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SHORT COMMUNICATION

Evaluation of the anti-inflammatory activities of solvent fractions of *Commelina ascendens*

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ABSTRACT

To evaluate the anti-inflammatory properties of the fractions of *Commelina ascendens* in rats. Egg albumin induced paw oedema and cotton pellet induced granuloma in rats were used to determine the anti-inflammatory activities of n-hexane (HF), ethylacetate (EF) and butanol (BF) fractions of *Commelina ascendens*. BF (200 mg/kg) significantly inhibited ($p<0.05$) oedema induced by egg albumin by producing 3.22 % protection at 4 h post-treatment. EF, HF and BF significantly ($p<0.05$) decreased the granuloma tissue caused by cotton pellet. The phytochemical screening of HF, EF and BF revealed the presence of saponin, tannins, flavonoids, steroids, carbohydrates. The extract did not show any sign of toxicity up to 5000 mg/kg body weight of mice. The results obtained justified the use of this plant in traditional medicine for the treatment of inflammation and may provide a basis for the development of anti-inflammatory agents.

Keywords: *Commelina ascendens*, egg albumin, cotton pellet, inflammation

INTRODUCTION

The search for new pharmacologically active agents has led to the screening of natural plants that are used for the treatment of human diseases.

Inflammation is a highly regulated protective response which helps in eliminating the initial cause of cell injury and initiates the process of repair (Brown et al., 2007). Inflammatory processes has led to tissue injury in autoimmune diseases like rheumatoid arthritis and other inflammatory conditions. The current available drugs for the treatment of inflammatory conditions and rheumatoid arthritis includes steroids, analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), disease –modifying anti-rheumatic drugs (DMARDs) and immunosuppressive agents, which have side effects on the gastrointestinal tract, blood cell count, hair and immune system (Tristano, 2009). These side effects has led to the search for new drug moiety, which are cheap and has minimal side effects. Natural phytochemical constituents derivable from medicinal plants could serve as a better alternative strategy for the effective treatment

of rheumatoid arthritis (Khanna et al., 2007).

One of such plants with putative anti-inflammatory activity is Commelina ascendens JK Morton. *Commelina ascendens* J.K. Morton belongs to the Commelinaceae family. It is found in the primary and secondary lowland rain-forest, often by rivers or streams (Morton, 1956). It is a scandent herb of 8ft length. It has stems rooting at lower nodes, lanceolate leaves up to 11 cm long and 3 cm broad, and pale blue flowers opening early in the day and fading within three hours of dawn (about 7-9 am) (Morton, 1956, Hutchinson and Dalziel, 1954). It is used traditionally in the eastern part of Nigeria for the treatment of boils, skin ulcers, cuts, wounds etc. Although the description of the plant is well documented, information on its pharmacological and phytochemical properties is sparse. The anti-inflammatory study of crude extract of the plant has been documented (Onwuka et al., 2018). This study aimed to validate the anti-inflammatory activity of the n-hexane, ethylacetate and butanol fractions of the plant.

MATERIALS AND METHODS

Collection, authentication and preparation of plant material. The fresh aerial part of *Commelina ascendens* were collected at Enugu and authenticated by Mr. A. Ozioko, a taxonomisst at Bioresources Development and Conservation Programme (BDCP) Center, Nsukka, Enugu State, Nigeria. A dried voucher specimen was preserved in the herbarium of the International Centre for Drug Development (Inter-CEDD) Nsukka, Enugu State with voucher number: InterCEDD/16295

The fresh aerial parts of *Commelina ascendens* were cleaned, cut into smaller pieces and dried under the shade to minimize loss of volatile compound and reduced to a coarse powder using milling machine. The coarse powder (3.1 kg) was held in reserve in an air tight container before use. About 3.1 kg of the powdered material was extracted by cold maceration in 10 L of a 1:1 mixture of Methanol-Dichloromethane for 72 hours with intermittent vigorous shaking every two hours. The extract was strained with a muslin cloth and filtered with Whatman No. 1 filter paper. The extract was concentrated in an electric rotary evaporator set at 40^0C to obtain the methanol-dichloromethane extract (CAE). The concentrated extract was stored in a sealed amber bottle and stored at 4^0C in a refrigerator before use.

