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EFFECTS OF METHANOL EXTRACT OF TOASTED AFRICAN YAM BEAN SEEDS (SPHENOSTYLIS STENOCARPA) ON ANTI-INFLAMMATORY PROPERTIES

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Abstract

This study was aimed at evaluating the effects of methanol extract of toasted African Yam Bean seeds on in vivo and in vitro anti-inflammatory studies. Qualitative phytochemical composition, Gas Chromatography-Molecular Spectrometry (GC-MS) quantification, membrane stabilization, egg albumin denaturation and egg albumin induced paw oedema in albino rats was done using standard laboratory procedures. The circumference of the hind paw was measured before induction and after topical application at 30 minutes, 1 hour and 2 hours. Preliminary phytochemical analysis revealed moderate composition of Alkaloids, Flavanoids, phenolics compounds and high composition of Steroids. The fatty acid methyl esters composed of alpha-D-Glucopyranoside methyl, Cyclopentaneundecanoic acid, Oxalic acid monoamide N-(2-fluorophenyl)-dodecyl ester, Dodecanoic acid but-3-enyl ester, 3-(Prop-2-enoyloxy) dodecane Methoxyacetic acid tetradecyl ester and 2-Heptanol-4-methyl. African Yam Bean seeds extract exhibited membrane-stabilizing property, as it significantly ($p < 0.05$) reduced the levels of haemolysis of Human Red Blood Cells exposed to hypotonic solution with high % inhibition of 79.81 %, 73.40 %, 81.41 % and 96.47 % at a dose-dependent level of (0.06 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1.0 mg/ml respectively of extract) compared to indomethacin 1.0 mg/ml with 71.79 % inhibition. The African Yam Bean seeds extract also significantly ($p < 0.05$) inhibited albumin denaturation with the highest inhibition having absorbances of 0.05 ± 0.01 and 0.04 ± 0.08, in also a dose dependent level. However, indomethacin at 1.0 mg/ml gave an absorbance of 0.36 ± 0.01 which was significantly different ($p<0.05$) when compared to all the tested groups. The African Yam Bean seeds extracts was found to significantly ($p < 0.05$) reduce the oedema induced by the phlogistic agent in rats in a dose-dependent manner (5 %, 4 % and 2 % respectively) and the reductions was during later phases of the inflammation (2 hours) compared to indomethacin. Methanol extract of toasted African Yam Bean seeds revealed a potent anti-inflammatory activity due to the presence of rich phytochemicals and fatty acids that may be exploited by pharmaceutical industries for the management of some inflammatory disorders.

Keywords: *African Yam Bean seeds, (sphenostylis stenocarpa) Phytochemical and Anti-inflammation*

Introduction

Inflammation is one of the most central processes required in defense of animal cells against certain injuries or microbial infections. Nevertheless, inflammation regularly progresses to acute or chronic. Chronic inflammation is caused due to a variety of diseases including neurodegenerative disorders, cancer, and cardiovascular diseases [1].

Mechanism of inflammation represents a chain of organized, dynamic responses including both cellular and vascular events with specific hormonal secretions. These pathways involve changing physical location of white blood cells (monocytes, basophils, eosinophils, and neutrophils), plasma, and fluids at inflamed site. A group of secreted mediators and other signaling molecules (e.g., histamine, prostaglandins, leukotrienes, oxygen- and nitrogen-derived free radicals, and serotonin) are released by immune defense cells principally in the mechanism which can contribute in the event of inflammation [2].

A variety of chemical mediators from circulatory system, inflammatory cells, and injured tissue actively contribute to and adjust the inflammatory response. The released chemical mediators include vasoactive amines such as histamine and serotonin, peptide (e.g., bradykinin), and eicosanoids (e.g., thromboxanes, leukotrienes, and prostaglandins) [3]. Mechanism of inflammation represents a chain of organized, dynamic responses including both cellular and vascular events with specific humoral secretions. These pathways involve changing physical location of white blood cells (monocytes, basophils, eosinophils, and neutrophils), plasma, and fluids at inflamed site. A group of secreted mediators and other signaling molecules (e.g., histamine, prostaglandins, leukotrienes, oxygen- and nitrogen-derived free radicals, and serotonin) are released by immune defense cells principally in the mechanism which can contribute in the event of inflammation [4].

