Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Medicinal Plants

ISSN 1819-3455
DOI: 10.3923/rjmp.2019.32.38

Research Article
In vitro Anti-inflammatory Activity of *Cymbopogon citratus* Leaves

Olaoluwa Temitope Talabi, Inijesu Eunice Taiwo, Olusola Gisanrin and Oluwaseyi Adegoke Adetunji

Department of Biochemistry, Benjamin Carson School of Medicine, Babcock University, Ilishan-Remo, Ogun State, Nigeria

Abstract

Background and Objective: *Cymbopogon citratus* is a commonly used source of herbal medicine throughout Africa in the treatment of different kind of ailments. This study was designed to determine the anti-inflammatory activity of aqueous and hexane fractions of *C. citratus* leaves *in vitro*. **Materials and Methods:** The aqueous and hexane fractions were obtained from solvent partitioning of 70% methanol leaf extract and were used in standard anti-inflammatory assays, erythrocyte membrane stabilization assay, anti-denaturation assay and anti-proteinase assay using *in vitro* methods. Quantitative phytochemical constituents were also determined using standard quantitative methods. **Results:** The *C. citratus* leaves hexane fraction with an IC_{50} of 56.88, 625.00 and 480.77 μg mL^{-1} showed a higher stabilization of human red blood cell (HRBC) membrane, anti-denaturation of protein and anti-proteinase activities, respectively when compared with aqueous fraction with IC_{50} of 89.45, 793.65 and 568.18 μg mL^{-1}. Phytochemical analysis of aqueous and hexane fractions of *C. citratus* leaves showed the presence of flavonoids, phenols, alkaloids, tannins, and saponins. **Conclusion:** This study therefore suggested that the hexane fraction of *C. citratus* leaves may be considered a conceivable basis of anti-inflammatory driver for pharmaceutical drug development.

Key words: Anti-inflammatory, *cymbopogon citratus*, medicinal plant, herbal medicine, phytochemical, erythropoietic activity, drug development

Citation: Olaoluwa Temitope Talabi, Inijesu Eunice Taiwo, Olusola Gisanrin and Oluwaseyi Adegoke Adetunji, 2019. *In vitro* anti-inflammatory activity of *Cymbopogon citratus* leaves. Res. J. Med. Plants, 13: 32-38.

Corresponding Author: Olaoluwa Temitope Talabi, Department of Biochemistry, Benjamin Carson School of Medicine, Babcock University, Ilishan-Remo, Ogun State, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Herbalism or botanical medicine, also known as herbal medicine, is one of the oldest forms of healthcare known to mankind. Throughout history, all cultures make use of herbs for their therapeutic values[1]. An herb is a plant or plant-part cherished for its medicinal, aromatic or savory qualities, and containing therapeutically active chemical substances[2]. A lot of the medicinal compounds being used today to treat diseases like diabetes, jaundice, hormonal imbalances, diabetes, inflammation, cancer etc., have been derived from plant source. Medicinal plants are commonly and plentifully available especially in the tropic zone. A wide variety of chemicals from which anti-inflammatory agents can be discovered are sourced from medicinal plants[3].

Inflammation is a substantially defensive response that is considered as a complicated sequence of occurrences that arises when the body is injured either by chemical or mechanical agents by a self-destructive process. There is a disproportionate stimulation of phagocytes and free radicals production in many inflammatory disorders, which leads to protein denaturation, vascular permeability and membrane alteration[4]. It is the beginning of a healing process in which the body attempts to protect itself, and to remove harmful stimuli, damaged cells, irritants, or pathogens and begin a healing process[5].

