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Anti-inflammatory actions and *Salmonella typhimurium*-bacteraemia clearance by methanol extract of *Curcuma longa* Linn. (Turmeric)

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ABSTRACT

Aims: Rhizome of turmeric is known to possess therapeutic activities and has been used in medical practice as an anti-diabetic, hypolipidemic, hepatoprotective, anti-diarrheal, and anti-asthma agent. This study was designed to investigate the anti-inflammatory and antimicrobial activities of *Curcuma longa*.
Methodology and results: Rhizomes of *Curcuma longa* (Turmeric) purchased from markets in Ado-Ekiti, Nigeria, were analysed for anti-inflammatory and anti-microbial properties, as well as phytochemical constituents. The *in vitro* anti-inflammatory activities of the *C. longa* methanol extract (CLME) were evaluated by albumin denaturation, proteinase inhibitory activity, membrane stabilization, and anti-lipoxygenase activity, at different concentrations using Aspirin, Diclofenac sodium and Indomethacin as standard drugs. The *in vitro* antimicrobial activities of CLME were carried out on five pathogenic microbes namely *Escherichia coli* ATCC 29929, *Staphylococcus aureus* ATCC 29293, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 4252 and *Candida albicans* ATCC 10231, using both agar well diffusion and broth dilution techniques. A *S. typhimurium* infected rat model was used for *in vivo* antimicrobial studies. Phytochemical analyses showed that *C. longa* rhizomes contain high concentrations of alkaloids, flavonoid and saponins, with moderate levels of phenols, tannin and ferric reducing antioxidant power. CLME was found to be rich in alkaloids, tannins, phenols, steroids, saponins, terpenoids, flavonoids and reducing sugars. CLME showed potent anti-inflammatory activities, and the results compared favourably with the standard anti-inflammatory drugs used. *C. longa* methanol extract significantly inhibited albumin denaturation and proteinase activity, stabilized membrane of red blood cell from haemolysis in heat and hypotonic conditions, as well inhibited lipoxygenase activity; all of which are associated with inflammatory processes. CLME was found to possess high *in vitro* antimicrobial activities against the five microorganisms tested. Rats orally infected with *S. typhimurium*, demonstrated bacteraemia five days post infection, with a total clearance of bacteraemia within 3-5 days following oral administration of CLME. The infected rats treated with CLME equally showed significant improvement in some haematological indices compared to infected rats that were not treated with CLME.
Conclusion, significance and impact of study: The results also showed that methanol extract of *C. longa* rhizome effectively cured with *S. typhimurium* infected rats. The overall results suggest that *Curcuma longa* is a potential source of anti-inflammatory and antimicrobial agents.

Keywords: Anti-inflammation, antimicrobial, bacteraemia, *Curcuma longa*, *Salmonella typhimurium*

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal values of plants lie in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant women and nursing mothers for medicinal purposes (Falodun *et al.,* 2003). In addition, the use of herbal medicine for the treatment of diseases and infections is as old as mankind. The World Health Organization supports the use of traditional medicine provided they are proven to be efficacious and safe (WHO, 2016). In developing countries, a huge number of people live in extreme poverty and some are suffering and dying for want of safe water and medicine, they have no alternative to primary health care (Fazly-Bazzaz *et al.,* 2005).

Curcuma longa Linn or turmeric (from Zingiberaceae family) is highly regarded as a universal panacea in the herbal medicine with a wide spectrum of pharmacological activities (Nasri *et al*., 2014). Turmeric is a plant distributed throughout tropical and subtropical regions of

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the world. Turmeric is an essential spice all over the world with a distinguished human use particularly among the Eastern people (Ravindran *et al.,* 2007). Apart from the uses as spice, it is used as traditional medicine in Asian countries such as India, Bangladesh and Pakistan because of its beneficial properties (Chattopadhyay *et al.,* 2004). Current traditional medicine claims its powder to be effective against gastrointestinal diseases, especially for biliary and hepatic disorder, diabetic wounds, rheumatism, inflammation, sinusitis, anorexia, coryza and cough. The colouring principle of turmeric is called curcumin, which has yellow colour and is the essential component of this plant. Recent studies have authenticated turmeric as anticancer, anti-diabetic, antioxidant, hypolipidemic, anti-inflammatory, anti-microbial, anti-fertility, anti-venom, hepatoprotective, nephroprotective, anticoagulant etc. The plant was also shown to possess anti-HIV activity to combat AIDS. These medicinal properties of turmeric caused it to be considered as a spice with multifunctional medicinal properties (Shpitz *et al.,* 2006).

The present study was designed to investigate the *in vitro* anti-inflammatory and both *in vitro* and *in vivo* antimicrobial activities of *Curcuma longa*.

MATERIALS AND METHODS

Collection of plant samples

The rhizomes of *Curcuma longa* used for this study were purchased in the month of October 2016 from the local markets of Ado-Ekiti, Nigeria, and transported to the Microbiology Laboratory of Afe Babalola University, Ado-Ekiti (ABUAD), Nigeria. Dr. O. T. Ogunmefun, a botanist in the Department of Biological Sciences, Afe Babalola University, identified the plants.

