Original Article

TIPS Trends in Pharmaceutical Sciences 2021: 7(4): 269-278.

Membrane Stabilization and Inhibition of Protein Denaturation as Mechanisms of the Anti-Inflammatory Activity of some Plant Species

Esho Babatunde Akinyemi[1,2], Samuel Babatunde[1*], Akinwunmi Kemi Feyisayo[3], Oluyemi Wande Michael[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.
[2]Department of Science Laboratory Technology, Faculty of Pure and Applied Science, Osun State College of Technology, Esa-oke, Nigeria.
[3]Department of Biochemistry and Molecular Biology, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria.

Abstract

Several reports have shown the potential of medicinal plants as vital sources of anti-inflammatory agents and this study is aimed at investigating the anti-inflammatory potential of selected medicinal plants used for the treatment of inflammation in folk medicine. The methanol and acetone extract of *Lecaniodiscus cupanioides*, *Talinum fruticosum*, *Ocimum gratissimum*, *Senna occidentalis* and *Senna alata* were subjected to anti-inflammatory assays using membrane stability and inhibition of albumin denaturation methods and absorbance measured at 560 and 660 nm respectively. The mean IC_{50} and SEM values were determined using normalized response variable through non-linear regression XY analysis on prism graphpad® (7.0). In the membrane stability experiment, the extracts tested showed varying levels of erythrocyte lysis inhibition within the range of (IC_{50}: 89.08±6.339 µg/mL≤IC_{50}≤278.3±5.678 µg/mL) while the standard drug (ibuprofen) has an IC_{50}: 61.93±8.359 µg/mL, *Lecaniodiscus cupanioides* acetone (IC_{50} of 89.08±6.339 µg/mL) extract showed to be most active when compared to other extracts. In the inhibition of albumin denaturation experiment, the tested extracts showed different level of activity within the range of (IC_{50}:22.89±5.52 µg/mL≤IC_{50}≤210.6±6.71 µg/mL). The acetone extracts of *Senna occidentalis* (22.89±5.52 µg/mL) and *Lecaniodiscus cupanioides* (80.9±9.11 µg/mL) showed significant activity with the lowest IC_{50} compared to other extracts while the standard drug (aspirin) had an IC_{50} of (4.055±2.72 µg/mL). The findings of this study shows that *Lecaniodiscus cupanioides* acetone extract has the most significant anti-inflammatory properties, therefore justifying the traditional use of the plant in the treatment of inflammatory injury and tissue damage.

Keywords: *Lecaniodiscus cupanioides*, Anti-inflammatory, Membrane Stability, Inhibition of Albumin Denaturation.

Please cite this article as: Akinyemi EB, Babatunde S, Feyisayo AK, Michael OW. Membrane Stabilization and Inhibition of Protein Denaturation as Mechanisms of the Anti-Inflammatory Activity of some Plant Species. Trends in Pharmaceutical Sciences. 2021;7(4):269-278.doi: 10.30476/TIPS.2021.93160.1118

1. Introduction

Inflammation is a pathological response of the body immune system to foreigners such as infection, noxious chemicals, drugs, virus and bacteria (1). Anytime an injury occurs it leads to the damage of tissues and cells, and the body respond by releasing inflammatory mediators such as histamine, chemokines, cytokines and serotonin to prevent further damage and commence the healing

Corresponding Author: Samuel Babatunde, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.
Email: tundebsamuel@gmail.com

Received: 08/07/2021; Accepted: 01/12/2021

process. The release of membrane phospholipids which are catalyzed by phospholipases to release arachidonic acids can be triggered (1). Inflammation is characterized by redness, edema, fever, pain, and loss of function (2). Inflammation may be associated with general "flu-like" symptoms such as fever, fatigue, cold, loss of appetite and muscular stiffness (2). Inflammation is responsible for several diseases such as rheumatoid arthritis, hemorrhoids, asthma, stroke, heart attack, bronchitis, inflammation of the gum (gingivitis) and mouth (stomatitis). Studies had revealed that medicinal plants contain principles that possess ability to facilitate the stability of biological membranes or inhibit the denaturation of albumin when subjected to induced lysis, hypotonic solutions and heat (4,5).

Lecaniodiscus cupanioides Planch. Ex Benth, a tropical plant widely distributed in Africa and Asia, is ethnomedicinally reputed to be useful in the treatment of wounds and sores, abdominal swelling caused by liver abscess, fevers, measles, hepatomegaly and burns, among others (6). The plant is locally used in the management of arthritis in Ibadan, Nigeria (7).

Talinum fruticosum (L.) juss. formerly known as *Talinum triangulare* (Family: *Portulaceae*), is commonly called waterleaf. The extract from the leaves and roots is used locally to cure asthma, the leaves are used in the treatment of kidney disorders, gout and rheumatoid arthritis (8). The aerial parts had been reported to possess anti-inflammatory activities because it treated formalin induced rats, showing moderate reduction in the cartilage and osteoblast (9).

