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Inhibition of Hydrogen Peroxide Induced Hemolysis of Four Potential Medicinal Plants Extract

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Abstract: Medicinal plants are used as drug in traditional system of medicine. They chemical composition in these plants mediates its activity. Red blood cells are targets for free radical as results of polyunsaturated fatty acids (PUFA) and redox activity. The study evaluates *Balanites aegyptica* leaf extract (BALE)*, Leptadenia hastate* leaf extract (LHLE)*, Annona senegalenses* leaf extract (ASLE) and *Nympheae spp* leaf extract (NSLE) for the inhibition of hydrogen peroxide induced hemolysis on blood groups A+, B+, AB+ and O+ at concentration 20-100 µg/ml. Our previous data revealed strong antioxidant activity of these plants extract. Phytochemical screening was carried out as reported by Harborne. The results showed that flavonoids, phenols, alkaloids and saponins were present in the leaf extracts. Tannins are present in BALE while sterol is present in LHLE and ASLE. The extracts significantly inhibited hemolysis extrapolated using percentage inhibition as presented in the figures and IC_{50}.The 50 % scavenging activity of the extracts showed that BALE: 42.65 µg/ml, ASLE: 46.90 µg/ml and NSLE: 45.15 µg/ml significantly ($p< 0.05$) inhibited hemolysis on AB+ compared to L-ascorbic acid IC_{50}:47.76 µg/ml. L-ascorbic acid IC_{50}:47.76 µg/ml significantly ($p< 0.05$) decreased hemolysis compared to LHLE: 50.26 µg/ml. LHLE significantly ($p< 0.05$) decreased hemolysis with IC_{50}: 82.95 µg/ml on A+, 50.26 µg/ml on B+ and 51.81 µg/ml on O+ compared to BALE (IC_{50}: 42.65 µg/ml) with significant ($p< 0.05$) decrease on AB+, other extracts and L-ascorbic acid. The findings support claims for using the plants in treating many ailments traditionally. The plants have potential to be harnessed as an alternative medicine, starting material or chemical composition of drug.

Key words: Medicinal Plants · Extracts · Hemolysis · Inhibition

INTRODUCTION

Medicinal plants are known to be the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. The pharmacological properties of these plants are enormous. It played an important role in the health of millions of people in the world especially in the rural areas. Chemical compounds in plants mediate their effects on human body by binding to a receptor molecule an identical process with synthetic drugs. This shows that they do not differ greatly from synthetic drugs in terms of their mechanism of action [2]. These plants preparations have relatively lower incidences of adverse reactions compared to conventional drugs and reduced cost. It is considered as an alternative to synthetic drugs [3]. The human body is made up of abundant red blood cells or erythrocytes. The possess desirable physiological and morphological characteristics which are exploited extensively in drug delivery [4]. Oxidative damage to the erythrocyte membrane may be implicated in hemolysis associated with some hemoglobinopathies, oxidative drugs, transition metal excess, radiation and deficiencies in some erythrocyte antioxidant systems [5]. Our previous study on these medicinal plants revealed various degree of antioxidant activity determined using DPPH radical scavenging

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activity, Ferric reducing antioxidant power, hydrogen peroxide radical scavenging activity and ABTS radical scavenging activity. It is against this background that this study evaluates four traditionally used medicinal plants for the inhibition of hemolysis induced by hydrogen peroxide on human erythrocytes.

Medicinal Plants of the Study: *Balanites aegyptiaca* is a medicinal plant which belongs to *Zygophyllaceae* family popularly named as the 'desert date' and Adu'a in Hausa [6]. It contains a wide variety of compounds and has wide range of biological and pharmacological properties. It is used in folk medicine for the treatment of various diseases such as intestinal worm infections, wound healing, syphilis, dysentery, constipation, diarrhea and fever [7]. It is known to have antioxidant, anti-inflammatory, antimicrobial, anthelminthic, antinoceceptive, antiviral, anticancer, antidiabetic and cytotoxic activities [8]. It also has antiasthmetic, antifertility, antidysentric properties and purgative [9].

