Trends in
Natural Products Research

Fractions of *Monodora myristica* (Gaertn.) Dunal (Annonaceae) root bark possesses anti-inflammatory activity

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Keywords: Carrageenan, formaldehyde, granuloma, *Monodora myristica*, xylene

Abstract: This evaluated the acute and chronic anti-inflammatory activity of *Monodora myristica* root bark extract and fractions in rodents. The methanol crude extract (CE) as well as the hexane (HF), ethyl acetate (EAF) and methanol (MF) fractions were prepared and screened for anti-inflammatory activity employing carrageenan induced rat paw edema, xylene induced topical ear edema, formaldehyde induced arthritis (FIA) and the cotton pellet induced granuloma (CPIG) models. Phytochemical screening as well as acute toxicity (LD_{50}) tests were equally evaluated. The EAF (400 mg/kg and 800 mg/kg) and MF (200 mg/kg) elicited significant ($p < 0.05$) and time-course inhibition of carrageenan induced rat paw edema. All the fractions exhibited significant ($p < 0.05$) inhibition of xylene induced topical ear edema. The extract and fractions evoked moderate inhibition of formaldehyde induced arthritis and the cotton pellet induced granuloma. The fractions gave positive reaction for alkaloids, glycosides, saponins, flavonoids, tannin, acid compounds, resins, steroids, terpenoids, protein, reducing sugars, carbohydrates, fats and oil in varying degrees. The acute toxicity screening showed no mortality at 5000 mg/kg of the extract and fractions after 48 hours of observation period. The results suggests that the root bark of *M. myristica* possesses anti-inflammatory effect which corroborates its folkloric use in managing edematous conditions.

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DOI: 10.48245/tnpr-2734391.2023.4.203
Page No.: 61-70
Volume: 4, Issue 2, 2023
Trends in Natural Products Research

INTRODUCTION

Recent clinical studies have indicated that deaths associated with COVID are mostly related to the cytokine storm leading to overwhelming inflammation of cells, tissues, and the airways (Moore and June, 2020). Thus, agents that inhibit inflammation may likely reduce mortality at the moment. Dexamethasone has been found useful as it has a potent anti-inflammatory effect (Ruat*et al*., 2020) but its adverse effects over prolonged use pose a major concern. Hence, natural products which could have good safety and efficacy profile would be a welcome development. One of such plants whose anti-inflammatory effects have not been fully elucidated is *Monodora myristica* (Gaertn.) Dunal.
Monodora. myristica tree grows in the forests of Nigeria, Cameroon, Angola, Uganda and West Kenya (Weiss, 2002; Burubai, 2007). It is a tropical and a perennial berry plant which is edible (Burubai, 2007). The tree serves as an ornamental as a result of its large orchid-like flowers (Weiss, 2002). The seeds have an aroma and taste similar to nutmeg thus serves as spice in West African dishes (Weiss, 2002). After the seeds are dried, they may be sold or ground for culinary purposes or used to make necklaces. Seeds of *M. myristica* have demonstrated antioxidant activity (Irondi *et al*., 2023; Lawyer and Orishi, 2023) and antibacterial activity (Onikanni *et al*., 2023). Extract of *M. myristica* seeds have been reported to demonstrated potent *in vitro and in vivo* anti-inflammatory activity (Akinwunmi and Oyedapo, 2015; Ishola *et al*., 2016).
This current study aimed to further explore the earlier works of Isiogugu and colleagues (Isiogugu *et al*., 2018) by evaluating the acute and chronic anti-inflammatory activity of *Monodora myristica* root bark extract and fractions in rodents.

MATRIALS AND METHODS

Animals

Mature Sprague-Dawley rats (100-150 g) and albino mice (20-30 g) of either gender and non-pregnant females were used. These animals were sourced from the Department of Pharmacology and Toxicology Animal Facility, University of Nigeria, Nsukka. All animals were fed standard pellets and water. Animal experiments were carried out in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH publication #85-23, revised in 1985). Ethical clearance was approved by the Faculty of the Pharmaceutical Sciences Ethical Committee.