Ocimum gratissimum (L.) is a herbaceous plant species that belongs to *Lamiale*'s family. The plant is indigenous to tropical areas especially India and it is also in West Africa, and it is used in the treatment of epilepsy and high fever. It had been reportedly used in treatment of blocked nostrils, abdominal pains, eyes and ears infections, coughs, barrenness, fever, convulsions, tooth-ache, regulation of menstruation pain and as a cure for prolapse of the rectum antipyretic, anti-inflammatory activity (10) and hemorrhoids (11). The leaves of *O. gratissimum* had been reported to prevent inflammation of the gum (gingivitis) and mouth (stomatitis), it also contains pharmacological principles capable of inhibiting the effect of formalin-induced paw edema in rats (12, 13).

Senna occidentalis (L.) Link formerly known as *Cassia occidentalis* Linn. (English: - Coffee Senna, Foetid Cassia, Negro Coffee, Family: *Calsalpiniaceae*). A paste of the leaves is used for treating piles, bronchitis and asthma. Alcoholic extract of leaves is used in intestinal and bronchial muscle relaxant (14). The leaves of *Senna occidentalis* had been reported to have comparative activities as conventional anti-inflammatory agents when assayed with male albino rats using carrageenan-induced rat paw edema model.

Senna alata (L.) Roxb. formally known as Cassia alata, and commonly known as Candlestick Senna, Wild Senna, Ringworm Cassia and King of the Forrest, belongs to the family Caesalpiniaceae. *S. alata* is traditionally used in the treatment of ringworm, diarrhea diseases, gastro-intestinal, upper respiratory tract infections, asthma, bronchitis and parasitic skin diseases (15, 16). The leaves were reportedly effective in the treatment of chronic inflammation such as arthritis (17), it also possesses anti-inflammatory, anti-mutagenic, analgesic, antimicrobial properties (18, 19).

The anti-inflammatory agents exert their effects through a variety of mechanisms including inhibition of cotton pellet granulation, uncoupling of oxidative phosphorylation, inhibition of denaturation of protein, stimulation and inactivation of adenosine triphosphate phosphatase, erythrocyte membrane stabilization, lysosomal membrane stabilization, fibrinolytic assay proteinase inhibition (20) and inhibition of some enzymes that are involved in inflammation. The plant extracts in this study were evaluated for their anti-inflammatory activities using heat induced egg albumin denaturation bio assay. This is widely used, validated, sensitive, quick and reliable *in vitro* technique to investigate anti-inflammatory activity of natural products (21). The rationale behind this assay is that denaturation of albumin proteins leads to the formation of antigens which initiates type III hypersensitive reaction leading to inflammation. Therefore, inhibition of its denaturation process by an agent indicates its anti-inflammation properties; the higher the degree of inhibition the

greater would be its anti-inflammation potential. Similarly, assay to examine the stabilization of the lysosomal membrane was employed in this study. Exposure of red blood cells to injurious substances such as hypotonic medium, heat, methyl salicylate or phenylhydrazine results in the lysis of the membranes, accompanied by haemolysis and oxidation of haemoglobin (22). Since human red blood cell (HRBC) membranes are similar to lysosomal membrane components (23), the inhibition of hypotonicity and heat induced red blood cell membrane lysis was taken as a measure of the mechanism of anti-inflammatory activity of garden egg extract. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Injury to red cell membrane will render the cell more susceptible to secondary damage through free radical induced lipid peroxidation (24). Membrane stabilization leads to the prevention of leakage of serum protein and fluids into the tissues during a period of increased permeability caused by inflammatory mediators.

This study was therefore aimed at further screening the extracts of the plants to evaluate their anti-inflammatory potentials using membrane stability and inhibition of albumin denaturation methods.

2. Materail and Methods

2.1. Sample collection and Extraction

Five plants were collected and identified by the curator of the botanical garden, University of Ibadan, Nigeria. Voucher specimen were deposited at the Forestry Research Institute of Nigeria (FRIN), Ibadan Herbarium. The following herbarium number were issued to the plants:

The leaves of *Lecaniodiscus cupanioides*, *Senna alata*, *Senna occidentalis* and the whole plants of *Ocimum gratissimum* and *Talinum fruticosum* were air-dried and grounded to powder. The grounded plants (200 g) were extracted with both acetone (aqueous) and methanol for 72 hours using cold maceration method. The extracts were filtered, concentrated and stored until it was used for analysis.

2.2 Reagents and Chemicals

All the reagents and chemicals used were of analytical grades and were obtained from Merck (Darmstadt, Germany), Sigma Aldrich (St. Lous, MO), British Drug House (BDH) England. Solutions, buffers and reagents used were prepared with distilled water and stored in the refrigerator.

2.3 Investigations of Anti-inflammatory Activities

2.3.1 Membrane Stabilizing Potential

The red blood cell was prepared as previously described (5). Typically, fresh bovine blood was collected into an anticoagulant (3.8% trisodium citrate) in a clean sterile bottle, mixed by invasion and brought to the laboratory in an ice bucket. Bovine blood was washed with normal saline by centrifugation at 4000 rpm for 10 min at room temperature using a centrifuge and the supernatant was carefully decanted. The process of washing and centrifuging was repeated until the supernatant became clear. The clear supernatant was decanted and 2% (w/v) red blood cells was prepared from the packed cell with normal saline and kept in the refrigerator.

