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(RESEARCH ARTICLE)

Membrane stabilizing activity of extracts and non-polar fractions of *Cola lepidota* K. Schum. fruit. (Malvaceae): A comparative study

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Abstract

Cola lepidota (Malvaceae), is a wild, under-utilized edible indigenous fruit tree found in the humid West and Central African forest. The present study investigates the membrane stabilizing (MS) activity of the extracts and partitioned fractions obtained from the pulp and seed of *C. lepidota*. Dried pulp and seeds were identified by a Botanist, pulverized and stored. The phytochemical constituents of the crude extract were analysed using standard method. Each sample was extracted using absolute ethanol and fractionated using N-hexane and dichloromethane. The MS activity of the crude extracts and their partitioned fractons were determined using the heat induced and hypotonic solution induced human erythrocytes hemolysis model at doses of 250, 500, 1000 and 2000 µg/ml. Qualitative phytochemical screening indicated the presence of flavonoids, glycosides, alkaloids, tannins, phenols and terpenoids as the major phytochemical constituents.The pulp ethanol crude extract (PECE), at 250, 500, 1000 and 2000 µg/ml inhibited heat induced lysis of the human red cell membrane with values of (37.89 , 42.90, 53.27, 67.33) %, while the seed were 7.39 %, 16.04 %, 20.53 % and 35.64 % respectively. The percentage inhibitions of lysis induced by hypotonic solution at 250, 500, 1000 and 2000 µg/ml of the fruit pulp were 43.07 %, 47.19 %, 46.88 % and 63.42 % while the seed were 38.18 %, 39.08 %, 47.50 % and 42.32 % respectively. The results obtained were comparable to that of the reference standards aspirin and diclofenac. The DCM fraction of the pulp showed a better activity among the fractions. It can be inferred that the extracts and fractions of *C. lepidota* pulp and seed contains principles that are capable of stabilizing human red blood cell membranes against hypotonic solution and heat induced lysis.

Keywords: *Cola lepidota*; Membrane stabilization; Pulp; Seed; Fruit; Phytochemicals

1. Introduction

Inflammation is one of the fastest growing metabolic disease in the world and uncontrolled inflammatory response is the main cause of a vast continuum of disorders including allergies, cardiovascular dysfunctions, metabolic syndrome, cancer, and autoimmune diseases imposing a huge economic burden on individuals, family and consequently on society

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[1]. The inflammatory mediators are released when inflammation response is triggered causing increased permeability of blood vessels and then leading to the leakage of plasma proteins into the injured tissues. Acute inflammation can be triggered by various stimuli and while it is characterized by a rapid response of hosts to the site of infection or trauma tissue, i.e. delivery of leukocytes and plasma proteins such as antibodies, to the site of inflammation, chronic inflammation may progress after acute inflammatory processes, which last for several weeks, months and even years [2]. Chronic inflammation is a prolonged inflammatory response that leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissues from the inflammatory process [3]. These effects can only be prevented through membrane stabilization. Membrane stabilization can be said to be the ability of a cell membrane to resist lysis under hyposmotic conditions thus it leads to prevention of leakage of serum protein and fluids into the tissues during a period of increased permeability caused by inflammatory mediators [4]. It has been recognized to be a possible mechanism of action of most anti-inflammatory drugs thus they have a growing role in pain management, both acute and chronic. They have been employed for many years in treating painful conditions such as headache, trigeminal neuralgia, perioperative settings as well as in acute pain situations. As knowledge of the multifactorial nature of inflammation related diseases increases so does the need for more challenging and appropriate therapies advance. Some of the established medications for controlling and suppressing inflammation crises include: steroids, non –steroids anti-inflammatory drugs (NSAIDS), and immunosuppressants. The need for natural anti-inflammatory agent has increased recently due to the established importance as against NSAIDS adverse effects. Studies have shown that diverse source of natural membrane stabilizers abounds such as extracts and natural products from plants and fungi like mushroom [5] including endophytic fungi [6,7]. There is a renewed interest in the last decade to search for phytochemicals of native and naturalized plants for pharmaceutical and nutritional purposes which stem from the fact that plant derived products have great potential as sources of pharmaceuticals [8,9,10,11]. *Cola lepidota* belongs to the group of Monkey kola, a member of the family Malvaceae and subfamily Sterculiaceae which is a wild, under-utilized, and important edible indigenous fruit tree found in the humid. Monkey cola fruit is consumed by men, women and children alike because of their natural tasty pulp, especially that of the species *Cola lepidota* and *Cola pachycarpa* [12]. The yellowish aril (waxy mesocarp) forms the edible part of the follicle [13]. In Nigeria, *Cola lepidota* is employed in folk medicine as febrifuges, for pulmonary problems and cancer related ailments [13] as well as a stimulant, to prevent dysentery, headache and to suppress sleep [14]. Nutritionally, they are believed to contain beta carotene which acts as antioxidant. Recent findings have revealed that the fruits contain bioactive compounds such as polyphenols, alkaloids, saponins and anthraquinones etc which have some medicinal potential [15]. Some studies have proven that seeds of *C. lepidota* can be used as an antioxidant [13]. The aim of the study was to investigate the membrane stabilization potential of *Cola lepidota* fruit pulp and seed extracts using an in vitro hemolytic assay as literature search has revealed that there is paucity of information as regards the membrane stabilizing effect of *Cola lepidota* fruit (pulp and seed [14].