The conventional names for symptoms of inflammation are derived from Latin: Dolor (pain), Rubor (redness), Calor (heat), Functio laesa (loss of function) and Tumor (swelling)[4]. Inflammation is of two types; acute and chronic. Cells already present in all tissues, mainly resident macrophages, dendritic cells, histiocytes, Kupffer cells and mastocytes instigates acute inflammation[5]. It starts fast (rapid onset) and speedily turns out be severe. It takes few days for signs and symptoms to be present, but in some cases, it may persist for a few weeks. Examples include acute bronchitis infection ingrown toenail, a scratch/cut on the skin, acute appendicitis, sore throat from a cold or flu, exercise (especially intense training), acute infective meningitis, acute dermatitis, acute tonsillitis, acute sinusitis, or a blow[6]. Chronic inflammation lasts for several months and even years. An autoimmune response to self-antigen is one of the most causes[7]. Examples of chronic inflammatory diseases and conditions include: asthma, tuberculosis, chronic peptic ulcer, chronic periodontitis, chronic active hepatitis rheumatoid arthritis, ulcerative colitis and Crohn's disease and chronic sinusitis[8]. Non-steroidal anti-inflammatory drugs (NSAID) are widely used anti-inflammatory medications. Their main mechanism of action involves inhibiting the cyclooxygenase (COX) enzyme[9].

Herbal plants are considered useful means to prevent and or ameliorate disorders such as arthritis. It is generally assumed that the active constituents contributing to these protective effects are the phytochemical, anti-inflammatory constituents and minerals[10]. Among these herbal resources, *Cymbopogon citrates* leaves were selected for this study. This study was aimed to determine the anti-inflammatory actions of aqueous and hexane fractions of *C. citratus* leaves *in vitro*.

MATERIALS AND METHODS

Collection of plant material: *Cymbopogon citratus* plant leaves popularly known by the Yorubas as Koriko-oba and by the Igbos as Acharaehi were gotten from a farm at Ogijo in Sagamu Local Government Area, Ogun State, Nigeria. The plant was identified and authenticated at the Department of Agricultural science, Babcock University, Ilishan-Remo, Ogun State, Nigeria. The study was carried out in Babcock University, Biochemistry Department, Benjamin Carson (Snr) Medical School. This research project was conducted from January, 2016 to March, 2016.

Sample extraction and solvent partitioning: The leaves and root were plucked, the root was then cut away from the leaves. The chopped leaves were thoroughly rinsed and were oven-dried at 40°C for 3 days and pulverized using mechanical grinder. Using 1600 mL of 70% methanol, 200 g of powdered leaf samples were extracted with intermittent shaking for 48 h. The Whatman No.1 filter paper was used for filtration and the filtrate was subsequently concentrated using rotary evaporator at 40°C (Stuart Rotary Evaporator (RE300)[10]. The concentrates were reformed with distilled water in a ratio of 1:1 (concentrate: distilled water) and partitioned using n-hexane. The fractions obtained were concentrated again in a rotary evaporator at 40°C. The fractions were dried further in a water bath at 40°C and preserved in the refrigerator at 4°C until further use[10]. The leave fractions were then subjected to standard quantitative phytochemical and anti-inflammatory evaluation methods.

Quantitative determination of phytochemicals

Determination of total phenolic content: The total phenolic content of the aqueous and hexane fractions was determined by a modification of the Satoskar *et al.*[11] Folin Ciocalteu assay. The principle is based on reduction/ color change of phosphomolybdic-phosphotungstic acid (Folin Ciocalteu reagent) to a blue-colored complex in an alkaline solution which occurs in the presence of phenolic compounds.

Determination of total flavonoid content: The total flavonoid content was determined by the aluminum chloride colorimetric method[12]. Acid stable complex was formed with the C-4 keto group and any of C-5 or C-3 hydroxyl group of flavonols and flavones in addition with aluminum chloride. Acid labile complexes were also formed with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids by aluminum chloride. The calibration curve was built using quercetin as the standard material at various concentrations.

Determination of total tannin content: The modified vanillin-HCl methanol method as described by Okwu and Okwu[13] was used to determine the tannin content of the samples. Tannins are intricate group of plant secondary metabolites which are soluble and are distinguished from other polyphenolic compound by their ability to precipitate protein.

Determination of total alkaloid content: The alkaloid content of *C. citratus* was determined using the Koncic *et al.*[14] method. The alkaloids content of *C. citratus* extract was determined by a gravimetric method.

Determination of total saponin content: The total saponin content was determined using Mohammed *et al.*[15] method.

Methods for anti-inflammatory assay

Anti-denaturation studies: The *C. citratus* fractions effects on protein denaturation were analyzed using Harbone[16] revised method.

