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Full Length Research Paper

Red blood cell membrane stabilizing potentials of extracts of *Lantana camara* and its fractions

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Anti-inflammatory activity of extract of *Lantana camara*, Linn and its fractions was investigated using stabilization of red blood cell membrane lysing technique. Phytochemically, whole plant extract (WPE) and ethanol fraction (EF) gave positive reactions for the presence of saponins, tannins, flavonoids and cardiac glycosides. The ethyl acetate fraction (EAF) gave reactions for the presence of flavonoids while butanol fraction (BF) gave positive test for the presence of saponins. The percentage membrane stability exhibited by the extract and various fractions was concentration dependent and compared favorably with those of standard drugs (Ibuprofen and Indomethacin). The results revealed that both ethanol and ethyl acetate fractions contained principles that protected the erythrocyte membranes effectively. Moreover, ethyl acetate fraction provided highest protection against induced lyses and exhibited both monophasic and biphasic responses at all the concentrations assayed. The possible mechanism of action of the extract and fractions is described and discussed.

Key words: Membrane, stabilization, anti- inflammatory, *Lantana camara.*

INTRODUCTION

Studies have revealed that several herbal derived drugs have been demonstrated to contain principles that possess ability to facilitate the stability of biological membranes when exposed to induced lyses. Some of these include extracts of *Lannea coromandelica* (Gandhidasan et al., 1991), *Gmelina asiatica* (Ismail et al., 1997)*, Gynandropis gynendra* (*Sadique* et al., *1989*)*, Theobroma cacao, Aspilia africanum, Plumbago zeylanica* and *Olax subscorpiodies* (Oyedapo and Famurewa, 1995; Oyedapo, 1996; Oyedapo et al., 1997, 2004).

The plant, *Lantana camara*, L, is a cosmopolitan shrub of Verbenaceae family. It is a native of America while a few varieties are indigenous to tropical Asia and Africa. It is a heavily branched rugged evergreen shrub which grows as compact clumps, dense thickets and as a scrambling vine (Scheper, 1996; Chhabra et al., 1993). The plant has long been recognized as highly toxic to grazing animals, causing death in children when large quantities of unripe barriers were taken (Chhabra et al., 1993; Saini et al., 2007). It contains toxic constituents such as triterpene acids, lantadene A (rehmanic acid), lantadene, their reduced forms, essential oils, quinine – like alkaloid (lantanine), saponin and flavonoids (Oyedeji et al., 2004; Pino et al., 2004; Oliver-Bever, 1983; Saleh, 1974; Raghu et al., 2004).

Studies on the biological activities of *L. camara*, L revealed that the plant is capable of exhibiting anti-inflammatory (Oyedapo et al., 1999), cytotoxicity, antimalarial, antifungal, anti-microbial, insecticidal, and nematicidal and wound healing (Raghu et al., 2004; Archhireddy et al., 1984; Begun et al., 1995; Shama et al., 1999; Day et al., 2003; Nayak et al., 2008). It is employed in traditional African folk medicine for the treatment and management of various forms of ailments. The stem and leaf extract as well as seed oil are employed in treating varying forms of skin diseases such as skin itches and as antiseptic for wound dressing (Saxena and Sharma, 1999). Moreover, the whole plant

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extracts are used for the treatment of different forms of cancer, chicken pox, measles, asthma, ulcers, swellings, tumors, high blood pressure, bilious fevers etc (Day et al., 2003; Begun et al., 1995).

This present study reported biological activity of the extract of *L. camara* and its fractions on the bovine red blood cells exposed to both heat and hypotonic induced lyses with a view to further evaluate the anti-inflammatory property of the plant using bovine red blood stabilization bio-assay (Oyedapo et al., 1999).

MATERIALS AND METHODS

Plant materials

Fresh leaves of *L. camara* were collected in June 2005, along Ibadan road in Ile-Ife, Osun State, Nigeria. Plant identification and authentication was done by Prof. J. O. Faluyi, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The fresh leaves were shade dried and pulverized using electric Karasa blender.