Solvent-guided fractionation of CAE. Methanol-dichloromethane extract CAE (63 g) was subjected to solvent-solvent fractionation using solvents of increasing polarity in the following order: n-Hexane, ethylacetate and butanol. The fractionation was accomplished by mixing the aqueous CAE (63 g) in a separating funnel in order of increasing polarity using separating funnel. The fractions were collected and concentrated using rotary evaporator at 40^0C to obtain n-Hexane (HF), Ethylacetate (EF) and Butanol (BF) fractions.

Phytochemical screening. The fractions were subjected to phytochemical screening according to standard procedures (Evans, 2009; Harbourne 1998).

Experimental Animals. Adult Swiss albino rats (150-200 g) (12 weeks) and Swiss albino mice (17-25 g) (6 weeks) of both sexes were obtained from the laboratory animal facility of the department of pharmacology and toxicology, University of Nigeria, Nsukka. The animals were kept in individual steel cages within the facility and allowed free

access to clean water and livestock pellets. The animals were kept in a well-ventilated room with a 12/12 h light/dark conditions and ambient room temperature. Animal experiments were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub. No. 85-23, revised 1985) and following the University Ethics Committee on the use of laboratory animals.

Acute toxicity tests (LD50). The mean lethal dose (LD_{50}) of the methanol-dichloromethane extract (CAE) of Commelina ascendens in mice was estimated using the method described by Lorke (Lorke, 1983). A total of 13 mice of either sex were fasted overnight before the study. The study was carried out in two stages. In stage one, nine mice were divided into 3 groups (n=3), received oral administration of 10, 100 and 100 mg/kg of CAE (prepared in 3% tween 80) and were observed for 24 hours for some deaths. At the end of 24 hours, no death was recorded. A fresh batch of mice divided into four groups (n=1) received 1600, 2900, 3600 and 5000 mg/kg of CAE in the second stage of the study and were observed for 24 hours for death.

Anti-inflammatory activity test. The n-hexane, ethylacetate and butanol (HF, EF and BF) fractions were prepared as suspension in 3% Tween 80 and tested orally for the anti-inflammatory activity using two anti-inflammatory models.

Egg Albumin Induced Paw Oedema in Rat. This test was performed according to the method described by Winter et al., (Winter et al., 1962). Briefly, rats were randomized into eight groups (n=5). Group I received 2 ml/kg of 3% tween 80, group II received 10 mg/kg indomethacin, groups III and IV received HF (100 mg/kg and 200 mg/kg), groups V and VI received EF (100 mg/kg and 200 mg/kg) while groups VII and VIII received BF (100 mg/kg and 200 mg/kg).

Thirty minutes after the administrations, inflammation was induced by subplantar injection of 0.1 ml of fresh undiluted egg albumin (Okoli and Akah, 2000). Oedema was assessed in terms of volume of distilled water displaced by the paw before and at 0.5, 1, 2, 3, 4, 5 and 6 hours after the induction of inflammation. The level of inhibition of oedema was calculated for each extract using the relation (Perez, 1996):

Inhibition (%) = $100[1 - \frac{a-x}{b-y}]$

Where a = mean paw volume of treated animals after egg albumin injection

x = mean paw volume of treated animals before egg albumin injection

b = mean paw volume of control animals after egg albumin injection

y = mean paw volume of control animals before egg albumin injection

Cotton Pellet Induced Granuloma in Rats. Implantation of cotton pellets followed the method described by Rathi et al., (Rathi et al., 2004). Briefly, rats were randomized into eight groups (n=5). The animals were then anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (10 mg/kg) before two sterile cotton pellets (20 mg each) were surgically implanted subcutaneously in both axilla regions of each rat following a single incision, which was thereafter closed by interrupted sutures. The animals were placed individually in a metal cage after grouping to avoid them biting each other. After the surgery, the animals were allowed to recover and treatment commenced

the next day. The rats received oral treatment of tween 80, diclofenac potassium, HF, EF and BF once daily for seven days. Group I received 2 ml/kg of 3% tween 80 (negative control), group II served as the positive control and received 10 mg/kg diclofenac potassium, groups III and IV were administered HF (100 mg/kg and 200 mg/kg), groups V and VI were administered EF (100 mg/kg and 200 mg/kg) while groups VII and VIII received BF (100 mg/kg and 200 mg/kg). On the 8th day, the animals were sacrificed with chloroform. The implanted cotton pellets together with the granuloma tissues were carefully removed. The pellets were weighed immediately for wet weight. Then, pellets were dried in an oven at 60 º C until a constant weight was obtained.