Since the inception of African tradition with herbal therapy which has become an integral part of our primitive primary Health care in Africa, plants are the primary source of Africa medicine. But some claims about plants by some herbal medical practitioners seem to be false [5]. African Yam bean (AYB) *(Sphenostylis stenocarpa)* is an herbaceous (tuberous) leguminous plant occurring throughout tropical Africa [6] and grows during the wet season [7]. It is often cited among the lesser-known and underexploited species [8]. It is cultivated both for the seeds and tubers because of its valuable and prominent source as plant protein [9]. Nigeria is very significant for AYB production where extensive cultivation had been reported in the eastern, western and northern areas of the country, where it is known as Geri-Geri in Hausa (Northern, Nigeria) and Ahuma in Tiv (North Central, Nigeria).

The research hypothesis states that the H_0: Methanol extract of AYB seeds (*Sphenostylis stenocarpa)* has no anti-inflammatory properties and the H_1: Methanol extract of AYB seeds (*Sphenostylis stenocarpa)* has anti-inflammatory properties.

Materials and Methods

Sample collection

African Yam Bean (AYB) seeds (Brown coloured) was used for the study. The AYB was purchased from Wurukum Market, Makurdi L.G.A of Benue State, Nigeria.

Preparation of African Yam Bean Extracts

Brown coloured AYB seeds (1.4 kg) were handpicked and sorted to removes stones, damaged seeds and other extraneous materials. 700 g of the seeds were toasted in a stainless-steel pan using hot plate at 80 °C, they were stirred continuously using a wooden spoon for 5 minutes until cracking. The seeds were allowed to cool and milled into fine flour. The toasted AYB seeds were pounded into fine flour and was stored in an air-tight container and kept in the cupboard. 260 g of the powdered sample was soaked in 1000 ml of 70 % methanol extraction solvent. The solution was left overnight for 24 hours and decanted, after which the solvent was filtered using Whateman No 1 filter paper. The filtrate was concentrated using a water bath at 68 °C and the extract was stored in the refrigerator and later used for the analysis.

Preliminary Phytochemicals Screening

The stock solution of AYB crude extracts was prepared by dissolving 1 g of AYB crude extract in

100 ml of ethanol. The obtained stock solution was used for phytochemical screening following the methodology of Nwankwo and Ekeanyanwu [10].

Test for alkaloids

The crude extract was dissolved ammonia solution (3 ml) and allowed to stand for few minutes to evaluate free alkaloids. Chloroform (10 ml) was added to the solution, it was shaken and filtered. The chloroform was evaporated from the crude extract under the water bath at 51 °C then 3 ml of Mayer's reagent was added. A cream colour precipitation was obtained showing the presence of alkaloids.

Test for phenolics

To the test solution, a few drops of 10 % lead acetate solution were added. Formation of white precipitate indicated the presence of phenolic compounds.

Test for flavonoids

The stock solution (1 ml) was taken in a test tube; few drops of dilute NaOH solution were added. An intense yellow colour was formed in the test tube. It became colourless on addition of a few drops of dilute hydrochloric acid which indicated the presence of flavonoids.

Test for steroids

The crude plant extracts (1 mg) was dissolved in chloroform (10 ml) in a test tube this was followed by addition of concentrated sulphuric acid to the test tube by sides. The upper layer in the test tube was turned into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

GC-MS Quantification Method

Analysis of Fatty Acid Methyl Esters (FAMEs) was performed by using GC-MS (QP2010 PLUS Shimadzu) equipped with a split injector. Separations were achieved using an SP^{TM} 2560 Sigma Aldrich capillary column (100 m × 0.25 mm ID, 0.20 µm film thickness). Helium was used as the carrier gas at flow rates of 1.80 ml/min and a split ratio of 20:1. The injector temperature was 250 °C. The oven temperature was programmed at 70 °C for a hold time of 1min and increased to 280 °C for a hold time of 5 min. The injection volume was 2 µl. MS spectra were obtained at a scan range of 30-400 m/z, interface temperature of 250 °C, ion source temperature of 200 °C, solvent cut time of 2.50 min, event time of 0.50 sec. and scan speed of 666 u/sec. Ionization energy (EI) of 70 eV was used for the mass spectroscopy detector at a scan rate of 1 sec. FAME peaks were identified by comparing their mass spectra with those of compounds obtained from the NIST mass spectral library (NIST 05) database.