Collection and preparation of plant material

The root of *M. myristica* was sourced from the Nru village in Nsukka LGA, Enugu State Nigeria in December, 2018. Mr Alfred O. Ozioko of the International Centre for Ethnomedicines and Drug Development (INTERCEDD) Nsukka, Enugu State performed the identification and authentication (voucher number: Intercedd/16087). The plant name was checked with http://www.theplantlist.org/ on May 16. 2020. The root bark was separated from the root wood, cleaned and dried under shade for 14 days to a constant mass and then ground into a powder with the aid of a milling machine.

Extraction of the plant material

The milled coarse root bark powder (5.0 kg) was extracted by cold maceration in methanol at room temperature for 48 hours with intermittent shaking. It was then filtered and the filtrate was concentrated to dryness using a rotary evaporator under reduced temperature and pressure. This yielded the crude extract (CE).

Solvent-guided fractionation of the crude extract

The crude extract (70 g) was triturated with silica gel (100 mesh size) of equal mass in a mortar to obtain a fine mixture. The mixture was allowed to air dry and was placed into the silica gel glass column (60 cm in length and 7.5 cm in diameter). The solvent-guided fractionation was initiated by introducing n-hexane into the column and allowing it to drain. The eluent was then collected into a conical flask while more solvent was continuously added until the eluting solvent became clear indicating that n-hexane had exhausted what it was capable of eluting. The same procedure was repeated using ethyl acetate and then methanol in increasing order of polarity to obtain their respective solvent fractions. Solvent fractions were concentrated by open air evaporation to yielded n-hexane fraction (HF), ethyl acetate fraction (EAF) and methanol fractions (MF).

Acute toxicity test

Acute toxicity (LD_{50}) of CE, HF, EAF and MF were carried out as previously outlined by Lorke (1983). The albino mice were placed in 3 groups (n = 3) and were administered the following; group 1 received 10 mg/kg of CE, group 2 received 100 mg/kg of CE and group 3 received 1000 mg/kg of CE. These were administered via the oral route. The animals were constantly observed for signs of toxicity for the first 2 hours, intermittently for the next 4 hours and then overnight and the observation was recorded at the end of 24 hours. From the results obtained, the second phase of the acute toxicity test was performed using 3 groups (n = 1). One group received a dose of 1600 mg/kg of CE, another group received a dose of 2900 mg/kg of CE and the last

group received a dose of 5000 mg/kg of CE. The mice were continuously monitored for signs of toxicity for another 24 hours. Thereafter the mice were sacrificed. This was repeated for the fractions (HF, EAF and MF). The LD_{50} was calculated as

$$LD_{50} = \sqrt{x_1 x_2}$$

Where x_1= least lethal dose after a 24 hours observation period and x_2= the highest non-lethal dose after a 24 hours observation period.

Phytochemical screening

Phytochemical screening was performed on CE, HF, EAF and MF as outlined by Trease and Evans (1983).

Carrageenan- induced rat paw edema

The method of Winter *et al* (1962) was adopted with modifications. Fourteen groups of Sprague-Dawley rats (n = 6) were used. The zero-time volume of the water displaced by the left hind paw of the rats were determined. Groups 1, 2 and 3 were treated with 200, 400 and 800 mg/kg of CE respectively, groups 4, 5 and 6 were treated with 200, 400 and 800 mg/kg of HF respectively, groups 7, 8 and 9 were treated with 200, 400 and 800 mg/kg of EAF respectively, groups 10, 11 and 12 were treated with 200, 400 and 800 mg/kg of MF respectively while groups 13 and 14 were treated with 5 ml/kg of the vehicle (distilled water) and 50 mg/kg of piroxicam (standard drug) respectively via the oral route. Inflammation was induced 1 hour later by injecting 0.1 ml of 1 % w/v carrageenan in normal saline into the sub plantar region of the left hind paw of each rat. After 30 minutes, the volume of water displaced by the left hind paw of each rat was taken and this was repeated every 1 hour for 6 hours. Edema was assessed in terms of the difference between the zero-time volume of the water displaced by the left hind paw (V_0) and the volume of water displaced by the left hind paw at the different time intervals (V_t) after carrageenan injection. The level of inhibition was calculated using the relation (Perez, 1996);

