*Asian Journal of Research in Medical and Pharmaceutical Sciences*

*3(1): 1-8, 2018; Article no.AJRIMPS.39303*
*ISSN: 2457-0745*

# Inhibition of the Activities of Pro-inflammatory Enzymes and Total Phenolic Contents of Leaf Extracts of *Strychnos spinosa*

**Adamu I. Isa[1,2*], Adebayo S. Adeniyi[2,3], Dzoyem J. Paul[2,4] and Jacobus N. Eloff[2]**

*[1]Department of Physiology, Faculty of Medicine, University of Bisha, Kingdom of Saudi Arabia.*
*[2]Phytomedicine Programme, Faculty of Veterinary Sciences, University of Pretoria, Private Bag X04, Ondersterpoort, 0110, South Africa.*
*[3]Department of Biomedical Sciences, Faculty of Science, Tshwane University of Technology, Private Bag X680, Pretoria, 0001, South Africa.*
*[4]Department of Biochemistry, Faculty of Science, University of Dschang, P.O.Box 67, Dschang, Cameroon.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author AII designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors ASA and DJP managed the analyses of the study. Author JNE managed the literature searches. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/AJRIMPS/2018/39303
Editor(s):
(1) Lokendra Bahadur Sapkota, Assistant Professor, Department of Biochemistry, Chitwan Medical College, Bharatpur, Nepal.
Reviewers:
(1) M. Sasikala, Karpagam College of Pharmacy, India.
(2) Ramaiah Maddi, Hindu College of Pharmacy, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/23340

***Original Research Article***

***Received 10th November 2017***
***Accepted 5th February 2018***
***Published 26th February 2018***

## ABSTRACT

**Aims:** *Strychnos spinosa* Lam. is a deciduous tree used in folkloric medicine to treat inflammatory-related conditions. The aim of this study was to establish the effect of leaf extracts of *S. spinosa* on the activity of pro-inflammatory cyclooxygenase (COX) enzymes and to determine the total phenolic contents of *S. spinosa* leaves.
**Study Design:** An *in vitro* study.
**Place and Duration of Study:** Department of Paraclinical Sciences, Faculty of Veterinary Sciences, University of Pretoria, Pretoria between June 2013 and November 2013.

**Corresponding author: E-mail: aiisa@ub.edu.sa;*

**Methodology:** Extracts were obtained by maceration with acetone, methanol and dichloromethane/methanol (1/1). Fractions were prepared by liquid–liquid fractionation of the acetone extract. COX activity was evaluated using a COX inhibitor screening assay kit, Cayman, MI, USA. The total phenolic content of the extracts were determined using the Folin–Ciocalteau method.

**Results:** The extracts significantly ($P$ = .05) inhibited COX enzyme activity. The n-butanol, water and hexane fractions selectively inhibited COX-1, with $IC_{50}$ values of 14.66 ± 0.01, 15.25 ± 0.20 and 14.93 ± 0.01 pg/mL, respectively. The dichloromethane/methanol extract (Dcm/MetE) and methanol extract (MetE) selectively inhibited COX-2, with $IC_{50}$ values of 15.51 ± 0.05 and 14.47 ± 0.12 pg/mL, respectively. The alkaloid fraction inhibited both COX-1 and COX-2, with $IC_{50}$ values of 15.42 ± 0.01 and 14.81±0.11 pg/mL, respectively. The water fraction had the highest phenolic content (78 ± 3.71 mg/g galic acetone extract , and the acetone extract had the lowest (8.0 ± 0.01 mg GAE/g).

**Conclusion:** The selective inhibition of COX-1 and COX-2 by the extracts point to the potential of *S. spinosa* as a potent anti-inflammatory agent. The results support the use of leaf extracts of *S. spinosa* in traditional medicine for the treatment of inflammation-related conditions.