Leptadenia hastata belongs to the family *Asclepiadaceae* [10]. In Nigeria, it is used traditionally for the treatment of hypertension, catarrh, skin diseases and diabetes [11]. In Nigeria, local healers in the northern use the decoction for the management of diabetes, scabies treatment, hypertension, catarrah, rheumatism and skin diseases [12].

Annona senegalensis belong to the family *Annonaceae.* It is locally called wild custard apple in English and Gwándàn dààjìì in Hausa [13]. The plant parts are used in treating guinea worms, diarrhea, gastroenteritis, snake bites, toothache, respiratory infections and malaria [14]. The root decoction is used to treatment of pneumonia, chest colds, venereal diseases, stomach ache and dizziness. The plant has antioxidant, antimicrobial, antidiarrheal, antiinflammatory, antiparasitic, anticonvulsant, antitrypanosomal, antisnake venom and antinociceptive [15].

Nymphaea spp belongs to Nymphaeaceae family [16]. It is known to have soothing and tranquilizing effects, detoxicant, aphrodisiac effect, astringent and diuretic properties [17]. The plant is reported for the treatment of dyspepsia, enteritis, diarrhea, urinary problems, fever and heart palpitations in Ayurveda medicine. The plant parts are known for pharmacological activities including diabetes, eruptive fever and liver disorders [18, 19]. The study is the first to report on the antihemolytic activity of these medicinal plants. The study evaluated four medicinal plants for its inhibition on hemolysis induced hydrogen peroxide on human erythrocytes group A+, B+, AB+ and O+ by methanol leaf extract of *Balanites aegyptica, Leptadenia hastate, Annona senegalenses* and *Nympheae spp*.

MATERIALS AND METHODS

Collection of Plant Materials: *Balanites aegyptica, Leptadenia hastate, Annona senegalenses* and *Nympheae spp*. were collected from Sangere village (near Modibbo Adama University of Technology, Yola), Girei Local Government Area, Adamawa State. Sangere village is located on latitude 90° 11' 15'' N and longitude 120° 20' 29'' E on the North bank of river Benue retrieved using Google earth map. The plants were taken to the Department of Plant Science, Modibbo Adama University of Technology, Yola for authentication and kept in herbarium.

Preparation of Plant Materials: The fresh leaves of *Balanites aegyptica, Leptadenia hastate, Annona senegalenses* and *Nympheae spp*. As shown in Plate I was chopped into pieces washed and rinsed with distilled water. The leaves were shade dried for two weeks at room temperature. The dried plant material was pulverized using laboratory mortar and pestle. The powdered samples were sieved using a fine sieve and stored in an air tight container.

Extraction of Plant Extracts: For each plant material, dried and powdered material was extracted with water: methanol (30:70 V/V) for 48 hours. Each powdered dry leaves sample 20 grams was subjected to soxhelet extraction with 300 ml methanol as solvent. The extract was carried out for 3 hours, 10 cycles and temperature was maintained at 65°C. The color the extract was seen green and dark green. The dried samples were weighed and kept in a dry place until being used for the assay.

Phytochemical Screening of Extract: Each plant extract was utilized for screening of different phytochemicals such as alkaloids (wagner's test, hager's test), flavonoids (shinoda test), tannins ($FeCl_3$ test), sterols (Salkowski method) and phenols (Folin's test) [20].

Collection of Human Red Blood Cell: Blood was obtained by venipuncture from healthy volunteers with blood group A+, B+, AB+ and O+ between the of 25-30 years in heparinized tubes. The blood was centrifuged for 15 minutes at 3500 rpm. Plasma and buffy coats were removed. Red blood cells (RBCs) was suspended in

10 volumes of 0.9 % NaCl and centrifuged at 2, 500 rpm for 5 minutes. The RBCs was washed three times with the same solution. During the washing, the packed cells was re-suspended in 10 volumes of phosphate buffered saline (PBS, pH 7.4) and utilized for the assay. Ethical consideration for blood sample collection were in compliance with the guidelines for the collection of blood samples in research involving humans in the department of Biochemistry, Modibbo Adamawa University of Technology, Yola.

