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Evaluation of Membrane Stabilizing, Proteinase and Lipoxygenase Inhibitory Activities of Ethanol Extract of Root and Stem of *Sphenocentrum jollyanum* Pierre

Olorunnisola Olubukola Sinbad[1], Fadahunsi Olumide Samuel[1*], Adetutu Adewale[1] and Olasunkanmi Adedoyin[2]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
[2]Department of Science Laboratory Technology, Federal Polytechnic, Ilaro, Ogun State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OOS designed the study and wrote the protocol. Author FOS managed the analyses of the study and the literature searches. Authors AA and OA performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aimed at evaluating the anti-inflammatory potential of ethanol extract of the stem and root of *Sphenocentrum jollyanum.*
Study Design: Red blood cell Membrane stabilization, anti-lipoxygenase and proteinase inhibitory activities of the extracts were assayed *in-vitro* as a measure of anti-inflammatory potential of ethanol extract of root and stem of *Sphenocentrum jollyanum* Pierre.
Place and Duration of Study: All the work was carried out in the Department of Biochemistry, Faculty of Basic Medical Science, Ladoke Akintola University of Technology, Ogbomoso, Nigeria between April 2015-February, 2016.
Methodology: Four hundred grams of the stem and root was extracted in batches with 90%

**Corresponding author: E-mail: fadahunsiolumide5@mail.com, fadahunsiolumide5@gmail.com;*

ethanol in a soxhlet apparatus and concentrated using a rotatory evaporator. Inhibitory effect of the extracts on erythrocytes membrane stabilization, trypsin and lipoxygenase (*in vitro*) were used to assess anti-inflammatory properties according to standard procedures. The reactions were performed in triplicates and changes in optical density of test samples and control were measured using a 96-well micro plate reader Spectra Max 384 plus (Molecular Devices, USA) and inhibitions were calculated.

Results: The result of this study revealed that the extraction yield of the stem and root was 32.00 g and 26.00 g respectively, while the stem extract exhibited a significantly ($p<0.05$) higher dose dependent erythrocyte membrane stabilization when compared with the root extract with IC_{50} of 325±13.81 µg/ml and 541±6.33 µg/ml respectively. The Proteinase inhibitory assay showed that the stem extract demonstrated a stronger inhibitory activity to the root with IC_{50} 1000±3.35 µg/ml and 1080±2.67 µg/ml. Lipoxygenase inhibition activity assay revealed that the stem and the root extract of *Sphenocentrum jollyanum* inhibited 50% of lipoxygenase at concentration of 426±6.33 µg/ml and 541±6.67 µg/ml respectively. Hence, the results of this study showed that S.J have some bioactive compounds which are effective in management of inflammatory disorders and thus validates the folkloric use of the plant.

Keywords: Sphenocentrum jollyanum pierre; stem and root extract; membrane stabilizing; proteinase; lipoxygenase inhibitory activity.

1. INTRODUCTION

Inflammation can be defined as a cascade of reactions in which the organism's immune system reacts to invading foreign agents and simultaneously initiate the healing process of any damaged tissue. These often result in the discomfort of the host [1,2]. The pathogenesis of different inflammatory diseases has been linked to membrane destabilization of the cells of the immune system (mast cells, neutrophils, basophils, eosinophils and leukocytes), which involves the release of their lysosomal constituents (myeloperoxidase, serine proteases, phospholipases, and lipoxygenses) in response to stimuli. These degradative enzymes damage endothelial, epithelial cells and destroy connective tissues thus resulting in different physiological responses which are evident in the classical signs of inflammation **(**pain, swelling, heat and redness) [3]. Lipoxygenses and cycloxygenases are well documented and reported in the generation of reactive oxygen species and inflammatory disorders [4]. They mediate the release of prostaglandins, thromboxanes, leukorienes and other ecosanoids [5,6] which play predominant roles in pathophysiology of many acute and chronic inflammatory diseases such as asthma, atherosclerosis, rheumatoid arthritis, inflammatory bowel diseases, dermatitis, and cancer [7,8]. Non steroidal anti-inflammatory drugs (NSAIDS) and corticosteroids have been effective in suppressing inflammatory responses but their prolonged administration can result to drug resistance and significant harmful effects such as increased risk of infection, cancer, cardiovascular diseases [9] damage to connective tissue and adrenal gland atrophy [10,11]. Hence, there is a dire need for development of natural alternative therapies that do not produce significant side effects. Medicinal plants have been the source of traditional drugs and therapies since time immemorial. According to the report of Gurib-Fakim [12], Mantle et al. [13], Annan and Houghton [14], about 80 percent of people in Africa and Asia relies on medicinal plants as alternative to modern medicine in the management of various health disorders.