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

Where a = mean volume of water displaced by treated rats after carrageenan injection; x = mean volume of water displaced by treated rats before carrageenan injection; b = mean volume of water displaced by control rats after carrageenan injection; y = mean volume of water displaced by control rats before carrageenan injection.

Xylene induced- topical ear edema

The method described by Tubaro *et al* (1985) and later modified by Atta and Alkohafi, (1998) was adopted with modifications. Six groups of albino mice (n = 5) were used. Group 1, 2, 3 and 4 received 5 mg/ear of CE, HF, EAF and MF respectively while group 5 and 6 received 5 mg/ear of the standard drug and 5 ml/ear of the vehicle respectively. These treatments were applied on the anterior surface of the right ear. Topical inflammation was instantly induced on the posterior surface of the same ear by application of xylene (0.1 ml/ear). Two hours after the induction of inflammation, the mice were sacrificed by over dose of chloroform. Circular section (6 mm diameter) of both the right ear (treated) and left ear (untreated) were punched out using a cork borer and weighed. Edema was measured as the weight difference between the two earplugs (Ibrahim *et al*., 2012). The anti-inflammatory activity was evaluated as percentage inhibition in the treated animals relative to control animals (Tubaro *et al*., 1985; Asuzu *et al.*, 1999) using the relation:

$$\text{Oedema inhibition } (\%) \; = \; 100(1 - (\frac{\text{Rt} - \text{Lt}}{\text{Rc} - \text{Lc}})$$

Where R_t = mean weight of the right earplug of treated animals; L_t = mean weight of the left earplug of treated animals; R_c = mean weight of the right earplug of control animals; L_c = mean weight of the left earplug of control animals.

Formaldehyde- induced arthritis

Ten groups of Sprague-Dawley rats (n = 6) were used for this study (Selye 1949). The zero-time volume of the water displaced by the left hind paw of the rats were determined. Groups 1 and 2 received 200 and 400 mg/kg of CE respectively, groups 3 and 4 received 200 and 400 mg/kg of HF respectively, groups 5 and 6 received 200 and 400 mg/kg of EAF respectively, groups 7 and 8 received 200 and 400 mg/kg of MF respectively, while groups 9 and 10 received 5 ml/kg of the vehicle and 10 mg/kg of the standard drug via the oral route respectively. Two hours later, inflammation was induced by injecting 0.1 ml of 2 % w/v formaldehyde into the sub-plantar region of the left hind paw of each rat. After 4 hours, the volume of water displaced by the left hind paw of each rat was then determined. On the 2nd day, the rats were treated with the extract, fractions, vehicle as well as piroxicam and soon thereafter, the volume of water displaced by the left hind paw of each rat was determined. On the 3rd day, the rats were treated with the extract, fractions, vehicle as well as piroxicam and soon thereafter, the volume of water displaced by the left hind paw of each rat was determined also and inflammation was subsequently re-induced. On the 4th day, the rats were treated with the extract, fractions, vehicle as well as piroxicam and the volume of water displaced by the left hind paw of each rat determined. This was then repeated on day 5, 6, 7, 8, 9 and 10. The animals were then sacrificed afterwards by overdose of chloroform. Edema was assessed in terms of the differences

between the zero-time volume of water displaced by the left hind paw (V_o) and the volume of water displaced by the left hind paw at the different time intervals (V_t) after 0.1ml of 2 % w/v formaldehyde injection. The % inhibition was calculated using the relation;

$$\% \text{ inhibition } = 100[1 - (\frac{c - x}{d - y})]$$

Where c = mean volume of water displaced by the left hind paw by the treated rats after formaldehyde injection; x = mean volume of water displaced by the left hind paw by treated rats before formaldehyde injection; d = mean volume of water displaced by the left hind paw by control rats after formaldehyde injection; y = mean volume of water displaced by the left hind paw by control rats before formaldehyde injection.