Erythrocyte stabilization assay: To study the *in vitro* anti-inflammatory activity, the human red blood cell (HRBC) membrane stabilization method has been used[17]. The HRBC or erythrocyte membrane is comparable to the lysosomal membrane and its balance infers that the extract may as well keep steady lysosomal membranes[18]. Hemolysis of the red blood cells was induced by hypotonicity. Inhibition of Hemolysis by the drug (test extract and standard) was calculated as a percentage of control, which represented 100% hemolysis.

***In vitro* anti-proteinase activity:** This assay was performed using Gandhidasan *et al.*[19] described method.

Statistical analysis: The statistical package for social sciences (SPSS) for Windows and Microsoft Excel worksheet was used in analyzing the results. Linear regression from SPSS was used to ascertain 50% inhibitory concentration (IC_{50}) values for the fractions. Also, the mean and standard deviation were determined using SPPS. Microsoft excel was used in plotting the standard curve and bar graph. Values were reported as mean+standard deviation as three replicate analysis.

RESULTS

Quantitative phytochemicals evaluation: Table 1 shows that the aqueous and hexane fraction of *C. citratus* leaves (1 mg mL^{-1}) contains the total phenol content of 0.41±0.06 mg gallic acid equivalent GAE g^{-1} and 0.32±0.07 mg GAE g^{-1}, total flavonoid content of 0.20±0.02 mg quercetin equivalent QUE g^{-1} and 0.12±0.04 mg QUE g^{-1}, respectively. Also, it shows that the sample contains 0.25±0.01 mg tannin acid equivalent TAE g^{-1} of sample.

Determination of anti-inflammatory activity *in vitro*: Figure 1 shows that the diclofenac sodium, aqueous and hexane leaf fractions of *C. citratus* stimulated protein denaturation in a reverse concentration dependent manner. The hexane fraction induced protein denaturation by 52.88 and 23.60% at the lowest (10 µg mL^{-1}) and highest (50 µg mL^{-1}) concentrations, respectively while the aqueous induced protein denaturation by 30.77 and 18.01% at the lowest (10 µg mL^{-1}) and highest (50 µg mL^{-1}) concentrations, respectively. Diclofenac sodium induced protein denaturation by 79.81 and 34.16% at the lowest (10 µg mL^{-1}) and highest (50 µg mL^{-1}) concentrations, respectively. Furthermore, the IC_{50} of diclofenac sodium, aqueous and hexane fractions were found to be 38.43, 56.88 and 89.45 µg mL^{-1}, respectively (Table 2).

Table 1: Quantitative phytochemical evaluation of *C. citratus* leaf

	Quantity present	
Fractions of *C. Citratus* leaf (1mg mL^{-1})	Total phenol (Mean±SD mg (GAE) g^{-1})	Total flavonoid (Mean±SD mg (QUE) g^{-1})
Aqueous	0.41±0.06, R^2 = 0.9863	0.20±0.02, R^2 = 0.971
Hexane	0.32±0.07, R^2 = 0.9863	0.12±0.04, R^2 = 0.971
Tannin (Mean±SD mg (TAE) g^{-1})		
C. Citratus leaf (0.20 g)	0.25±0.01, R^2 = 0.9922	

R^2: Level of correlation

Table 2: *Cymbopogon citratus* leaf 50% inhibitory concentration (IC_{50}) of anti-denaturation assay, erythrocyte stabilization assay and trypsin inhibitory assay

	IC_{50} (µg mL^{-1})		
Fractions of *C. citratus* leaf	Anti-denaturation assay (10-50 µg mL^{-1})	Erythrocyte stabilization assay (50-450 µg mL^{-1})	Anti-proteinase assay (50-500 µg mL^{-1})
Diclofenac	38.43	454.55	333.33
Hexane	56.88	625.00	480.77
Aqueous	89.45	793.65	568.18

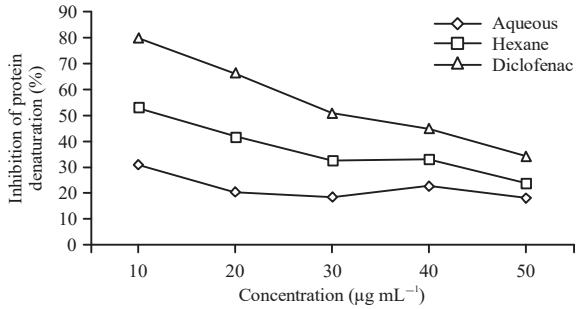

Fig. 1. Thermally induced inhibition (%) by protein denaturation of aqueous and hexane fractions of *C. citratus* leaf