***In vitro* Antihemolytic Activity:** Inhibition of H_2O_2 induced red blood cell hemolysis of methanol extracts and L-ascorbic acid was examined by the *in vitro* method described previously by Tavazzi [21]. The erythrocytes from human blood was separated by centrifugation and washed with saline or isotonic sodium phosphate buffer (pH 7.4) until the supernatant is colorless. The erythrocyte was diluted with saline or phosphate buffer to give a 4 % suspension. Varying amounts of sample (20, 40, 60 80, 100 µg/ml) and L-ascorbic acid with saline or buffer was added to 2 ml of the suspension of erythrocytes. The entire mixture was made to 3.5 ml volume with either normal saline. The mixture was preincubated for 120 min and then 0.5 ml H_2O_2 solutions of appropriate concentration in saline or buffer was added. Hydrogen peroxide concentration in the mixture was adjusted after 120 minutes so as to bring 90 % hemolysis of the blood cells. The extent of hemolysis was estimated by measuring the absorbance at 540 nm corresponding to hemoglobin liberation after incubation and centrifugation at 5 min at ×1000 g. The 50 % radical scavenging activity by the extracts and l-ascorbic acid (IC_{50}) was extrapolated from reference inhibition curve. Antihemolytic activity was expressed as the inhibition percentage and was calculated using the following formula:

$$\text{Percentage Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of Control}} \times 100$$

Statistical Analysis: Data in three replicates was collected and analyzed using analysis of variance. Results of the findings were expressed as Mean ± SD. Statistical analysis of data was carried out using One Way ANOVA. The results $p < 0.05$ was regarded statistically significant.

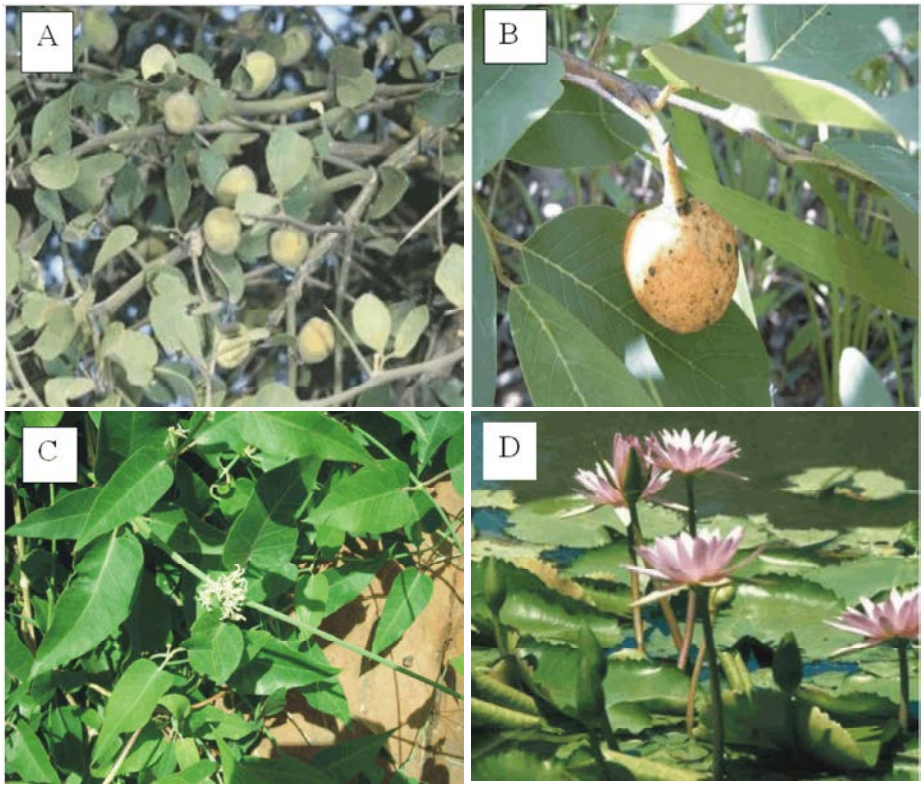

Plate 1: Plant library showing A: *Balanites aegyptica* leaves and fruits B: *Annona senegalenses* leaves and fruit C: *Leptadenia hastate* leaves D: *Nympheae spp* leaves

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening: Qualitative phytochemical screening of the medicinal plants was determined and presented in Table 1. The results showed the presence of flavonoids, phenols, alkaloids and saponins in the leaf extracts of *Balanites aegyptiaca, Leptadenia hastate*, *Annona senegalensis* and *Nymphaea spp*. Tannins was only present in the leaf extract of *Balanites aegyptiaca*. Sterols were present in *Leptadenia hastate* and *Annona senegalensis* leaf extracts. The presence of these secondary metabolites served a prerequisite for various biological and pharmacological activities of the extracts.

