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In-vitro Antioxidant Activity and Ameliorative Effect of Butanol extract of *Curcuma longa* on Osmotic-induced Haemolysis

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Abstract

Antioxidants are widely distributed in plants that are either used as food or medicine. These natural antioxidants, especially carotenoids, and polyphenols exhibit a wide range of biological effects which include; anti-inflammatory, anti-aging, anti-atherosclerosis and anticancer. Extraction and proper assessment of antioxidants from plants are crucial to explore the potential antioxidant sources and promote the application in functional foods, additives and pharmaceutical. The *in-vitro* antioxidant and ameliorating effect of butanol extract of Turmeric (*C. longa*) on osmotic-induced haemolysis was examined. Various experimental model including iron (III) reducing capacity, total antioxidant capacity, DPPH scavenging activity and *in-vitro* inhibition of osmotic-induced haemolysis were used for the characterization of antioxidant activity and ameliorating effect of the extract. The extract showed various degree of efficacy in each assay in dosed depended manner. In addition, at concentration of 1000μg/ml, the DPPH radical scavenging activity of the extract was found to compare well with that of the standard compound of ascorbic and gallic acids. From the obtained results, *C. longa* may be considered to be a source of antioxidant which can prevent oxidative stress.

Keywords: Turmeric; *In-vitro*; Antioxidant; Ameliorative; Haemolysis; Radicals; Scavenging

Background

Electron fluxes are constant within cellular metabolism. Donating or accepting electrons, either naked or as hydrogen atoms, is one of the most important properties of bioenergetic networks. These redox reactions fulfill key physiological phenomena such as cellular growing, phenotypic differentiation, nutritional adaptations and redox-dependent cellular signaling, but when they became unregulated, serious pathologies such as degenerative diseases and metabolic disorders arise (Méndez et al., 2017).

An imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products result in a phenomenon called oxidative stress. ROS can play several physiological roles (i.e., cell signaling), and they are normally generated as by-products of oxygen metabolism (Pizzino et al., 2017).

Oxidative stress plays an essential role in the pathogenesis of chronic diseases such as cardiovascular diseases, diabetes, neurodegenerative diseases, and cancer. Long term exposure to increased levels of pro-oxidant factors can cause structural defects at a mitochondrial DNA level, as well as functional alteration of several enzymes and cellular structures leading to aberrations in gene expression (Sharifi-rad et al., 2020).

The modern lifestyle associated with processed food, exposure to a wide range of chemicals and lack of exercise plays an important role in oxidative stress induction. However, the use of medicinal plants with antioxidant properties has been exploited for their ability to treat or prevent several human pathologies in which oxidative stress seems to be one of the causes (Sharifi-rad et al., 2020).

Plants have been used as a primary source of medicine since ancient times and about 80% of the world's population use herbal medicine to treat different ailments (Tugume & Nyakoojo, 2019).

Natural antioxidants are widely distributed in medicinal plants. These natural antioxidants, especially polyphenols and carotenoids, exhibit a wide range of biological effects, including anti-inflammatory, anti-aging, anti-atherosclerosis and anticancer. The effective extraction and proper assessment of antioxidants from food and medicinal plants are crucial to explore the potential antioxidant sources and promote the application in functional foods, pharmaceuticals and food additives (Xu et al., 2017).

The use of herbal medicine, as one element of complementary and alternative medicine, is increasing worldwide. Little is known about the reasons for and factors associated with its use (Welz et al., 2018).

Turmeric (*Curcuma longa L.*) is a rhizomatous herbaceous perennial plant widely cultivated in the tropical and subtropical regions of the world (A. Jorge et al., 2018).

Curcumin, a yellow polyphenolic pigment from the *Curcuma longa L.* (turmeric) rhizome, has been used for centuries for culinary and food coloring purposes, and as an ingredient for various medicinal preparations, widely used in Ayurveda and Chinese medicine. In recent decades, their biological activities have been extensively studied (Sharifi-Rad et al., 2020).

Numerous studies have shown that curcumin possesses a wide spectrum of biological and pharmacological properties, acting, for example, as anti-inflammatory, anti-angiogenic and anti-neoplastic, while no toxicity is associated with the compound (Praditya et al., 2019).

B Curcumin has already acknowledged immense interest from both medical and scientific research because of its multifaceted activity. To date, the promising effects of curcumin were perceived against numerous inflammatory diseases (Dhar & Bhattacharjee, 2021).

Therefore, it was imperative to assay for the antioxidant property and ameliorating effect on the most measured index in a diseased condition (induced-osmotic haemolysis) of the active ingredient of this much important and valued plant (*C. longa L*).