Calculation. The final dry weight was calculated after deducting cotton pellet weight and taken as a measure of granuloma tissue formation (Jian- Yu et al., 2011).

Wet weight of cotton pellet = weight of the cotton pellet (wet) - weight of the cotton pellet
Dry weight of cotton pellet = weight of the cotton pellet (dry) - weight of the cotton pellet.
The increase in the wet weight and dry weight of the granuloma tissue formed was calculated relative to the control (3% tween 80), thus:

$$\% \text{ Inhibition} = \left[\frac{Wc - Wd}{Wc}\right] \times 100$$

Wc = Difference in pellet weight of the control group

Wd = Difference in pellet weight of the drug treated group

Statistical analysis. Data obtained was analyzed by One way ANOVA followed by Dunnett's multiple comparisons post-hoc test using Graphpad Prism version 5.0. The values were expressed as Mean ± standard error of mean (SEM). $p<0.05$ was considered statistically significant.

RESULTS

The methanol-dichloromethane extract of *Commelina ascendens* yielded 3.7% w/w of CAE while the yield of the various solvent fractions HF, EF and BF were 28.6% w/w, 12.7% w/w and 10.3% w/w. The phytochemical screening of the fractions showed the varying presence of saponin, alkaloids, tannins, flavonoids, steroids, carbohydrates. The oral administration of CAE up to 5000 mg/kg dose did not produce lethality or sign of acute toxicity in mice after 48 h.

Effect of the fractions of Commelina ascendens on egg albumin induced rat paw ooedema. The fractions of Commelina ascendens inhibited rat paw oedema induced by egg albumin. The butanol fraction (200 mg/kg) significantly inhibited ($p<0.05$) oedema induced by egg albumin by producing 3.22 % protection at 4 h post-treatment. At 5h, HF (100 mg/kg and 200 mg/kg), EF (200 mg/kg) and BF (20 mg/kg) significantly ($p < 0.05$) inhibited paw oedema by 16.13% , 19.35%, 32.26% and 3.23% respectively when compared to the control (3% tween 80) (Table 1).

TABLE 1: The effect of the fractions of *Commelina ascendens* on egg albumin induced rat paw oedema

TREATMENT	DOSE (mg/kg)	Oedema volume (ml) (% inhibition)						
		0.5h	1h	2h	3h	4h	5h	6h
Control (3% Tween80)	2 ml/kg	1.78±0.17	1.72±0.15	1.64±0.15	1.58±0.12	1.58±0.13	1.58±0.11	1.44± 0.13
HF	100	1.54±0.04 (4.88)	1.51±0.05 (1.32)	1.46±0.06 (NI)	1.38±0.08 (NI)	1.42±0.04 (NI)	1.28± 0.04* (16.13)	1.22±0.02 (4.17)
HF	200	1.62±0.02 (NI)	1.58±0.02 (NI)	1.46±0.02 (NI)	1.44±0.02 (NI)	1.37±0.03 (NI)	1.28±0.05* (19.35)	1.28±0.04 (NI)
EF	100	2.16±0.07 (NI)	2.12±0.07 (NI)	1.98±0.05 (NI)	1.72±0.07 (NI)	1.56±0.07 (25.81)	1.38±0.07 (54.84)	1.30±0.04 (58.33)
EF	200	1.94±0.07 (NI)	1.84±0.10 (NI)	1.74±0.10 (NI)	1.45±0.08 (8.06)	1.38±0.07 (19.35)	1.30±0.05* (32.26)	1.24±0.08 (25.00)
BF	100	2.23±0.11 (NI)	2.13±0.07 (NI)	2.00±0.08 (NI)	1.75±0.11 (6.58)	1.62±0.07 (6.45)	1.44±0.10 (35.48)	1.38±0.09 (29.16)
BF	200	1.84±0.09 (NI)	1.72±0.10 (NI)	1.65±0.09 (NI)	1.38±0.07 (NI)	1.28±0.05* (3.22)	1.28±0.05* (3.23)	1.18±0.08 (NI)

Values expressed as Mean ± SEM; n=5 animals per group. Results were analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett' post hoc test.* $p < 0.05$. NI – no inhibition
HF: n-Hexane Fraction EF: Ethylacetate Fraction BF: Butanol Fraction