Reagents and chemicals

Potassium dihydrogen orthophosphate, sodium monohydrogen orthophosphate, sodium carbonate, trisodium citrate, sodium chloride are products of British Drug House (BDH) Chemicals Limited, Poole, England. Ethanol, methanol, Ethylacetate, and Petroleum ether are products of Sigma Aldrich Germany. All the reagents were of analytical grades. All the solutions, reagents and buffers were prepared with glass distilled water. Indomethacin capsules and Ibruprofen tablets (400 mg) standard non-steroidal anti-inflammatory drugs were purchased from a pharmaceutical shop at Obafemi Obafemi University, Ile-Ife, Nigeria.

Spectroscopic readings

The absorbance readings were recorded on Cam-Spec M201 Vis-Spectrophotometer Model, (Cambridge CB 4BG) and Pharmacia Biotech Novaspec II Spectrophotometer (Pharmacia Chemical Limited, Uppsala, Sweden).

Preparation of extracts and fractionation

The extracts of *L. camara* were prepared and fractionated according to a procedure that was based on those earlier reported by Oyedapo and Amos (1997) and Adeoye and Oyedapo (2004). Fresh aerial parts of plant were collected, washed clean under running water and cut into tiny pieces. 500.0 g of the leaf was crushed in a mortar to form paste which was followed by squeezing of its juice into a clean beaker. The filtrate was subjected to centrifugation at 4,000 rpm for 10 min on Bench Centrifuge Model 800D (Microfield Instrument, Essex, England). The dark green solution was obtained and allowed to settle to give slight yellow solution.

The solution was evaporated to dryness at 35°C under reduced pressure on Edwards High Vacuum Pump (Edwards Vacuum Components, Crawley England) to afford a light yellow residue that was termed whole plant extract (WPE). 50 g of the pulverized dried leaf was extracted with 80% (v/v) ethanol over a period of 5 days after which it was filtered using a piece of white cotton gauze. The residue was washed with the same solvent system, filtrates were combined, and evaporated to complete dryness under reduced pressure at 35°C as above to yield brown residue which was termed ethanol extract (EE).

Ethanolic extract (15.0 g) was` dissolved in hot distilled water (100 ml) in a separating funnel, followed by partionning with petroleum ether (50 ml x 5) to remove chlorophyll and other lipid materials. The aqueous layer was collected and partitioned with ethyl acetate (50 ml x 5). The ethyl acetate fractions were pooled together and then subjected to evaporation under reduced pressure to yield ethylacetate fraction (EAF).

Finally, the aqueous fraction was equally partitioned with n-butanol (50 ml x 5), fractions were pooled and subjected to evaporation to complete dryness to give butanol fraction (BF). The extraction was carried out five times to afford enough samples for analysis. The solid residues were weighed, pooled together and kept in a air-tight container in the desiccators until required for further analyses and biological assays.

Phytochemical screening

Extracts (WPE and EE) of *L. camara* and the fractions (EAF and BF) were screened for the presence of secondary metabolites (Trease and Evans, 2002; Oyedapo et al., 1999). Alkaloids (Mayer's, Wagner's, Dragendorf reagents and Picric acid), saponins (froth and haemolytic tests), flavonoids (ethanolic KOH/ ethylacetate), cardiac glycosides (chloroform/H_2SO_4), tannins and phenols (ferric chloride reagent) and anthraquinones (ethanolic NaOH).

Preparation of drugs

Standard drugs (Ibuprofen and Indomethacin, 2.5 mg/ml) and various extracts/fractions were prepared in isosaline (0.85% w/v NaCl) to final concentrations of 1.0 - 5.0 mg/ml.

Preparation of bovine red blood cell

Fresh bovine blood samples were collected into an anticoagulant (containing dextrose (2%), sodium citrate (0.8%), citric acid (0.05%) and sodium chloride (0.42%). Blood samples were centrifuged at 3000 rpm on a Bench centrifuge Model 800D for 10 min at room temperature. The supernatants (plasma and leucocytes) were carefully removed while the packed red blood cell was washed in fresh normal saline (0.85% w/v NaCl). The process of washing and centrifugation were repeated five times until the supernatants were clear. Then, bovine erythrocytes (2% v/v) were prepared as reported previously (Oyedapo et al., 2004).