Till this present day, medicinal plants continue to play a major role in providing raw materials for modern drug development which can be linked to their diverse bioactive compounds and phytochemical constituents [15]. *Sphenocentrum jollyanum* Pierre is a shrub that belongs to the family of Menispamacea. It predominantly grows in West-African Countries of Nigeria, Ghana, Sierra-Leone and Cameroon [16]. Interestingly, traditional usage and scientific research on the plant has been richly documented. Various biological and pharmacological activities of its various parts have been reported, they include anti-oxidant, anti-angiogenic [16,17], anti-malaria, anti-diabetes [18,19] hypoglycemic, hypolipidemic [20,21] anti-inflammatory [22] and enhancement of reproductive activity in male albino rats [23]. Although, thorough extensive studies have been carried out on extracts of *S. jollyanum*, but from information available to us, there is paucity of scientific documentation on the membrane stabilization, lipoxygenase and

protease inhibition activity of the root and stem extracts. Hence, evaluation of the above mentioned parameters will be of great importance to knowledge.

2. MATERIALS AND METHODS

2.1 Plant Materials

The root and stem of *Sphenocentrum jollyanum* (SJ) were collected from the Botanical garden of University of Ibadan, Nigeria and were identified by Professor A.J Ogunkunle of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology. Voucher samples (LHO 240 and LHO 241) of the plant were also deposited at the University herbarium. The stem and root were rinsed with clean water to remove sandy particles and air dried for two weeks after which they were chopped into pieces and pounded using a wooden mortar and pestle. This was later blended into powder with electrical blender (Tower brand, 220-240v 50/60HZ 500W) and milling machine (Honda, Japan).

2.2 Preparation of Ethanol Extracts

Blended plant material (400 g) was loaded in a soxhlet extractor in batches for 5hours each and subjected to extraction with 90% v/v ethanol. The extracts were concentrated at 45°C using a rotary evaporator and the dried extracts were kept in a refrigerator (4°C) till it was needed.

2.3 Membrane Stabilization Assay

Membrane stabilization method was carried out according to the method of [24,25] with slight modification. Briefly, blood was collected from male albino rats and transferred to the centrifuge tubes. The blood was centrifuged at 3000 rpm for 10 min and was washed three times with equal volume of normal saline. The volume of blood was measured and re-constituted as 10% v/v suspension with normal saline. Various concentrations of extracts were prepared (100 ,150, 200, 250 and 300 µg/ml) adding distilled water and to each concentrations, 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of red blood cell suspension were added. The mixture was incubated at 37°C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatants solution was estimated. The reactions were performed in triplicates in 96-well micro plate reader Spectra Max 384 plus (Molecular Devices, USA) at 560 nm and mean value was considered. Diclofenac sodium (100 µg /ml) was used as reference standard. The percentage (%) of HRBC membrane stabilization or protection was calculated using the following formula:

% inhibition of hemolysis = Absorbance of control - Absorbance of sample / Absorbance of Control X 100

2.4 Protease Inhibition Activity Assay

The test was performed according to the modified method of [26,25]. The reaction mixture (2 ml) containing 0.03 mg trypsin, 0.5 ml of 10 mM Tri HCl buffer (pH7.4) and different concentrations of the extracts. The reaction mixture was incubated at 37°C for 5 min after which 1 ml of 0.8% (W/V) casein was added. The mixture was incubated for an additional 20 min, 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm using 96-well micro plate reader Spectra Max 384 plus (Molecular Devices, USA). The experiment was performed in triplicate and the percentage protein inhibitory activity was calculated by;

Percentage inhibition = Absorbance of control –Absorbance of sample/ Absorbance of control X 100