Preparation of plant extracts

The rhizomes of *C. longa* were washed to remove sand, sliced, air dried and ground into fine powder using an electrical blender. Plant extract was prepared by cold percolation method as described by Akinpelu and Onakoya (2006). An amount of 200 g of the plant powder was soaked in 600 mL of methanol in glass containers and properly covered. The mixture was allowed to stand for 48 hours at room temperature, to permit full extraction of the active ingredients. The fluid was then filtered using Whatman No 1 filter paper into beakers. The extract was concentrated in a rotary evaporator, followed by drying at 45 °C in a water bath.

Phytochemical screening of plant extracts

A preliminary phytochemical screening of the plant extract was carried out using standard phytochemical procedures. The concentrations of alkaloids, flavonoids, steroids, saponins, phenols, tannins, glycosides, cardiac glycosides and reducing sugar in turmeric powder were determined following the methods of Harborne (1998) and Mayuri (2012).

Test for flavonoids:

A 5 mg weight of the compound was dissolved in water, warmed and filtered. Ten percent (10%) aqueous sodium hydroxide was added to 2 mL of the solution to produce a yellow coloration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.

Tannins:

One gram of the sample was extracted with 25 mL of the solvent mixture of 80 : 20 acetone : 10% glacial acetic acid for 5 hours. The supernatant was filtered and the absorbance of the filtrate as well as the reagent blank measured at 500 nm absorbance. A standard graph was produced with 10, 20, 30, 40, 50 mg/100 g of tannic acid. The concentration of tannin was read off taking into consideration dilution factors.

Alkaloids:

One gram of the sample (W) was added to 20 mL of 10% acetic acid in ethanol, shaken, allowed to stand for 4 hours and filtered. The filtrate was allowed to evaporate to about a quarter of its original volume and one drop of concentration ammonia added. The precipitate formed was filtered through a weighed (W1) filter paper. The filter paper dried in the oven at 60 °C, weighed when it has attained a constant weight (W2).

$$\text{Alkaloid (\%)} = \frac{W2 - W1}{W} \times 100$$

Phenols/Phenolics/Phenolic Acids:

An aliquot of 2 mL of the extract was mixed with 0.5 mL of Folin-Ciocalteau reagent and 1.5 mL sodium carbonate (20%). The mixture was stirred for 15 seconds and allowed to stand at 40 °C for 30 min to develop colour. The absorbance was then measured A_{765}. Expressed as GAE/g (Gallic Acid Equivalent).

Saponins:

One gram of sample was added to 5 mL of 20% ethanol in a conical flask and placed in a water bath at 55 °C for 4 h. Filtering and washing the residue with 20% ethanol twice and reducing the extract to about 5 mL over water bath at 90 °C. The extract was further purified by successive treatment with petroleum ether, butanol and 5% sodium chloride. After discarding the sodium chloride layer, the solution was dried in a water bath at 90 °C, later transferred in a crucible and dried in an oven until a constant weight was obtained.

$$\text{Saponin (\%)} = \frac{\text{Weight of Saponin}}{\text{Weight of the Sample}} \times 100$$

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Steroids:

Five grams of the sample was added to 100 mL of water and drops of 0.1 M ammonium hydroxide were added to take the pH to 9.1, then 2 mL pet-ether, 3 mL acetic anhydride and concentrated H_2SO_4 were added, and the absorbance measured at 420 nm.

Cardiac glycosides/cardenolides:

One gram of the sample was extracted with 40 mL water and placed in the water bath at 100 °C for 15 min, then 1 mL of the extract plus 5 mL water added to 2 mL glacial acetic acid plus one drop of $FeCl_3$. Then 1 mL of concentrated H_2SO_4 was added and measurement of absorbance of the resulting solutions was taken at 410 nm.

Ferric reducing antioxidant power (FRAP) assay:

The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method employing an easily reduced oxidant, Fe (III). Reduction of a ferric tri-pyridyltriazine complex to ferrous (2,4,6-Tripyridyl-s-triazine)$_2$ i.e. Ferric (III) [colourless] to Ferrous (II) [blue] can be monitored by measuring absorbance at 593 nm. The absorption readings are related to the reducing power of the electron-donating antioxidants present in the test compound. Hence the FRAP assay can rank the reducing power and the antioxidant potential of the compound. The FRAP equation is:

$$\text{FRAP value of sample (µM)} = \frac{\text{Absorbance (Sample)} \times \text{FRAP value of standard (µM)}}{\text{Absorbance (Std)}}$$

Assessment of *in vitro* anti-inflammatory activity of *Curcuma longa*

The *in vitro* anti-inflammatory activity of the Methanol extract of *Curcuma longa* (CLME) was evaluated using albumin denaturation, proteinase inhibitory activity, membrane stabilization, and anti-lipoxygenase activity.