Cotton pellet- induced granuloma

The method described by D'Arcy *et al* (1960) and modified by Gepdiremen *et al* (2004) was adopted with modifications. Sprague-Dawley rats were randomly placed into 10 groups (n = 6). Groups 1 and 2 received 200 and 400 mg/kg of CE respectively, group 3, 4 and 5 received 200, 400 and 800 mg/kg of EAF respectively, groups 6, 7 and 8 received 200, 400 and 800 mg/kg of MF respectively, while groups 9 and 10 received 5 ml/kg of the vehicle and 10 mg/kg of the standard drug respectively via the oral route. After 1 hour, cotton pellets of 0.03 g (30 mg) autoclaved at 160º for 30 minutes were implanted one on each side of the subcutaneous dead space in the depilated axial region of rats under xylazine (administered as 10 mg/kg) and ketamine (administered as 50 mg/kg) anaesthesia. The wounds were sutured with silk and mopped with an alcoholic swab and the animals placed in their individual cages. The treatment with CE, EAF, MF, vehicle and piroxicam were repeated on days 2, 3, 4, 5, 6, 7 and on day 8. The animals were sacrificed on day 9 by overdose of chloroform and the cotton pellets coated with granuloma tissue were surgically removed. The cotton pellets were then dried at 60^0C to a constant mass and weighed. The granuloma formed was determined as the difference between the final and initial weights of the cotton pellets.

Statistical analysis

The data obtained were s analyzed using GraphPad Prism version 8.30. Result were expressed as Mean ± Standard Error of Mean (S.E.M). Differences between the treated and control groups were evaluated further using LSD Post hoc test and considered significant at $p < 0.05$, 0.01 and 0.001.

RESULTS

Extractive yield

The extraction of *M. myristica* root bark gave a yield of 113.86 g (2.28%) of the crude extract (CE). Fractionation of 70 g of the crude extract yielded 10.96 g of HF (15.66 % w/w), 25.30 g of EAF (36.14 % w/w) and 20.65 g of MF (29.50 % w/w).

Acute toxicity

The crude extract and fractions showed no lethality and sign of acute intoxication at 5000 mg/kg.

Phytochemical analysis

The crude extract and solvent fractions gave positive reactions to for steroids, reducing sugars, tannin, resins, flavonoids, alkaloids, proteins, glycosides, saponins, carbohydrates, terpenoids, acidic compounds and fats and oil (Table 1).

Effect on Carrageenan- induced rat paw edema

The CE, HF, EAF and MF elicited a significant ($p < 0.05$) non-dose dependent varying degrees of inhibition of carrageenan induced oedema at different time intervals with EAF (400 mg/kg) and MF (200 mg/kg) producing the greatest inhibition (Table 2).

Effect on Xylene- induced topical ear edema

The HF elicited the greatest % inhibition of xylene induced ear oedema (64.94) which was also significant ($p < 0.001$) when compared to control group (Table 3).

Effect on Formaldehyde- induced arthritis

The CE, HF, EAF and MF elicited a non-dose dependent and varying degrees of reduction of the volume of oedema produced in formaldehyde induced arthritis (Table 4).

Effect on Cotton pellet- induced granuloma

The CE, HF, EAF and MF elicited a non-dose dependent and varying degrees of reduction of the volume of edema produced in formaldehyde induced arthritis (Table 5).