Materials and Methods

Collection and Extraction plant material

Rhizomes of Turmeric (*Curcuma longa*) were obtained from Kaura local government area of Kaduna State and was identified at the Herbarium of Biology Department Ahmadu Bello University Zaria. Specimen voucher of the plant is 1071.

Fresh rhizomes (200 g) of turmeric was pounded to puree an extracted with 200 ml of petroleum ether at 40°C, after which puree was again re-extracted using 200 ml of n-butanol at 60°C. The solvent was completely removed by rotary evaporator (NYC R-205D) and obtained a brownish gummy exudate. The extract was stored in the refrigerator until use.

Collection Treatment of Blood sample

Fresh blood samples from sheep, cattle and goat were collected in separate EDTA bottles from abattoir in Kakuri Kaduna State Nigeria. after swirling to mix, 2 ml of each blood sample was transferred into centrifuge tube using a clean pipette. Then 5 ml of phosphate buffer saline (PBS), pH 7.4 was added to each tube and centrifuge at 2000 rpm for 10 min. this process was repeated twice. The washed erythrocyte was suspended in 4 ml PBS.

Counting of erythrocyte was done as follows; Washed erythrocyte was drawn up to 0.5 mark of diluting pipette and Toisson's reagent drawn up to 10mark of the haemocytometer. The solution inside the erythrocyte diluting pipette was mixed homogenously and poured on a glass slide mounted on a microscope then viewed under 40X magnification. Only erythrocyte in the square marked R were counted.

Fragility Test

Test tubes containing 5 ml of different concentrations (0.00-0 85%) of PBS, 10^5 washed erythrocytes were added. The mixture was incubated in a water bath at 37°C for 30 min. After incubation, the mixture was centrifuged at 2000 rpm for 10 min, the supernatant was poured into a cuvette and absorbance was taken at 540 nm against PBS as blank. Percent haemolysis was calculated as;

% Haemolysis = Absorbance of individual tubes/Absorbance of 100% haemolysis X 100

Retardation of Osmotic-induced haemolysis by aqueous butanol extract of C. longa

From the fragility curve, the effective concentration that will cause 50% haemolysis (EC_{50}) was extrapolated. The EC_{50} was used to incubated the was erythrocyte of each animal with varying concentration of aqueous n-butanol extract of *C. longa*. The test tubes were as follows.

1. Control tube containing 10^5 of washed erythrocytes in distilled water.
2. Control tube containing 10^5 of washed erythrocytes in phosphate buffer, pH 7.4.
3. Treated tubes containing 5 ml of EC_{50} saline with 10^5 of washed erythrocyte.
4. Tube containing different concentration of aqueous butanol extract (1000-1600 µg/ml) was added to erythrocyte suspension. The total volume was made to 5 ml by adding phosphate buffer, pH 7.4.

The extract was prepared in phosphate buffer, pH 7.4. all tubes were incubated at 37°C for 60 min. Tubes were centrifuge at 2000 rpm for 10 min and the colour density of the supernatant was measured at 540 nm. Percent retardation was calculated as mean ± standard deviation by the formular:

Percent Retardation = A - B/A x 100.
Where A -= Haemolysis by EC_{50} saline concentration.
B = Haemolysis caused by the concurrent addition of extract.

Antioxidant activity test

Determination of total antioxidant capacity

The total antioxidant activity of butanol extract of *C. longa* was evaluated by the phosphomolybdate method as described by Prieto et al., (Prieto et al., 1999). The absorbance was measured at 695 nm using spectrophotometer (Jenway UV/Vis 6505) against blank after cooling to room temperature.

Reducing power assay

To assay for the reducing power different concentrations aqueous butanol extract of *C. longa* (31.25 - 10000 µg/ml) in 1 ml distilled water with 2.5 ml phosphate buffer (pH 6.6, 0.2 M) and 2.5 ml of potassium ferricyanide [1% $K_3Fe(CN)_6$] were incubated at 50^0C for 20 mins. To a portion, 2.5 ml of 10% trichloroacetic acid (TCA) was added to the mixture and then centrifuged at 3000 rpm for 10 mins as described by Oyaizu (Oyaizu, 1986).

The upper layer of the mixture (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of iron chloride (1 % $FeCl_3$). The absorption was measured at 700 nm using spectrophotometer (Jenway UV/Vis 6505). Ascorbic and gallic acid were used as standards. Phosphate buffer (pH 6.6) was used as blank. The absorbance of the final mixtures was in triplicate and the result expressed as mean ± standard deviation.

