help the researcher to uncover the critical area of *Plasmodium* parasites resistance and malaria transmission-blocking that many researchers were not able to explore.

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REFERENCES

1. WHO., 2009. Report of the WHO interregional workshop on the use of traditional medicine in primary health care: Ulaanbaatar, Mongolia, 23-26 August 2007. World Health Organization, Rome, Italy, May 22, 2009 pp: 1-84. http://apps.who.int/iris/bitstream/10665/44008/1/9789241597425_eng.pdf
2. Firenzuoli, F. and L. Gori, 2007. Herbal medicine today: Clinical and research issues. Evid. Based Complement. Alternat. Med., 4(Suppl. 1): 37-40.
3. Schmelzer, G.H. and A. Gurib-Fakim, 2008. Plant Resources of Tropical Africa: Medicinal Plants 1. PROTA Foundation, Wageningen/Backhuys Publishers, Leiden, Netherlands, pp: 191.
4. Akoègninou, A., W.J. van der Burg and L.J.G. van der Maesen, 2006. Flore Analytique du Bénin. (Wageningen Agricultural University Papers 06.2), Backhuys Publishers, Leiden, ISBN: 9789057821813, Pages: 1034.
5. Adomou, A.C., H. Yedomonhan, B. Djossa, S.I. Legba, M. Oumorou and A. Akoegninou, 2012. Etude Ethnobotanique des plantes médicinales vendues dans le marché d'Abomey-Calavi au Bénin. Int. J. Biol. Chem. Sci., 6: 745-772.
6. Hermans, M., A. Akoègninou and J. van der Maesen, 2004. Medicinal plants used to treat Malaria in Southern Benin. Econ. Bot., 58: S239-S252.
7. Yetein, M.H., L.G. Houessou, T.O. Lougbegnon, O. Teka and B. Tente, 2013. Ethnobotanical study of medicinal plants used for the treatment of malaria in plateau of Allada, Benin (West Africa). J. Ethnopharmacol., 146: 154-163.
8. Nondo, R.S.O., D. Zofou, M.J. Moshi, P. Erasto and S. Wanji *et al*., 2015. Ethnobotanical survey and *in vitro* antiplasmodial activity of medicinal plants used to treat malaria in Kagera and Lindi regions, Tanzania. J. Med. Plants Res., 9: 179-192.
9. Omokhua, G.E., 2011. Medicinal and Socio-cultural importance of *Costus afer* (*Ker gravl*) in Nigeria. Afr. Res. Rev., 5: 282-287.