*Keywords: Strychnos spinosa; anti-inflammatory; cyclooxygenase; phenolics.*

## 1. INTRODUCTION

*Strychnos* species are used in traditional medicine to treat snakebites, ulcers, wounds, headaches, gastric and intestinal problems, venereal diseases, leprosy, diarrhoea and fevers [1]. Analgesic uses of the fruit of the plant in folk medicine has been reported [2]. Anti-inflammatory and anti-microbial activities of *Strychnos* species have also been reported [3-5]. Cyclooxygenases are key enzymes in the synthesis of prostanoid and eicosanoids from polyunsaturated fatty acids, which are involved in various inflammatory and allergic disorders [6,7]. COX enzymes are membrane-bound heme proteins that exist in two distinct isoforms: a constitutive form (COX-1) and an inducible form (COX-2). COX-1 is expressed in resting cells of most tissues. It functions as a housekeeping enzyme and is responsible for maintaining homeostasis (gastric and renal integrity) and normal production of eicosanoids [7]. COX-2 is predominantly found in brain and kidney and is virtually absent in most other tissues [8,9]. COX-2 expression in tissues is significantly up-regulated in various acute and chronic inflammatory conditions [8,9]. Although conventional anti-inflammatory drugs can inhibit inflammation, they cannot cure chronic inflammatory disorders [9]. Therefore, there is a need for new, safe anti-inflammatory agents. A previous study showed that phenolics and flavonoids were excellent anti-inflammatory agents [10], indicating that natural products may play a significant role in human health in relation to the prevention and treatment of inflammatory conditions. Ongoing studies are investigating the potential of plant constituents used in herbal and traditional medicine as anti-inflammatory agents [11,12]. The aim of this study was to establish the effect of leaf extracts of *S. spinosa* on the activity of pro-inflammatory COX enzymes and to determine the total phenolic contents of *S. spinosa* leaves.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Preparation of Plant Material

Fresh leaves of *S. spinosa,* known as '*Kwokwa'* or '*Kokyi'* in Hausa in Nigeria [1], were collected in January 2013 from Sakara village, Zaria, Nigeria. The leaves were authenticated by a botanist (Mal Musa Muhammad) at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria. A voucher specimen (No. 900161) was deposited.at the herbarium section, Department of Biological Sciences. Ahmadu Bello University, Zaria.

#### 2.1.1 Preparation of extracts from *S. spinosa* leaves

The leaves were removed from the stem, packed in a well-perforated bag and air-dried under shade at room temperature for 2 weeks. The dried leaves were ground, powdered and kept in an air-tight polyethylene bag until required.

#### 2.1.2 Acetone, methanol and dichloro-methane/methanol extraction

The dried leaves were ground into a powder, and the powder (2 kg) was macerated three times in acetone (6 L) to obtain an acetone extract

(AcetE), followed by filtration and removal of the solvent in vacuum. The residues were macerated in methanol (6 L), following the same procedure as described for acetone extraction above to obtain a methanol extract (MetE). Part of the dried powdered leaves (1 kg) was macerated in a mixture of dichloromethane/methanol (50/50, v/v) (3 L) three times to obtain a dichloromethane/methanol extract (DcmMetE). After filtration and concentration of the extract to solid form, as shown in Fig. 1, part of the acetone extract (70 g) was dissolved in a mixture (50/50, v/v) of chloroform and water to obtain water and chloroform components. n-Butanol was added to the water component to obtain n-butanol and water fractions (n-ButF and WatF, respectively), and the chloroform component was concentrated to dryness and further dissolved in 10% water in methanol. A hexane fraction (HexF) and residue of 10% water in methanol were obtained after the addition of n-hexane. The proportion of water in methanol was increased to obtain a 35% water in methanol component. A chloroform fraction (ChlF) and 35% WatF were obtained following the addition of chloroform. From a comparative thin layer chromatography (TLC), water and 35% water in methanol ractions were combined into one fraction.

#### 2.1.3 Alkaloids extraction

The leaves of *S. spinosa* (1 kg) were macerated with a mixture (96:3:1, v/v) of Ethyl acetate/Ethanol Ammonium Hydroxide (EtOAc-EtOH-$NH_4OH$) (600 mL) and then percolated with EtOAc. The extract was obtained after removal of the solvent using a rotary evaporator under reduced pressure. The extract was further dissolved in EtOAc and extracted with 4% acetic acid to afford a EtOAc fraction. The acidic solution (pH 3-4) was basified to pH (8-9) with sodium carbonate and extracted three times with dichloromethane (DCM) to obtained crude alkaloids after removal of the solvent in vacuum..