The red blood cell membrane stabilizing activity assay was carried out using the method summarized above (5), with ibuprofen as reference drug. The assay mixture consisted of 1 ml hyposaline, 0.5 ml of 0.1 M phosphate buffer (pH 7.4), varying concentration of the extracts (50 µg-300 µg) and 0.5 ml of 2% (v/v) erythrocyte

Table 1. The plants and their voucher numbers.

S/N	Botanical Name	Herbarium Number
1	Lecaniodiscus cupanioides Planch ex Benth	FHI 112846
2	Senna alata (L.) Roxb	FHI 112183
3	Talinum fruiticosum (L.) Juss	FHI 112845
4	Ocimum gratissimum L.	FHI 112184
5	Senna Occidentalis (L.) link	FHI 112185

suspension. The reaction mixture was made up to 3.0 ml with normal saline.

The drug control contained all reagents as above without 2% (v/v) erythrocyte suspension while the blood control contained all the reagents except the drug or the extract. The blood control represents 100% lysis. The reaction mixture was incubated at 56 °C for 30 minutes. The tubes were cooled and centrifuged at 3,500 rpm for 10 minutes. The supernatant was collected and the absorbance of the released haemoglobin was read at 560 nm against reagent blank. The same procedure was employed with standard anti-inflammatory drug ibuprofen (1 mg/mL). The percentage membrane stability was calculated using the expression below (Equation 1):

$$\%\ stability = 100 - \frac{(Abs\ of\ test\ drug - Abs\ of\ drug\ control) \times 100}{Abs\ of\ blood\ control} \quad \text{Eq.1}$$

2.3.2 Inhibition of Denaturation of Albumin

The ability of the plant extracts to inhibit the denaturation of albumin was investigated by the method of Mizushima and kobayashi (4) as reported by Sakat (20) with minor modification. Varying concentration of the extracts (50 µg-300 µg) were prepared and the volumes were made up to 2.5 ml with 0.85% NaCl. This was followed by the addition of 0.5 ml albumin (1.5 mg/ml). The mixture was incubated at 37 °C for 20 minutes and further incubated at 57 °C for 20 minutes. The tubes were cooled and 2.5 ml of 0.5 M sodium phosphate buffer (pH 6.3) was added. The turbidity was measured spectrophotometrically at 660 nm. The experiment was carried out in triplicates and aspirin was used in place of the extract as the standard drug. The percentage inhibition of albumin denaturation was calculated using the expression below in (Equation 2).

$$\%\ inhibition = \frac{Abs\ of\ control - Abs\ of\ test\ drug}{Abs\ of\ Control} \times 100 \quad \text{Eq. 2}$$

2.4. Statistical analysis

All data were analyzed and the results obtained were expressed as mean±standard error of mean from triplicate experiments. IC50 values were determined using normalized response variable through non-linear regression XY analysis on prism graphpad® (7.0).

3. Results and discussion

The importance of the use of appropriate solvents for the extraction of bioactive constituents from plant cannot be over-emphasized, the ability to get those bioactive principles into solutions form the basis for the extraction process and thence the choice of solvents should be most appropriate. The solvent of choice should be able to dissolve secondary metabolites and other materials present in the plant. (25).

The results obtained from this experiment are presented in Table 2 below, the results showed the mean IC50 for all the extracts and the test drugs used as references. From the results for membrane stability assay, the various extracts that were tested showed different levels of anti-inflammatory activities by being able to stabilize the membrane against hypotonic solution and heat induced lysis of the red blood cells. The extracts with best activities are *L. cupanioides* acetone (89.08±6.339 µg/mL) and *T. fruticosum* acetone (91.18±7.793 µg/mL) these extracts showed comparable activities with the standard ibuprofen drug (61.93±8.359 µg/mL). The outcome from inhibition of albumin denaturation assay also indicated that the extracts had anti-inflammatory potential; the extracts protected the albumin from being denatured when subjected to hypotonic solution and heat. The extracts with best activities for inhibition of albumin denaturation method are *S. occidentalis* acetone (22.89±5.52 µg/mL) and *L. cupanioides* acetone (80.9±9.11 µg/mL), these extracts demonstrated good level of activity but the reference aspirin drug (4.055±2.72 µg/mL) was far more active. The other extracts had good IC50; SAA (210.6±6.71 µg/mL), SAM (95.5±8.91 µg/mL), TFA (139.9±13.4 µg/mL), TFM (96.22±8.02 µg/mL), SOM (117.2±9.17 µg/mL), OGA (132±6.61 µg/mL), OGM (143.6±6.14 µg/mL), LCM (95.8 ± 9.14 µg/mL).

The results of albumin denaturation showed that all the extracts had good inhibition while the standard drug (aspirin) had the best inhibition. The data in Figure 1 showed that the percentage inhibition of albumin denaturation for

Table 2. Mean IC50±SEM for inhibitions of Albumin Denaturation and Membrane Stability.