Inhibition of albumin denaturation

Determination of the anti-inflammatory activity of the methanol extract of *C. longa* by inhibition of albumin denaturation technique was carried out according to Mizushima and Kobayashi (1968) and Sakat *et al.* (2010). The reaction mixture consisted of 100 µL (100-500 µg/mL) test extract and 500 µL of 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted to 6.3 using small amount of 1 N HCl. The sample extracts were then incubated at 37 °C for 20 min and then heated to 51 °C for 20 min. After cooling the samples, the turbidity was measured at 660 nm (Spectrophotometer Model Spectronic 20). The experiments were performed in triplicate. The percentage inhibitions of protein denaturation were then calculated as follows:

$$\text{Percentage inhibition of protein denaturation} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Proteinase inhibitory action

The test was performed according to the methods described by Oyedepo and Famurewa (1995), and Sakat *et al*. (2010). The reaction mixture (2 mL), contained 0.06 mg trypsin, 1 mL 20 mM Tris-HCl buffer (pH 7.4) and 1 mL of CLME at different concentrations (100-500 µg/mL). The mixture was incubated at 37 °C for 5 min and then 1 mL of 0.8% (w/v) casein was added. The mixture was then incubated for an additional 20 min. A 2 mL volume of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiments were performed in triplicate. The percentage inhibition of proteinase inhibitory activity was then calculated as:

$$\text{Percentage of proteinase inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Membrane stabilization

Blood was collected from a healthy human volunteer who had not taken any Non Steroidal Anti-Inflammatory Drugs (NSAIDs) for 2 weeks prior to the experiment and transferred into centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and washed three times with equal volume of normal saline. The volume of blood was then measured and reconstituted as 10% v/v suspension with normal saline.

Heat Induced haemolysis

The reaction mixture (2 mL) consisted of 1 mL of CLME at different concentrations (100-500 µg/mL) and 1 mL of 10% red blood cells (RBCs) suspension. Instead of test sample, only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 °C for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants were taken at 560 nm. The experiments were performed in triplicates for all the test samples (Sakat *et al*., 2010). The Percentage inhibition of haemolysis was calculated as follows:

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$$\text{Percentage inhibition of haemolysis} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Hypotonicity induced haemolysis

A volume of 0.5 mL of different concentration of CLME (100-500 µg/mL), standard drug (100 µg/mL), and control (distilled water instead of hyposaline to produce 100% haemolysis) were separately mixed with 1 mL of phosphate buffer (pH 7.4, 0.15 M), 2 mL of hyposaline (0.36%) and 0.5 mL of HRBC suspension. Diclofenac sodium (100 µg/mL) was used as a standard drug. All the assay mixtures were incubated at 37 °C for 30 min and centrifuged at 3000 rpm. The supernatant liquid was decanted and the haemoglobin content estimated by a spectrophotometer at 560 nm. The percentage haemolysis was estimated by assuming the haemolysis produced in the control as 100% (Sakat *et al*., 2010).

$$\text{Percentage protection} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Anti-lipoxygenase activity

Anti-Lipoxygenase activity was studied using linoleic acid as substrate and lipoxidase as enzyme. Test sample was dissolved in 0.25 mL of 2 M borate buffer (pH 9.0) and 0.25 mL of lipoxidase enzyme solution (20,000 U/mL) was added, followed by incubation at 25 °C for 5 min. Afterwards, 1.0 mL of Linoleic acid solution (0.6 mM) was added and mixed well. Absorbance was measured at 234 nm. Indomethacin was used as reference standard (Sakat *et al*., 2010). The percent inhibition was then calculated from the following equation:

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

A dose-response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

***In vitro* antimicrobial activities of CLME**

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the CLME on *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 29929, *Staphylococcus aureus* ATCC 29293, *Klebsiella pneumoniae* ATCC 4252 and *Candida albicans* ATCC 10231, by the macrodilution (tube) method as described by Clinical and Laboratory Standards Institutes, CLSI (2012, 2016).

Different concentrations (0.04-10.0) mg/mL of the extract was prepared by serial dilution in Mueller Hinton broth medium (Khan *et al.,* 2007; CLSI, 2012). To each test tube was added 1 mL of each of the standardized (0.5 McFarland standard) test organism and 1 mL of the turmeric extract. Two Mueller Hinton broth tubes, with and without microbial inoculation, were used as the growth and sterility controls. The tubes were incubated aerobically at 37 °C for 24 h. Following the incubation period, the tubes were observed for the MICs by checking concentration of the first tube in the series (ascending extract concentrations) that showed no visible trace of growth. The first tube in the series with no visible growth after the incubation period was taken as the MIC.

The minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a particular bacterium. The tubes from the broth dilution MIC tests were subcultured to agar plates that do not contain test agents. The MBC's were identified by determining the lowest concentration of antimicrobial agent that reduced the viability of the initial microbial inoculum by ≥ 99.9% (French, 2006; CLSI, 2012).