Table 3: Effect of the extract and fractions on xylene induced topical ear edema

Treatment	Dose (mg/ear)	Weight of right ear (mg)	Weight of left ear (mg)	Difference = edema (mg)	% Inhibition
Control	-	23.33 ± 2.40	7.17 ± 0.60	16.17 ± 2.01	-
Piroxicam	5	16.00 ± 1.32	8.00 ± 0.93	8.00 ± 1.77	50.52**
CE		16.8 ± 2.38	12.4 ± 0.22	4.40 ± 2.11	58.1**
HF	5	17.50 ± 1.71	11.83 ± 1.01	5.67 ± 0.80	64.94***
EAF	5	19.17 ± 1.08	9.50 ± 0.56	9.67 ± 1.76	40.20*
MF	5	14.50 ± 1.34	7.33 ± 0.42	7.17 ± 1.45	55.66**

n = 6; values shown for weight and differences in weight are mean ± SEM (one-way ANOVA); *, **, *** $p <$ 0.05, 0.01, 0.001; CE = crude extract; HF = hexane fraction; EAF= ethyl acetate fraction; MF= methanol fraction

Table 1: Phytochemical constituents of the extract and fractions

Phytochemical constituents	Relative abundance			
	CE	**HF**	**EAF**	**MF**
Alkaloids	++	-	-	+++
Glycosides	+++	-	-	++++
Saponins	+	-	-	+
Flavonoids	+	-	+++	+
Tannins	++	-	-	++
Acid compounds	+	-	-	+
Resins	+++	+++	++	-
Steroids	+++	++++	-	+
Terpenoids	++++	+++	+++	+
Proteins	+++	-	+	-
Reducing sugars	+++	-	-	+++
Carbohydrates	++++	-	-	++++
Fats and oil	+	+++	+	-

+ = mildly present; ++ = moderately present; +++ = highly present; ++++ = abundantly present; - = absent; HF = hexane fraction; EAF = ethylacetate fraction; MF = methanol fraction; CE = crude extract

Table 4: Effect of the extract and fractions on AUC of formaldehyde induced arthritis

Treatment	Dose (mg/kg)	AUC	% Inhibition
Control	-	6.59 ± 0.19	-
Piroxicam	10	4.72 ± 0.13	28.38
CE	200	5.22 ± 0.13	20.79
	400	5.50 ± 0.13	16.54
HF	200	6.29 ± 0.36	4.55
	400	4.88 ± 0.24	25.95
EAF	200	5.74 ± 0.19	12.90
	400	5.90 ± 0.23	10.47
MF	200	6.18 ± 0.28	6.22
	400	5.83 ± 0.23	11.53

n = 6; values shown for AUC are mean ± SEM (one-way ANOVA); AUC = Area under curve; CE = crude extract; HF, EAF and MF = hexane, ethyl acetate and methanol fraction respectively

Table 2: Effect of the extract and fractions on carrageenan induced rat paw edema