Effect of the fraction of Commelina ascendens on cotton pellet induced granuloma in rats. The oral administration of HF (100 mg/kg), EF (100 mg/kg) and BF (100 and 200 mg/kg) significantly ($p<0.05$) decreased the wet weight of the cotton pellets granuloma of rats when compared to the control. Treatment with HF (100 and 200 mg/kg) and EF (100 mg/kg) significantly ($p<0.05$) while EF (200 mg/kg) and BF (100 and 200 mg/kg) significantly ($p<0.01$) decreased the dry weight of the cotton pellets when compared with the control (Figure 1).

DISCUSSION

The phytochemical screening results revealed that all the fractions contain appreciable amounts of saponin, tannin, alkaloids, flavonoids and steroids. Flavonoids have been reported to possess potent inhibitory effect on enzymes involved in the production of the chemical mediators of inflammation and metabolism of arachidonic acid (Oweyele et al., 2005; Metowogo et al., 2008; Kumar et al., 2011; Saleem et al., 2011; Vijayalakshmi et al., 2011). The fractions of *Commelina ascendens w*ere tested at two different dose levels (100 and 200 mg/kg). The n-hexane, ethylacetate and butanol fractions of *Commelina ascendens* were found to inhibit oedema induced by cotton pellet and egg albumin.

Oedema that develops as a result of egg albumin administration is a biphasic event. According to Wallace (2002), the early phase of egg albumin induced oedema lasts for 2 h with release of inflammatory mediators like histamine and serotonin while the last phase occurs from 3 – 5 h after administration of irritant with release of bradykinin, protease, prostaglandins and lysosomes. The suppression of oedema in the second phase of inflammation suggests that the fractions of *Commelina ascendens* may act through suppression of the prostaglandins, protease and kinnins released due to egg albumin.

