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(RESEARCH ARTICLE)

Investigation of the chemical composition and anti-inflammatory effects of the crude methanolic extract and fractions of *Artocarpus heterophyllus* (Fam. Moraceae)

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Abstract

Inflammation is a local response of living tissues in mammals towards injury and is normally linked to the pathogenesis of several diseases. In this study, we investigated the anti-inflammatory activity of the methanol extract and the n-hexane, ethyl acetate and butanol fractions of the Jack fruit leaves. Methanolic cold maceration was carried out to get the extract, followed by liquid-liquid fractionation. Two models, viz. xylene-induced ear edema in mice and egg albumin-induced paw edema in rats were used to evaluate them.

The anti-inflammatory activity of the extract and fractions was screened for anti-inflammatory activity using xylene-induced ear edema in mice and egg albumin-induced paw edema in rats. The chemical constituents of the extract and fractions were detected by dereplication using HPLC-DAD.

Results showed that for the xylene-induced topical edema model, only the 250µg/ear n-butanol fraction exhibited good anti-inflammatory activity at 21.21% when compared with the piroxicam (positive control) at 32.21%. In the egg albumin-induced paw edema both the methanol extract and n-butanol fraction at 250mg/kg and 500mg/kg showed good anti-inflammatory activity after 2hours post-administration, with maximum effect at 6 hours. Specifically, at 6 hours the methanol extract at 250 mg/kg exhibited 41.21% inhibition; the butanol fraction at the same dose exhibited 43.63% inhibition when compared with piroxicam (500 mg/kg) with 38.39% inhibition. The HPLC-DAD analysis of the extract and fractions revealed the presence of six (6) compounds: catechin (1), septicine (2), 5-isoprunetin 8C glucoside (3), naamine (4), protocatechuic acid (5), and vitexin (6). These compounds have been reported to display anti-inflammatory activity. These findings suggest that the leaf extract possess an anti-inflammatory phytocompounds which authenticates their ethno-medicinal usage claim by local people in different parts of Nigeria.

Keywords: Inflammation; Phytocompound; Paw Edema; Anti-Inflammatory Activity

1. Introduction

Inflammatory diseases, such as rheumatism and arthritis, have continued to be a significant cause of debilitation, morbidity, and mortality globally (Okolo et al., 2021). Most contemporary drugs are derived from plants that have ethnomedicinal relevance (Kehrer *et al.,* 1994). Most available therapeutic agents for the treatment of inflammatory diseases lack specificity and had posed various adverse effects ranging from gastrointestinal irritations, gastric ulcers, nephrotoxicity, hypertension among others (Rang et al., 2003). According to several authors (Bello et al., 2008; Bobbio *et al*., 1978 and Khan *et al*., 2003), medicinal plant species has therapeutic value and their usage in treatment of certain

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aliments dated back to the apothecary. Researchers (Shirajum *et al*., 2015; Chawdhary *et al*., 1997; Tulyathan *et al*., 2002 and Charles, 2006; Jayasuriya, 2013) state that Bioactive compounds from medicinal plants are preferred as drug candidates to their synthetic congeners because of their efficiency, cultural acceptability and little or no side effects associated with the synthetic drugs. The *Artocarpus heterophyllus* is a species of tree of the mulberry family (Moraceae) is also known as jackfruit (Eng.), Kathal, Panas (Hindi), Kanthal (Beng.), Palaa (Tamil), Phanas (Guj & Mar) &Chakka (Malayalam) (Rahman et al., 1999). According to Prakash *et al*., 2007, part of the whole plant used ethnomedicinally includes; seeds, fruits, bark, root, leaves and latex.

Phytochemically, the bioactive compounds found in the plant are vast and includes several flavones colouring matters like morin, dihydromorin, cynomacurin, artocarpin, etc., (Rama *et al*., 1973). The leaves and stem show the presence of sapogenins, cycloartenone, cycloartenol, β-sitosterol and tannins, they show estrogenic activity (Prakash 2007). The root of *Artocarpus heterophyllus* have been shown to contain compounds like β-sitosterol, ursolic acid, Betulinic acid and cycloartenone (Dayal, 1974).