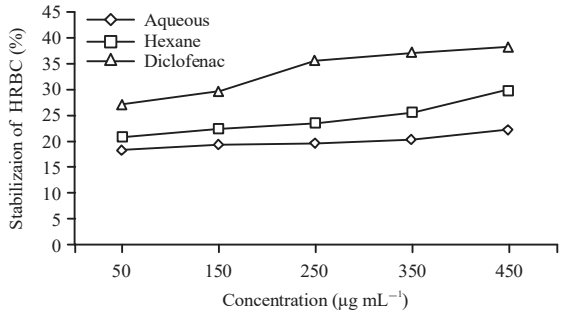

Fig. 2. Human red blood cell (HRBC) percentage stabilization at varying concentrations between aqueous and hexane fractions of *C. citratus* leaf

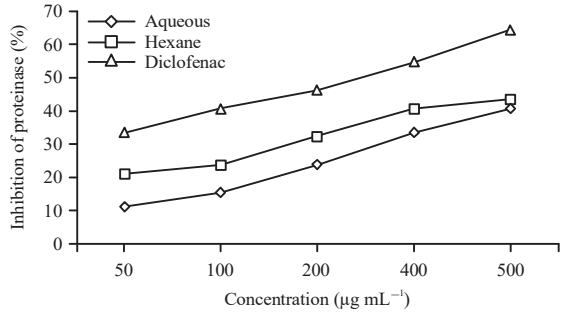

Fig. 3: Percentage proteinase inhibitory action of aqueous and hexane fractions of *C. citratus* leaf

The hemolysis of the human red blood cell membrane is also inhibited by the hexane fraction by 30.07 and 21.02% at the highest (450 µg mL^{-1}) and lowest concentrations (50 µg mL^{-1}), respectively while the aqueous inhibition of human red blood cell membrane hemolysis is by 22.38 and 18.35% at the highest (450 µg mL^{-1}) and lowest concentrations (50 µg mL^{-1}), respectively (Fig. 2). Diclofenac sodium inhibited human red blood cell membrane hemolysis by 38.46 and 27.22% at the highest (450 µg mL^{-1}) and lowest concentrations (50 µg mL^{-1}), respectively. Furthermore, the IC_{50} of diclofenac sodium, aqueous and hexane fractions were found to be 454.55, 625.00 and 793.65 µg mL^{-1}, respectively (Table 2).

Figure 3 shows the inhibition of trypsin activity by diclofenac sodium, aqueous and hexane leaf fractions of *C. citratus*is in a concentration dependent manner. The hexane fraction inhibited trypsin activity by 43.66 and 21.13% at the highest (500 µg mL^{-1}) and lowest concentration (50 µg mL^{-1}), respectively while the aqueous inhibited trypsin activity by 40.85 and 11.27% at the highest (500 µg mL^{-1}) and lowest concentration (50 µg mL^{-1}), respectively. Diclofenac sodium inhibited trypsin activity by 64.79 and 33.80% at the highest (500 µg mL^{-1}) and lowest concentration (50 µg mL^{-1}), respectively. Furthermore, the IC_{50} of diclofenac sodium, aqueous and hexane fractions were found to be 333.33, 480.77 and 568.18 µg mL^{-1}, respectively (Table 2).

DISCUSSION

There has been a tremendous increase in recent years in the exploration of natural products with anti-inflammatory activity. Diverse bioactive components with various biological activities have been ascribed to herbal extracts, especially crude drugs[20].

In this study, quantitative phytochemical analysis revealed that the aqueous and hexane fractions of *C. citratus* contained phenols, flavonoids, tannins, alkaloids, and saponins. Research has shown that flavonoids, saponins and tannins have been accounted for their anti-inflammatory and analgesic activities and are capable of significantly interference with inflammatory mediators[21-23]. Alkaloids are also known to possess anti-inflammatory effects[9]. Secondary

metabolites such as flavonoid and phenolic compounds work as antioxidant that prevent oxidative cell damage, with anti-inflammatory, anti-allergic and anti-thrombotic[24,25]. Earlier studies have reported plant extracts possessing phytochemicals with anti-inflammatory properties may as well have antioxidant activities against chain reactions triggered by reactive oxygen species associated with inflammation[26,27].