EXTRACT	Albumin Denaturation	Membrane Stability
	MEAN IC50±SEM (µg/mL)	MEAN IC50±SEM (µg/mL)
LCM	95.8±9.14	154.5±8.053
LCA	80.9±9.11	89.08±6.339
OGM	143.6±6.14	278.3±5.678
OGA	132±6.61	125.8±9.564
SOM	117.2±9.17	222.3±9.613
SOA	22.89±5.52	275.9±10.09
TFM	96.22±8.02	159.9±9.165
TFA	139.9±13.4	91.18±7.793
SAM	95.5±8.91	166.0±9.264
Aspirin	4.055±2.72	----------
Ibuprofen	----------	61.93±8.359

KEYS: LCM = *Lecaniodiscus cupanioides* methanol extract, LCA = *Lecaniodiscus cupanioides* acetone extract, OGM = *Ocimum gratissimum* methanol extract, OGA = *Ocimum gratissimum* acetone extract, SOM = *Senna occidentalis* methanol extract, SOA = *Senna occidentalis* acetone extract, TFM = *Talinum fruticosum* methanol extract, TFA = *Talinum fruticosum* acetone extract, SAM = *Senna occidentalis* methanol extract.

aspirin in the following concentrations 25, 50, 100, 150, 200 and 250 µg/mL were 68.67, 75.39, 76.81, 82.18, 85.07, and 86.13% respectively; and the two promising extracts: SOA- 25, 50, 100, 150, 200 and 250 µg/mL were 43.48, 74.51, 73.4, 74.69, 77.87, and 79.76% respectively, LCA- 25, 50, 100, 150, 200 and 250 µg/mL were 23.77, 31.26, 73.45, 57.2, 65.48, and 77.46%. The inhibition showed a dose-dependent relationship for the aspirin and dose-independent relationship for the extracts (Figure 1). We found a linear correlation between aspirin, SOA, and LCA concentration and albumin denaturation inhibition percentage, with regression equations, y=0.07321x+69.59, y=0.1088x+56.58, and y=0.2120x+27.10, respectively, and correlation coefficient, R2=0.9033, 0.4921, and 0.6835, respectively (Figure 3). It can be seen that the relationship between the extracts and the albumin denaturation inhibition percentage is parabolic and largely dose-independent. This could be because the extracts contain complex matrices of so many other compounds that may interfere with the activity or cause inflammatory response.

The results of membrane stability as-

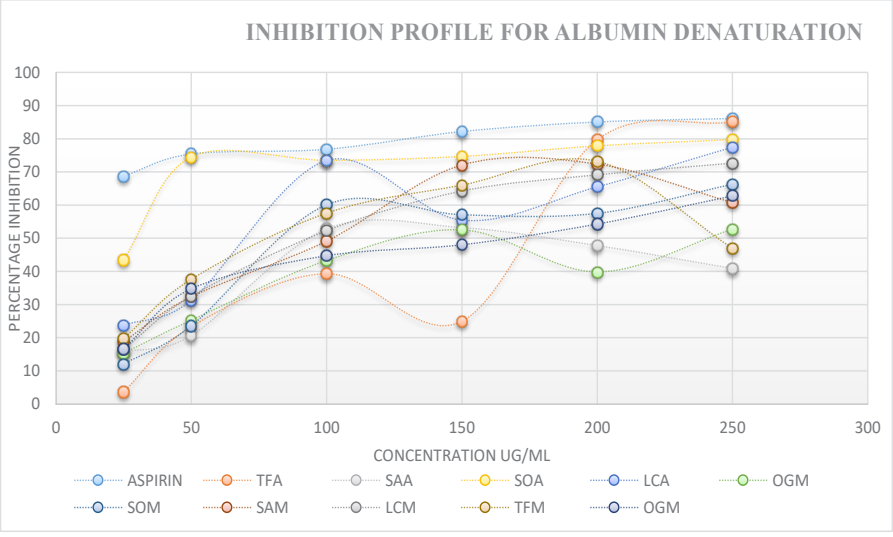

Figure 1. Inhibition of albumin denaturation percentage of various concentrations of the extracts.

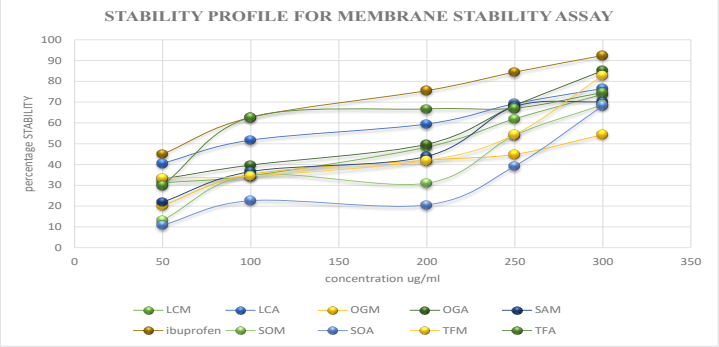

Figure 2. Membrane stability of percentage of various concentrations of the extracts.