***In vitro* Anti-inflammatory Studies**

Determination of the Effect of the Methanol extract of African Yam Beans on Hypotonicity-Induced Haemolysis of Human Red Blood Cells Membrane Stabilization

This was determined using modifications of the method of [11].

Procedure

The reaction mixture was consisting of test extracts and 3% tween 80 which serve as the hypotonic solution. An aliquot (1 ml) of the AYB extracts were made up to 4.9 ml with the vehicle (3 % tween 80 dissolved in distilled water) in different test tubes at various concentrations. A control tube contained 4.9 ml of the vehicle while another contained 4.9 ml of 3 % tween 80 dissolved in normal saline (isotonic solution). HRBCs suspension (0.1 ml) was added to each tube, and after gentle mixing, the mixtures were incubated for 1 hr at room temperature (37 °C) and centrifuged at 3,000 rpm for 10 min. The absorbance of the supernatant measured at 418 nm using a spectrophotometer. The tests were carried out in triplicates. The percentage inhibition of haemolysis was calculated using the relation below:

$$\% \text{ Inhibition of Haemolysis} = \left(1 - \frac{OD_2 - 0D_1}{OD_3 - OD_1}\right) \times 100$$

Inhibition of Albumin Denaturation

The anti-inflammatory activity of AYB extract was studied using inhibition of albumin denaturation technique which was studied according to [12 and 13] followed with minor modifications. The reaction mixture was consisting of test extracts and 1 % aqueous solution of egg albumin fraction. pH of the reaction was adjusted using small amount of 1N HCl (to pH 6.8). The extracts were incubated at 37 °c for 20mins and then heated to 51°c for another 20 mins. After cooling, the sample turbidity was measured at 660nm. The experiment was performed in triplicate.The percentage inhibition of protein denaturation

was calculated as follows: -Percentage inhibition= $\frac{(Abs\ control - Abs\ sample)}{Abs\ control}$ X 100

***In vivo* Anti-inflammatory Study**

Determination of the Effect of the AYB methanol extract on Egg Albumin Induced Paw Oedema in Albino Rats

This was done according to modification of the method of [14]. The increase in the right hind paw size of the rats induced by the sub-plantar injection of fresh egg albumin was used as a measure of acute inflammation [15].

Experimental Design

A total of fifteen (15) male albino rats were used for the study. They were divided into five (5) Groups of three (3) rats each and treated as follows:

Group 1: Received no treatment and vehicle (control)
Group 2: Received 5 % AYB Extract (v/v in petroleum jelly)
Group 3: Received 4 % AYB Extract (v/v in petroleum jelly)
Group 4: Received 2 % AYB Extract (v/v in petroleum jelly)
Group 5: Received 4 % Indomethacin (w/v in petroleum jelly)

Procedure

Rats were fasted for 18 h before the experiment to ensure uniform hydration and minimize variability in oedematous response, after which the right hind paw size of the rats at time zero (before the induction of oedema) was measured using a thread and ruler [16]. This was followed by topical application of test substances as outlined above. One (1) hour after application of ointment, acute inflammation was induced by injecting 0.1 ml of undiluted fresh egg albumin into the sub-plantar surface of the right hind paw of rats. The increase in the right hind paw size of rats was subsequently measured at 0.5 hr, 1 hr, and 2 hrs after fresh egg albumin injection. The difference between the paw size of the injected paws at time zero and at different times after fresh egg albumin injection was used to assess the formation of oedema. These values were used in the calculation of the percentage inhibition of oedema for each dose of the AYB extract and for indomethacin at the different time intervals using the relation below:

Paw oedema = (V_t-V_o)
V_o = Paw oedema at time zero
V_t = Paw oedema at time t (0.5, 1 and 2)

Percentage inhibition of oedema $= \frac{(Vt-Vo)control - (Vt-Vo)\ treated\ Groups}{(Vt-Vo)control} \times 100$

Statistical Analysis

The data obtained were analyzed and results expressed as mean ± Standard Error of Mean (SEM) using Statistical Product and Service Solutions (SPSS), version 23.0. Tests of statistical significance were carried out using one-way Analysis of Variance (ANOVA). Mean values with ($p < 0.05$) were considered statistically significant.

Results

Qualitative Phytochemical screening of toasted AYB Extract

The Qualitative phytochemical screening result shows that phytochemicals such as Alkaloids, Phenolics and Flavanoids were moderately found, while Steroids was largely found in AYB seed extract as shown in table one (1).