Also, the presence of alkaloids in the leaves showed that aqueous and hexane fractions of *C. citrates* leaf has anti-microbial activities. It also showed that they have pharmacological effects and can be used as medications and as recreational drugs[28]. Saponins are also known to produce inhibitory effect on inflammation. They could be used in the treatment of cough and asthma [29].

In this current study, the aqueous fraction, hexane fraction of *C. citratus* and standard drug inhibited protein denaturation. The *C. citratus* of leaf hexane fraction revealed the most anti-denaturation of protein property in contrast to the aqueous fraction, hence the anti-inflammatory property of *C. citratus* leaf may possess a non-polar chemical structure. Denaturation of proteins is a well-documented cause of inflammation, which leads to inflammatory diseases like rheumatoid arthritis[30,31]. Thus, the hexane fraction of *C. citratus* could be used as an anti- rheumatoid arthritis agent. Reports have also been generated on the ability of compounds capable of inhibiting thermally-induced protein denaturation to serve a potential therapeutic value as anti-inflammatory agent[32,33].

The erythrocyte membrane stabilization assay showed that the aqueous fraction, hexane fraction and also the standard drug makes the human erythrocyte membrane stable as against hypotonicity-induced hemolysis. From the assay *C. citratus* leaf hexane fraction showed a higher ability in the stabilization of human erythrocyte against hypotonic induced hemolysis when compared to the aqueous fraction. The components of erythrocyte membrane have been shown to be similar to that of lysosomal membrane, therefore, agents capable of stabilizing human erythrocyte from hypotonic induced hemolysis could be a good source of anti-inflammation[34,35]. This showed that *C. citratus* leaf hexane fraction may be considered a good source of human erythrocyte stabilizer.

Furthermore, arthritic reactions have been associated with proteinases. Neutrophils are rich source of proteinase having many serine proteinases in their lysosomal granules [3]. The leukocytes proteinase is reported to play an important role in the existence of tissue damage during inflammatory reactions and significant level of defense was provided by proteinase inhibitors[36]. This study also indicated that the aqueous fraction, hexane fraction and diclofenac sodium were able to inhibit proteinase activity. Also, the hexane fraction showed the highest proteinase inhibiting activity. This may be due to presence of the non-polar chemical structure of the hexane fraction of *C. citratus* leaf, indicating that it may be able to play a role in the repair of tissues during inflammation. This showed that *C. citratus* leaf hexane fraction has a higher anti-inflammatory activity when compared to the aqueous fraction. These anti-inflammatory activities may be due to the presence of polyphenolic compounds (alkaloids, flavonoids, tannins, steroids and phenols) earlier observed. Present study gives an idea that the *C. citratus* leaf hexane fraction, if purified, may be used in designing a potent anti-inflammatory drug which can be used for treatment of various diseases like cancer, neurological disorder, aging and inflammation.

CONCLUSION

This study indicated that aqueous and hexane fractions of *Cymbopogon citratus* leaf possess anti-inflammatory properties, with the hexane fraction exhibiting a higher anti-inflammatory property than the aqueous fraction. And this may be because of the phytochemical constituents present in the fraction. It is recommended that further studies should be carried out on the hexane fraction of *Cymbopogon citratus* with the aim of changing the chemical compounds present in it to drugs or herbs that used in the treatment of inflammatory diseases.

SIGNIFICANCE STATEMENT

This study discovers that the aqueous and hexane fractions of *Cymbopogon citratus* leaf possess anti-inflammatory properties, with the hexane fraction exhibiting higher anti-inflammatory property than the aqueous fraction. This may be beneficial in the alleviation or repair of inflamed tissues. If further research is on the purification of the hexane fraction of *Cymbopogon citratus* leaf is achieved, the same may serve as a potent anti-inflammatory drug which can be used for treatment of various diseases like cancer, neurological disorder, aging and inflammation. This study will help the researcher to uncover critical area of anti-inflammatory drug discovery, thus, a new and possibly more affordable anti-inflammatory drug, may be arrived at.