Assay of membrane stabilizing activity

The membrane stabilizing activity assay was carried out as previously described (Sadique et al., 1989; Oyedapo et al., 2004) using 2% (v/v) bovine erythrocyte suspension while Ibruprofen and Indomethacin were used as standard drugs. The assay mixtures consisted of 2 ml of hyposaline (0.25% w/v) sodium chloride, 1.0 ml of 0.15 M sodium phosphate buffer, pH 7.4, 0.5 ml of 2% (v/v) bovine erythrocyte suspension, 0.0 - 1.0 ml of drugs (standard, extracts/fractions) and final reaction mixtures were made up to 4.5 ml with isosaline. Drugs were omitted in the blood control, while the drug control did not contain the erythrocyte suspension. The reaction mixtures were incubated at 56°C for 30 min on a water bath, followed by centrifugation at 5000 rpm on Gallenkamp Bench Centrifuge for 10 min at room temperature. The absorbance of the

released heamoglobin was read at 560 nm. The percentage membrane stability was estimated using the expression:

$$\frac{100 - \{\text{Abs of test drug-Abs of drug control}\}}{\{\text{Abs of blood control}\}} \times 100$$

where the blood control represents 100% lysis or zero percent stability.

Statistical analysis

Each value represented the mean ± sem of 4 consistent readings. The significance of the differences between controls, tests and fractions were analyzed using Students' t-test and analysis of variance. Values of $P \leq 0.05$ were considered to be statistically significant (Argyrous, 2005).

RESULTS AND DISCUSSION

During inflammation, lysosomal hydrolytic enzymes are released into the cites which causes damages of the surrounding organelles and tissues with attendance variety of disorders (Sadique et al., 1989). Various methods were employed to screen and study drugs, chemicals, herbal preparations that exhibit anti-inflammatory properties or potentials. These techniques include uncoupling of oxidative phosphorylation (ATP biogenesis linked to respiration), inhibition of denaturation of protein, erythrocyte membrane stabilization, lysosomal membrane stabilization, fibrinolytic assays and platelet aggregation (Kalyanpur et al., 1968; Lee and Thong, 1970; Swingle, 1974; Kumar and Sadique, 1987; Pal and Chaudhuri, 1992; Oyedapo et al., 1999). In the present study, stabilization of erythrocyte membranes exposed to both heat and hypotonic induced lyses was employed due to its simplicity and reproducibility.

Analyses of the various extracts and fractions of *L. camara* phytochemically revealed that whole plant extract (WPE), ethanolic extract (EE), and ethylacetate fraction (EAF) gave positive tests for the presence of flavonoid, while saponin detected in both WPE and butanol fractions (BF) amongst other phytochemicals, a confirmation of our earlier observations (Oyedapo et al., 1999).

In Figures 1a - e are the membranes stabilizing profiles of various extracts/fractions of *L. camara* on bovine red blood cell exposed to both heat and hypotonic induced lyses. The whole plant extract exhibited a minimum membrane stability of 3.65 ± 0.85% and maximum activity of 27.95 ± 1.27%. The mode of response of the erythrocyte was both monophasic and biphasic (Figure 1a). Ethanol extract exerted a minimum membrane stability of 2.86 ± 1.58% and maximum activity of 91.76 ± 6.12%, respectively.

The response of red blood cell was monophasic and biphasic at lower and higher concentrations of the extract, respectively (Figure 1b).

Ethyl acetate fraction (EAF) gave membrane stability of 13.52 ± 5.20 and 95.98 ± 3.15% as minimum and maximum percentage activity respectively. The response of the red blood cells was also monophasic and biphasic to the fractions (Figure 1c).

Moreover, butanol fraction also exerted minimum and maximum percentage stability activities of 4.84 ± 1.05 and 59.57 ± 4.19%, respectively. The response of the red blood cell was mainly monophasic at all the tested concentrations (Figure 1d).

Finally, the standard anti-inflammatory drugs (Ibuprofen and Indomethacin) at 2 mg/ml exerted maximum membrane stabilities of 19.93 ± 1.03 and 56.83 ± 3.32%, respectively. The response was biphasic on the bovine red blood cells. Earlier studies revealed that indomethacin inhibited haemolysis of red blood cells at a concentration of 2 µg/ml (5 µM), although the dose-response curve shifts as the cells age after collection and storage (Swingle, 1974). The results revealed that both ethanolic and ethylacetate fractions contained principles that protected the erythrocytes membranes effectively. Moreover, ethylacetate fraction provided highest protection against induced lyses.