***In vivo* antibacterial activity**

The procedure was carried out using 25 male Wistar rats (7-8 weeks old) weighing 150-175 g. The rats were maintained in the Afe Babalola University Animal House, inside standard cages, with food and water provided *ad libitum*. Optimum conditions including temperature, light and ventilation, were provided throughout the period of experimentation. The rats were distributed into five groups (A-E) of five animals each. Administration of plant extract, CLME (the MIC value and double folds of it) and test organisms were done orally by intubation using a gavage needle (Pan *et al.,* 2014).

The inhibition of the growth of test organisms in rats was then determined by monitoring test organism in the blood of the rats. Briefly *S. typhimurium* (ATCC 14028) was grown overnight and diluted in normal saline solution (0.9% NaCl) to achieve a final concentration of approximately 1.5×10^8 CFU/mL of test organism (Pan *et al.,* 2014). Animals were administered orally, using gavage needle, with the test organism. Groups A, B, C and D were administered with 1 mL of test organism (*S. typhimurium*), while Group E rats were not administered with the organism.

Demonstration of establishment of bacteraemia in the rats was carried out on the fifth day post infection; blood samples of rats from all groups were collected via the retro-orbital plexus bleeding, inoculated on *Salmonella-Shigella* agar plates, and incubated overnight at 37 °C. The plates were then observed for bacterial growth after 24 hours. On the 6th day, 1 mL of 0.56, 1.12 and 2.24 mg/mL of the methanol extract *C. longa* was administered to rats in Groups A, B and C respectively, for three consecutive days, with the exception of Group D (the positive) and Group E (negative control). On the 10th day of *S typhimurium* inoculation, i.e. 4 days following treatment with CLME, blood samples were collected from all the rats via the retro-orbital plexus bleeding and inoculated on *Salmonella-Shigella* agar plates. Plates were observed for bacterial growth. Haematological

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analyses of the blood samples were carried out using the Haematological Analyser (Count-Maxcom, China), to determine the white blood cell count (WBC), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), platelet (PLT), mean platelet volume (MPV), mean corpuscular volume (MCV), granulocyte and lymphocyte concentrations, mean corpuscular haemoglobin concentration (MCHC), Width distribution of platelets (PDW), plateletcrit (PCT), red cell distribution width, covariance (RDW_CV) and red cell distribution width, standard deviation (RDW_SD).

Statistical analysis

Results were expressed as mean ± standard deviation. The significant differences between experimental groups were determined by One-Way Analysis Of Variance (ANOVA), followed by Dunnet Multiple comparison test (control vs test) using the Graph Pad Instat software.

RESULTS

Phytochemical composition of Turmeric (*Curcuma longa* Linn.)

The extractive yield of 250 g of dried pulverized rhizomes of *C. longa* was 3.30%. Quantitative phytochemical analyses of the dried *Curcuma longa* rhizomes revealed 0.729 mg/g concentration of phenol; 0.542 mg/g of tannin; 21.334 mg/g of flavonoids; 9.500 mg/g of saponins; and 30.600 mg/g of alkaloids (Table 1). The ferric reducing antioxidant power (FRAP) of *Curcuma longa* was found to be 15.220 Mg GAE/g of plant sample (Table 1).

Table 1: Phytochemical composition of *Curcuma longa*.

Parameter	Qualitative (Methanol extract)	Quantitative (of the powder)
Phenol	+	72.9 mg/100g
Tannin	+	54.2 mg/100g
Flavonoid	+	2133.4 mg/100g
Saponin	-	950.0 mg/100g
Alkaloid	+	3060.0 mg/100g
Terpenoids	-	ND
Steroids	-	ND
Glycosides	-	ND
Cardiac glycosides	-	ND
FRAP	+	15.220 Mg GAE/g

+, present; -, absent; ND, not done.

***In vitro* anti-inflammatory activity of *Curcuma longa* methanol extract**

Curcuma longa methanol extract (CLME) was found to exhibit anti-inflammatory activities through albumin denaturation, proteinase inhibitory activity, membrane stabilization, and anti-lipoxygenase activity.

CLME was effective in inhibiting heat induced albumin denaturation at different concentrations as shown in Figure 1. The CLME showed maximum inhibition of 87.4% with 500 µg/mL concentration. Aspirin, a standard anti-inflammatory drug, showed the maximum inhibition of 80.7% at a concentration of 100 µg/mL.

The methanol extracts of *Curcuma longa* exhibited significant anti-proteinase activity at different concentrations as shown in Figure 2. The methanol extract of *Curcuma longa* showed maximum inhibition of 61% with 500 µg/mL concentration. Aspirin showed the maximum inhibition of 62.2% with 100 µg/mL concentration.