Fatty Acid Ester Profile of Methanol Extract of toasted African Yam Bean Seeds

The relative percentage composition of the fatty acids ester of the studied African Yam Bean Seeds is shown in Table 3 and Table 4. The AYB methanol extract showed a high percentage of unsaturated fatty acids methyl esters which are: alpha. -D-Glucopyranoside, methyl, Cyclopentaneundecanoic acid, Oxalic acid, monoamide, N-(2-fluorophenyl)-, dodecyl ester, Oxalic acid, Dodecanoic acid, but-3-enyl ester; 3-(Prop-2-enoyloxy) dodecane and Methoxyacetic acid tetradecyl ester; 2-Heptanol, 4-methyl which is a fatty acyl.

Chromatogram of Fatty Acid Methyl Esters of toasted African Yam Bean

The chromatogram shows the presence of fatty acids methyl esters in abundance per given time.

Discussion

In the present study, the anti-inflammatory effect of action of the methanol extract of *Sphenostylis stenocarpa* seed have been established using *in vivo* (albumin-induced rat paw oedema) and *in*

vitro (membrane stabilization and inhibition of albumin denaturation) approaches.

The preliminary phytochemical screening shows that the methanol extract of toasted AYB (*S. stenocarpa*) contains moderately phytochemical compounds such as alkaloids, flavonoids, phenolics compound; and also, highly contains steroids. These phytochemicals have been implicated to have analgesics and anti-inflammatory activities [17]. The African yam bean (*S. stenocarpa*) seed gave a high composition of fatty acid esters for the AYB (*S. stenocarpa*) sample. The Fatty acids found include alpha-D-Glucopyranoside methyl, Cyclopentaneundecanoic acid; Oxalic acid, monoamide-N-(2-fluorophenyl)-, dodecyl ester; Dodecanoic acid, but-3-enyl ester; Methoxyacetic acid, tetradecyl ester; 3-(Prop-2-enoyloxy) dodecane and 2-Heptanol, 4-methyl for the toasted AYB sample. Studies have shown that these fatty acid esters have antiperspirant, drug for dermatological and disorder of senses drug, cytotoxicity against tumour and antioxidative properties [18, 19 and 20].

Human Red Blood Cells (HRBCs) are analogous to lysosomal membrane [21]. The HRBCs membrane is made up of majorly polyunsaturated fatty acids which makes the cells highly susceptible to oxidative damage [22] leading to haemolysis by which haemoglobin and other internal cell components are released into the surrounding fluids. Red blood cell membrane can be lysed due to its exposure to injurious substances such as hypotonic medium, heat, and methyl salicylate or phenyl hydrazine. This study demonstrated the capability of the toasted AYB sample to inhibit the lyses of HRBCs membrane induced by hypotonic solution. The stabilization of erythrocyte membranes by the AYB sample thus, implies that it may as well stabilize lysosomal membranes. The AYB sample perhaps stabilized the RBC membrane; this may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, steroids, phenols and also Cyclopentaneundecanoic acid. The extract serves as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the hypotonic induced membrane stabilization. Stabilization of the membrane also prevents the leakage of serum protein and fluids into the tissues during a period of increased vascular permeability caused by inflammatory mediators [23].

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat [24]. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of toasted AYB extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced egg albumin denaturation. Maximum inhibition of 0.28 ± 0.02 absorbance was observed at 1.0 mg/ml. Indomethacin, a standard anti-inflammation drug showed the maximum inhibition 0.36 ± 0 .01 absorbance at the concentration of 1.0 mg/ml compared with other test groups. This may be due to the presence of Cyclopentaneundecanoic acid found in toasted AYB extract which acts as anti-oxidant (anti-oxidant play the role of reducing not only the catalytic activity of iNOS but also its protein and mRNA levels, thereby lowering the NO production and ROS that avail them during inflammation). Swelling (tumour/oedema) is one of the classical signs of inflammation. Oedema is caused by accumulation of fluid [24]. The topical anti-inflammatory activity of the toasted AYB extract was confirmed by measuring its ability to reduce local oedema induced in the albino rat right paw by injection of an irritant/phlogistic agent [25]. Carrageenan induction of inflammation involves three distinct phases of mediators' release including histamine and 5-hydroxytryptamine in the first phase, kinins in the second phase and prostaglandin in the third phase [26]. Prostaglandin in particular, is known to cause or enhance the cardinal signs of inflammation. Fresh egg albumin-induced inflammation appears to be similar to carrageenan induced inflammation in rats [27]. The toasted AYB extract at 5 %, 4 % and 2 % showed good anti-inflammatory activity as it significantly ($p < 0.05$) inhibited the increase in paw circumference from 30 minutes to 2 hours which covers the three phases for carrageenan induced oedema namely, histamine and 5-hydroxytryptamine (5- HT) release in the first (early) phase, kinins release in the second phase and prostaglandins release in the third (late)