Treatment	Edema (ml)							
	0 h	0.5 h	1 h	2 h	3 h	4 h	5 h	6 h
Control	0.47 ± 0.03	0.9 ± 0.05	1.35 ± 0.12	1.50 ± 0.1	1.6 ± 0.1	1.72 ± 0.1	1.8 ± 0.06	1.83 ± 0.07
Piroxicam 50 mg/kg	0.45 ± 0.03	0.57 ± 0.06 (55.8)*	0.77 ± 0.08 (67.05)**	0.93 ± 0.15 (56.31)*	0.95 ± 0.13 (58.41)**	1.05 ± 0.16 (54.4)**	1.05 ± 0.15 (57.14)**	1.05 ± 0.15 (58.09)***
CE 200 mg/kg	0.53 ± 0.05	0.73 ± 0.04 (53.49)	1.1 ± 0.1 (35.23)	1.35 ± 0.12 (20.39)	1.37 ± 0.11 (25.64)	1.52 ± 0.12 (20.8)	1.8 ± 0.08 (19.55)	1.5 ± 0.11 (28.68)
400 mg/kg	0.4 ± 0.04	0.63 ± 0.04 (46.51)**	0.8 ± 0.09 (54.55)**	1.08 ± 0.16 (33.98)	1.17 ± 0.11 (31.86)	1.25 ± 0.09 (32.0)	1.35 ± 0.09 (26.32)*	1.2 ± 0.12 (41.18)***
800 mg/kg	0.53 ± 0.05	0.85 ± 0.06 (25.58)	1.18 ± 0.15 (26.14)	1.25 ± 0.08 (30.1)	1.42 ± 0.1 (21.24)	1.48 ± 0.12 (24.0)	1.63 ± 0.1 (17.29)	1.51 ± 0.14 (22.79)
HF 200 mg/kg	0.47 ± 0.07	0.73 ± 0.09 (39.53)	1.08 ± 0.15 (30.68)	1.3 ± 0.2 (19.42)	1.5 ± 0.2 (8.85)	1.5 ± 0.15 (17.6)	1.58 ± 0.07 (16.54)	1.5 ± 0.11 (24.26)
400 mg/kg	0.47 ± 0.07	0.77 ± 0.06 (48.84)	1.5 ± 0.11 (48.86)	1.25 ± 0.15 (32.04)	1.42 ± 0.12 (23.01)	1.53 ± 0.1 (21.6)	1.55 ± 0.07 (24.81)	1.48 ± 0.07 (31.62)
800 mg/kg	0.53 ± 0.02	0.75 ± 0.05 (41.86)	1.08 ± 0.11 (38.64)	1.25 ± 0.1 (30.1)	1.47 ± 0.1 (16.81)	1.58 ± 0.09 (16.0)	1.50 ± 0.16 (27.07)	1.45 ± 0.11 (32.35)
EAF 200 mg/kg	0.55 ± 0.02	0.78 ± 0.02 (53.49)	0.98 ± 0.05 (54.55)	1.2 ± 0.07 (39.81)	1.38 ± 0.05 (29.2)	1.48 ± 0.07 (28.0)	1.45 ± 0.07 (34.59)	1.43 ± 0.07 (37.5)
400 mg/kg	0.55 ± 0.02	0.73 ± 0.03 (58.14)	0.95 ± 0.08 (54.55)	1.2 ± 0.08 (36.89)	1.28 ± 0.08 (35.4)	1.4 ± 0.06 (32.0)	1.35 ± 0.03 (39.85) *	1.32 ± 0.03 (43.38) *
800 mg/kg	0.555 ± 0.03	0.8 ± 0.04 (41.86)	0.97 ± 0.07 (52.27)	1.18 ± 0.13 (38.83)	1.27 ± 0.13 (36.28)	1.38 ± 0.16 (33.6)	1.35 ± 0.13 (39.85) *	1.3 ± 0.13 (44.85)**
MF 200 mg/kg	0.57 ± 0.02	0.78 ± 0.05 (51.16)	1.2 ± 0.04 (51.14)	1.18 ± 0.11 (40.78)	1.32 ± 0.06 (33.63)	1.47 ± 0.12 (28.0)	1.37 ± 0.1 (39.85) *	1.32 ± 0.08 (44.85)*
400 mg/kg	0.57 ± 0.04	0.82 ± 0.05 (41.86)	1.23 ± 0.14 (25.00)	1.47 ± 0.14 (12.62)	1.57 ± 0.08 (11.5)	1.62 ± 0.09 (16.0)	1.55 ± 0.1 (26.32)	1.52 ± 0.1 (30.15)
800 mg/kg	0.48 ± 0.04	0.73 ± 0.05 (41.86)	1.23 ± 0.21 (14.77)	1.38 ± 1.18 (12.62)	1.57 ± 0.17 (3.54)	1.60 ± 0.14 (10.4)	1.6 ± 0.14 (15.17)	1.48 ± 0.12 (26.47)

n = 6; values shown are mean ± SEM (one-way ANOVA); *, **, *** $p < 0.05, 0.01, 0.001$ values in parenthesis represent % inhibition; ME = crude extract; EAF = ethyl acetate fraction; MF = Methanol fraction