### 2.2 Instruments and Reagents

COX-inhibitor screening kit (Catalog No. 560131, Cayman Chemical, USA), Indomethacin (Sigma), Folin–Ciocalteu reagent, gallic acid, were purchased from Sigma-Aldrich St.Louis, MO, USA.

#### 2.2.1 Determination of total phenolic constituents

The total phenolic constituents of the extracts were determined using the Folin–Ciocalteau method as described earlier [13], with some modifications. The crude extracts and fractions were dispensed into different test tubes at a concentration of 1:1 (mg/mL) plant material:extracting solvent (50 µL), and the volume was increased to 500 µL using distilled water. Folin–Ciocalteau reagent (250 µL) diluted with distilled water (1:1) and 1,250 µL of 20% sodium carbonate solution were added to the extracts and fractions. The mixture was vortexed and incubated at room temperature for 40 min. The absorbance was then read at 725 nm. The amount of polyphenols (expressed as milligrams of gallic acid/g dry weight) was calculated from a prepared standard curve for gallic acid (0.0019–0.25 mg/mL of gallic acid).

#### 2.2.2 Anti-inflammatory assays

##### *2.2.2.1 Cyclooxygenase 1 (COX-1) and Cyclooxygenase (COX-2) inhibition assay*

The inhibition of COX-1 and COX-2 activity in extracts and fractions from the leaves of *S. spinosa* was determined using a COX-inhibitor screening kit Cayman, MI, USA according to the manufacturer's instructions. The enzyme immunoassay used to determine COX-1 and COX-2 inhibitory activity has been described elsewhere [14]. The assay measures the production of $PGF_{2\alpha}$, which is generated by Tin(II) chloride ($SnCl_{2)}$ in the presence of $PGH_2$. The initial reactions took place in test tubes (initial reaction test tubes) heated at 37°C. In background test tubes, reaction buffer and heme were mixed together. In 100% activity test tubes containing reaction buffer and heme, the enzyme in question (COX-1 or COX-2) and solvent were added. In sample test tubes, the following was added: buffer, heme, different concentrations of the COX inhibitor and COX enzymes. All the test tubes (initial reaction test tubes, 100% activity test tubes, background test tubes, and sample test tubes) were incubated for 15 min at 37°C, followed by the addition of arachidonic acid and incubation for another 2 min. One molar hydrochloric acid (HCl) was added to each test tube to stop the reaction, and stannous chloride was used to trap the reaction product and reduce it to a more stable form. The test tubes were then incubated for 5 min at room temperature. The test tubes were diluted, with reaction buffer and the backgrounds (inactivated COX-1 and COX-2) were left as they were (backgrounds). A 96-well plate coated with mouse anti-rabbit immunoglobulin G (IgG) was provided. In the wells, nonspecific binding, maximum binding,

standards and inhibitor dilutions were added with tracer and antiserum. The plate was incubated at room temperature for 18 h, washed five times with wash buffer, developed with Ellman's reagent (MO, USA) and read on a microplate reader at 410 nm. The stock solution of the extracts and fractions were dissolved in dimethyl sulhoxide (DMSO) to give a final concentration of 15.6–200 pg/mL. The percent inhibition of enzyme (COX-1/COX-2) activity was calculated by a comparison of of treated and untreated groups. The concentration of the test compound causing 50% inhibition ($IC_{50}$, pg/mL) was calculated from the concentration–inhibition response curve. Indomethacin (1 mg/mL) was used as a reference standard.

## 3. RESULTS AND DISCUSSION

### 3.1 Phenolic Composition of the Fractions and Extracts of *S. spinosa* Leaves

All the extracts and fractions of *S. spinosa* contained phenolic compounds. However, the quantity varied between samples (8.0 ± 0.26–78.0 ± 2.30. mg GAE/g plant material) (Fig. 1). The MetE contained a significantly (*P* P = .05) higher amount (70 ± 0.27 mg GAE/g) of phenolic compound than the AcetE (8.0 ± 0.01 mg GAE/g) and DcmMetE (61.0 ± 0.9 mg GAE/g). Similarly, there was a significant (*P* = *.05*) difference in the phenolic content of the MetE and AcetE. There were no significant differences in the phenolic contents of the n-ButF, WatF and ethyl acetate fraction (EtAcF) (58.0 ± 1.00 mg GAE/g, 78.0 ± 1.30 GAE/g and 32.0 ± 1.1 GAE/g, respectively). However, there were significant (*P* P = .05) differences when compared with the HexF (8.0 ± 0.80 mg GAE/g) and ChlF (48.0 ± 0.89 mg GAE/g).