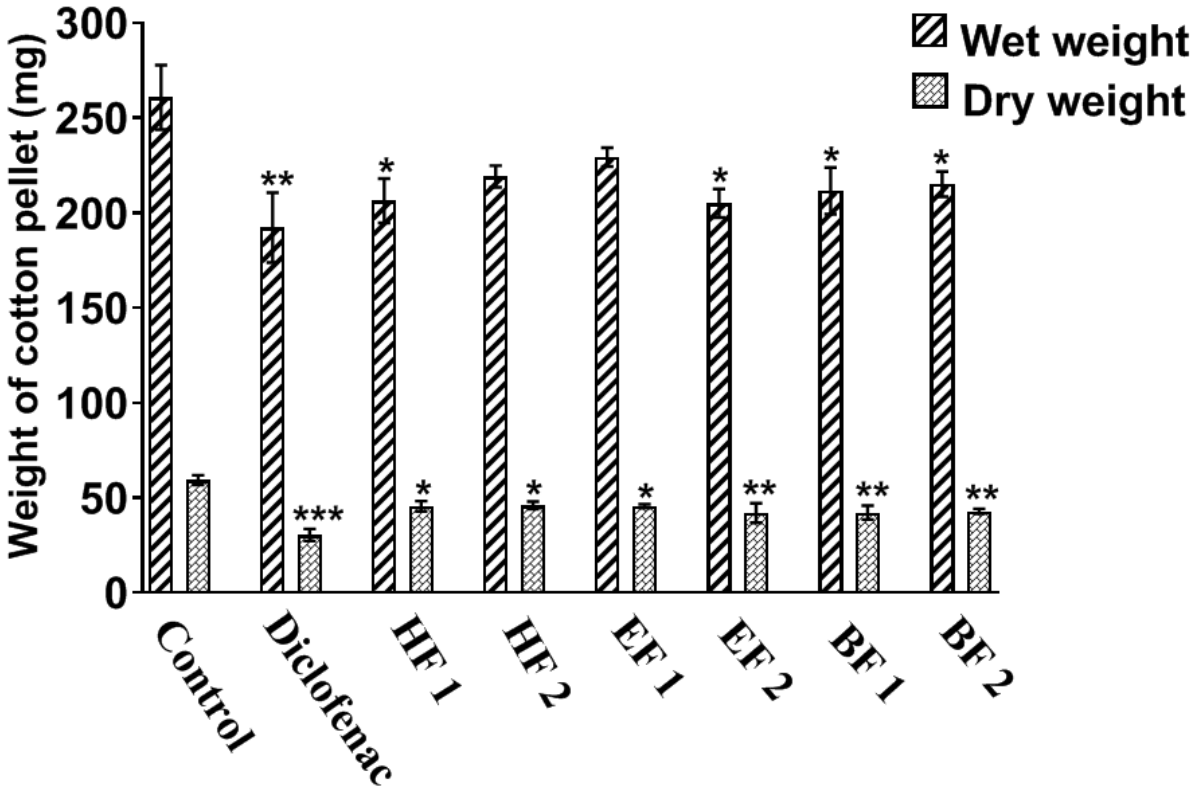

Figure 1: Effect of fractions of *Commelina ascendens* on cotton pellet induced granuloma in rats. n= 5, values are expressed in Mean + S.E.M. *p<0.05, **p<0.01, ***p<0.001, HF 1= n-Hexane fraction 100 mg/kg, HF 2= n-Hexane fraction 200 mg/kg, EF 1= Ethylacetate fraction 100 mg/kg, EF 2= Ethylacetate fraction 200 mg/kg, BF 1= Butanol fraction 100mg/kg, BF 2= Butanol fraction 200 mg/kg.

According to Panthong *et al*, (2004) antiproliferative effects of drugs can be evaluated by cotton pellet test. The transudative, exudative and proliferative phases of chronic inflammation can be evaluated using Cotton pellet induced granuloma test (Swingle and Shideman, 1972, Boonyarikpunchai et al., 2014). The transudative phase occurs within 3 h after cotton pellet implantation and is characterized by increased vascular permeability which results to leakage of fluid from blood vessels. The exudative is characterized by protein leakage around the granuloma and it occurs from 3 -72 h after cotton implantation. The last phase, proliferative phase lasts from 3 to 6 days and is characterized by the development of granuloma tissue as a result of the release of pro-inflammatory mediators (Boonyarikpunchai et al., 2014; Pingsusaen et al., 2015). The moist weight of the pellets correlates with transude, the dry weight of the pellet correlates with the amount of granulomatous tissue formed (Paschapur et al., 2009).

The n-hexane, ethylacetate and butanol fractions of *Commelina ascendens* reduced both the wet and dry weight of the granuloma formed. This suggests that the fractions are effective against the proliferative phase of inflammatory processes. This result also denotes possible activity of *Commelina ascendens* fractions in rheumatoid arthritis.

CONCLUSION

This study has demonstrated that *Commelina ascendens* fractions possess anti-inflammatory properties which may be mediated through mechanisms involving inhibition of release of inflammatory substances like his-

tamine, prostaglandins, kinnins and inhibition of chronic inflammation. This study reveals that *Commelina ascendens* is a potential candidate for the treatment of inflammation related diseases. Further studies need to be carried out to determine the anti-inflammatory mechanism of action of *Commelina ascendens.*

CONFLICT OF INTEREST

The author declares no conflicts of interest.

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REFERENCES

Aggarwal BB. Natural products as a gold mine for arthritis treatment. Curr Opin Pharmacol 2007; 7(3):344-51.

Boonyarikpunchai W, Sukrong S, Towiwat P. Antinociceptive and anti-inflammatory effects of rosmarinic acid isolated from Thunbergia laurifolia Lindl. Pharmacol Biochem Behav 2014; 124:67-73.

Brown KL, Cosseau C, Gardy JL, Hancock RE. Complexities of targeting innate immunity to treat infection. Trends Immunol 2007; 28(6):260–6.

Evans WC. Trease and Evans Pharmacognosy (16th ed) Edinburgh: WB Saunders; 2009

Harbourne JB. Textbook of Phytochemical Methods: A guide to modern techniques of Plant Analysis. Chapman and Hall Ltd: London. 1998; p.12.

Hutchinson J, Dalziel JM. Flora of West Tropical Africa, Vol 3, Part 1, by Royal botanic gardens publishers; 1954.

Jian-Yu S, Qing-Chan L, Liang Z. Evaluation of the in vivo anti-inflammatory activity of a flavone glycoside from Cancrinia discoidea (ledeb.) poljak. EXCLI J 2011; 10:110-116.