2.5 Anti-lipoxygenase Activity Assay

Anti-lipoxygense activity was assayed according to the method of [27]. Briefly, linoleic acid was used as substrate and lipoxidase as the enzyme. Test samples were dissolved in 0.25 ml of 2 M borate buffer (pH 9.0), 0.25 ml of lipoxidase enzyme. The solution was incubated for 5 min at 25°C, after which 1.0 ml of linoleic acid solution (0.6 mm) was added and properly agitated. Absorbance was measured at 234 nm, and indomethacin was used as reference standard. The percentage inhibition was calculated from the following equation,

% inhibition = Absorbance of control – Absorption of extract / Absorbance of control × 100

2.6 Statistical Analysis

All data were presented as mean ± standard error of mean (SEM) of triplicates and IC_{50} µg/ml was determined. Independent T- Test was used to determine the significant difference between

groups and control using the software Graph Pad prism 5.0 (Graph Pad Software Inc. California, USA).

3. RESULTS AND DISCUSSION

3.1 Extraction of Yield

The ethanol extraction of 400 grams of stem and root of *S. Jollynum* gave a yield of 32.00 g and 26.00 g respectively (Table 1).

Table 1. Yield in (grams) of ethanol extract from 400 grams of *S. jollyanum* stem and root

Plant organ	Yield in grams
Stem	32.00 g
Root	26.00 g

3.2 *In-vitro* Anti--Inflammatory Assays

3.2.1 Membrane stabilization activity of ethanol extracts of stem and root of *S. jollyanum*

Membrane stabilization assay is a widely used technique in the screening of test compounds and plant extracts for anti-inflammatory potentials. In this study, the ethanol extract of the stem and root of *S. jollyanum* showed considerable dose-dependent membrane stabilization activity. The ethanol extract of the stem showed a significant ($P<0.05$) higher membrane stabilizing ability when compared with the ethanol extract of the root. The highest percentage erythrocyte stabilization of 42.60% and 30.20% respectively was observed at concentration of 300 µg/ml with IC_{50} (µg/ml) values of 355±13.81 µg/ml and 541±6.33 µg/ml respectively. However, the standard drug diclofenac exhibited the highest erythrocyte membrane stabilization activity with IC_{50} of 51±6.12 (Table 2). Previous phytochemical investigations reported that *S. jollaynum* stem extracts constitutes in abundance bioactive compounds such as saponins, tannins, flavonoids, alkaloids and terpenes [16]. Polyphenolic compounds have been reported to inhibit the tissue damaging effect of photolytic, bactericidal enzymes and reactive oxygen species that are released by the polymorpho nuclear cells in response to invading foreign agents [28]. Flavonoids and alkaloids have also been reported to modulate cellular activities of inflammatory related cells by stabilizing their membranes, thus preventing de-granulation [29] [30] and therefore impairing lysosomal enzyme release of arachidonic acid, elactase and glucoronidase [31]. Also, Fawole et al. [32] and [33] reported that flavonoids and saponin are known to posses anti-inflammatory activity. Hence, the likely mode of action of the extracts observed in (Table 2) may be through the above mentioned biochemical processes. Furthermore, the result obtained in this study is in conformity with earlier submissions of [34-36] that reported significant activities of *Solanum aethiopicum*, *Calliandra surinamensis* extracts and honey respectively against hypotonic and heat induced lysis of red blood cells. In addition, works by Sacchin et al. [37] and [38] reported that *Erythrina indica* and *Lagenaria breviflora* extracts significantly inhibited erythrocytes membrane disruption.

3.2.2 Proteinase inhibition activity of ethanol extracts of stem and root of *S. jollyanum*

Proteinases such as trypsin and other serine proteases are essential enzymes that mediate the hydrolytic breakdown of peptide bonds in proteins [39]. They have been extensively studied with respect to their roles in inflammation, cardiovascular diseases, tissue remodeling, matrix destruction, auto immune arthritis and skin allergies [40]. Therefore, therapeutic inhibition of these enzymes will be of novel contribution. The result from the present study indicates that the ethanol extract of the stem exhibited a significantly ($P<0.05$) higher percentage inhibition when compared to the root extracts with IC_{50} values of 1000±3.35 µg/ml and 1080±2.67 µg/ml respectively. It was also observed that inhibition was concentration dependent and the standard drug Indomethacin demonstrated the highest Proteinase inhibition (Table 3). Various plants and natural products have been recorded to demonstrate considerable proteinase inhibition. According Gulnaz et al. [41], ethanolic extract of *Randia uliginosa* exhibited significant proteinse inhibition, while [42-44] also have similar observations.