Table 5: Effect of the extract and fractions on cotton pellet induced granuloma

Treatment	Dose (mg/kg)	Weight of granuloma (mg)	% Inhibition
Control	-	70.83 ± 5.81	-
Piroxicam	10	60.00 ± 0.86	15.29
CE	200	61.00 ± 1.55	13.88
	400	61.83 ± 1.74	12.71
	800	62.67 ± 3.42	11.52
HF	200	55.01 ± 2.14	22.34
	400	40.20 ± 1.77	43.24
	800	60.53 ± 3.56	14.54
EAF	200	61.67 ± 6.16	12.93
	400	58.17 ± 3.70	17.87
	800	56.00 ± 1.93	20.94
MF	200	44.00 ± 8.03	37.88
	400	51.50 ± 2.63	27.29
	800	67.33 ± 7.58	4.94

n = 6, values shown for granuloma are mean ± SEM (one-way ANOVA), CE = crude extract, EAF = ethyl acetate fraction, MF = methanol fraction

DISCUSSION

The screening for the anti-inflammatory effects of the fractions of *M. myristica* root bark indicated that they inhibited acute (topical and systemic) and chronic inflammation. The fractions were more effective in inhibiting topical than systemic acute inflammation. The fractions elicited varying degrees of inhibition of carrageenan-induced inflammation. Carrageenan is used to facilitate the release of pro-inflammatory mediators such as prostaglandins, leukotrienes, histamine, bradykinin, TNF-α (Loram *et al*., 2007). Carrageenan causes an inflammatory response which peaks at about 3 h, which may lead to hyperalgesia (Romero *et al*., 2005). Carrageenan stimulates release of interleukin 6 (IL-6), IL-1 β, tumor necrosis factor alpha (TNF α), and cytokine-induced neutrophil chemoattractant (Loram *et al*., 2007). Some authors have suggested that the inflammation due to carrageenan injection manifests in three stages; the first stage (occurs between0- 90 min) and is linked with the release of histamine and serotonin, the second stage (occurs between 90–150 min) and is reliant on kinin release and the third stage (occurs after 150 min), and is dependent on prostaglandin release (Guo *et al*., 2011; Rock *et al*., 2018). Thus, ability to reduce volume of oedema after carrageenan injection is an indication that EAF and MF possess moderate anti-inflammatory activity against carrageenan induced rat paw edema model. Judging from the result, we can infer that the extract and fractions is quite active against the third stage of acute inflammation (stage 3) and we can further postulate this is probably due to inhibition of prostaglandin release.

Xylene induced topical ear oedema has always been employed in the screening for acute anti-inflammatory activity (Bralley *et al*., 2008). Xylene-induced ear oedema is initiated by inflammatory mediators (histamine, serotonin, and bradykinin). Xylene-induced ear oedema involves vasodilation and elevated vascular permeability usually associated with substance P (Richardson and Vasko, 2002). Xylene may cause elevated capillary permeability and leukocyte infiltration in mice. Ear oedema may trigger the release of inflammatory mediators, thereby promoting vasodilation and elevated vascular permeability. Severe vasodilation and oedema of skin accompanied by infiltration of inflammatory cells are classic signs of acute inflammation upon topical application of xylene. Xylene-induced mice ear oedema is an indication of oedema which occurred in early phase of acute inflammation, resulting in the release of inflammatory mediators (Jumping *et al*., 2005). The propensity to reduce the level of oedema produced upon topical application of xylene indicates that HF, EAF and MF possess anti-inflammatory activity against the xylene induced topical ear edema model. Judging from the result, we can infer that the extract and fractions is quite active against the early stage of acute inflammation (stage 1 and 2) and we can

further postulate this is probably due to inhibition of histamine release.