say showed a dose-dependent response for all the extracts and the standard drug (ibuprofen), the extracts with the lowest IC50 are LCA and TFA having a value of (89.08±6.339 µg/mL) and (91.18±7.793 µg/mL) respectively, while the ibuprofen had an IC50 of (61.93±8.359 µg/mL). All the extracts had good stability and good IC50, such as LCM (154.5±8.053 µg/mL), OGM (278.3±5.678 µg/mL), OGA (125.8±9.564 µg/mL), SOM (222.3±9.613 µg/mL), SOA (275.9±10.09 µg/mL), SAM (166.0±9.264 µg/mL). The data in Figure 2 showed that the percentage membrane stability for ibuprofen in the following concentrations 50, 100, 200, 250, and 300 µg/mL were 45.04, 62.74, 75.53, 84.43, and 92.4% respectively; and the two promising extracts: LCA- 50, 100, 200, 250, and 300 µg/mL were 40.59, 51.71, 59.46, 69.23, and 76.55% respectively, TFA- 50, 100, 200, 250, and 300 µg/mL were 30.03, 62.74, 66.73, 67.09, and 74.61%. The inhibition showed a dose-dependent relationship for the ibuprofen, a similar relationship observed in LCA and TFA (Figure 2). We found a linear correlation between ibuprofen, LCA, and TFA concentration and membrane stability percentage, with regression equations, y=0.1777x+40.02, y=0.1350x+35.19, and y=0.1410x+34.87, respectively, and correlation coefficient, R2=0.9697, 0.9704, and 0.7014, respectively (Figure 4). Our findings following the experiments from the two methods adjured the most active extract to be LCA.

Compounds with anti-inflammatory potential acts by interfering with inflammatory pathways at the early stage; which eventually prevent the release of phospholipase that can trigger the formation of inflammatory mediators (26). If, inflammation is not prevented, lysomal hydrolytic enzymes are released into the cells and cause damages to the surrounding organelles and tissues (27). Several methods had been reportedly used to screen drugs, plants extracts and chemicals that were considered to possibly have anti-inflammatory properties. Anti-inflammatory agents control the biochemical processes involved during the inflammatory response by stabilizing the membranes of lysosomes (28). The erythrocyte membrane is

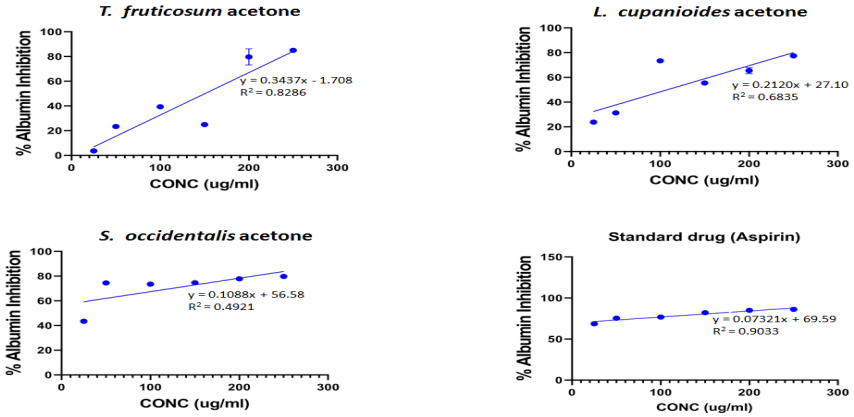

Figure 3. Chart showing the relationship between promising extracts concentration versus albumin denaturation inhibition percentage.

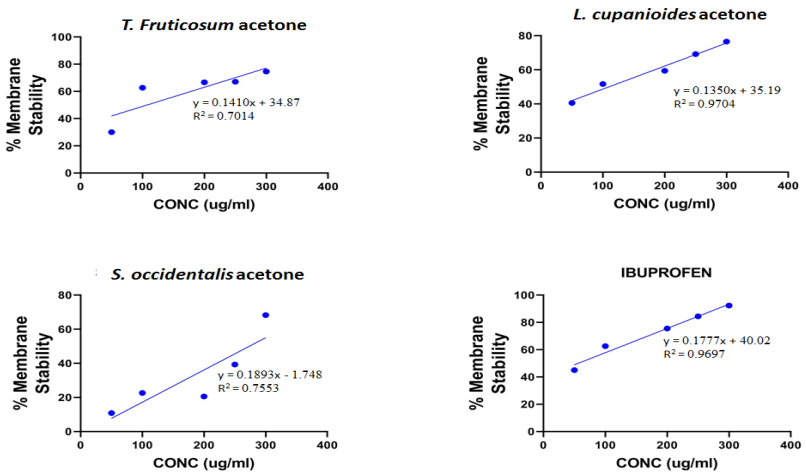

Figure 4. Chart showing the relationship between promising extracts concentration versus membrane stability percentage.

analogous to the lysosomal membrane, and its stabilization implies that LCA may as well stabilize lysosomal membranes comparably with ibuprofen (28). Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophils such as bactericidal enzymes and proteases, which cause further inflammation and damage on extracellular release (29). The extracts in this study perhaps stabilized the red blood cell membrane by preventing the release of lytic enzymes and active mediators of inflammation. The anti-inflammatory activities are probably due to their inhibitory effect on enzymes involved in the production of the chemical mediators of inflammation and metabolism of arachidonic (30-31).