3.2.3 Anti-lipoxygenase activity of ethanol extracts of stem and root of *S. jollyanum*

Lipoxygenases (LOXs) are family of non-heme iron-containing enzymes that have been implicated in the metabolism of arachidonic, linoleic and other polyunsaturated fatty acid into biologically active metabolites which are known mediators of inflammatory and immune responses [45,7]. Hence, the inhibition of these

enzymes becomes imperative, and could pave way for discovery of new anti-inflammatory agents. In this study, it was observed that the stem and root extracts of *S. jollyanum* exhibited a significant (P<0.05) concentration dependent lipoxygenase inhibition. It was also observed that the stem extract at concentration of 300 µg/ml demonstrated the stronger percentage LOX inhibition of 40.45±0.55% with IC_{50} of 426±6.33 µg/ml when compared to the root extract. However, Indomethacin demonstrated the highest LOX inhibition (Table 4). This observed LOX inhibitory activity might be due to synergistic effect of the prevalent phytochemicals (flavonoids, alkaloids, tannins and saponins) according to the phytochemistry report of [16]. Secondary metabolites inhibits the expression of LOX and other related enzymes by suppressing the activation of transcription factors, inhibition of phospholipases A2 [46,46], modulating arachidonic acid [47,48,49] . Csaki et al. [50], reported that isolated compounds such as flavonoids from natural products have been implicated in prevention and regulation of inflammation. According to Kim et al. [40], bioactive principles in plants have the ability to protect against disease such as inflammation and cancer. Bioactive compounds have been reported to inhibit pro oxidants such as LOX of its radical scavenging ability and promote anti-oxidant enzymes activity of super oxide dismutase, catalase and glutathione peroxidase [51,52]. Quite a number of plant

Table 2. IC_{50} (µg/ml) and percentage (%) membrane stabilization activity of ethanol extracts of *S. jollaynum* stem and root

% Membrane Stabilization			
Concentration (µg/ml)	**Stem**	**Root**	**Diclofenac**
100	18.59±0.02	14.60±0.01	54.60±0.03
150	21.69±0.09	18.40±0.02	55.00±0.06
200	33.00±0.01	22.86±0.06	62.50±0.05
250	38.61±0.11	27.20±0.09	65.67±0.09
300	42.60±0.07	30.20±0.01	68.00±0.04
IC_{50} (µg/ml)	325±13.81*	541±6.33*	51±6.12

*Values represent mean ± SEM (n=3) *p<0.05 considered as IC_{50} significant when compared to the Standard Drug (Diclofenac)*

Table 3. IC_{50} (µg/ml) and percentage (%) Proteinase inhibition activity of ethanol extracts of *S. jollaynum* stem and root

% Proteinase Inhibition			
Concentration (µg/ml)	**Stem**	**Root**	**Indomethacin**
100	12.10±0.07	7.64±0.08	40.00±0.05
150	15.31±0.01	8.65±0.05	43.20±0.06
200	17.50±0.04	10.42±0.03	47.00±0.05
250	19.94±0.01	12.25±0.04	51.00±0.06
300	20.05±0.03	16.90±0.01	54.00±0.03
IC_{50} (µg/ml)	1000±3.35*	1080±2.67*	246±5.66

*Values represent mean ± SEM (n=3) *p<0.05 considered as IC_{50} values significant when compared to the Standard Drug (Indomethacin)*

Table 4. IC_{50} (µg/ml) and percentage (%) lipoxygenase inhibitory activity of ethanol extracts of *S. jollaynum* stem and root

% Lipoxygenase Inhibition			
Concentration (µg/ml)	**Stem**	**Root**	**Indomethacin**
100	24.79±0.51	14.6±0.03	45.50±0.06
150	28.21±0.60	19.40±0.35	47.60±0.05
200	31.12±0.96	22.86±0.92	51.60±0.73
250	36.70±0.83	27.20±0.43	56.40±0.35
300	40.45±0.55	30..20±0.19	59.80±0.77
IC_{50} (µg/ml)	426±6.33*	541±6.67*	172±5.97

*Values represent mean ± SEM (n=3) *p<0.05 considered as IC_{50} significant when compared to the Standard Drug (Indomethacin)*

extracts have been able to demonstrate lipoxygenase inhibition. Ali et al. [53] reported the anti-lipoxygenase of some indigeneous medicinal plants, while [54-57] reported the lipoxygenase inhibitory activities of *Caralluma arabica, Fraxinus rhynchophylla, Thespesia lampas and M. malabaricum with* IC_{50} values of 30.77 µg/ml, 62.6 ug/ml, 586.5 µg/ml and 29.87 µg/ml respectively.