Antihemolytic Activity of Potential Medicinal Plants of the Study: In this study, erythrocytes are treated with hydrogen peroxide (toxicant) which induced hemolysis to a greater extent. Erythrocytes are main target for free radicals due to the presence of membrane concentration of polyunsaturated fatty acids (PUFA) and oxygen transport associated with redox active hemoglobin molecules. This could be attributed to the oxidizing nature of hydrogen peroxide with respect to the destruction of cell membrane and subsequent liberation of hemoglobin from the cells [22]. Inhibition of hemolysis on human erythrocytes blood groups A+, AB+, B+ and O+ was studied using four medicinal plants extracts and L-ascorbic acid as shown in Table 2 and Figure 1-4. The results in Figure 1 revealed that *Balanites aegyptiaca* leaf extract (BALE) significantly inhibited hemolysis on blood groups AB+ compared to other blood groups and L-ascorbic acid. Extrapolated IC_{50} value Table 2 revealed that *Balanites aegyptiaca* leaf extract (BALE) has lower IC_{50}: 42.65 µg/ml compared to L-ascorbic acid IC_{50}: 47.76 µg/ml. Figure 2 showed antihemolytic activity of *Annona seneglensis* leaf extract (ASLE). The results revealed that inhibition of hemolysis was recorded higher on blood group AB+ compared to other blood group and L-ascorbic acid. The results also showed lower IC_{50}: 46.90 µg/ml on *Annona seneglensis* leaf extract compared to L-ascorbic acid IC_{50}: 47.76 µg/ml. Figure 3 showed that antihemolytic activity of *Nymphaea Spp* leaf extract (NSLE). The result revealed significant inhibition of hemolysis on blood group AB+ compared to other blood group. The result further revealed lower IC_{50}: 45.15 µg/ml compared to L-ascorbic acid IC_{50}: 47.76 µg/ml while Figure 4 revealed that *Leptadenia hastate* leaf extract (LHLE) significantly inhibited blood group B+ compared to other blood groups. L-ascorbic acid has lower IC_{50}: 47.76 µg/ml and significantly inhibited hemolysis compared with *Leptadenia hastate* leaf extract with higher IC_{50}: 50.26 µg/ml. Our findings further revealed that *Leptadenia hastate* leaf extract (LHLE) significantly decreased hemolysis with IC_{50}: 82.95 µg/ml A+, IC_{50}: 50.26 µg/ml on B+ and IC_{50}: 51.81 µg/ml on O+ compared to *Balanites aegyptiaca* leaf extract (BALE) that has significant decrease on hemolysis with IC_{50}: 42.65 µg/ml, other extracts and L-ascorbic acid. The results revealed that the plants extract was capable of inhibiting hydrogen peroxide induced hemolysis on blood groups AB+ compared to other blood group. Lipid oxidation of human erythrocyte

Table 1: Qualitative Phytochemical Screening of Four Medicinal Plants

Extract/ Phytochemicals	Flavonoids	Phenols	Alkaloids	Tannins	Saponins	Sterols
BALE	+	+	+	+	+	-
LHLE	+	+	+	-	+	+
ASLE	+	+	+	-	+	+
NSLE	+	+	+	-	+	-

Key: +: Present; -: Absent

BALE: *Balanites aegyptiaca* Leaf Extract; LHLE: *Leptadenia hastate* Leaf Extract; ASLE: *Annona senegalensis* Leaf Extract; NSLE: *Nymphaea spp* Leaf Extract.