10. Adjanohoun, E., V. Adjakidje, M.R.A. Ahyi, L. Akéassi and A. Akoegninou *et al*., 1989. Contribution Aux Études Ethnobotaniques et Floristiques En Republique Du Bénin. Agence de Coopération Culturelle et Technique, Paris, Pages: 895.
11. Anyasor, G.N., F. Onajobi, O. Osilesi, O. Adebawo and E.M. Oboutor, 2014. Anti-inflammatory and antioxidant activities of Costus afer Ker Gawl. hexane leaf fraction in arthritic rat models. J. Ethnopharmacol., 155: 543-551.
12. Ezejiofor, A.N., Z.N. Igweze, N.A. Udowelle and O.E. Orisakwe, 2017. Histopathological and biochemical assessments of *Costus afer* stem on alloxan-induced diabetic rats. J. Basic Clin. Physiol. Pharmacol., 28: 383-391.
13. Ukpabi, C.F., K.N. Agbafor, O.K. Ndukwe, A. Agwu and S.N. Nwachukwu, 2012. Phytochemical composition of *Costus afer* extract and its alleviation of carbon tetrachloride-induced hepatic oxidative stress and toxicity. Int. J. Mod. Bot., 2: 120-126.
14. Anyasor, G.N., F.D. Onajobi, O. Osilesi and O. Adebawo, 2014. Proximate composition, mineral content and *in vitro* antioxidant activity of leaf and stem of Costus afer (Ginger lily). J. Intercult. Ethnopharmacol., 3: 128-134.
15. Anyasor, G.N., K.O. Ogunwenmo, O.A. Oyelana and B.E. Akpofunure, 2010. Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of Costus afer Ker Gawl. (Costaceae). Afr. J. Biotechnol., 9: 4880-4884.
16. Atere, T.G., O.A. Akinloye, R.N. Ugbaja, D.A. Ojo and G. Dealtry, 2018. *In vitro* antioxidant capacity and free radical scavenging evaluation of standardized extract of *Costus afer* leaf. Food Sci. Hum. Wellness, 7: 266-272.
17. Tchamgoue, A.D., L.R. Tchokouaha, P.A. Tarkang, J.R. Kuiate and G.A. Agbor, 2015. *Costus afer* possesses carbohydrate hydrolyzing enzymes inhibitory activity and antioxidant capacity *in vitro*. Evidence-Based Complement. Altern. Med., Vol. 2015. 10.1155/2015/987984.
18. Wagner, H. and S. Bladt, 2001. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd Edn., Springer, Berlin, ISBN: 3540586768, Pages: 384..
19. Shah, M.D. and M.A. Hossain, 2014. Total flavonoids content and biochemical screening of the leaves of tropical endemic medicinal plant *Merremia borneensis*. Arabian J. Chem., 7: 1034-1038.
20. Amoussa, A.M.O., A. Sanni and L. Lagnika, 2015. Antioxidant activity and total phenolic, flavonoid and flavonol contents of the bark extracts of *Acacia ataxacantha*. J. Pharm. Phytochem., 4: 172-178.
21. El Jemli, M., R. Kamal, I. Marmouzi, A. Zerrouki, Y. Cherrah and K. Alaoui, 2016. Radical-scavenging activity and ferric reducing ability of *Juniperus thurifera* (L.), *J. oxycedrus* (L.), *J. phoenicea* (L.) and *Tetraclinis articulata* (L.). Adv. Pharmacol. Sci., Vol. 2016. 10.1155/2016/6392656.
22. Kumar, R.S., B. Rajkapoor and P. Perumal, 2012. Antioxidant activities of Indigofera cassioides Rottl. Ex. DC. Using various *in vitro* assay models. Asian Pac. J. Trop. Biomed., 2: 256-261.
23. Ruch, R.J., S.J. Cheng and J.E. Klaunig, 1989. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 10: 1003-1008.
24. Trager, W. and J.B. Jensen, 1976. Human malaria parasites in continuous culture. Sciences, 193: 673-675.
25. Desjardins, R.E., C.J. Canfield, J.D. Haynes and J.D. Chulay, 1979. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. Antimicrob. Agents Chemother., 16: 710-718.
26. Sarr, S.O., S. Perrotey, I. Fall, S. Ennahar and M. Zhao *et al*., 2011. *Icacina senegalensis* (Icacinaceae), traditionally used for the treatment of malaria, inhibits *in vitro Plasmodium falciparum* growth without host cell toxicity. Malaria J., Vol. 10 10.1186/1475-2875-10-85.
27. OECD., 2002. OECD Guidelines for the Testing of Chemicals/Section 4: Health Effects Test No 423: Acute Oral Toxicity-Acute Toxic Class Method. OECD., Paris.
28. Clarkson, C., V.J. Maharaj, N.R. Crouch, O.M. Grace and P. Pillay *et al*., 2004. *In vitro* antiplasmodial activity of medicinal plants native to or naturalised in South Africa. J. Ethnopharmacol., 92: 177-191.
29. Bero, J., H. Ganfon, M.C. Jonville, M. Frederich and F. Gbaguidi *et al*., 2009. *In vitro* antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria. J. Ethnopharmacol., 133: 439-444.
30. Ferreira, J.F.S., D.L. Luthria, T. Sasaki and A. Heyerick, 2010. Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. Molecules, 15: 3135-3170.
31. Bero, J., M. Frederich and J. Quetin-Leclercq, 2009. Antimalarial compounds isolated from plants used in traditional medicine. J. Pharm. Pharmacol., 61: 1401-1433.
32. Mbah, J.A., G. Ndikum, D. Zofou, M.N. Ngemenya and S.M.N. Efange, 2011. Antiplasmodial triterpenes from the stem bark of *Baillonella toxisperma*. ISESCO J. Sci. Tech., 7: 84-87.
33. Osman, C.P., N.H. Ismail, R. Ahmad, N. Ahmat, K. Awang and F.M. Jaafar, 2010. Anthraquinones with antiplasmodial activity from the roots of *Rennellia elliptica* Korth. (Rubiaceae). Molecules, 15: 7218-7226.
34. Chabir, N., H. Ibrahim, H. Romdhane, A. Valentin, B. Moukarzel, M. Mars and J. Bouajila, 2014. Seeds of *Peganum harmala* L. chemical analysis, antimalarial and antioxidant activities and cytotoxicity against human breast cancer cells. Med. Chem., 11: 94-101.
35. Ezerioha, C.E. and H.D. Kagbo, 2017. Evaluation of antimalarial effect of methanol root extract of *Costus lucanusianus* in the treatment of malaria-infested albino mice. Innov. Pharm. Pharmacother., 5: 137-140.

36. Percario, S., D.R. Moreira, B.A.Q. Gomes, M.E.S. Ferreira and A.C.M. Goncalves *et al*., 2012. Oxidative stress in malaria. Int. J. Mol. Sci., 13: 16346-16372.
37. Tekwan, B.L. and L.A. Walker, 2005. Targeting the hemozoin synthesis pathway for new antimalarial drug discovery: Technologies for *in vitro* β-hematin formation assay. Comb. Chem. High Throughput Screen, 8: 63-79.
38. Huang, D., B. Ou and R.L. Prior, 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem., 53: 1841-1856.
39. Leopoldini, M., T. Marino, N. Russo and M. Toscano, 2004. Antioxidant properties of phenolic compounds: H-atom versus electron transfer mechanism. J. Phys. Chem. A, 108: 4916-4922.
40. Anyasor, G.N., O. Funmilayo, O. Odutola, A. Olugbenga and E.M. Oboutor, 2014. Chemical constituents in n-butanol fractions of *Castus afer* ker Gawl leaf and stem. J. Intercult. Ethnopharmacol., 3: 78-84.
41. Santos-Filho, S.D., 2016. Erythrocyte membrane and hemolysis: Effects of natural products. Int. J. Life Sci. Technol., 9: 28-35.
42. NRC., 1996. Guide for the Care and Use of Laboratory Animals. The National Academies Press, Washington, DC., USA., ISBN-13: 9780309053778, Pages: 125.
43. Ramaiah, S.K., 2011. Preclinical safety assessment: Current gaps, challenges and approaches in identifying translatable biomarkers of drug-induced liver injury. Clin. Lab. Med., 31: 161-172.
44. Alarifi, S., A. Al-Doaiss, S. Alkahtani, S.A. Al-Farraj and M.S. Al-Eissa *et al*., 2012. Blood chemical changes and renal histological alterations induced by gentamicin in rats. Saudi J. Biol. Sci., 19: 103-110.