ACKNOWLEDGMENT

We acknowledged Prof. D.S. Daramola of the Department of Agricultural Science, Babcock University, Nigeria who authenticated the samples used in the study.

REFERENCES

1. Benzie, I.F.F. and S. Wachtel-Galor, 2011. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd Edn., CRC Press, Boca Raton, FL, USA., ISBN-13: 9781439807163, Pages: 499.
2. Ackerknecht, E.H., 1973. Therapeutics: From the Primitives to the Twentieth Century. Hafner Press, New York, pp: 526.
3. Padmanabhan, P. and S.N. Jangle, 2012. Evaluation of *in-vitro* anti-inflammatory activity of herbal preparation, a combination of four medicinal plants. Int. J. Applied Basic Med. Res., 2: 109-116.
4. Umapathy, E., E.J. Ndebia, A. Meeme, B. Adam, P. Menziwa, B.N. Nkeh-Chungag and J.E. Iputo, 2010. An experimental evaluation of Albuca setosa aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. J. Med. Plant Res., 4: 789-795.
5. Cotton, R.S., V. Kumar and S.L. Robbins, 1994. Robbins Pathologic Basis of Disease. 5th Edn., W.B. Sauderers Company, Philadelphia, pp: 53-54.
6. Ritchie, A.C., 1990. Boyd's Textbook of Pathology. Lea and Feberger, UK., pp: 60-88.
7. Janeway, C.A.Jr., P. Travers, M. Walport and M.J. Shlomchik, 2001. Immunobiology: The Immune System in Health and Disease. 5th Edn., Garland Science, New York, ISBN-10: 0-8153-3642-X.
8. Dey, N.C., S. Debashish and P.K. Pey, 1994. Textbook of Pathology. New Central Book Agency Ltd., USA., pp: 61-65.
9. Rubin, E., 2001. Essential Pathology. 2nd Edn., Lippincort Williams and Wilkins, Philadelphia, pp: 24-46.
10. Moldoveanu, S. and V. David, 2015. Chapter 6-Solvent Extraction. In: Modern Sample Preparation for Chromatography, Moldoveanu, S. and V. David (Eds.)., Elsevier, USA., ISBN: 978-0-444-54319-6, pp: 131-189.
11. Satoskar, R.S., S.D. Bhandarkar and S.S. Ainapur, 2003. Angiotensin, Kinins, Leukotrienes Prostaglandins and Cutokiones. 18th Edn., Popular Prakashanpvt. Ltd., Bombay India, pp: 333-339.
12. Shi, J.Y., X.B. Zou, J.W. Zhao, H. Mel, K.L. Wang, X. Wang and H. Chen, 2012. Determination of total flavonoids content in fresh *Ginkgo biloba* leaf with different colors using near infrared spectroscopy. Spectrochim. Acta Part A: Mol. Biomol. Spectrosc., 94: 271-276.
13. Okwu, D.E. and M.E. Okwu, 2004. Chemical composition of *Spondias mombin* Linn plant parts. J. Sust. Agric. Environ., 6: 140-147.
14. Koncic, M.Z., D. Kremer, J. Gruz, M. Strnad and G. Bisevac *et al*., 2010. Antioxidant and antimicrobial properties of *Moltkia petraea* (Tratt.) Griseb. flower, leaf and stem infusions. Food Chem. Toxicol., 48: 1537-1542.
15. Mohammed, N.A., I.A.M. Ahmed and E.E. Babiker, 2010. Nutritional evaluation of sorghum flour (*Sorghum bicolor* L. Moench) during processing of injera. Int. J. Biol. Life Sci., 6: 35-39.
16. Harborne, J.B., 1973. Phytochemical Methods. Chapman and Hall Ltd., London, UK., pp: 49-188.
17. Obadoni, B.O. and P.O. Ochuko, 2002. Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. Global J. Pure Applied Sci., 8: 203-208.
18. Sakat, S.S., A.R. Juvekar and M.N. Gambhire, 2010. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* linn. Int. J. Pharm. Pharm. Sci., 2: 146-155.
19. Gandhidasan, R., A. Thamaraichelvan and S. Babura, 1991. Anti-inflammatory action of *Lannea coromandelica* by HRBC membrane stabilization. Fithotherpia, 62: 82-83.
20. Chou, C.T., 1997. The antiinflammatory effect of an extract of *Tripterygium wilfordii* Hook F on adjuvant induced paw oedema in rats and inflammatory mediators release. Phytother. Res., 11: 152-154.
21. Oyedapo, O.O. and A.J. Famurewa, 1995. Antiprotease and membrane stabilizing activities of extracts of *Fagara zanthoxyloides*, *Olax subscorpioides* and *Tetrapleura tetraptera*. Int. J. Pharmacogn., 33: 65-69.
22. Chung, H.J., H.S. Lee, J.S. Shin, S.H. Lee, B.M. Park, Y.S. Youn and S.K. Lee, 2010. Modulation of acute and chronic inflammatory processes by a traditional medicine preparation GCSB-5 both *in vitro* and *in vivo* animal models. J. Ethnopharmacol., 130: 450-459.
23. Ramesh, M., Y.N. Rao, A.V.N.A. Rao, M.C. Prabhakar, C.S. Rao, N. Muralidhar and B.M. Reddy, 1998. Antinociceptive and anti-inflammatory activity of a flavonoid isolated from *Caralluma attenuata*. J. Ethnopharmacol., 62: 63-66.
24. Narayana, K.R., M.S. Reddy, M.R. Chaluvadi and D.R. Krishna, 2001. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian J. Pharmacol., 33: 2-16.
25. Ullah H.M.A., S. Zaman, F. Juhara, L. Akter, S.M. Tareq, E.H. Masum and R. Bhattacharjee, 2014. Evaluation of antinociceptive, *in-vivo* and *in-vitro* anti-inflammatory activity of ethanolic extract of *Curcuma zedoaria* rhizome. BMC Complement. Altern. Med., Vol. 14. 10.1186/1472-6882-14-346.
26. Trease, E. and W.C. Evans, 2008. Phenols and Phenolic Glycosides. Elsevier Ltd., India, pp: 214-252.
27. Arts, I.C.W. and P.C.H. Hollman, 2005. Polyphenols and disease risk in epidemiologic studies. Am. J. Clin. Nutr., 81: 317S-325S.
28. Ayoola, G.A., H.A. Coker, S.A. Adesegun, A.A. Adepoju-Bello, K. Obaweya, E.C. Ezennia and T.O. Atangbayila, 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop. J. Pharm. Res., 7: 1019-1024.