2. Materials and Methods

2.1. Reagents and Instruments

All the reagents and solvents were of analytical grade and are products of JHD. Standard drugs used include aspirin, diclofenac.

2.2. Plant Procurement and Preparation

The fruits of *Cola lepidota* were obtained in mid-June from Onitsha main market, Anambra state, Nigeria. The fruits were authenticated and deposited in Herbarium of Pharmacognosy and Traditional Medicine Department at Chukwuemeka Odumegwu Ojukwu University, Igbariam Campus, Anambra State. The fruits were first washed with water and the back (epicarp) removed. The samples (pulp, and seed of the *Cola lepidota* fruit were separated, cut into pieces and air dried at room temperature to a constant weight, then each of the samples were pulverised using a milling machine and kept in separate clean bags till further use.

2.3. Extraction and partitioning of Samples

A 400 g of the dried pulverised *C. lepidota* pulp and seed were weighed out and poured into separate glass jar and each macerated with 1200 mL of absolute ethanol. The mixtures were stirred and allowed to stay for 24 hours with intermittent agitation of the sample. After 24 hours the filtrates obtained were filtered using Whatman's filter paper No. 1 attached to glass funnel. The residues were transferred back into clean dry beakers and the process repeated for the next 48 hours at 24 hours interval. The obtained extracts were concentrated until it remained at least one-tenth of its original volume using rotary evaporator (45 ^{0}C) The dried concentrates obtained were placed in desiccator until its weight became constant and it was put in an air-tight container until further use. About 21.95 g and 15.00 g of the ethanol crude extracts of the pulp (PECE) and seed (SECE) respectively were subjected to vigorous solvent-solvent partitioning using non-polar solvents (n-hexane and dichloromethane (DCM)) to yield n-hexane fractions, n-hexane

partitioned fraction from pulp NPFP and n-hexane partitioned fraction from seed (NPFS) respectively; and DCM fractions, DCM partitioned fraction from pulp (DPFP) and DCM partitioned fraction from seed (NPFS) respectively.

2.4. Phytochemical Screening

Extracts of *C. lepidota* fruit pulp and seeds were screened for the presence of secondary metabolites [16]. The phytochemicals analysed include alkaloids, flavonoids, phenols, terpenoids, tannins, cardiac glycosides and anthraquinones.

2.5. Membrane Stabilization Activity

This was carried out using the stabilization of human red blood cell (HRBC) membrane model described by Ikpeazu *et al.,* [17] and Eze *et al.,* [18]. Briefly, Blood sample (5 ml) was collected from a healthy male donor (that has not received anti-inflammatory drug in the past 10 days) and transferred into ethylene diamine tetraacetate (EDTA) centrifuge tube. The tube was centrifuged at 3000 rpm for 10 mins. The HRBC was repeatedly washed three times with equal volume of normal saline by centrifugation until the supernatant was clear. The volume of the red blood pellets was noted and reconstituted as a 40% v/v suspension in an isotonic buffer solution of pH 7.4 made of 10 mM sodium phosphate buffer which contained 0.2 g of NaH_2PO_4, 1.15 g of Na_2HPO_4, and 9 g of NaCl in 1 litre of distilled water. The re-suspended supernatant was used for the assay. Two milliliters (2 ml) of various concentrations (2000, 1000, 500, 250 µg/ml) of the test samples were prepared in the isotonic phosphate buffer solution (two sets) in pairs/ inside the tube. Distilled water (one set) and the red blood cell suspension (30 µl) were added to each pair of the test tubes containing the test samples and controls and was mixed gently. One pair of the isotonic solution was heated for 20 min at a temperature at 54°C. The other prepared in distilled was maintained at room temperature for 20 min. After 20 minutes, the tubes were centrifuged at 2000 rpm for 5 min and the hemoglobin content of the supernatants was determined spectrophotometrically at 540 nm. Diclofenac sodium (100 μg/ml) and aspirin were used as the reference drugs and positive control. The % inhibition of hemolysis by the test samples, as a function of anti-inflammatory activity, was calculated using the following,