Polyphenolic compounds are important bioactive components of medicinal plant extracts and exhibit various pharmacological properties [15]. Phenolics are one of the main classes of secondary metabolites, and several thousand, including over 8,150 flavonoids, different compounds have been identified, with a large range of structures (monomeric, dimeric and polymeric) [16]. Many classes of phenolics are categorized on the basis of their basic skeleton. Phenolic phytochemicals are primarily natural antioxidants, which act as reducing agents, metal chelators and single oxygen quenchers [16].

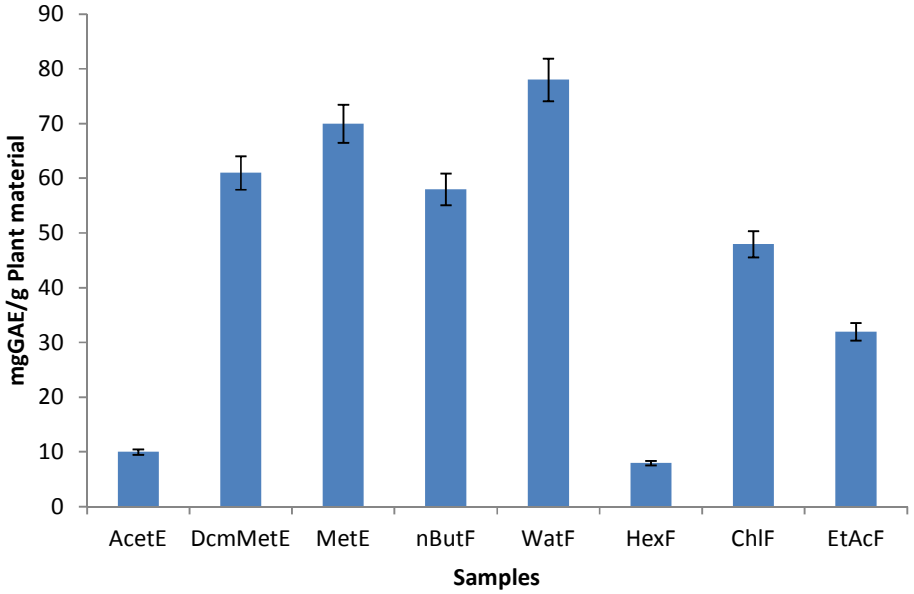


**Fig. 1. Total phenolic content of *S. spinosa* leaf extracts and fractions**

*Acetone extract, (AcetE); dichloromethane/methanol extract, (DcmMetE); methanol extract, (MetE); n-butanol fraction, (n-ButF); water fraction, (WatF); hexane fraction, (HexF); chloroform fraction, (ChlF); ethyl acetate fraction, (EtAcF). The different superscript letters denote a significant difference. (P = .05).*

Phenolic-enriched extracts have been reported to confer a wide range of physiological and health benefits [17]. For example, polyphenolic compounds have anti-allergenic, antiviral, antibacterial, antifungal [18] anti-secretory, anti-spasmolytic, anti-motility, anti-inflammatory, immuno-modulatory and parasitic activities. In traditional medicine, water or ethanolic solutions are the main solvents used in the preparation of plant extracts [18]. These solvents mainly extract polar compounds, primarily phenolic compounds [19]. In this study, the WatF had the highest content of phenolic compounds. The latter may explain its high anti-oxidant and anti-inflammatory activities when compared with those of the other extracts and fractions in this study.

Although the presence of secondary metabolites, such as triterpenoids, in addition to sterols, essential oils [20,21] secoiridoids [22] and monoterpenes [23], have been described in *S. spinosa*, there are no reports of the presence of phenolic and flavonoids in *S. spinosa* leaves.