The methanol extract of *C. longa* was also effective in inhibiting the heat-induced haemolysis of red blood cells (RBC), as shown in Figure 3. The results showed that at concentration range of 300-500 µg/mL, methanol extract of *C. longa* significantly protects the erythrocyte membrane against lysis induced by heat. Aspirin at 100 µg/mL equally offered a significant protection against damaging effect of heat-induced haemolysis.

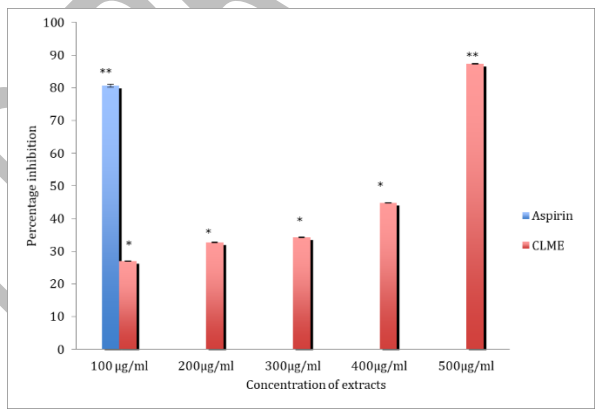

Figure 1: Effect of *Curcuma longa* methanol extract on heat induced protein denaturation. Experimental groups compared with control: **p ≤ 0.01 was considered as highly significant; *p ≤ 0.05 considered significant; and ^{ns}p > 0.05 considered not significant.

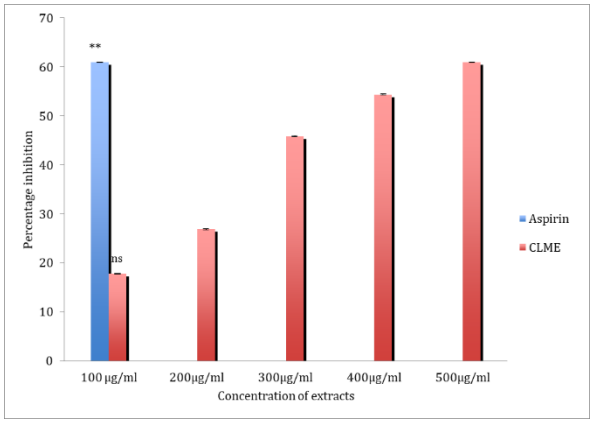

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Figure 2: Percentage effect of *Curcuma longa* methanol extract on proteinase inhibitory action. Experimental groups compared with control: **p ≤ 0.01 was considered as highly significant; *p ≤ 0.05 considered significant; and ^{ns}p > 0.05 considered not significant.

The results also showed that CLME at concentration range of 200-500 µg/mL protected significantly (p < 0.01) the erythrocyte membrane against lysis induced by hypotonic solution (Figure 4). At a concentration of 500 µg/mL *Curcuma longa* showed maximum protection of 78.9%, whereas Diclofenac sodium (100 µg/mL) showed 57.3% inhibition of RBC haemolysis when compared with control.

CLME tested at 100, 200, 300, 400, 500 µg/mL for lipoxygenase inhibitory action, showed 11.9, 19.7, 25.1, 47.5, and 61.4% inhibition of lipoxygenase respectively. The standard indomethacin, showed 84.8% inhibition at a concentration of 100 µg/mL (Figure 5).

In vitro antimicrobial activity of *Curcuma longa* Methanol Extract (CLME)

CLME showed high antimicrobial activities against *S. typhimurium* ATCC 14028, *Escherichia coli* ATCC 29929, *Staphylococcus aureus* ATCC 29293, *Klebsiella pneumoniae* ATCC 4252 and *Candida albicans* ATCC 10231. The MICs and MBCs of the plant extract against the five pathogenic microorganisms are shown in Table 2.

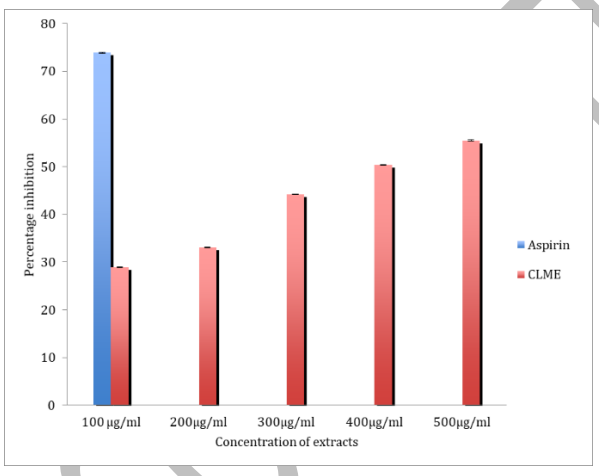

Figure 3: Effect of *Curcuma longa* methanol extract on heat induced haemolysis of erythrocyte. Experimental groups compared with control: **p ≤ 0.01 was considered as highly significant; *p ≤ 0.05 considered significant; and ^{ns}p > 0.05 considered not significant.