4. CONCLUSION

From the results of this study, it is suggested that *S. jollyanum* could really have anti-inflammatory properties.

ETHICAL APPROVAL

As per international standard or university standard, Ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Medzhitov R. New adventures of an old flame. Cell. 2010;140:771–6.
2. Ferrero-Miliani L, Nielson OH, Andersen PS, Girardin SE. Importance of NOD2 and NALP3 in interleukin-1βgeneration. Clin Exp Immunol. 2007;147(2):227–235.
3. Vadivu R, Lakshmi KS. *In-vitro* and *in-vivo* anti-inflammatory activity of leaves of *Symplocos cochinchnensis* (Lour) Moore ssp. Laurina. Bangl. J. Pharmacol. 2008;3:121–124.
4. Steinhilber D. 5 Lipoxygenase: A target for anti-inflammatory drugs. Curr. Med. Chem. 1996;6:69-83.
5. Wisastra R, Dekker FJ. Inflammation, cancer and oxidative lipoxygenase activity are intimately linked, Cancers (Basel). 2004;6:1500-21.
6. Kumaraswamy MV, Satish S. Antioxidant and Anti-Lipoxygenase activity of *Thespesia lampas* Dalz & Gibs. Adv Biol Res (Rennes). 2008;2:56–9.
7. Sircar JC, Schwender CF, Johnson EA. Soybean lipoxygenase inhibition bynonsteroidal anti inflammatory drugs. Prostagalndins. 1983;25(3):393-396.
8. Bhattacharjee S. Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transduction in plants. Curr. Sci. 2007;89(7):1113-1121.
9. Reddy VJS, Rao PGD, Lakshmi GR. A review on antiarthritic activity of some medicinal plaants. J Glob Trends Pharm Sci. 2014;5:2061–73.
10. Jackson L, Evers BM. Chronic inflammation and pathogenesis of GI and pancreatic cancers. Cancer Treat. Res. 2006;130:39–65.
11. Schottenfeld D, Beebe V, Dimmer J. Chronic inflammation: A common and important factor in the pathogenesis of neoplasia. CA Cancer J. Clin. 2006;56: 69–83.
12. Gurib-Fakim A. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine. 2006;27:1 93.
13. Mantle D, Gok MA, Lennard TW. Adverse and beneficial effects of plant extracts on skin and skin disorders. Adverse drug react. Toxicol. Rev. 2001;20(2):89-103.
14. Annan K, Houghton PJ. Antibacterial, antioxidant and fibroblast growth stimulation of aqueous extracts of *Ficus asperifolia* Miq and *Gossypiumar boreum L*, wound-healing plants of Ghana. J Ethnopharmacol. 2008;119(1):141–144.
15. Yao LH, Jiang YM, Shi J, Tomas-Barberan FA, Datta N, Singanusong R. Flavonoids in food and their health benefits. Plant Foods Hum. Nutr. 2004;59:113–122.
16. Nia R, Paper DH, Essien EE. Evaluation of the anti-oxidant and anti-angiogenic effects of *Sphenocentrum jollyanum* Pierre. African Journal of Biomedical Research. 2004;7:129-132.
17. Olorunnisola OS, Afolayan AJ. *In-vivo* antioxidant and biochemical evaluation of *Sphenocentrum jollyanum* leaf extract in *p. berghei* infected mice. Journal of Pharmaceutical Science. 2013;26:445-450.
18. Olorunnisola OS, Afolayan AJ. *In-vivo* antimalaria of methanolic leaf and root extracts *Sphenocentrum jollyanum*. African Journal of Pharmacy and Pharmacology. 2011;4(14):1669-1673.
19. Mbaka GO, Adeyemi OO, Anunobi CC. Anti-hyperglycaemic effects of ethanol leaf extract of *Sphenocentrum jollyanum* in normal and alloxan-induced diabetic rabbits. Global Journal of Pharmacology. 2008;2(3):46-51.
20. Mbaka GO, Adeyemi O, Osinubi A, Noronha C, Okanlawon A. The effect of aqueous extract of *Sphenocentrum*

jollyanum on blood glucose level of rabbits. Journal of Medicinal Plants Research. 2009;3(11):870-874