Ethnobotanically, the leaves are useful in fever, boils, wounds and skin diseases. The fruits are acrid, astringent, and carminative, laxative, and possess aphrodisiac properties. The seeds used to treat constipation. The stem wood is used as sedative and in convulsions treatment (Hemborn, 1996). The latex is useful antibacterial agent especially for pharyngitis treatment (Sato and Fujiwara, 1996, Vaidya Gogte, 2000) and also in treatment of swelling (anti-inflammatory agent (Gupta and Tandon, 2004). The ash of Jackfruit leaves is used in ulcers treatment. The root is a remedy for skin diseases and asthma. A lot of pharmacological investigations have been carried out based on the constituents of the plant and they include Anti-inflammatory Effect (Fang et al., 2008), Antioxidant Effect (Ko et al., 1998), Antifungal Effect (Trindade et al., 2006), Immunomodulatory effect of Jacalin (Kabir, 1998), Antidiabetic Effect (Fernando et al., 1991), Antibacterial Effect (Khan et al., 2003), and Anthelmintic Effect (Sharma &Trivedi, 1995).Hence, this study is aimed at investigating the ant inflammatory properties of the extract and fractions of *Artocarpus heterophyllus* using xylene-induced ear edema and egg albumin-induced paw edema as well as detect the chemical constituents by dereplication using HPLC-DAD.

2. Materials and Methods

2.1. Experimental animals

Albino Swiss mice (15–25 g) and Wistar rats (120-150 g) of both sexes were obtained from the colony breed of the animal house of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu, Anambra State. Animals were handled in compliance with the National Institute of Health guidelines for the care and use of laboratory animals (Pub. No. 85-23, revised 1985), as approved by the Faculty.

2.2. Plant Materials

Plant material (leaves) was collected from Abba town in Njikoka LGA in Anambra State, Nigeria. The leaves were identified and authenticated by Mrs. Amaka Roseline Onwuyuli and given a voucher number (PCG/474/009). The leaves were sun-dried for 72 hours and thereafter pulverized.

2.3. Extraction

About 1 kg of the pulverized leaves were extracted in 2 L of methanol by cold maceration for 48 hours with intermittent shaking. The maceration process was repeated twice for maximal extraction, and the extracts were filtered using a Buckner funnel. The filtrate recovered was concentrated to dryness using a rotary evaporator at 50°C.

2.3.1. Liquid-liquid pre-chromatographic fractionation

The liquid- liquid fractionation was carried out using the method described by Okoye et al. (2004). The crude methanol extract (10g) was dissolved in 250mL of distilled water and subjected to liquid-liquid fractionation using a 500mL Pyrex separation funnel. The solvents used were n-hexane, ethyl acetate, and n-butanol in increasing order of their polarities. The fractions so obtained were filtered twice using Whatman No. 1 filter paper in a Buckner funnel and concentrated in-vaccuo using the rotary evaporator set at a revolution of 70 RPM and a temperature of 50º C. The fractions obtained were stored at 4ºC for future analysis.

High Performance Liquid Chromatography- Diode Array Detector (HPLC-DAD) Analysis.

The extracts and fractions were subjected to HPLC analysis using the method described by Eboh *et al*. (2022).

About 2 mg of each dried extract and fraction were reconstituted in 2 mL of HPLC-grade methanol, sonicated for 10 min, centrifuged at 3000 rpm for 5 min, and filtered using Whatman No. 1 filter paper. Subsequently, 100 µL of the filtrate containing dissolved samples were each added into an HPLC vial containing 500 µL of HPLC-grade methanol. The diluted samples were then subjected to HPLC analysis using a Dionex P580 HPLC system with a photodiode array detector (UVD340S, Dionex Softron GmbH, Germering, Germany). The components were detected at 235 nm, 254 nm, 280 nm, and 340 nm. The separation column (length internal diameter: 125 X 4 mm; length X internal diameter) was prefilled with Eurospher-10 C18 (Knauer, Germany) column, and the eluent was a linear gradient of nanopure water (adjusted to pH 2 with formic acid) and methanol as the mobile phase (injection volume of 30 µL of sample).