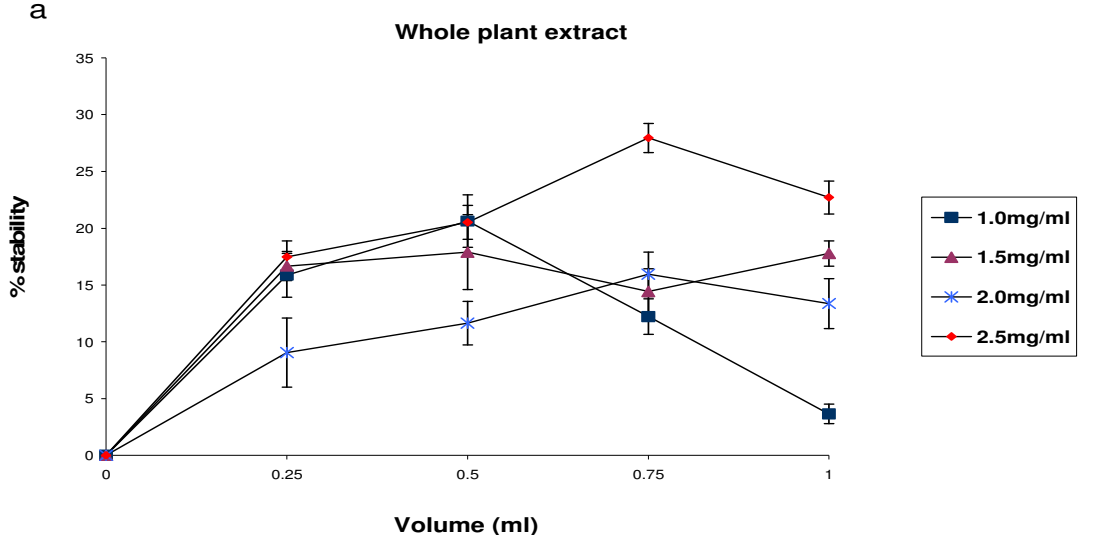
Also, it was noted that all the extracts and fractions showed dose dependent membrane stabilizing activity over all the concentration ranges. The activities of the extracts/fractions were higher than that of the standard drugs even at lower concentration ranges.

The mode of action of the extracts, fractions and standard anti-inflammatory drugs could be connected with binding to the erythrocyte membranes with subsequent alteration of the surface charges of the cells. This might have prevented physical interaction with aggregating agents or promote dispersal by mutual repulsion of like charges which are involved in the haemolysis of red blood cells. It has been reported that certain saponins and flavonoids exerted profound stabilizing effect on lysosomal membrane both *in vivo* and *in vitro,* while tannins and saponins posses ability to bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules (Hess and Milloning, 1972; Van-Cangeghen, 1972; Sadique et al., 1989; Pathak et al., 1991; Middleton, 1996; El-Shabrany et al., 1997; Oyedapo et al., 2004).

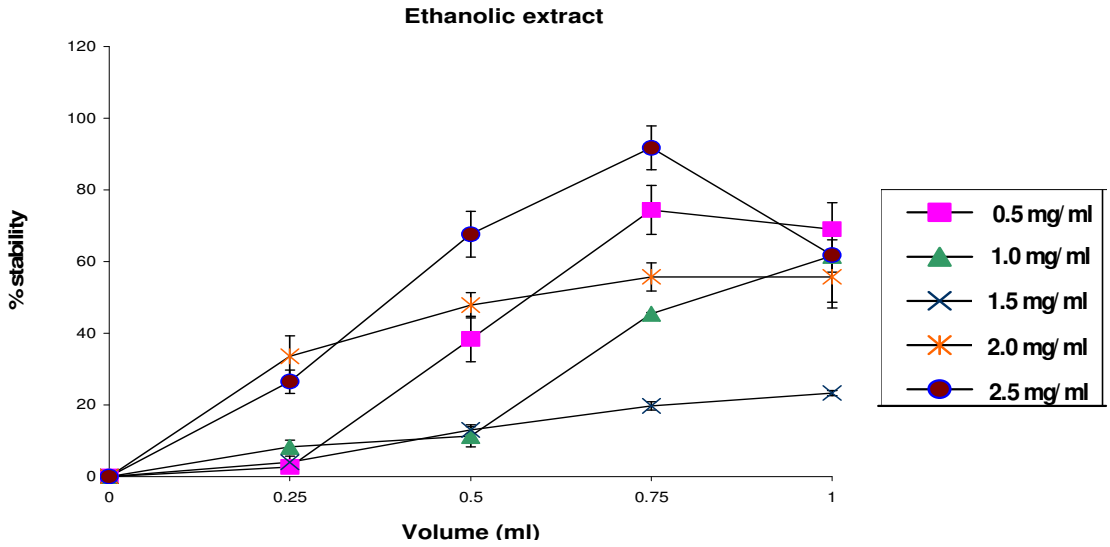
It was noted that ethanolic extract and ethylacetate which gave positive tests for flavonoids exhibited highest membrane stabilities of 91.76 ± 6.12 and 95.98 ± 3.10%, respectively. This implied that the membrane stabilizing activities of these fractions were aided by the presence of flavonoid in them. The lowest activities observed with whole plant extract could be due to the masking of the action of the above named molecules that are associated with membrane stabilizing activities by other phyto-constituents.