The formalin-induced arthritis model is used to investigate an agent with supposed anti-proliferative activity since this screening is linked with the proliferative phase of inflammation (Banerjee *et al*., 2000). Formaldehyde induced arthritis model has been extensively utilized in screening for activity on chronic inflammation since the effects are similar to that which occur in rheumatoid arthritis (Akah*et al*., 2007). It has been reported that sub-plantar injection of formalin also progresses in two phases. In the first phase (neurogenic phase), substance P as well as bradykinin are released, but in the second phase, there is release of histamine, serotonin and prostaglandins (Sreejamole*et al*., 2011). Thus, weak reduction in the volume of oedema produced after formaldehyde injection indicates that the extract and fractions possess mild anti-inflammatory activity against formaldehyde induced arthritis. Judging from the result, we can infer that the extract and fractions is less active against chronic inflammation and we can further postulate this is probably due to weak inhibition of substance P and bradykinin release.

In cotton pellet induced granuloma, inflammation occurs in two phases, namely the transudative and proliferative phases. The production of granuloma due to implantation of cotton pellet has been extensively utilized as a chronic inflammation model to evaluate the transudative as well as the proliferative phases of the inflammation. This model is based on implantation of a foreign body (cotton pellets) in rodents leading to a granuloma formation around the pellets which eventually results in inflammation observed in the proliferative phase (Kou *et al*., 2003). The weight of the implanted cotton pellet (after drying to a constant mass) is an indication of the amount of granuloma produced (Kumar *et al*., 2016). The ability to prevent or minimize granuloma formation is an indication of suppression of the mechanisms associated with granulomatous and chronic inflammation. Thus, the inability to significantly reduce granuloma formation indicates that the extract and fractions possesses mild anti-inflammatory activity against cotton pellet induced granuloma. Judging from the result, we can infer that the extract and fractions is less active against chronic inflammation and we can further postulate this is probably due to weak inhibition of substance P and bradykinin release.

Several studies have reported that some phytoconstituents such as sterols (Akihisa *et al*., 2007), flavonoids (Hämäläinen*et al*., 2007), tannins (Owoyele*et al*., 2010), alkaloids (Barbosa-Filho *et al*., 2006) and glycosides (Liu, 2011) possess anti-inflammatory activity. Also, flavonoids have shown anti-inflammatory effect in proliferative phase as well as exudative phase of inflammation thereby inhibiting the release of histamine, cytokine, prostaglandins and leukotrienes (Park *et al*., 2008; Permender*et al*., 2009). The presence of these phytoconstituents could be responsible for the anti-inflammatory activity of *M. myristica* root bark and thus justifies its traditional use in managing acute inflammatory conditions.

CONCLUSION

This study concludes that the root back of *M. myristica* possess moderate anti-inflammatory activity with the hexane fraction (HF) being the most active. Thus, *M. myristica* roots may be used to ameliorate the inflammation associated with COVID-19 and other disorders.

Limitation

This work was limited by poor yield of the HF. In the course of this work, only two dose levels (200 mg/kg and 400 mg/kg) were employed in the formaldehyde induced arthritis. This was to accommodate the remaining quantity of the HF.

Acknowledgement

The authors wish to acknowledge Prof. Udegbunam and Dr. Nnamdi Okereke (both of Faculty of Veterinary Medicine, University of Nigeria) for their support in this work.

Conflict of Interest

The authors have declared no conflict of interest associated with this work.

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CITATION: Isiogugu ON, Peter IE, Ofokansi MN, Alozie EM, Abonyi UC, Ogbue CO, and Ezike AC. (2023) Fractions of *Monodora myristica* (Gaertn.) Dunal (Annonaceae) root bark possesses anti-inflammatory activity
Trend Nat Prod Res 4(2). 61-70. DOI: 10.48245/tnpr-2734391.2023.4.203