Albumin Denaturation assay is based on monitoring the loss of biological properties of protein molecules when subjected to denaturation conditions such as elevated temperature and hypotonic solution. Albumin denaturation had been documented as one of the causes of inflammation (20) and the ability of plants extracts to inhibit this process of inflammation is being monitored in this experiment. Although there are possibilities that the plant extracts stop the release of lysosomal content of neutrophils at the site of inflammation (32). The content of the lysosomal membrane includes proteinases and bacterial enzymes, these can damage the tissues and cause inflammation if released (33). The rationale behind this assay is that denaturation of albumin proteins leads to the formation of antigens which initiates type III hypersensitive reaction leading to inflammation (22). Therefore, inhibition of its denaturation process by an agent indicates its anti-inflammation properties; higher the degree of inhibition greater would be its anti-inflammation potential.

All the extracts tested are active and the results are able to confirm the traditional claims that the selected plants were used to treat various inflammatory conditions such as; rheumatoid arthritis, asthma, gingivitis, pains etc (8-11). It was observed that only LCA extract had a very good IC50 in both assay methods, despite the differences in the mechanisms of action LCA prevent the denaturation of albumin at low concentration and likewise stabilize the red blood cells against lysis at low concentration.

These results had been able to show that there could be variation in the inhibitory responses obtained, when a plant is extracted with different solvent, this could be attributed to the differences in the soluble matrix present along the region of polarities (21).

This result is suggesting a correlation between *in vitro* and *in vivo* studies, in previous *in vivo* study; *Senna/Cassia occidentalis*, *Ocimum gratissimum* and *Talinum fruticosum* were adjured to be active when assayed in male albino rats using carrageenan- induced rat paw oedema and formalin induced rats' treatment respectively (8).

The phytochemical studies on *Lecaniodiscus cupanioides*, *Talinum fruticosum* and *Senna*

occidentalis showed that they all possess flavonoids, terpenoids and tannins. It had been reported that flavonoids and terpenoids are responsible for acute anti-inflammatory effect (25). Some other studies have confirmed that flavonoids possess anti-inflammatory properties (17, 25, 39)

The anti-inflammatory response exhibited by *L. cupanioides*, *O. gratissimum*, *S. occidentalis*, *T. fruticosum* and *S. Alata* extracts might be due to the presence of flavonoids, triterpenes and tannins (34), these extracts act by preventing the lysis of the lysosomal membrane, a higher intensity of anti-inflammatory properties may arise due to the presence of saponins, tannins, cardiac glycosides, steroids and alkaloids. Triterpenoids had been suggested to be biologically active in producing anti-inflammatory effects, it is also on record that flavonoids inhibit inflammation by inhibiting signal transducer and activator of transcription 1 (STAT-1) and nuclear factor kappa beta (NF-kβ) activations (27). The presence of phytochemicals such as flavonoids and triterpenoids in L. cupanioides and T. fruticosum may be responsible for the swift anti-inflammatory activities experienced.

The ethyl-acetate extract of *Ocimum gratissimum* had been previously shown to significantly inhibit inflammatory process in formalin-induced paw oedema in rats (11), phytochemicals study reveals the presence of tannins, flavonoids, phenols and alkaloids in *O. gratissimum*, the presence of those phytochemicals contributed to the observed activity in this experiment. *Lecaniodiscus cupanioides* has not been reported to possess anti-inflammatory activities but there are reports of how it protected abdominal swelling caused by liver abscess (35). The plant also possesses flavonoids, triterpenoids, saponins etc, these could be responsible for the observed activity and the plant had been suggested to contain some significant bioactive constituents capable of inhibiting the rupturing of the membrane (36). These results agreed with an earlier report by Akinwumi *et al* where a flavonoids rich seed (Monodora myristica) had been found to be very active as an anti-inflammatory agent. (37).

It was observed that the differences in the mechanism of action between membrane stability and albumin denaturation assays were responsible for the slight variation in the therapeutic response of the extracts.

In both assay methods, *Lecaniodiscus cupanioides* acetone proves to be the most active extract because it had the lowest IC_{50} in both methods, although the mechanism of action for both assays are different yet *Lecaniodiscus cupanioides* acetone was able to inhibit inflammatory process like the standard drugs. This finding shows that the acetone extract of *Lecaniodiscus cupanioides* contain a lot of bioactive constituents that are can adequately inhibit inflammation processes despite their different mechanism of action. The observed activity might be due to the presence of flavonoids, triterpenoids, tannins and saponins which might be acting synergistically.

4. Conclusion

This study has established that there is a scientific basis for the local application of these plant extracts in the treatment and management of inflammation associated ailments. Two of the plants (*Senna occidentalis* and *Lecaniodiscus cupanioides*) clearly demonstrated significantly higher activities comparable to the reference drugs. Further studies towards the isolation and structural elucidation of the bioactive compounds present in these extracts are ongoing.

Acknowledgments

The authors acknowledge the technical assistance of Mr. Ogundepo, Amadi Chizi and Esther in the course of this work.

Conflict of Interest

There is no conflict of interest in this study.