Khanna D, Sethi G, Ahn KS, Pandey MK, Kunnumakkara AB, Sung B, Aggarwal A, Aggarwal BB. Natural products as a gold mine for arthritis treatment. Curr Opin Pharmacol 2007; 7(3):344-51.

Kumar S, Kumar V, Prakash OM. Pharmacognostic study and anti-inflammatory activity of Callistemon lanceolatus leaf. Asian Pac J Trop Biomed 2011; 1(3):177-181.

Lorke D. A new approach to practical acute toxicity testing. Arch. Toxicol 1983; 53:275-287.

Metowogo K, Agbonon A, Eklu-Gadegbeku K, Aklikokou AK, Gbeassor M. Anti-ulcer and anti-inflammatory effects of hydroalcohol extract of Aloe buettneri A. Berger (Lilliaceae). Trop J Pharm Res 2008; 7(1):907.

Morton JK. Cytotaxonomic studies on the Gold Coast species of the genus Commelina Linn. J Linn Soc Bot, 1956; 55(361):507-531.

Okoli CO, Akah PA. A pilot evaluation of the anti-inflammatory activity of Culcasia scandens, a traditional anti-rheumatic agent. J Tradit Complement Med 2000; 6(5): 423-427.

Onwuka AM, Nworu CS, Peter IE, Mbaoji FN, Obi BC, Onyeto AC, Nwabunike IA, Isiogugu ON, Ofokansi MN, Charles O, Akah PA. Evaluation of the anti-inflammatory activity of the Commelina ascendens J.K Morton (Commelinaceae) aerial part extract. Academic Journal 2018; 86:EA232.

Oweyele VB, Oloriegbe YY, Balaogun EA, Soladoye AO. Analgesic and anti-inflammatory properties of Nelsonia canescens leaf extract. J Ethnopharmacol 2019; 99(1):153-156.

Panthong A, Kanjanapothi D, Taesotikul T, Phankummoon A, Panthong K, Reutrakul V. Anti-inflammatory activity of methanolic extract from Ventilago harmandianai Pierre. J Ethnopharmacol 2004; 91(2-3):237-242.

Paschapur MS, Patil M B, Kumar R, Patil SR. Evaluation of anti-inflammatory activity of ethanolic extract of Borassus flabellifer L. male flowers (inflorescences) in experimental animals. J Med Plants Res 2009; 3(2):49-54.

Perez GRM. Anti-inflammatory activity of Ambrosia artemisiifolia and Rheo spathacea. Phytomedicine 1996; 3(2):163-167.

Pingsusaen P, Kunanusorn P, Khonsung P, Chiranthanut N, Panthong A, Rujjanawate C. Investigation of anti-inflammatory, anti-nociceptive and antipyretic activities of Stahlianthus involucratus rhizome ethanol extract. J Ethnopharmacol 2015; 162:199-206.

Rathi B, Patil PA, Baheti AM. Evaluation of aqueous extract of pulp and seeds of Moringa oleifera for wound healing in albino rats. J Nat Remedies 2004; 4(2):145–149.

Saleem TKM, Azeem AK, Dilip C, Sankar C, Prasanth NV, Duraisami R. Anti-inflammatory activity of the leaf extracts of Gendarussa vulgaris Nees. Asian Pac J Trop Biomed 2011; 1(2): 147-149.

Swingle K, Shideman F. Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their modification by certain anti-inflammatory agents. J Pharmacol Exp Ther 1972; 183(1):226-234

Tristano AG. Tyrosine kinases as targets in rheumatoid arthritis. Int Immunopharmacol 2009; 9:1-9

Vijayalakshmi A, Ravichandiran V, Velraj M, Hemalatha S, Sudharani G, Jayakumari S. Anti-anaphylactic and anti-inflammatory activities of a bioactive alkaloid from the root bark of Plumeria acutifolia Poir. Asian Pac J Trop Biomed 2011; 1(5):401-405.

Wallace JM. Nutritional and botanical modulation of the inflammatory cascade: eicosanoids, cyclooxygenase and lipoxygenase-as an adjunct in cancer therapy. Integr Cancer Ther 2002; 1:7-37.

Winter C A, Risley E A, Nuss G W. Carrageenin-induced oedema in the hind paw of rat as an assay for anti-inflammatory drugs. Proc Soc Exp Bio Med 1962; 111: 544-547.

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