phase [26]. This shows that the extract had effect on all the phases of the inflammatory response. Toasted AYB seed extract at 2 % and 4 % showed a high significant ($p < 0.05$) decrease of oedema compared to indomethacin 4 % after 30 min of induction of oedema. The AYB extract at 2 %, 4 % and 5 % showed a higher significant ($p < 0.05$) decrease of oedema compared to indomethacin 4 % from1 hr to 2 hr in the inflammation model tested. The suppression of oedema in the second and third phase of inflammation suggests that the anti-inflammatory activity of the extract may also be due to the suppression of kinin and prostaglandin formation induced by egg albumin within this period. The inhibition of oedema by the extract might be due to the presence of phytochemical compounds such as phenolics, alkaloids, flavonoids and steroids which have an antioxidant and anti-inflammatory activities by reducing not only the catalytic activity of iNOS but also its protein and mRNA levels, thereby lowering the NO production; inhibition of cyclooxygenase and lipoxygenase activities in epidermal microsomes and cytosol; Inhibition of platelet aggregation induced by arachidonate and thromboxane B_2 production from arachidonate in human blood platelets; and inhibition of hepatic cytochrome P_{450}–linked alkoxyresonifin O-dealkylase activities which is responsible for oxidative metabolism of a wide array of carcinogenic aromatic hydrocarbons [28, 29, 30, and 31].

Conclusion

The present study, indicate that the methanol extracts of toasted African Yam Bean seed (*Sphenostylis stenocarpa*) possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, steroids, and phenols. Also, chemical compounds such as α-D-Glucopyranoside methyl, Cyclopentaneundecanoic acid, 2-heptanol-4methyl and Methoxyacetic acid-4-methyl teradecyl ester. This study reveals that toasted African Yam Bean Seeds (*Sphenostylis stenocarpa*) can be used in design of potent topical anti-inflammatory drug.

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References

1. Sofi, F., Fabbri, A. and Casini, A. (2016). Inflammation and Cardiovascular Disease and Protection by the Mediterranean Diet. Springer International publishing. 6: 89–96.

2. Headland, S. and Norling, L. (2015). The resolution of inflammation: principles and challenges. Journal Immunology. 27:149–160.

3. Halliwell, B. and Gutteridge, J.M. (2015). Free Radicals in Biology and Medicine. Oxford University Press, USA.6:674-693

4. Nigam, S., Honn, K., Marnett, L. and Walden, T. (2012). Eicosanoids and other Bioactive Lipids in Cancer, Inflammation and Radiation Injury: Proceedings of the 2nd International Conference September 17-21, 1991 Berlin, FRG. Springer Science and Business Media.71:45-53.

5. Ramzan, I. (2015). Phytotherapies: Efficacy, Safety, and Regulation. John Wiley and Sons, Hoboken, Newjessy. 43: 55-63.

6. Porter, D. (1992). Economic botany of Sphenostylis stenocarpa (Luguminosac). Economic Botany, 46 (3): 262-275.

7. Allen, O.N., and Allen, E.K. (1981). A Source of Book Characterization, Use and Nodulation. London: Macmillan Publishers.

8. Ene-Obong, H.N., and Carnovale, E. (1992). A comparison of the proximate mineral and amino acid composition of some known and lesser known legumes in Nigeria. Journal of Food Chemistry, 43: 169-175.

9. Amoatey, H. M., Klu, G. Y., Bansa, D. and Kumaga, F. K. (2000). African yam bean (Sphenostylis stenocarpa): A neglected crop in Ghana. Journal of Applied Ecology.1: 53-6.

10. Nwankwo, M. O. and Ekeanyanwu C. R. (2011). Nutritional Pharmacology and Phytochemicals. Lambart Publication Germany.