DPPH radical scavenging activity

Determination of free radicals scavenging activity of butanol extract *C. longa* using DPPH (1,1-Diphenyl-2-picryhydrazyl) was assayed as described by Blois (Blois, 1958) with slight modifications.

Various concentration (31.25 - 1000 µg/ml) of butanol extract in methanol was prepared. 1 ml of 0.3 mM DPPH in methanol was added to 2 ml solution of the extract and standards, the mixtures were then incubated in the dark at room temperature for 30 mins. Absorption was then measured at 518 nm using Spectrophotometer (Jenway UV/Vis 6505). The decrease in absorbance was then converted to percentage antioxidant activity (% AA) using the formular;

%AA = [(Absorbance of control - Absorbance of sample) / absorbance of control] x 100.

Blank was methanol (1 ml) plus sample solution (2.0 ml). Negative control was DPPH (1 ml, 0.25 mM) and methanol (2 ml), ascorbic and gallic acid were used as standards. The scavenging reaction between (DPPH-) and an antioxidant (H - A) can be written as;

$$[DPPH^-] + [H - A]\ DPPH\text{-}H^+\ [A^-]$$

Results

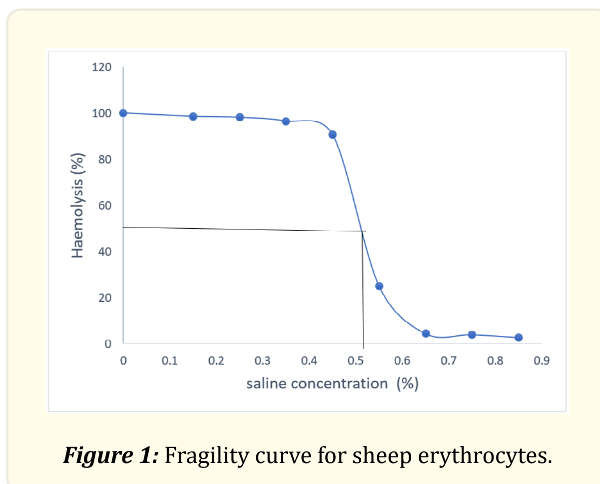

Figure 1: Fragility curve for sheep erythrocytes.

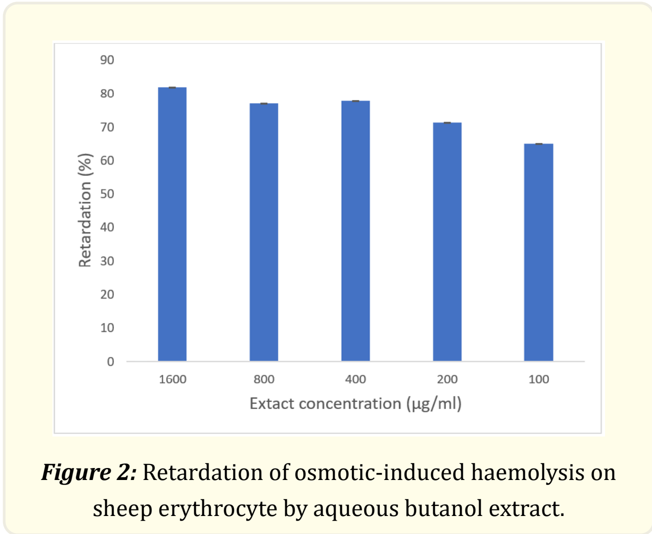

Figure 2: Retardation of osmotic-induced haemolysis on sheep erythrocyte by aqueous butanol extract.

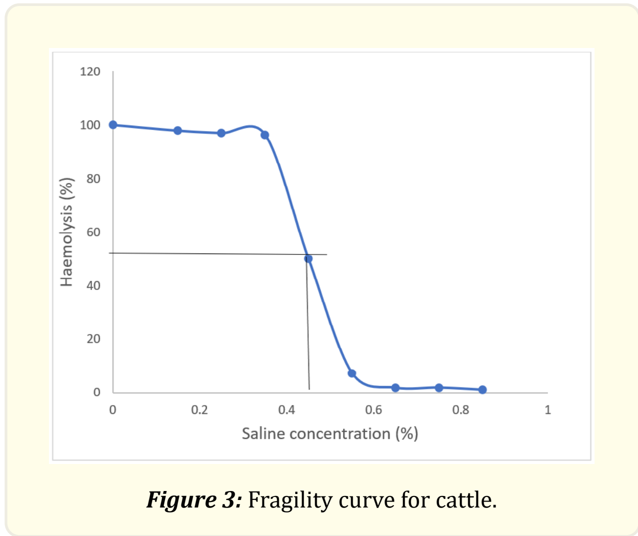

Figure 3: Fragility curve for cattle.