2.5.1. Heat-Induced Haemolysis

$$\% \text{ Inhibition } = \frac{\text{Control} - (\text{T1} - \text{T2})}{\text{Control}} \times 100$$

Where
Control = Absorbance of heated control
T1 = absorbance of test sample unheated,
T2 = absorbance of test sample heated,

2.5.2. Hypotonic-Induced Haemolysis

$$\% \text{ Inhibition } = \frac{\text{Control} - (\text{T1} - \text{T2})}{\text{Control}}$$

Where Control = Absorbance of control

T1 = Absorbance of test solution in hypotonic solution,
T2 = Absorbance of test solution at room temperature (isotonic)

3. Results

Table 1 Phytochemicals present in the ethanolic extract of *C. lepidota* fruit pulp and seed

Phytochemicals	Fruit Pulp	Seed
Alkaloids	_	_
Tannins	+	+
Flavonoids	+	+
Phenols	+	+
Terpenoids	_	+
Anthraquinone	+	_
Cardiac glycosides	+	+

(+): Present; (-): Absent

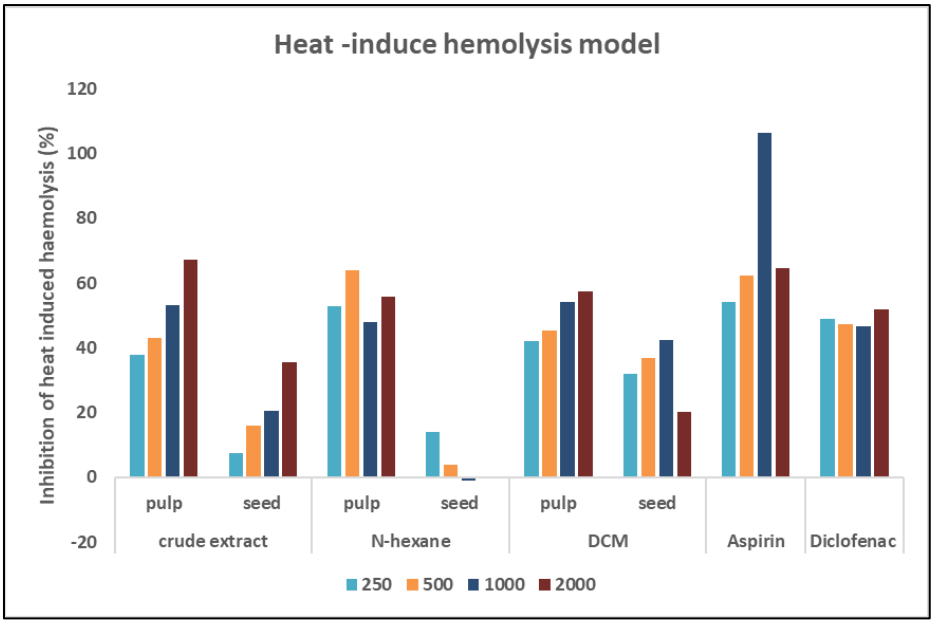

Figure 1 Membrane stabilizing effect of test drugs and standard on HRBC in a heat environment.