Table 2: IC_{50} (µg/ml) Values of Medicinal Plants Extracts and L-Ascorbic Acid

Extracts	IC_{50} (µg/ml)			
Concentration (µg/ml)	A+	B+	AB+	O+
BALE	102.10	72.47	42.65	64.37
ASLE	184.30	89.61	46.90	54.54
NSLE	188.82	86.37	45.15	55.08
LHLE	82.95	50.26	69.55	51.81
L-Ascorbic Acid	107.64	89.75	47.76	53.66

BALE: *Balanites aegyptiaca* Leaf Extract; LHLE: *Leptadenia hastate* Leaf Extract; ASLE: *Annona senegalensis* Leaf Extract; NSLE: *Nymphaea spp* Leaf Extract

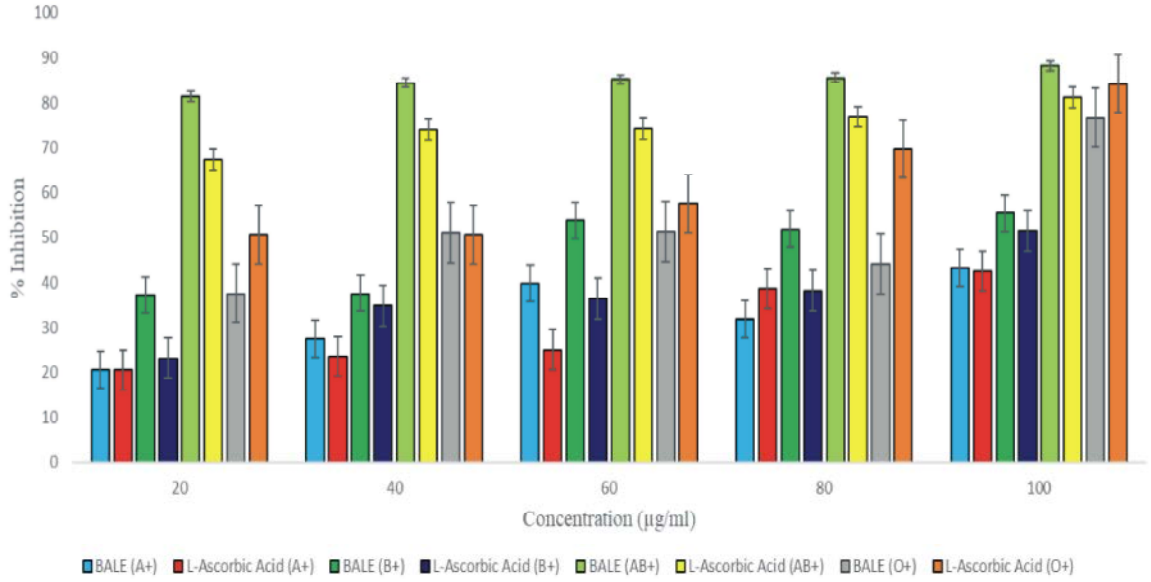

Fig. 1: Antihemolytic activity of *Balanite aegyptica* leaf extract (BALE) and L-ascorbic acid values are Mean ± SD for three determinations