11. Oyedepo, O. O., Akindele, V. R. and Okunfolami, O. K. (1997). Effect of extracts of Olaxsubsorpoides and Aspila africana on bovine red blood cells. Journal of Phytotherapy Research. 11: 305- 306.

12. Mizushima, Y. and Kobayashi, M. (1968). Interaction of anti-inflammatory drugs with serum preoteins, especially with some biologically active proteins. Journal of Pharmaceutical Pharmacology; 20:169-173.

13. Sakat, S., Juvekar A.R, Gambhire, M.N. (2010) In-vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata. Linn. International Journal of Pharmaceutical and Pharmacological Sciences, 2 (1): 146-155.

14. Winter, C. A., Risley, E. A. and Nuss, G. W. (1962). Carrageenin-induced oedema in hind paw of the Rat as an assay for anti-inflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine, 111: 544–547.

15. Akah, P. A. and Nwambie A. (1994). Evaluation of Nigerian traditional Medicines: 1 Plants used for rheumatic (inflammatory) disorders. Journal of Ethnopharmacology. 42: 179 – 182.

16. Okokon, J. and Nwafor, P (2010) Anti-inflammatory, analgesic and antipyretic activities of ethanolic root extract of Croton zambesicus. Pakistan Journal of Pharmacological Science, 23 (4): 383-390.

17. Charu, G. and Dhan P. (2015). Phytopharmaceuticals and their health benefits. Dream 2047. Research Gate Journal. Vol. 17 (5): 22-30.

18. O'Neil, M.J. (2013). The Merck Index-An Encyclopedia of Chemicals, Drugs, and Biological. Royal Society of Chemistry: Cambridge, UK. P. 413-527.

19. Lewis, R.J. Sr. (2007). Harley's Condensed Chemical Dictionary. John Wiley and Sons, Inc. New York.15th Edition. P 345-674.

20. Cosmas Samuel; Joshua Parker Elijah; Durojaye Olanrewaju Ayodeji; Ngwu Anthonia Ngozi; Ajima Judith Nnedimkpa; Didiugwu Chizoba Maryann; Eze Chinenyenwa Kayla; 1Okoli Paul Tobechukwu (2019). Lowering the blood brain barrier permeation property of 6-gingerol through a structural monosubstitution modification approach for the prevention of psychotropic side effects: an in-silico pharmacokinetics study. Pharmacologyonline, 1: 1-9.21.

21. Mounnissamy, V. M., Kavimani, S., Balu, V. and Quine, S. D. (2008). Evaluation of Anti-inflammatory and Membrane stabilizing property of Ethanol Extract of Cansjera rheedii J. Gmelin (Opiliaceae). Iranian Journal of pharmacology and Therapeutic, 6 (2): 235-237.

22. Nagamani, J. E, Divya, N. and Prabhu, S. (2012). Antioxidant and antihemolytic activities of Bombax Ceiba Pentandra spike and fruit extracts. International Journal of Pharmacy and Pharmaceutical Sciences, 4(5): 311-315.

23. Chaitanya, R., Sandhya, S., David, B., Vinod, K. R. and Murali, S. (2011). HRBC membrane stabilizing property of root, stem and leaf of Glochidion velutinum. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2 (1): 256–259.

24. Cotran, K. and Collins, C. (1998). Robbins Pathologic Basis of Disease. Philadelphia: W.B Saunders Company.

25. Omkar, A., Jeeja, T. and Chhaya, G. (2007). Evaluation of anti-inflammatory activity of Nyctanthes arbour-tristis and Onosma echioides. Pharmacognosy Magazine, 2 (8): 258 - 260.

26. Di Rosa, M., Giroud, J. P., Willoughby, D. A. (1971). Studies of the mediators of the acute inflammatory responses induced in rats in different sites by carrageenan and turpentine. Journal of Pathology, 104: 15–29.

27. Nwafor, P. A. and Okwuasaba, F. K. (2003). Antinociceptive and anti-inflammatory effects of methanolic extract of Asparagus pubescebs roots in rodents. Journal of Ethnopharmacology, 84: 125-129.

28. Bailey, J.M. (2013). Prostaglandins, Leukotrienes, Lipoxins, and PAF: Mechanism of Action, Molecular Biology, and Clinical Applications. Springer Science and Business Media. 12:654-662

29. Honn, K.V., Marnett, L.J., Nigam, S.,.Jones, R.L. and Wong, P.Y. (2013). Eicosanoids and other Bioactive Lipids in Cancer, Inflammation, and Radiation Injury. Springer Science and Business Media, New York. 3:243-249.