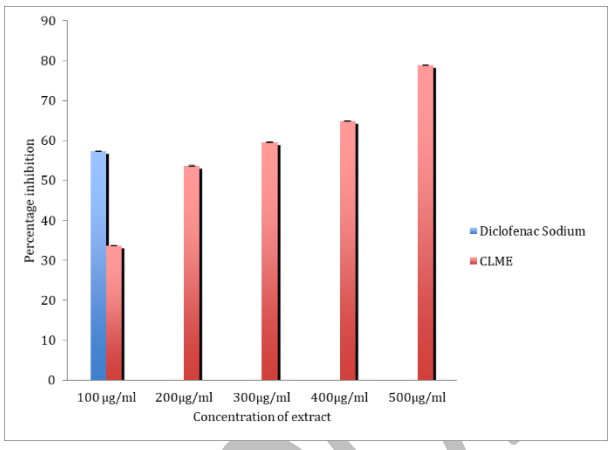

Figure 4: Effect of *Curcuma longa* methanol extract on hypotonicity induced haemolysis of erythrocyte. Experimental groups compared with control: **p ≤ 0.01 was considered as highly significant; *p ≤ 0.05 considered significant; and ^{ns}p > 0.05 considered not significant.

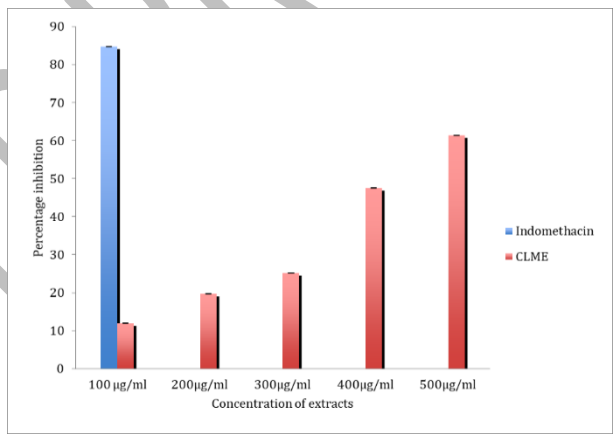

Figure 5: Effect of *Curcuma longa* methanol extract on lipoxygenase inhibitory action. Experimental groups compared with control: **p ≤ 0.01 was considered as highly significant; *p ≤ 0.05 considered significant; and ^{ns}p > 0.05 considered not significant.

Table 2: Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of *Curcuma longa* methanol extract on microorganisms.

Microorganisms	MIC (mg/mL)	MBC (mg/mL)
Escherichia coli ATCC 29929	0.14	0.56
Staphylococcus aureus ATCC 29293	0.56	0.75
Salmonella typhimurium ATCC 14028	0.56	0.75
Klebsiella pneumoniae ATCC 4252	0.14	0.56
Candida albicans ATCC 10231	0.14	0.56

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Table 3: Haematological parameters of rats infected with *S. typhimurium* and treated with *Curcuma longa*.

Biomarkers	*C. longa* 0.56 mg/mL	*C. longa* 1.12 mg/mL	*C. longa* 2.24 mg/mL	Infected with *S. typhimurium*	Control (No Infection)
Red Blood Cell (IL,10^3)	7.7±0.27^{abc}	7.9±0.84^{a}	8.0±0.63^{a}	6.5±1.12^{bc}	7.2±1.20^{abc}
Haemoglobin g/dL	16.8±0.29^{a}	17.2±1.97^{ab}	17.2±1.86^{ab}	14.8±0.49^{bc}	16.2±1.51^{ab}
Hematocrit (%)	50.9±1.27^{a}	49.2±3.12^{a}	49.9±2.05^{a}	41.2±6.51^{b}	48.7±5.25^{a}
Platelet (IL,10^3)	1044.5±366.9^{ab}	1028.6±258.9^{ab}	1066.0±219.86^{a}	816.5±170.4^{ab}	868.7±145.1^{b}
Mean Platelet Volume (PL)	7.3±1.27^{a}	7.6±1.07^{a}	7.6±1.07^{a}	7.9±0.57^{a}	8.0±0.40^{a}
Width distribution of platelets	8.2±0.64^{a}	11.3±5.47^{ab}	11.3±5.47^{ab}	13.4±5.95^{ab}	8.8±0.35^{b}
Platelet (%)	0.7±0.13^{a}	0.7±0.13^{ab}	0.7±0.13^{ab}	0.7±0.09^{b}	0.7±0.40^{ab}
Mean corpuscular volume (PL)	66.3±0.71^{a}	65.0±2.31^{ab}	65.0±2.31^{ab}	63.0±0.85^{b}	65.1±1.27^{a}
Mean corpuscular haemoglobin (pg)	21.9±0.42^{a}	21.5±0.81^{a}	21.5±0.81^{a}	22.8±3.11^{a}	22.7±1.96^{a}
Mean corpuscular haemoglobin concentration (g/l)	33.1±0.21^{a}	33.0±0.15^{a}	33.0±0.15^{a}	36.2±4.45^{a}	35.0±3.71^{a}
Red cell distribution width (covariance)	16.8±0.07^{a}	16.9±0.26^{a}	16.9±0.26^{a}	18.2±1.41^{a}	17.5±1.44^{a}
Red cell distribution width (standard deviation)	44.5±0.71^{a}	44.0±1.00^{a}	44.0±1.00^{a}	46.0±4.24^{a}	45.7±2.89^{a}
White Blood Cell (IL,10^3)	18.6±4.10^{a}	17.9±2.61^{a}	17.7±2.97^{a}	21.9±0.85^{b}	16.7±5.54^{a}
Lymphocytes (%)	80.1±3.2 5^{a}	77.1±1.81^{b}	75.1±5.80^{ab}	85.7±9.05^{ab}	65.8±6.97^{c}
Mid cells (%)	8.3±0.07^{a}	10.2±0.60^{a}	12.1±4.72^{a}	14.2±6.01^{a}	14.6±0.31^{b}
Granulocytes (%)	12.9±3.32^{a}	12.6±1.62^{a}	11.7±3.02^{a}	25.1±3.04^{b}	11.2±8.26^{a}