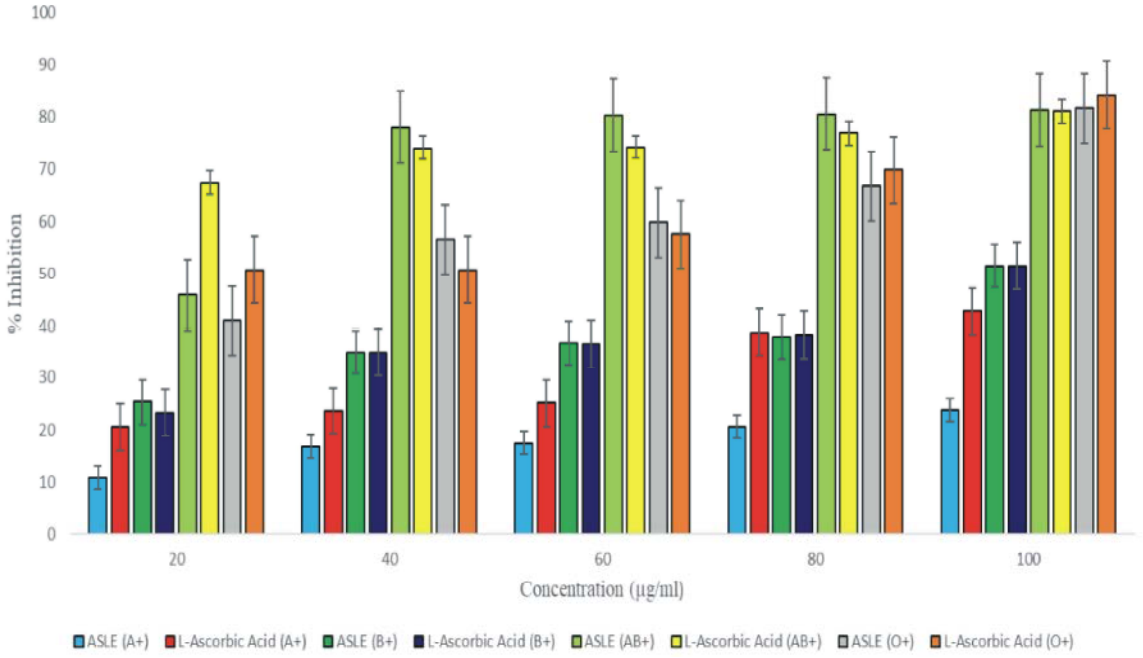

Fig. 2: Antihemolytic activity of *Annona seneglensis* of leaf extract (ASLE) and L-ascorbic acid values are Mean ± SD for three determinations

blood group A+, B+, AB+ and O+ membrane mediated by hydrogen peroxide induces membrane damage and subsequently hemolysis. Our findings also showed that the extracts have potential protective effect against hemolysis induced by hydrogen peroxide. Antihemolytic activity of the extracts may be attributed to radical scavenging activity of the bioactive components in the plants. Akinpelu *et al.* [23] reported that the chemical composition of plants produced definite physiological action on the human body. The antihemolytic activity could also be the expression of collaborative action of the various antioxidant mechanisms of the extracts as reported by Thagriki *et al.* [24].