30. Engelhardt, N. (2014). Synthese und Charakterisierung von Oberflächen-FunktionalisiertenPolymerenNanopartikeln auf Poly (2-oxazolin). Basic Doctoral Dissertation. Post Higher Medical Dissertation. 63: 395–408.

31. Moreno, J. J. (2017). Eicosanoid receptors: Targets for the treatment of disrupted intestinal epithelial homeostasis. European Journal of Pharmacology, 796: 7–19.

TABLE 1: Phytochemical Analysis of AYB Seeds Extract

Parameters	Relative (mg/100ml)
Alkaloids	++
Phenolics	++
Flavanoids	++
Steroids	+++

KEY: ++: Moderately present. +++: Highly present

TABLE 2: Bioactivity of Fatty Acids Methyl Esters identified in Methanol extract of AYB

Compound Name	Mol. Formular	Nature	Biological Activity	Reference
alpha.-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	Methyl glycosides	Cytoxicity against tumor Hesa cells, agonist ER stress response signaling pathway.	O'Neil, (2013)
Cyclopentaneundecanoic acid	$C_{17}H_{32}O_2$	Methyl ester	Antioxidant, drugs for dermatological disorders.	Lewis, (2007)
Oxalic acid, monoamide, N-(2-fluorophenyl)-, dodecyl ester	$C_{21}H_{38}O_4$	Heptyl ester	Not found	-
Dodecanoic acid, but-3-enyl ester	$C_{16}H_{30}O_2$	Fatty acid ester	Not found	-
Methoxyacetic acid, tetradecyl ester	$C_{17}H_{34}O_3$	Fatty acid ester	Anti-microbial.	Lewis, (2007)
2-Heptanol, 4-methyl-	$C_8H_{18}O$	Fatty Acyls	Drugs for disorder of senses, dermatological disorder; dentifrices, antiperspirant.	Lide and Milne, (1994)
3-(Prop-2-enoyloxy) dodecane	$C_{15}H_{20}O$	Fatty acid ester	Not found	-

TABLE 3: Effect of Methanol extract of toasted AYB Extract on Hypotonicity Induced Haemolysis of Human Red Blood Cell

Treatment	Concentrations	OD_{418nm}			Differences in OD Values	Percentage Inhibition of Haemolysis (%)
		Hypotonic Solution (OD_3)	Hypotonic Solution (OD_2)	Isotonic Solution (OD_1)	($OD_2 - OD_1$)	
Hypotonic Solution		0.48 ± 0.03				
Isotonic Solution				0.17 ± 0.01		
Group 1	1.0 mg/ml		0.18 ± 0.03^{b}		0.011	96.47
Group 2	0.5 mg/ml		0.23 ± 0.01^{c}		0.058	81.41
Group 3	0.25 mg/ml		0.25 ± 0.06^{e}		0.083	73.40
Group 4	0.125 mg/ml		0.23 ± 0.02^{d}		0.063	79.81
Group 5	0.06 mg/ml		0.42 ± 0.03^{f}		0.252	19.23
Indomethacin	0.5 mg/ml		0.26 ± 0.01^{a}		0.080	71.79

Results are expressed in means ± SEM; n = 3

Mean values having different letters as superscripts from top to bottom of the column are considered significant ($p < 0.05$)

TABLE 4: Effect of Methanol extract of toasted AYB Extract on Inhibition of Albumin Denaturation

		Absorbance at 660_{nm}
Treatment	**Concentration**	**Absorbance of Sample**
Group 1	1.0 mg/ml	0.28 ± 0.02^{c}
Group 2	0.5 mg/ml	0.13 ± 0.06^{b}
Group 3	0.25 mg/ml	0.04 ± 0.08^{a}
Group 4	0.125 mg/ml	0.05 ± 0.01^{a}
Indomethacin	1.0 mg/ml	$0.36 \pm 0 .01^{d}$

Results are expressed in means ± standard error of mean (SEM); n = 3

Mean values having different letters as superscripts from top to bottom of the column are considered significant ($p < 0.05$)

TABLE 5: Effect of Methanol extract of toasted AYB on Egg Albumin-Induced Paw Oedema in Albino Rats