29. Ukpabi, C.F., K.N. Agbafor, O.K. Ndukwe, A. Agwu and S.N. Nwachukwu, 2012. Phytochemical composition of *Costus afer* extract and Its alleviation of carbon tetrachloride-induced hepatic oxidative stress and toxicity. Int. J. Mod. Bot., 2: 120-126.
30. Herbert, R.B., 1999. The biosynthesis of plant alkaloids and nitrogenous microbial metabolites. Nat. Prod. Rep., 16: 199-208.
31. Gill, L.S., 1992. Ethnomedical Uses of Plants in Nigeria. University of Benin Press, Benin, Nigeria, ISBN-13: 9789782027207, Pages: 276.
32. Williams, L.A.D., A. O'Connar, L. Latore, O. Dennis and S. Ringer *et al*., 2008. The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. West Indian Med. J., 57: 327-331.
33. Pham, C.T., 2008. Neutrophil serine proteases fine-tune the inflammatory response. Int. J. Biochem. Cell Biol., 40: 1317-1333.
34. Twigg, M.S., S. Brockbank, P. Lowry, S.P. FitzGerald, C. Taggart and S. Weldon, 2015. The role of serine proteases and antiproteases in the cystic fibrosis lung. Mediat. Inflamm., Vol. 2015. 10.1155/2015/293053
35. Govindappa, M., S.S. Naga, T.S. Sadananda and C.P. Chandrappa, 2011. Antimicrobial, antioxidant and *in vitro* anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc. J. Pharmacogn. Phytother., 3: 43-51.
36. Williams, L.A., 2009. Further insight into the bovine serum albumin assay (the *in vitro* anti-inflammatory assay). West Indian Med. J., 58: 181-182.