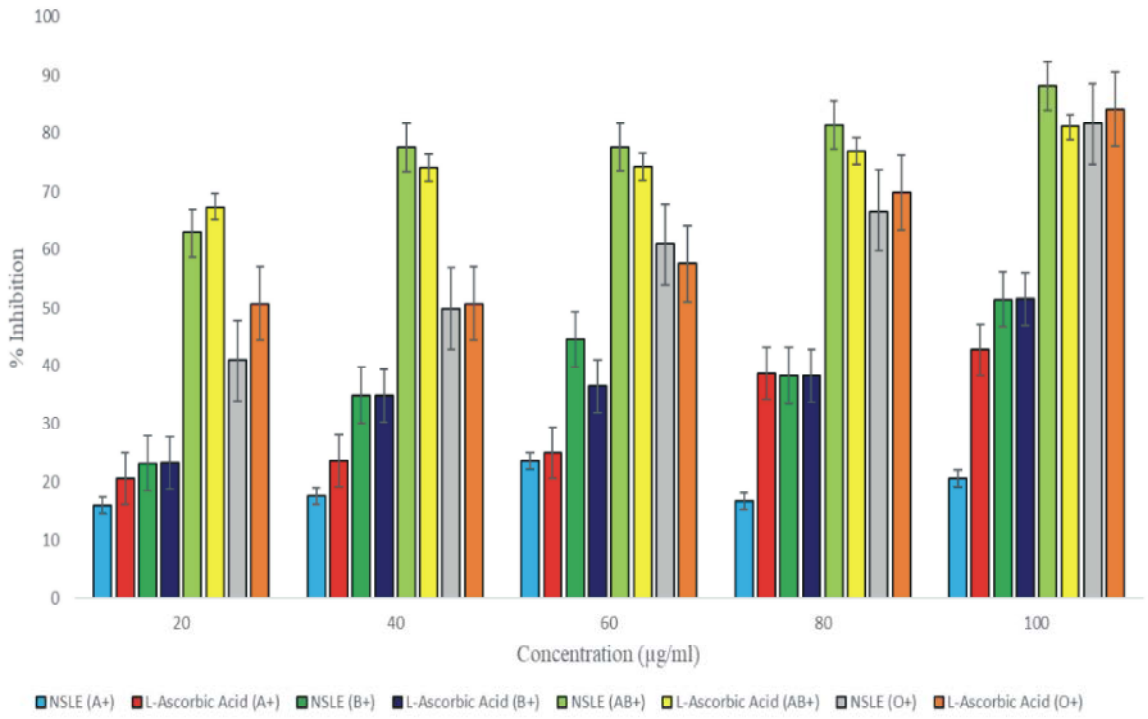

Fig. 3: Antihemolytic activity of *Nymphaea Spp* leaf extract (NSLE) and L-ascorbic acid values are Mean ± SD for three determinations

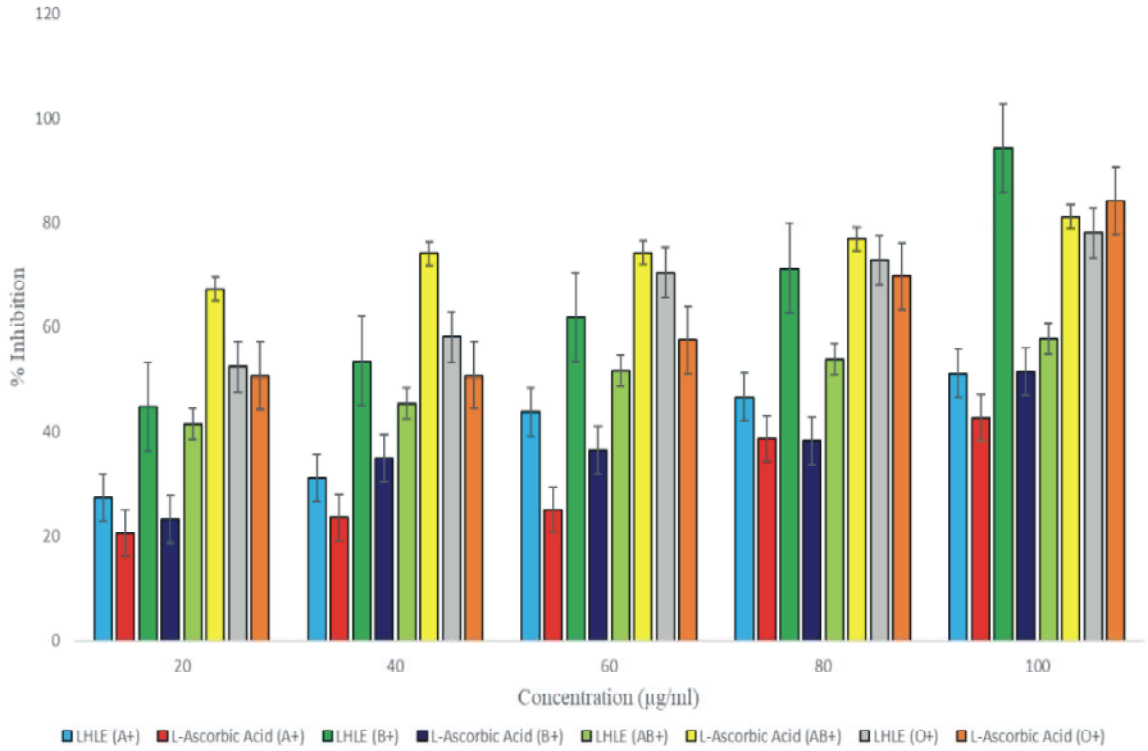

Fig. 4: Antihemolytic activity of *Leptadenia hastate* leaf extract (LHLE) and L-ascorbic acid values are Mean ± SD for three determinations

CONCLUSION

The study showed remarkable antihemolytic activity of the extracts (*Balanites aegyptica, Annona senegalenses* and *Nympheae spp*) compared to L-ascorbic acid. The plants extracts revealed the presence of flavonoids, phenols, alkaloids and saponins in the extracts of the plants. The presence of the chemical composition could be attributed to the bioactivity of the extracts.

Conflict of Interest: The authors declare that they have no conflict of interests.

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