21. Alese MO, Adewole OS, Ijomone OM, Ajayi SA, Alese OO. Hypolipidemic and hypoglycemic activities of methanolic extract of *Sphenocentrum jollyanum* on stretozocin induced diabetic wistar rats. European Journal of Medicinal Palnts. 2014;4(3):353-364.
22. Moody JO, Robert VA, Connolly JD, Houghton PJ. Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* Pierre (Menispermaceae). J Ethnopharmacol. 2006;104:87–91.
23. Raji Y, Fadare OO, Adisa RA, Salami SA. Comprehensive assessment of the effect of *Sphenocentrum jollyanum* root extract on male albino rats. Reprod. Med. Bio. 2006;5:283-292.
24. Sadique J, Al-Rqobahs WA, Bughaith GE, ElGindi AR. The bioactivity of certain medicinal plants on the stabilization of RBS membrane system. Fitoterapia. 1989;60:525-532.
25. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. International Journal of Pharma and Pharmacological Sciences. 2010;2(1):146-155.
26. Oyedepo OO, Femurewa AJ. Anti-protease and membrane stabilizing activities of extracts of Fagra zanthoxiloides, Olax subscorpioides and *Tetrapleura tetraptera*. Int J of Pharmacong. 1995;33:65-69.
27. Tappel AL. In methods in enzymology. edited. By Colowick SP and Kaplan N0, New York and London. Academic Press. 1962;536-539.
28. Laskey T, Zanbergen G, Solbach W. Neutrophil Granulocys as host cell and transport veichles for intracellular pathogens; apoptosis as infection-promoting factor. Immunology. 2008; 213(3-4):183-191.
29. Middleton EJR, Kandaswami C, Heoharides TC. The effect of plant flavonoids on mammalian cells, Implication for inflammation, heart disease and cancer. Pharmacological Reviews. 2000; 52:673-751.
30. Pečivová J, Mačičková T, Sviteková K, Nosá R. Quercetin inhibits degranulation and superoxide generation in PM Astimulated neutrophils. Interdiscip. Toxicol. 2012;5:81–83.
31. Kanashiro A, Souza JG, Kabeya LM, Azzolini AE, Lucisano-Valim YM. Stimulet neutrophils inhibited by flavonoids: Importance of the catechol group. Z. Naturforsch. 2007;62:357–361.
32. Fawole OA, Amoo SO, Ndhlala AR, Light ME, Finnie JF, Van Staden J. Anti-inflammatory anticholinesterase, antioxidant and phyto chemical properties of medicinal plants used for pain-related ailments in South Africa. J. Ethnopharmacol. 2010;127(2):235-241.
33. Gepdireman A, Mshvildadze V, Suleyman H, Elias R. Acute anti-inflammatory activity of four saponins isolated from ivy: Alphahederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F in carrageenan-induced rat paw edema. Phytomedicine. 2005;12(6-7):440-444.
34. Anosike CA, Obidoa O, Ezeanyika LU. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum)*. DARU Journal of Pharmaceutical Sciences. 2012;2:20-76.
35. Al Amin S, Shaikhul M, Abida S, Mohammad AK, Mohammad AR. *In vitro* membrane stabilizing activity, total phenolic content, cytotoxic, thrombolytic and antimicrobial activities of *Calliandra surinamensis* (Wall.). Journal of Pharmacognosy and Phytochemistry. 2012;1(3):340-44.
36. Honnayakanahalli M, Gowdam M, Sharanaiah U. Assessment of membrane stabilizing activity from honey. An *in-vitro* approach Acta Sci. Pol. Technol. Aliment. 2015;14(1):85–90.
37. Adedapo A, Adewuyi T, Sofidiya M. Phytochemistry, anti-inflammatory and analgesic activities of the aqueous leaf extract of *Lagenaria breviflora* (Cucurbitaceae) in laboratory animals. Int. J. Trop. Biol. 2013;61(1):281-290.
38. Sakat SS, Tupe P and Juvekar A. *In-vitro* anti inflammatory activity of aqueous and methanol extracts of *Erythrina indica* leaves. Pharmacology Online. 2009;3:221-229.
39. Joanitti GA, Frietas SM, Silva LP. Proteinacous protease inhibitors: Structural features and multiple functional faces. Current Enzyme Inhibition. 2006;2(3):199-217.