References

1. Iwalewa EO, McGaw LJ, Naidoo V, Eloff JN. Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. *Afr J Biotechnol.* 2007;6:2868-2885. doi: 10.5897/AJB2007.000-2457

2. Kim HP, Son KH, Chang HW, Kang SS.

Anti-inflammatory plant flavonoids and cellular action mechanisms. *J Pharmacol Sci*. 2004 Nov;96(3):229-45. doi: 10.1254/jphs.crj04003x. Epub 2004 Nov 12. PMID: 15539763.

3. Newman DJ, Cragg GM. Advanced preclinical and clinical trials of natural products and related compounds from marine sources. *Curr Med Chem.* 2004 Jul;11(13):1693-713. doi: 10.2174/0929867043364982. PMID: 15279577.

4. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *J Pharm Pharmacol.* 1968 Mar;20(3):169-73. doi: 10.1111/j.2042-7158.1968.tb09718.x. PMID: 4385045.

5. Oyedapo OO, Akinpelu BA, Akinwunmi KF, Adeyinka MO, Sipeolu FO. Red blood cell membrane stabilizing potentials of extracts of Lantana camara and its fractions. *Int J Plant Physiol Biochem.* 2010;2(4):46-51.

6. Nafiu MO, Abdulsalam TA, Akanji MA. Phytochemical Analysis and Antimalarial Activity Aqueous Extract of Lecaniodiscus cupanioides Root. *J Trop Med*. 2013;2013:605393. doi: 10.1155/2013/605393. Epub 2013 Aug 1. PMID: 23983718; PMCID: PMC3747395.

7. Gbadamosi IT, Oloyede AA. The mineral, proximate and phytochemical components of ten Nigerian medicinal plants used in the management of arthritis. *Afr J Pharm Pharmacol*. 2014;8:638-643. DOI: 10.5897/AJPP

8. Agnel Arul John N, Shobana, G. Anti-inflammatory activity of Talinum fruticosum L. on formalin induced paw oedema in albino rats. *J App Pharm Sci*. 2012;2:123-127.

9. Ueda-Nakamura T, Mendonça-Filho RR, Morgado-Díaz JA, Korehisa Maza P, Prado Dias Filho B, Aparício Garcia Cortez D, et al. Antileishmanial activity of Eugenol-rich essential oil from Ocimum gratissimum. *Parasitol Int*. 2006 Jun;55(2):99-105. doi: 10.1016/j.parint.2005.10.006. Epub 2005 Dec 15. PMID: 16343984.

10. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant Staphylococcus aureus activity. *BMC Complement Altern Med.* 2005 Mar 11;5:6. doi: 10.1186/1472-6882-5-6. PMID: 15762997; PMCID: PMC1079793.

11. Chinenye A-O, Emmanuel H, Hassan L.G, Awojide K.O. Phytochemical Screening and Evaluation of Anti-Inflammatory Activity Studies of Ethyl Acetate Leaves Extract from Ocimum gratissimum (L.). *J Dent Med Sci*. 2016;15:16-22.

12. Aguiyi JC, Obi CI, Gang SS, Igweh AC. Hypoglycaemic activity of Ocimum gratissimum in rats. *Fitoterapia*. 2000 Aug;71(4):444-6. doi: 10.1016/s0367-326x(00)00143-x. PMID: 10925022.

13. Ghani A. Medicinal Plants of Bangladesh with chemical constituents and uses. 2nd edn, Asiatic Society of Bangladesh. 2003.

14. Fernand VE, Dinh DT, Washington SJ, Fakayode SO, Losso JN, van Ravenswaay RO, et al. Determination of pharmacologically active compounds in root extracts of Cassia alata L. by use of high performance liquid chromatography. *Talanta*. 2008 Jan 15;74(4):896-902. doi: 10.1016/j.talanta.2007.07.033. Epub 2007 Aug 8. PMID: 18371725; PMCID: PMC2276639.

15. Akinmoladun AC, Obuotor EM, Farombi EO. Evaluation of antioxidant and free radical scavenging capacities of some Nigerian indigenous medicinal plants. *J Med Food.* 2010 Apr;13(2):444-51. doi: 10.1089/jmf.2008.0292. PMID: 20192848.

16. Khan MR, Kihara M, Omoloso AD. Antimicrobial activity of Cassia alata. *Fitoterapia*. 2001 Jun;72(5):561-4. doi: 10.1016/s0367-326x(00)00335-x. PMID: 11429256.

17. Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr.* 2005 Jan;81(1 Suppl):230S-242S. doi: 10.1093/ajcn/81.1.230S. PMID: 15640486.

18. Somchit MN, Reezal I, Nur IE, Mutalib AR. In vitro antimicrobial activity of ethanol and water extracts of Cassia alata. *J Ethnopharmacol.* 2003 Jan;84(1):1-4. doi: 10.1016/s0378-8741(02)00146-0. PMID: 12499068.

19. Lewis A, Levy A. Anti-inflammatory activities of Cassia alata leaf extract in complete Freund's adjuvant arthritis in rats. *West Indian Med J*. 2011 Dec;60(6):615-21. PMID: 22512217.