2.4. Pharmacological assay

2.4.1. Topical anti-inflammatory test induced by xylene on the mouse ear

This was carried out according to the methods described by Okoli et al. (2006 & Okoye et al., 2010). In this experiment, the effect of the methanol extract as well as the three fractions was evaluated. The mice were divided into six groups of six (6) animals each, where the treatment groups were the methanol extract and the fractions (n-hexane, ethyl acetate, and n-butanol) while the other groups were the positive control (piroxicam) and negative control (Tween 80 and distilled water).The treatment groups received 250 or 500 µg/ear on the anterior surface of the right ear. Topical inflammation was instantly induced on the posterior surface of the same ear by the application of 0.05mL of xylene. Control animals received only the vehicle (3%, v/v Tween 80). Two hours after induction of inflammation, the mice were killed by overdosing with ether anesthesia, and both ears were removed and ear plugs extracted with a 6 mm cork-borer. The anti-inflammatory activity was evaluated as percentage edema reduction/inhibition in the treated animals relative to control animals using the relation

Oedema reduction (%) / inhibition = [100 ((Rt - Lt)/(Rc-Lc))]

where Rt is the mean weight of right ear plugs of treated animals; Lt is the mean weight of left ear plugs of treated animals; Rc is the mean weight of right ear plugs of control animals; and Lc is the mean weight of left ear plugs of control animals.

2.4.2. Systemic anti-inflammatory test: egg albumin- induced paw edema in rats

This was done by using the egg albumen-induced rat paw edema model described by Osadebe and Okoye, (2003). The methanol extract and n-butanol fraction were administered at doses of 500 mg/kg and 250 mg/kg, while the positive control (piroxicam) and the negative control (8% Tween 20) were administered at 500 mg/kg and 0.2 mL respectively. The animals (n=5) were fasted for 6 hours and deprived of water only during the experiment to ensure uniform hydration and minimize variability in oedematous response (Osadebe and Okoye, 2003). All the test substances were solubilized in 8% Tween 20 and administered by intraperitoneal injection to the rats 30 min before induction of edema. The paw edema was induced in the planar region of the right hind paw by sub planter injection of the phylogistic agent (0.1ml of fresh undiluted egg albumen).

Paw volume was then measured by water displacement method using a Plethysmometer at 0,1,2,3,4,5 and 6 h after egg albumen injection. The anti-inflammatory activity was calculated at each time of observation as percent inhibition of edema in the animals treated with the substances under test in comparison to the vehicle-treated animals. The percentage inhibition of edema is calculated using the formula.

% Inhibition = V0 – V1/Vo

Where Vt is the volume of edema at the corresponding time and Vo is the volume in edema of control rats at the same time.

2.5. Data analysis

The results were analyzed using SPSS Version 16.00 and presented as mean±SEM. Significant between the control and the extract/fraction treated groups using one-way ANOVA (Turkey LSD Alpha Post Hoc Test). Differences between means were considered statistically significant at $P<0.05$.

3. Results

Table 1 Yield of extract and fractions

S/N	Solvent	Extract/Fractions
1	Methanol(crude)	2.857 g
2	n-hexane	0.820 g
3	Ethyl acetate	0.415 g
4	Butanol	1.610 g