On the basis of these results, it could be inferred that the extracts/fractions of *L. camara* contained principles that were capable of stabilizing bovine red blood cells

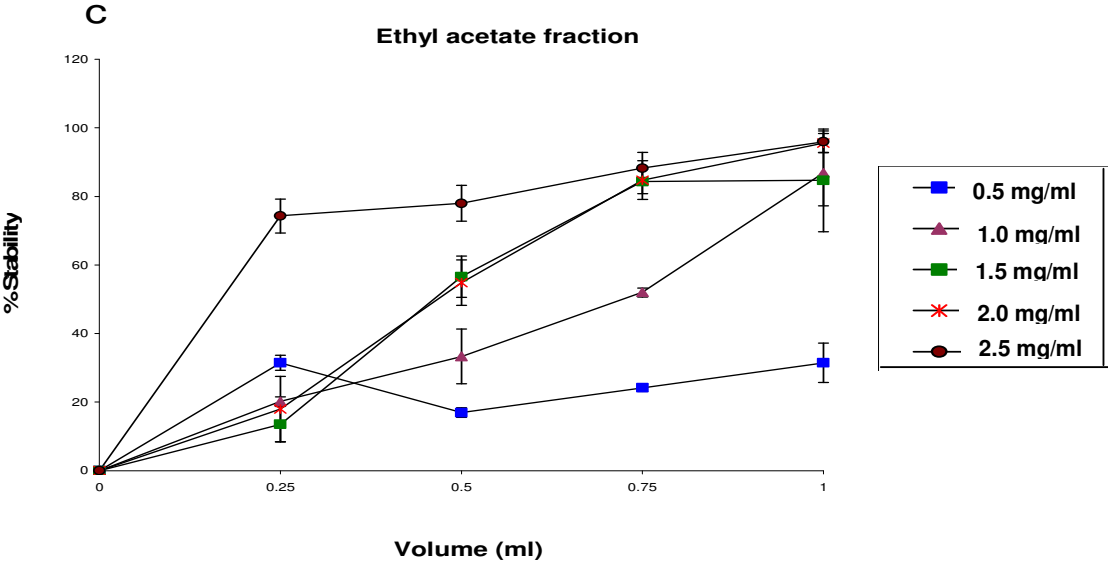
a


Whole plant extract
% stability
Volume (ml)
1.0mg/ml
1.5mg/ml
2.0mg/ml
2.5mg/ml

b


Ethanolic extract
% stability
Volume (ml)
0.5 mg/ ml
1.0 mg/ ml
1.5 mg/ ml
2.0 mg/ ml
2.5 mg/ ml

C


Ethyl acetate fraction
% Stability
Volume (ml)
0.5 mg/ml
1.0 mg/ml
1.5 mg/ml
2.0 mg/ml
2.5 mg/ml