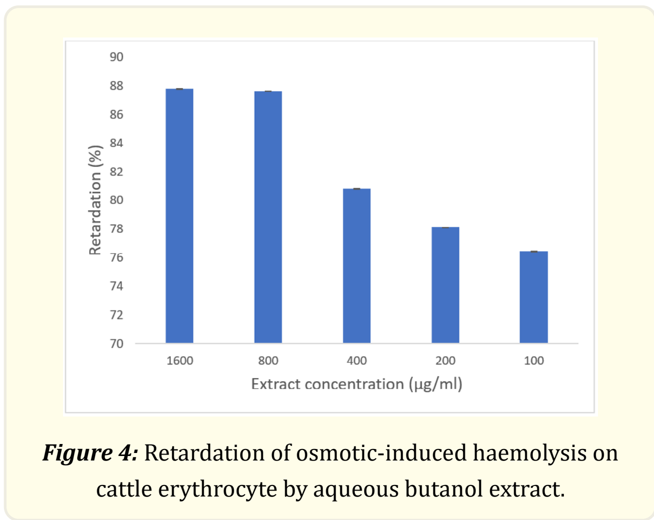

Figure 4: Retardation of osmotic-induced haemolysis on cattle erythrocyte by aqueous butanol extract.

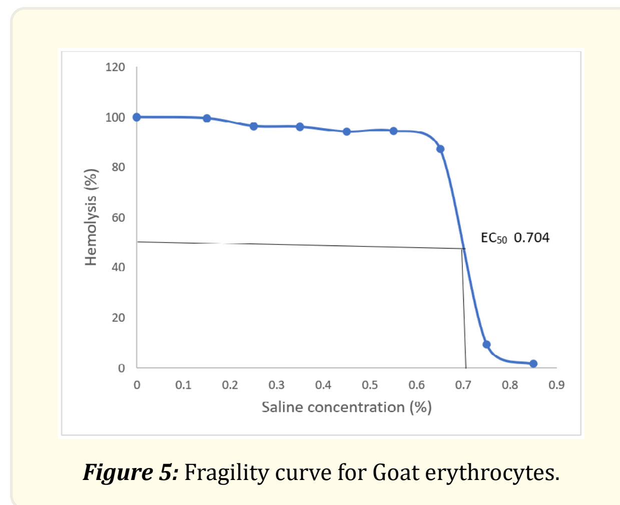

Figure 5: Fragility curve for Goat erythrocytes.

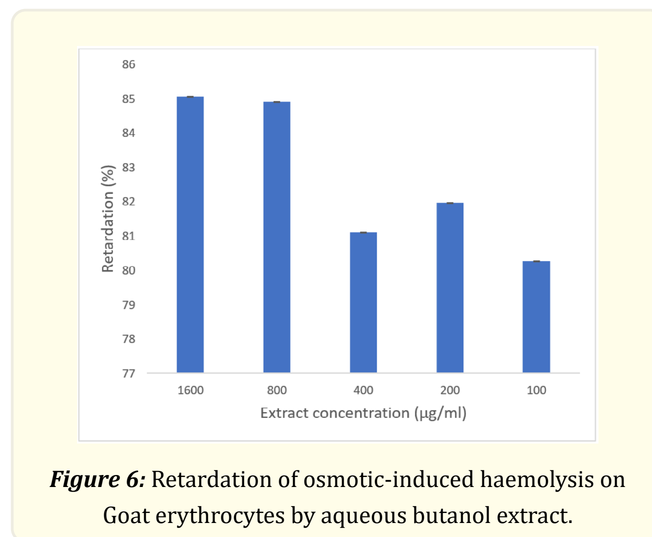

Figure 6: Retardation of osmotic-induced haemolysis on Goat erythrocytes by aqueous butanol extract.