GROUP	DOSE	MEAN PAW OEDEMA (mm)			
		Time 0	30min	1hr	2hr
Group 1		46.66 ± 3.28^{aA}	56.33 ± 3.17^{aAB}	60.00 ± 3.21^{bB}	47.33 ± 1.85^{aA}
Group 2	5 %	48.00 ± 2.08^{aA}	55.66 ± 0.66^{aC} ◊20.68	54.00 ± 1.73^{aBC} ◊55.00	50.00 ± 0.57^{aAB} ◊81.23
Group 3	4 %	45.00 ± 1.00^{aA}	56.66 ± 2.90^{aC} ◊37.93	52.00 ± 2.00^{aBC} ◊89.98	46.33 ± 0.88^{aAB} ◊90.62
Group 4	2 %	48.00 ± 1.52^{aA}	56.66 ± 1.85^{aB} ◊31.03	50.00 ± 1.65^{aA} ◊87.50	48.33 ± 1.20^{aA} ◊96.88
Group 5 (Standard Drug)	4 %	42.66 ± 2.90^{aA}	53.33 ± 2.18^{aC} ◊20.68	51.00 ± 0 .57^{aBC} ◊40.00	47.00 ± 0.57^{Aab} ◊62.50

Results are expressed as mean ± SEM; n = 3; ◊ = Percentage inhibition of oedema calculated relative to control

Mean values having different lower-case letters as superscripts from top to bottom of the column and those having different upper-case letters as superscripts across the row are considered significant ($p < 0.05$).

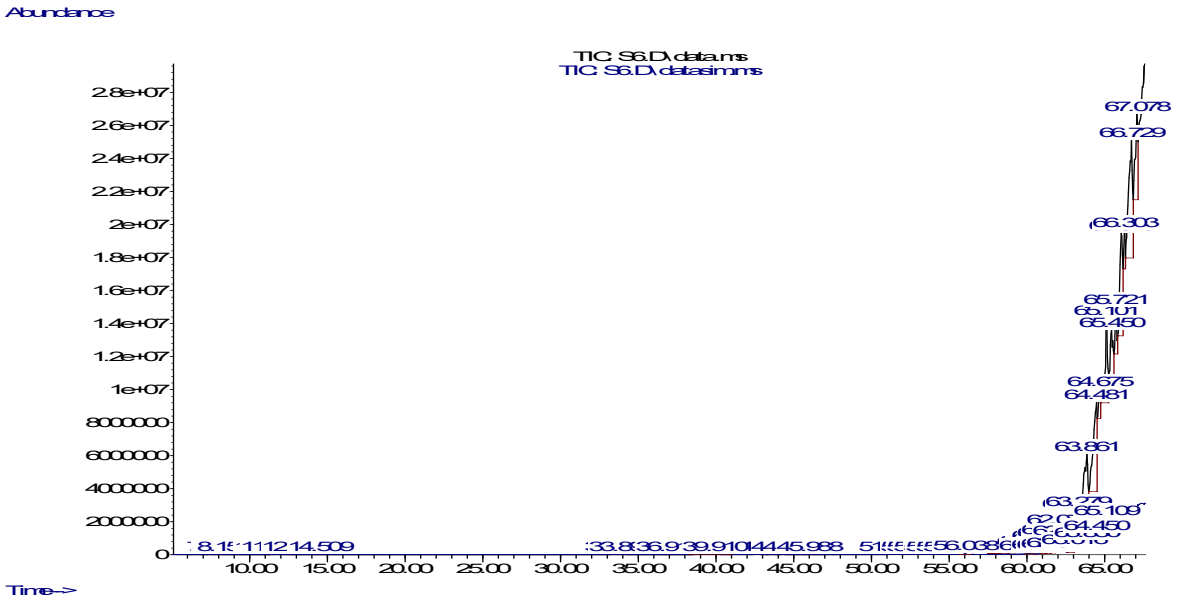
Abundance
TIC: S6.D\data.ms
TIC: S6.D\datasimms.ms
2.8e+07
2.6e+07
2.4e+07
2.2e+07
2e+07
1.8e+07
1.6e+07
1.4e+07
1.2e+07
1e+07
8000000
6000000
4000000
2000000
0
67.078
66.729
66.303
65.721
65.101
65.450
64.675
64.481
63.861
63.270
65.109
64.450
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00
65.00
Time-->

Figure 1.