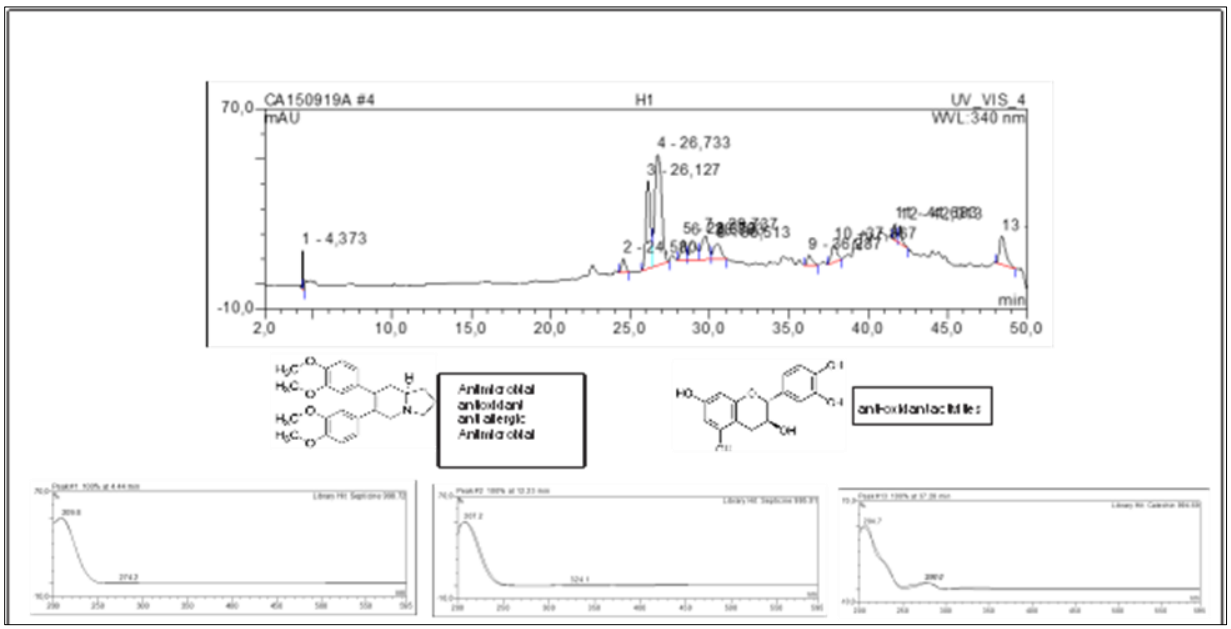

Figure 1 HPLC Chromatogram of n-Hexane fraction and the detected compounds

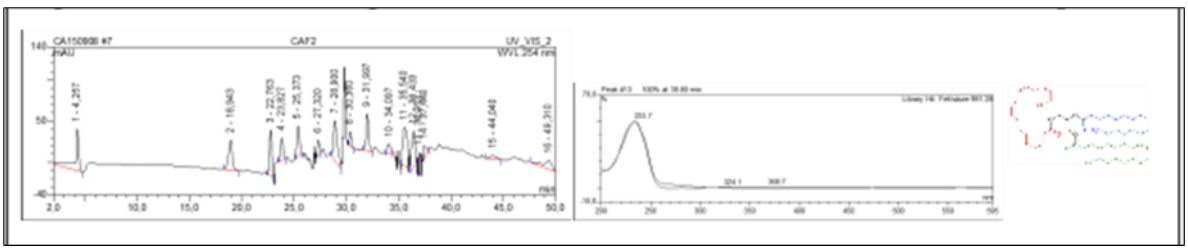

Figure 2 HPLC Chromatogram of Ethyl acetate fraction and the detected compounds

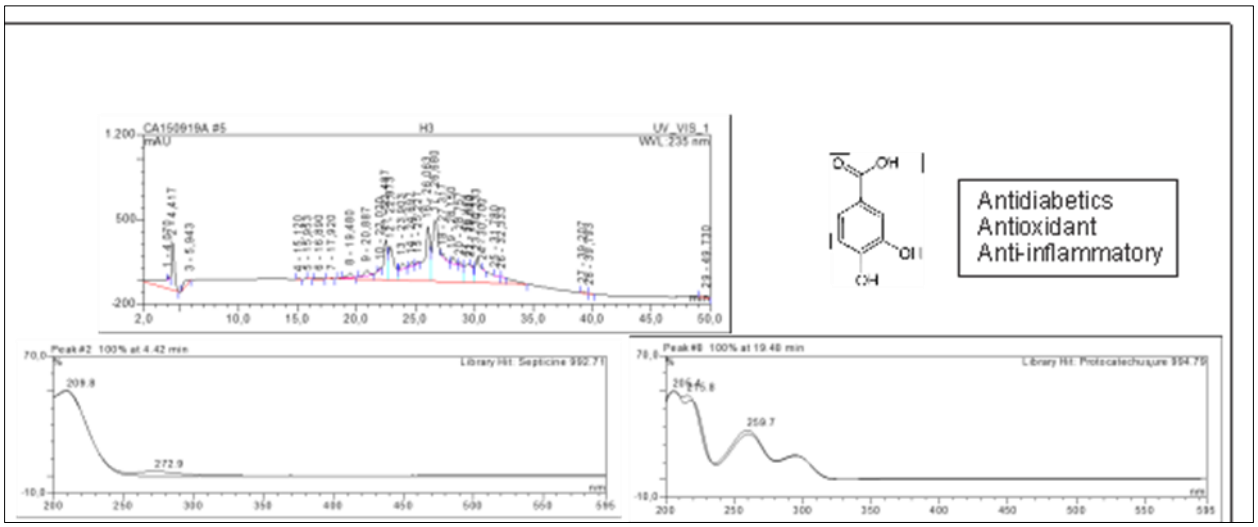

Figure 3 HPLC Chromatogram of n-Butanol fraction and the detected compounds

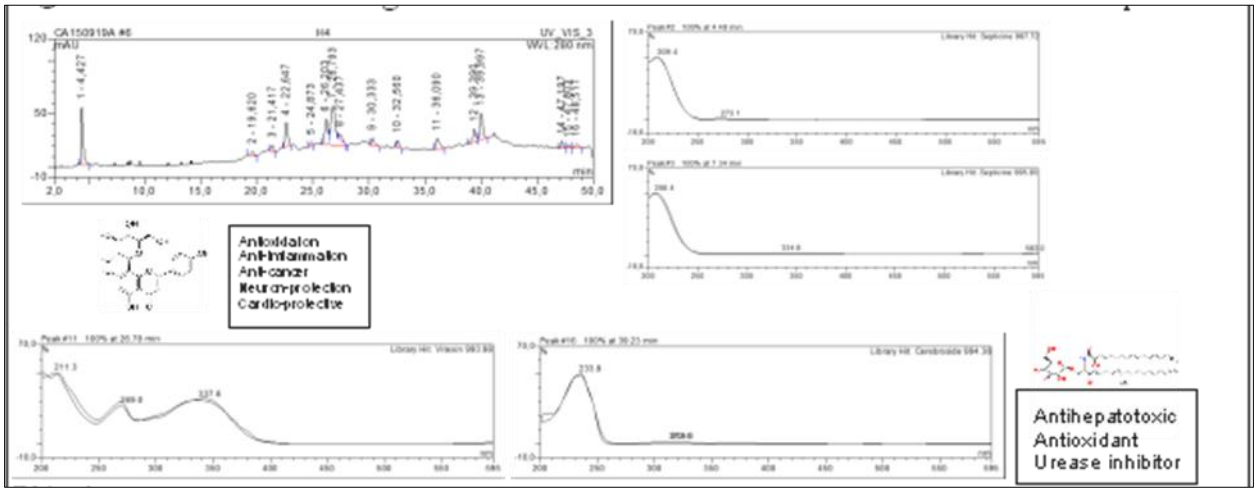

Figure 4 HPLC Chromatogram of methanol fraction and the detected compounds

Table 4 Effects of extract and fractions on xylene-induced topical edema

Groups	Weight of left ear (mg)	Weight of right ear(mg)	Difference	Inhibition (%)
Negative control	4.86 ± 0.53	5.96 ± 0.70	1.10 ± 0.50	0.00