### 3.2 COX Inhibition Assay

The effects of the extracts and fractions of *S. spinosa* on COX activity (COX-1 and COX-2) are presented in Fig. 2 and Table 1. The ButF, WatF and HexF selectively inhibited COX-1, with percentage inhibition of 71.08 ± 1.91%, 65.98 ± 1.62% and 68.65 ± 1.52%, respectively. The percentage inhibition did not differ significantly (*P = .05*) from that of indomethacin, which was used as the reference standard (80.12 ± 2.52%). MetE and DcmMetE selectively inhibited COX-2, with percentage inhibition of 72.06 ± 1.87% and 59.60 ± 1.33%, respectively. Indomethacin inhibited COX-2 (93.09 ± 3.20%), and the percentage inhibition did not differ significantly from the percentage inhibition of MetE and DcmMetE. The AlkE significantly inhibited both COX-1 and COX-2, with percentage inhibition of 60.87 ± 1.70% and 67.65 ± 2.00%, respectively, whereas indomethacin had an $IC_{50}$ of 3.50 ± 0.006 and 190.5 µM against COX-1 and COX-2, respectively.

The X-ray crystal structure of both enzymes suggests that the proteins share a very similar tertiary conformation [24]. The amino acids that serve as a substrate binding pocket and catalytic site are nearly identical in both enzymes [24].

Four levels of activity are defined in the COX assay, with activity below 20% considered insignificant, 20–40% considered low, 40–70% considered moderate and 70–100% considered high [25] Based on these criteria, the results in this study showed that the AlkE of *S. spinosa*

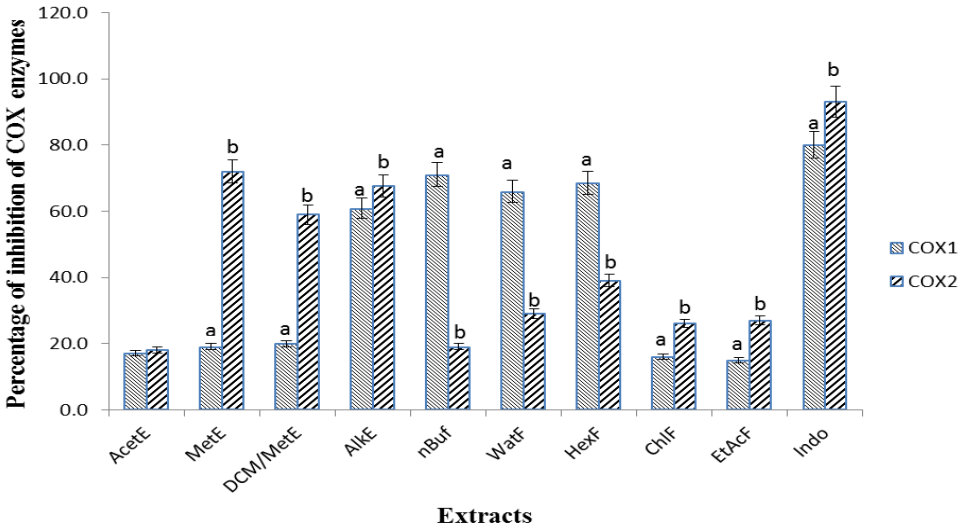


**Fig. 2. Inhibition of COX enzymes**

*[a,b] denote a significant difference.. Data represent mean ± SEM. COX-1, cyclooxygenase 1; COX-2, cCOX- 2; AcetE, acetone extract; MetE, methanol extract; DcmMetE, dichloromethane/methanol extract; AlkE, alkaloids extract; n-Buf, n-Butanol fraction; WatF, water fraction; HexF, n-hexane fraction; ChlF, chloroform fraction; EtAcF, ethyl acetate fraction; indo, indomethacin The different superscript letters denote a significant difference (P = .05).*

**Table 1. Anti-inflammatory activities of extracts and fractions from *S. Spinosa***

| Extracts | $IC_{50}$ (pg/mL) | |
|---|---|---|
| | COX 1 (units) | COX 2 (units) |
| AcetE | ND | ND |
| MetE | ND | 14.47 ± 0.12[a] |
| DCMMetE | ND | 15.51 ± 0.05[b] |
| AlkE | 15.42 ± 0.01 | 14.81 ± 0.11[a] |
| **Fractions** | | |
| nButF | 14.66 ± 0.01[a] | ND |
| WatF | 15.25 ± 0.20b | ND |
| HexF | 14.93 ± 0.01[a] | ND |
| ChlF | ND | ND |
| EtOAF | ND | ND |
| Indo | 3.5 ± 0.00[c] | 190 ± 0.01 |