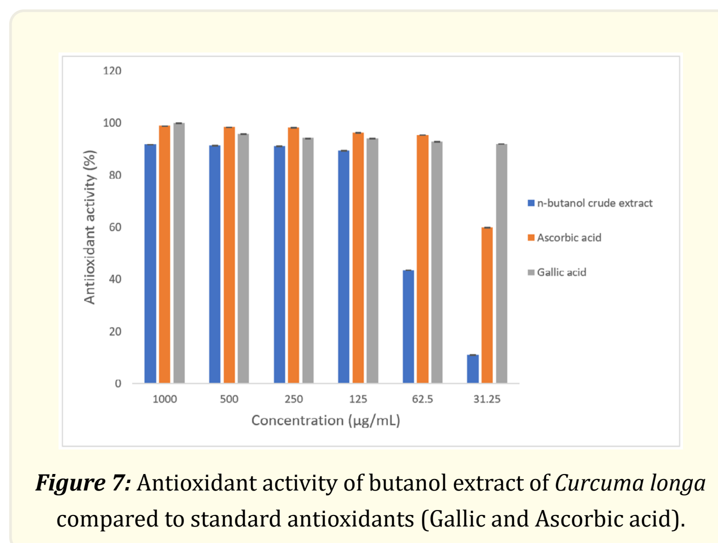

Figure 7: Antioxidant activity of butanol extract of *Curcuma longa* compared to standard antioxidants (Gallic and Ascorbic acid).

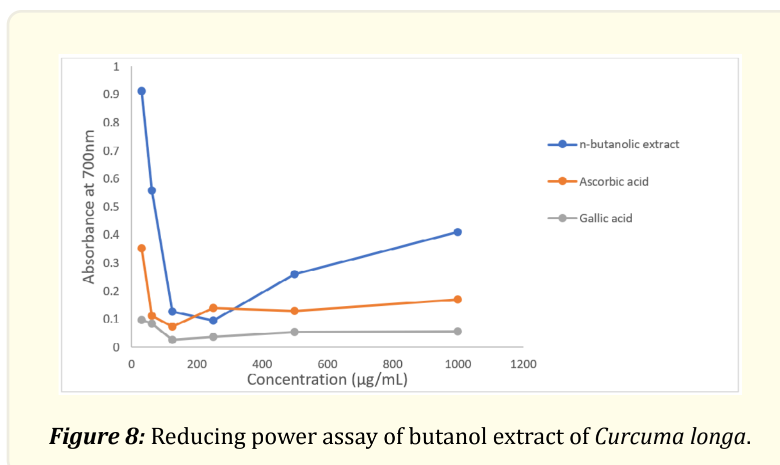

Figure 8: Reducing power assay of butanol extract of *Curcuma longa*.

Concentration (µg/mL)	*TAC of extract (Equivalent to ascorbic acid)*
1000	370
500	210
250	100
125	60
62.5	30
31.25	15

Table 1: Total antioxidant capacity (TAC) of butanol extract of *C. longa*.

Discussion

Haemolysis was observed in all erythrocytes of sheep, cattle and goat. The effective concentration of saline that causes 50% haemolysis (EC_{50}) differ in all animals (Figure 1, 3, and 5). The result showed that sheep erythrocytes happens to be more stable. This could be attributed to the variation in the composition of the lipid bilayer membrane of erythrocytes.

The aqueous butanol extract caused a retardation of osmotic induced haemolysis in all erythrocytes in all erythrocytes in a concentration-dependent manner (Figure 2, 4, and 6). This implies that extract has the ability to protect the integrity of the erythrocyte membrane at least *in-vitro*. This finding agrees with Neeta and Ramtej (Neeta & Ramtej, 2007) on aflatoxin-induced haemolysis.

On antioxidant activity, the result of DPPH scavenging activity shows that DPPH has high free radicals scavenging ability as compared to standard compounds, ascorbic and gallic acid at concentration of 1000 µg/ml (Figure 7).

For the reducing power assay, aqueous butanol extract of *C. longa* showed low activity in reducing $FeCl_3$ to $FeCl_2$. this could be due to the fact that the active component in the extract are insoluble in aqueous solvent.

The extract showed high total antioxidant capacity compared to ascorbic acid (Table 1). This high antioxidant activity (Aderogba et al., 2004). May be due to the presence of phytochemicals such as phenols, flavonoids, anthocyanins. The antioxidant mechanism of *C. longa* was attributed to its unique conjugated structure, which include two methoxylated phenols and enol form of β-diketone; the structure shows typical radical-trapping ability as a chain- breaking antioxidant.

Conclusion

The antioxidant activity and ameliorative effect of butanol extract of *C. longa* on osmotic induced haemolysis *in-vitro* were determined. On the basis of the result obtained, rhizomes of *C. longa* were found to be a source of antioxidant which can be tolerated at very high doses without any toxic effects even as evaluation studies have proven this. Therefor *C. longa* have the potential to be developed as modern medicine for the treatment of various diseases caused by oxidative stress.

Conflict of interest

The authors declare that there was no conflict of interest.

Author contribution

SKU collected samples; SKU, AK and MEE conducted experiment; SKU, MEE, AK and BT interpret results; MEE and AK wrote manuscript; BT supervise and directed study; All author read, approved final manuscript.

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