$^{a-d}$Values of haematological parameters compared between mice along rows with no common superscript are significantly different ($p \leq 0.05$)

***In vivo* antibacterial efficacy of CLME in rats**

Bacteraemia was established in the rats as early as the five days following oral administration of *S. typhimurium,* indicated by the growth of the organism on S-S agar inoculated with blood collected from test rats on the 6th day. Complete clearance of *S. typhimurium* from the blood of infected rats was achieved following 3 day treatment of with CLME.

The haematological profiles of the rat groups studied are presented in Table 3. The exasperated haematological parameters due to *S. typhimurium* infection were ameliorated following treatment with CLME. For CLME, there was a significant dose dependent reduction in the elevated WBC and granulocyte of rats infected with *S. typhimurium*. The reduction in RBC, HGB, HCT, were significantl ameliorated following treatment with CLME.

The PCT and MCH values of the rats were not affected by various treatments. There was however, an insignificant increase in the levels of RDW_CV, RDW_SD, MCHC, Lymph, and Mid in all treated infected rats treated with CLME.

DISCUSSION

Medicinal plants are important source for exploring new pharmacological agents and can be natural composite sources of new anti-infectious agents (Okeniyi *et al.,* 2012). *Curcuma longa* in previous studies had been found to possess *in vitro* antimicrobial and phytochemical qualities (Okeniyi *et al.,* 2012; Okiki *et al*., 2017). In this study, the medicinal potentials of *Curcuma longa* rhizomes were assessed through the phytochemical composition, and the antimicrobial and anti-inflammatory activities of the plants.

The high levels of alkaloids, tannins, flavonoids, phenols, and saponins, detected in *C. longa* rhizomes justify its uses as a medicinal plant, and can be useful in detecting lead compounds for the manufacture of both anti-inflammatory and anti-microbial drugs of plant origin. The cardio-protective, anti-cancer, anti-inflammatory, anti-microbial, anti-diabetic, anti-aging, neuro-protective and antioxidant properties, previously associated with the plant could be attributed to these phytochemicals present (Mizushima and Kobayashi, 1968). The high content of Ferric Reducing Antioxidant Power (FRAP) of *C. longa* rhizome also revealed its richness in antioxidant properties.

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structures by application of an external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological functions when denatured (Mizushima and

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Kobayashi, 1968). Denaturation of proteins is a well-documented cause of inflammation. Phenylbutazone, salicylic acid, flufenamic acid (anti-inflammatory drugs) etc., have shown dose dependent inhibition to thermally induce protein denaturation (Mizushima and Kobayashi, 1968). Varied concentrations of CLME showed anti-inflammation activity by inhibit protein denaturation in this study. The CLME produced 87.4% inhibition of heat induced albumin denaturation at 500 µg/mL, which compared favourably with Aspirin, a standard anti-inflammatory drug, showing 80.7% inhibition at a concentration of 100 µg/mL.

Neutrophils are known to be rich source of serine proteinase localized in the lysosomes. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Das and Chatterjee, 1995). The CLME showed maximum inhibition of 61% at 500 µg/mL concentration, and compared favourably with Aspirin, which showed maximum inhibition of 62.2% at 100 µg/mL.

The Human Red Blood Cell (HRBC) membrane stabilization has been used as a method to study the *in vitro* anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane (Rajendran and Lakshmi, 2008) and its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes released during inflammation produce various disorders. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation. Non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane (Vadivu and Lakshmi, 2008).