**Means with different superscript letters are significantly (P = .05) different.*

leaves moderately inhibited both COX-1 and COX-2 activity, whereas the ButF, WatF and HexF selectively and significantly (*P = .05*) inhibited COX-1. The MetE and DcmMetE selectively inhibited COX-2 activity. The latter was likely due to the high contents of phenolics and alkaloids in the plant [23]. Phenolicsalkaloids and triterpenoids have been reported to exhibit anti-inflammatory activity in previous studies [26]. The studies showed that they exerted anti-oxidative properties, reduced superoxide dismutase ($O^{2\cdot-}$) and malondialdehyde (MDA) production, and reduced plasma extravasations and cell migration, mainly of leukocytes. Furthermore, they potentiated the radical scavenging activity of superoxide dismutase SOD [23]. Reactive species are one of the most important mediators in provoking and sustaining inflammatory processes [23,26,27]. Consequently, their removal by antioxidants and radical scavengers, such as phenolic compounds, has the potential to alleviate inflammation [26,27]. Previous research reported that ROS played a regulatory role in the expression of COX, particularly COX-2 and subsequent synthesis of prostaglandin $E_2$ ($PGE_{2)}$, leading to inflammation [23]. Therefore, it is not surprising the AlkE inhibited both COX-1 and COX-2 in the present study. A previous study reported that alkaloids inhibited COX-1 and COX-2 enzymes [28]. Research also suggested that inhibition of COX-1 may lead to adverse effects, such as gastric ulceration, and increase risk of adverse cardiovascular events. As a result, much emphasis has been placed on the development of selective COX-2 inhibitors [27].

## 4. CONCLUSION

The selective inhibition of COX 1 and COX 2 by the extracts point to the potential of *S.spinosa* as a potent anti-inflammatory agent. The results support the use of extracts from *S. spinosa* leaves in traditional medicine for the treatment of inflammation-related conditions.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Niswanger HD. African ethnobotany poisons and drugs: Chemistry, pharmacology, toxicology. Chapman & Hall. London. 1996;941.
2. Msonthi JD, Galeffi C, Nicoletti M, Messana I. Research of african medicinal-plants kingiside aglucone, a natural secoiridoid from unripe fruits of strychnos-spinosa. Phytochemi. 1985;24:771-772.
3. Isa AI, Awouafack MD, Dzoyem JP, Aliyu M, Magaji RA, Ayo JO, Eloff JN. Some *Strychnos spinosa* (Loganiaceae) leaf extracts and fractions have good antimicrobial activities and low cytotoxicities. BMC Complem Altern M. 2014a;14:456.
4. Isa AI, Adebayo SA, Mohammed A, Magaji RA, Ayo JO, Suleiman MM. In-vitro lipoxygenase inhibitory activity and total flavonoid of *Strychnos spinosa* leaf

extracts and fractions. Niger J Pharm Sci. 2014b;13:35-42.