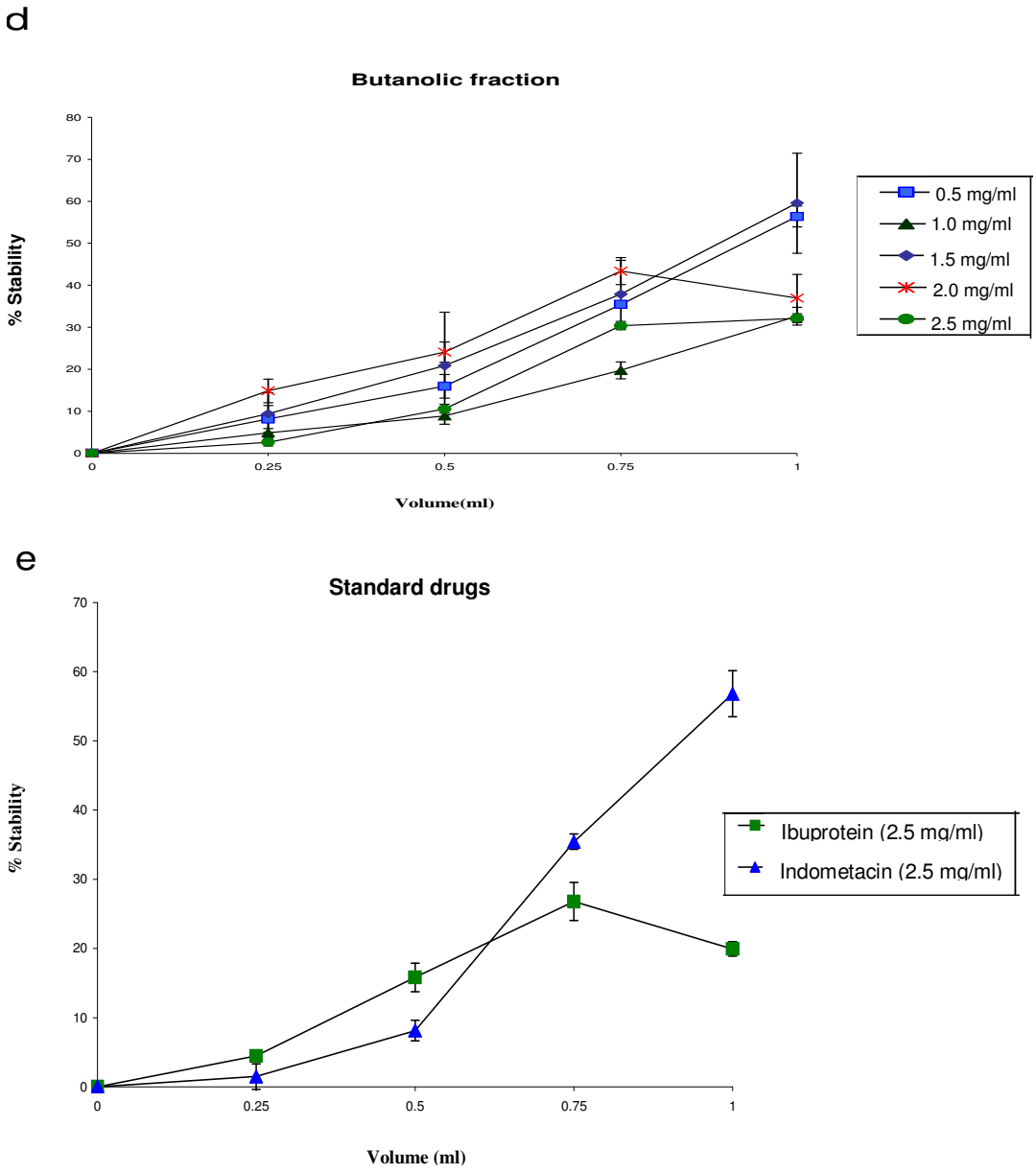


Figure 1(a - e). Membrane stabilizing profiles of (a) whole plant extract; (b) ethanolic extract; (c) ethylacetate fraction; (d) butanolic fraction of *L. camara*, L. and; (d) standard drugs (Ibuprofen and Indomethacin) on bovine red blood cells subjected to both heat and hypotonic induced lyses. Each value represents the mean of four readings ± sem.

membranes against heat and hypotonic- induced lyses. The plant therefore could be regarded as a natural source of membrane stabilizers and was capable of providing an alternative remedy for the management and treatment of inflammatory related disorders and diseases.

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