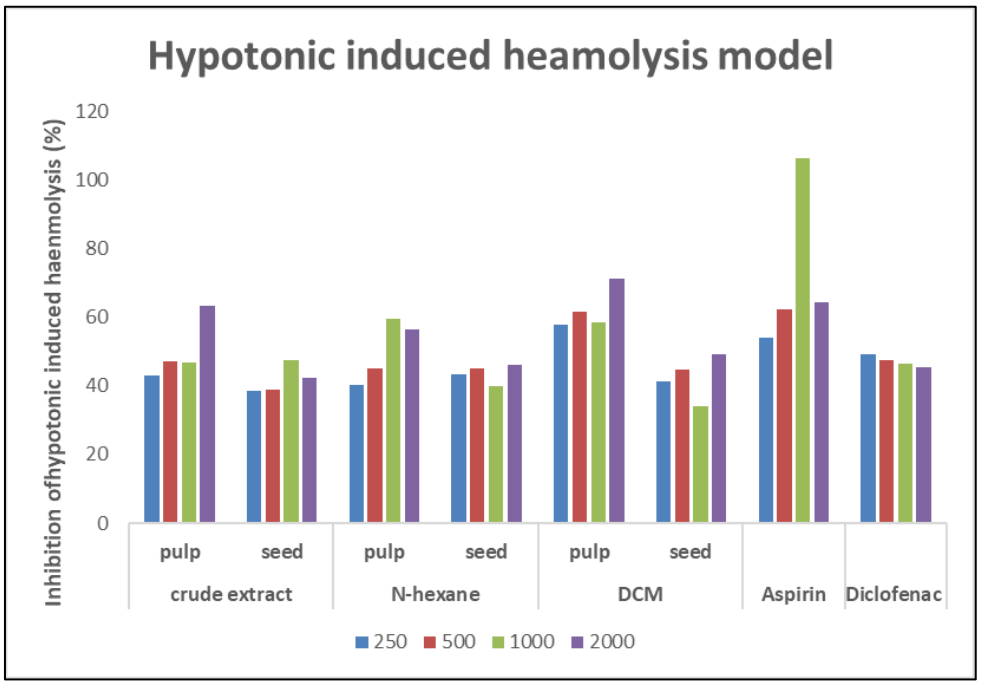

Figure 2 Membrane stabilising effect of test drugs and the standard in a hypotonic environment.

4. Discussion

Phytochemical investigation reveals that the pulp extract contains flavonoids, tannins, phenols, anthraquinone and cardiac glycosides excluding alkaloids and terpenoids while the seed contains tannins, flavonoids, terpenoids, phenols and cardiac glycosides except alkaloids and anthraquinones. The membrane stabilizing activities of the extracts are shown on Figure 1 and 2. The study on the stability of HRBC in a heat environment studied showed that at 250 µg /ml, the seed had a very poor activity as its inhibition was rated 7.39 %. At 500, 1000 and 2000 µg /ml, the pulp gave higher activities (42.90 %, 53.27 %, 67.33 %) more than the seed (16.04 %, 20.53 %, 35.64 %). On the other hand, the pulp had an activity comparable to that of diclofenac (51.22 %, 47.59 %) at 500 and 1000µg /ml respectively with a significant greater inhibition activity at 2000 µg /ml compared to the reference standards and the other extracts. From the result

of the inhibition of heat induced lysis obtained, the activity of the fruit pulp and seed were dose-dependent, the higher the concentration, the greater the activity of the extracts. The stability in hypotonic induced condition was also studied. At the different concentration, all the extracts had a good activity in inhibiting hypotonic induced haemolysis (Fig 2 & 3). At 250 and 500 µg /ml, the pulp had a greater activity, 43.07 % and 47.19 % respectively than the seed (38.64 %, 39.08 %) but has a comparable inhibition activity to that of standard, diclofenac. While at 2000 µg /ml, it also exhibited significant membrane stabilizing property than that of the standard diclofenac and comparable to aspirin. The results showed that although both crude extract are potent on human erythrocyte, adequately protecting it against heat and hypotonic induced lyses, the pulp crude extract had a better activity than that of the seed. On partitioning, the result showed that DCM fraction of the fruit pulp has a better activity than other fractions in both heat induced and hypotonic induced haemolysis assay. Membrane stabilization assay is a very common procedure to investigate the potential anti-inflammatory activity of a plant extract. When erythrocytes are exposed to hypotonic medium, heat, methyl salicylate, phenylhydrazine, etc., the lysis of their membrane occurs. It results in the leakage of serum protein and fluids into the tissues instigating inflammation. So, the compounds capable of membrane stabilization might be very suitable as anti-inflammatory agents (Chaity *et al.,* 2016).

5. Conclusion

Our study showed that the crude extracts has a significant membrane stabilizing property than that of the standard diclofenac at hypotonic environment and comparable activity in heat induced activity. The pulp crude extract as well as pulp DCM fraction showed a highly significant higher activity compared to the seed crude extract and other fractions respectively. Thus it can be inferred that the extracts of *C. lepidota* pulp and seed contains principles that are capable of stabilizing human red blood cells membranes against hypotonic solution and heat induced lysis. membranes against hypotonic solution and heat induced lysis. The plant therefore could be regarded as a natural source of membrane stabilizers and an alternative for management of inflammatory conditions.

Compliance with ethical standards

Disclosure of conflict of interest

All authors declare that there is no conflict of interest.

Authors Contribution

BOO, DIE, OEA, and CCO designed and executed the project while EEO and ECO did the membrane stabilization assay.

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