5. Isa AI, Dzoyem JP, Adebayo SA, Suleiman MM, Eloff JN. Nitric oxide inhibitory activity of *Strychnos spinosa* (Loganiaceae) leaf extracts and fractions. Afr J Tradit Complement Altern Med. 2016;13(6):22-26.
6. Martel-Pelletier JM, Lajeunesse D, Reboul P, Pelletier JP. Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs. Ann Rheum Dis. 2003;62:501–509.
7. Daniel M, Ana IR, Marta S, Raquel D, German G, Jawed A, Carlos MR de G Antonio. Regulation of heme oxygenase-1 expression through the phosphatidylinositol 3-kinase/akt pathway and the nrf-2 transcription factor in response to the antioxidant phytochemical carnosol. J Biol Chem. 2004;279:8919–8929.
8. Xu X. C. COX-2 inhibitors in cancer treatment and prevention, a recent development. Anticancer Drugs. 2002;13:127.
9. Jiménez P1, García A, Santander S, Piazuelo E. Prevention of cancer in the upper gastrointestinal Curr. Pharm. Des. 2007;13(22):2261-2273.
10. Hendra R, Ahmad S, Oskoueian E, Sukari A, Shukor MY. Antioxidant, antiinflammatory and cytotoxicity of *Phaleria macrocarpa* (Boerl) scheff fruit. Biomedcentral BMC Complem Altern M. 2011;11:110.
11. Nam NH. Naturally occurring NF-B inhibitors. Mini-Reviews in Medicinal Chemistry. 2006;6:945–951.
12. Read MA. Flavonoids: Naturally occurring anti-inflammatory agents. Am J Pathol. 1995;147:235–242.
13. Makkar HPS, Francis G, Becker K. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production system. Animal. 2007;1(9):1371-1391.
14. Vundac VB, Brantner AH. Plazibat M. Content of polyphenolic constituent and antioxidant activity of some stachys taxa. Food Chem. 2007;104:1277-1281.
15. Lattanzio V, Lattanzio VMT, Cardinali, A. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. Phytochem Advanc Res. 2006;23-67.
16. Pietta PG. Flavonoids as antioxidants. J. Nat. Prod. 2000;63:1035–1042.
17. Ahmed AS, Elgorashi EE, Moodley N, McGaw LJ, Naidoo V. Eloff JN. The antimicrobial, antioxidative, anti-inflammatory activity and cytotoxicity of different fractions of four South African bauhinia species used traditionally to treat diarrhea. J. Ethnopharmacol. 2012; 143:826–839.
18. Hoet S, Pieters L, Muccioli GG. Habib-Jiwan JL. Antitrypanosomal activity of triterpenoids and sterols from the leaves of *Strychnos spinosa* and related compounds. J. Nat. Prod. 2007;70:1360-136.
19. Ohri FC, Verpoorte R. Baerheim-svendsen A. 12-Hydroxy-11-methoxy-diaboline: A new alkaloid from *Strychnos spinosa* [stem bark]. Planta Med. 1984;50:446-7.
20. Itoh A, Oya N, Kawaguchi E. Nishio S. Secoiridoid glucosides from *Strychnos spinosa*. Nat. Prod. 2005;68:1434-6.
21. Adesogan EK, Morah FN. Stryspinolactone, an unusual monoterpene lactone from *Strychnos spinosa*. Phytochem. 1981;20:2585-86.
22. Al-Hourani BJ, Sharma SK, Mane JY, Baracos TJ, Kniess V, Suresh T. Pietzsch MJ, Wuest F. Synthesis and evaluation of 1, 5-diaryl-substituted tetrazoles as novel selective cyclooxygenase-2 (COX-2) inhibitors. Bioorganic Med. Chem. 2011;21:1823–1826.
23. Taylor JLS, Van Staden J. COX-1 inhibitory activity in extracts from Eucomis L'Herit. Species. J. Ethnopharmacol. 2001;75:257–265.
24. Nardi GM, Siqueira JM, Monache BF, Pizzolatti MG, Ckless K, Ribeiro-do-Valle RM. Antioxidant and anti-inflammatory effects of products from Croton celtidifolius Bailon on carrageenan-induced pleurisy in rats. Phytomed. 2007;14:115-122.
25. Delaporte RH, Sanchez GM, Cuellar AC, Giuliani A, Palazzo de Mello JC. Anti-inflammatory activity and lipid peroxidation inhibition of iridoid lamiide isolated from *Bouchea fluminensis* (vell.) Mold. (Verbenaceae). J. Ethnopharmacol. 2002; 82:127-130.

26. Geronikaki AA, Gavalas AM. Antioxidants and anti-inflammatory diseases: Synthetic and natural antioxidants with anti-inflammatory activity. Comb Chem High Throughput Screen. 2006;9:425-442.
27. Aderogba MA, Ndhlala AR, Rengasamy KRR, Staden JV. Antimicrobial and selected In Vitro enzyme inhibitory effects of leaf extracts, flavonols and indole alkaloids isolated from *Croton menyharthii*. Molecules. 2013;18:12633-12644.
28. Augusto LS, Josean FT, Marcelo Sobral DS, Margareth FFMD, PF AF, José MBF. Anti-Inflammatory activity of alkaloids: An update from 2000 to 2010, Molecules. 2011;6(10):8515-8534.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://www.sciencedomain.org/review-history/23340*