20. Sakat S, Juvekar AR, Gambhire MN. In-vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis cornicu-

lata Linn. *Int J Pharm Sci.* 2010;2:146-156. doi:10.1055/s-0029-1234983
21. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evaluation of in-vitro anti-inflammatory activity of coffee against the denaturation of protein. *Asian Pac J Trop Biomed.* 2012;2(1):S178-S180. https://doi.org/10.1016/S2221-1691(12)60154-3
22. Agrawal SS, Paridhavi M. Herbal drug technology. Edn 2, University press Pvt. Ltd. Hyderabad, 2007.
23. Ferrali M, Signorini C, Ciccoli L, Comporti M. Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenylhydrazine, divicine and isouramil. *Biochem J.* 1992 Jul 1;285 (Pt 1)(Pt 1):295-301. doi: 10.1042/bj2850295. PMID: 1637315; PMCID: PMC1132780.
24. Mounnissamy VM, Kavimani S, Balu V, Drlin QS. Evaluation of anti-inflammatory and membrane stabilizing properties of ethanol extract of Canjera rehedi. *Iran J Pharmacol Ther*. 2008; 6:235-237.
25. Akinpelu BA, Makinde AM, Isa MO, Taiwo OP, Ojelabi, OM & Oyedapo OO. In-vitro Evaluation of Membrane Stabilizing Potential of Selected Bryophyte Species. *Eur J Med Plants*. 2015;6(3):181-190 doi.org/10.9734/EJMP/2015/7629.
26. Aitdafoun M, Mounier C, Heymans F, Binisti C, Bon C, Godfroid JJ. 4-Alkoxybenzamidines as new potent phospholipase A2 inhibitors. *Biochem Pharmacol.* 1996 Mar 22;51(6):737-42. doi: 10.1016/0006-2952(95)02172-8. PMID: 8602868.
27. Sadique J, Chandra T, Thenmozhi V, Elango V. Biochemical modes of action of Cassia occidentalis and Cardiospermum halicacabum in inflammation. *J Ethnopharmacol.* 1987 Mar-Apr;19(2):201-12. doi: 10.1016/0378-8741(87)90042-0. PMID: 3613609.
28. Kumar V, Bhat ZA, Kumar D, Khan NA, Chashoo IA. Evaluation of anti-inflammatory potential of leaf extracts of Skimmia anquetilia. *Asian Pac J Trop Biomed.* 2012 Aug;2(8):627-30. doi: 10.1016/S2221-1691(12)60109-9. PMID: 23569983; PMCID: PMC3609364.
29. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils--a review. *Food Chem Toxicol.* 2008 Feb;46(2):446-75. doi: 10.1016/j.fct.2007.09.106. Epub 2007 Sep 29. PMID: 17996351.
30. Owoyele VB, Oloriegbe YY, Balogun EA, Soladoye AO. Analgesic and anti-inflammatory properties of Nelsonia canescens leaf extract. *J Ethnopharmacol*. 2005 May 13;99(1):153-6. doi: 10.1016/j.jep.2005.02.003. PMID: 15848036.
31. Metowogo K, Agbonon A, Eklu-Gadegbeku K, Aklikokou AK, Gbeassor M. Anti-ulcer and anti-inflammatory effects of hydr-alcohol extract of Aloe buettneri A. Berger (Lilliaceae). *Trop J Pharmaceut Res.* 2008;7(1):907-912. DOI: 10.4314/tjpr.v7i1.14676
32. Kalyanpur SG, Pohujani S, Nack SR, Seth UK. Study of biochemical effects of anti-inflammatory drugs in carrageenin-induced oedema and cotton pellet granuloma. *Biochem Pharmacol*. 1968; 17, 797-803. doi: 10.1016/0006-2952(68)90016-6
33. Govindappa M, Naga SS, Poojashri MN, Sadananda TS, Chandrappa CP. Antimicrobial, antioxidant and in-vitro anti-inflammatory activity of ethanol extract and active phytochemical screening of Wedelia trilobata (L). Hitch. *J Pharmacol Phytother.* 2011;3(3):43-51. https://doi.org/10.5897/JPP.9000012
34. Raju GS, Moghal MM, Hossain MS, Hassan MM, Billah MM, Ahamed SK, et al. Assessment of pharmacological activities of two medicinal plant of Bangladesh: Launaea sarmentosa and Aegialitis rotundifolia roxb in the management of pain, pyrexia and inflammation. *Biol Res*. 2014 Oct 28;47(1):55. doi: 10.1186/0717-6287-47-55. PMID: 25418519; PMCID: PMC4416252.
35. Yemitan OK, Adeyemi OO. CNS depressant activity of Lecaniodiscus cupanioides. *Fitoterapia*. 2005 Jul;76(5):412-8. doi: 10.1016/j.fitote.2005.02.010. PMID: 15955638.
36. Yemitan OK, Adeyemi OO, Adeogun OO. Analgesic activities of the aqueous root extract Lecaniodiscus cupanioides. *West Afr J Pharmacol Drug Res*. 2005;20:10-14. doi: 10.4314/wajpdr.v20i1.14738.
37. Akinwunmi KF, Oyedapo OO. In-vitro Anti-inflammatory Evaluation of African Nutmeg (Monodora myristica) Seeds.*Eur J Med Plants.* 2015;8(3):167-174. doi:10.9734/EJMP/2015/17853