40. Ong P. Emerging drugs for atopic dermatitis. Expert opin emerg drugs. 2009;1:165-179.
41. Gulnaz AR, Wethroe K, Jyoti BC. *In vitro* anthelminthic and anti-inflammatory activity of ethanolic extract of *Randia uliginosa DC* Leaf. Int. J. Curr. Microbiol. App. Sci, 20143(9):793-799.
42. 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.). Hitchc. Journal of Pharmacognosy and Phytotherapy. 2011;3(3):43-51.
43. Arkel HH, Khalid MF, Mohammad AZ, Bashir AG. Protease inhibitor associated antimicrobial activity of pea *Pisum sativum* L. cv. Int. J. Pure App. Biosci. 2016;4(1): 172-179.
44. Leelaprakash G, Mohan DS. *In vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare.* International Journal of Drug Development & Research. 2012;3(3):189-197.
45. Azila Y, Don MM. Evaluation of *Trametes Lactinea* extracts on the inhibition of hyaluronidase, lipoxygenase and xanthine oxidase. Journal of Physical Science. 2012;23:1-15.
46. Fu PK, Wu CL, Tsai TH, Hsei Cl. Anti Inflammatory and Anti- coagulative effects of Paenol on LPS–induced acute lung injury in rats. Evid Based Complement. Alternate Med. 2012;7:(8)3-7.
47. Kim YS, Young MR, Bobe G, Colburn NH, Milner JA. Bioactive food components, inflammatory targets and cancer prevention. Cancer PREV Res. 2009;2:200-208.
48. Kwak WJ. Papyriflavonol A from *Broussonetia papyrifera* inhibits the passive cutaneous anaphylaxis reaction and has a secretory phospholipase A2-inhibitory activity. Biol. Pharm. Bull. 2003;26:299–302.
49. Likhitiwitayawuid K, Sawasdee K, Kirtikara K. Flavonoids and stilbenoids with COX-1 and COX 2 inhibitory activity from Dracaena louureiri. Planta Med. 2002;68: 841-843.
50. Csaki C, Keshishzadeh N, Fischer K, Shakibaei M. Regulation of inflammation signaling by resveratrol in human chondrocytes *in vitro*. Biochem. Pharmacol. 2008;75:677–687.
51. Gulcin I. Antioxidant propetites of resveratrol; a structure- acivity insight, Innovat. Food Sci Emerg Tech. 2010;111: 210-218.
52. Shen SQ, Zhang Y, Xiang JJ, Xiong CL. Protective effect of curcumin against liver warm ishemia/ reperfusion injury in rat model is assoicated with regulation of heat shock protein and antioxidant enzymes. World Journal of Gastroenterol. 2007;13: 1953-1961.
53. Ali SM, Ashraf M, Irshad A, Shafia A, Muhammad Y, Abida L. Anti-lipoxygenase activity of some indigenous medicinal plants. Journal of Medicinal Plants Research. 2013;7(6):219-222.
54. Mohammad K, Hanan ME, Nael MF, Alaaeldin AH, Abdul RC, Ahmed HH. Antioxidant activity and lipoxygenase inhibitory effect of *Caralluma arabica* and related polyphenolic constituents. American Journal of Plant Sciences. 2014;5:1623-1631.
55. Man KH, Kyung SC, Sung JS. Inhibitory effect of lipoxygenase and dpph radical scavening activity of *Fraxinus rhynchophylla.* European Journal of Advanced Research in Biological and Life Sciences. 2015;3(3):56-62.
56. Kumaraswamy MV, Satish S. Antioxidant and anti-lipoxygenase activity of *Thespesia lampas* Dalz & Gibs. Advances in Biological Research. 2008;2(3-4):56-59.
57. Shailasree S, Sampatha KK, Niranjana SR, Prakash HS. *In vitro* antioxidant activity, lipoxygenase, cyclooxygenase-2 inhibition and dna protection properties of *memecylon* species. International Journal of Pharmacy and Pharmaceutical Sciences. 2013;5(2):257-262.

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