Stabilization of the RBCs membrane was studied to further establish the mechanism of anti- inflammatory action of CLME. The extract was effective in inhibiting the heat-induced haemolysis at different concentrations. The extract may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophil lysosomal constituents include bactericidal enzymes and protease, which upon extracellular release cause further tissue inflammation and damage (Chou, 1997). The plant extract (300-500 µg/mL) inhibited the heat-induced haemolysis of RBCs to varying degree as shown in Figure 2. CLME showed maximum inhibition with 55.5% at 500 µg/mL. Aspirin showed the maximum inhibition with 73.9% at 100 µg/mL. CLME (200-500 µg/mL) also showed inhibition of hypotonicity-induced hemolysis of RBCs (Figure 3) with maximum inhibition of 78.9% at 500 µg/mL. Although the precise mechanism of this membrane stabilization is yet to be elucidated, it is possible that the extracts produced this effect on surface area/volume ratio of the cells, which could be brought about by an expansion of membrane or the shrinkage of the cells and an interaction with membrane proteins (Shinde *et al.,* 1999). The significant membrane stabilizing activity of the methanol extracts may be due to the presence of polyphenolic contents. Several reports have shown that herbal drugs are capable of facilitating the stabilization of red blood cell membrane and possess anti-inflammatory activity (Sadique *et al.,* 1989).

The plant lipoxygenase (LOX) pathway is in many respects equivalent to the 'arachidonic acid cascades' in animals (Gardner, 1991). For this reason, the *in vitro* inhibition of lipoxygenase constitutes a good model for the screening of plants with anti-inflammatory potential (Abad *et al.,* 1995). LOXs are sensitive to antioxidants and most of their action may consist in inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy or lipid peroxy radical formed in course of enzyme peroxidation. This can limit the availability of lipid hydroperoxide substrate necessary for the catalytic cycle of LOX (Abad *et al.*, 1995). CLME showed significant inhibition ($p > 0.05$, at 300-500 µg/mL) of lipoxygenase activity. Lipoxgenases are lipid-peroxidizing enzymes involved in the biosynthesis of leukotriene from arachidonic acid, mediators of inflammatory and allergic reactions. These enzymes catalyse the addition of molecular oxygen to unsaturated fatty acids such as linoleic and arachidonic acids (Porta and Rocha-Sosa, 2002). There are four main isoenzymes already described, namely, 5-LOX, 8-LOX, 12-LOX and 15-LOX, depending on the site of oxidation in the unsaturated fatty acids (Porta and Rocha-Sosa, 2002). The common substrates for LOX are linoleic and arachidonic acids.

The results obtained from this study showed that methanol extract of *C. longa* rhizome effectively cured with *S. typhimurium* infected rats, clearance of bacteraemia within three days of administration, with improvement of haematological. Haematological parameters are important indices of the physiological and pathological status for both animals and humans (Adeneye *et al.,* 2006). Assessment of haematological parameters can be used to determine the extent of deleterious effects of foreign compounds including plant extracts on the blood. It can also be used to explain blood-relating functions of chemical compounds/plant extracts. Such laboratory investigations have been reported to be highly sensitive, accurate, and reliable and it remains the bedrock of ethical and rational research, disease diagnosis, prevention and treatment (Adeneye *et al.,* 2006).

Curcuma longa significantly boosted the WBC and granulocyte (Gran) concentrations of treated rats compared to rats infected with *S. typhimurium* ($p \leq 0.05$). The drop in WBC and granulocyte concentrations might have been due to immune-suppression caused by the infection (El-Demerdash, 2004). Similarly, *C. longa* also significantly boosted the RBC of treated rats with the drop in RBC in infected rats possibly as a result of red blood cell haemolysis caused by the bacterial infection. *C. longa* has been found to contain flavonoids (El-Demerdash, 2004), which are free radical scavengers. It

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is therefore possible that these components compete with haemoglobin (HGB) in RBC for oxygen, resulting in hypoxia, which then stimulates synthesis and RBC production. It is also possible that the end product of *C. longa* metabolism in the body stimulates the kidney directly to cause formation and secretion of erythropoietin, which is the humoral regulator of RBC production (Sanchez-Elsner *et al.,* 2004). There was also a dose-dependent increase in platelet count in rats treated with all concentrations of the extracts. Drop in platelet count in infected rats may also be as a result of anaemia caused by infection with *S. typhimurium* (White *et al.,* 2001). Treatment with *Curcuma longa* also significantly improved the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MHC), red cell distribution width (RDW_CV & RDW_SD), and plateletcrit (PCT), which could have resulted from iron deficiency caused by the bacterial infection. It is evident that *C. longa* has the ability to stabilize the cell membrane and restore various blood variables (Sharma *et al.,* 2011).

CONCLUSION

C. longa methanol extract significantly inhibited albumin denaturation and proteinase activity, stabilized membrane of red blood cell from haemolysis in heat and hypotonic conditions, as well inhibited lipoxygenase activity; all of which are associated with inflammatory processes. The results also showed that extract of *C. longa* rhizome effectively cured with *S. typhimurium* infected rats. The overall results suggest that *Curcuma longa* is a potential source of anti-inflammatory and antimicrobial agent.

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