Piroxicam	4.86 ± 0.69	5.60 ± 1.18	0.74 ± 0.50^{ns}	32.73
Methanol (500 µg/ear)	4.53 ± 0.31	5.83 ± 0.57	1.30 ± 0.75 ^{ns}	-18.18
Methanol fraction (250 µg/ear)	4.57 ± 0.15	6.53 ± 0.29	1.97 ± 0.42 ^{ns}	-78.79
Ethyl acetate fraction (500 µg /ear)	4.73 ± 0.21	6.60 ± 0.92	1.87 ± 0.93 ^{ns}	-69.70
Ethyl acetate fraction (250 µg /ear)	4.00 ± 0.26	6.73 ± 0.23	2.73 ± 0.21 ^{ns}	-148.48
n-hexane fraction (500 µg /ear)	4.50 ± 0.20	5.90 ± 1.21	1.40 ± 1.30 ^{ns}	-27.27
n-hexane fraction (250 µg ear)	4.37 ± 0.59	5.80 ± 0.87	1.43 ± 0.31 ^{ns}	-30.30
Butanol fraction (500 µg /ear)	4.30 ± 0.53	6.40 ± 1.54	2.10 ± 1.65 ^{ns}	-90.91
Butanol fraction (250 µg /ear)	4.47 ± 0.46	5.33 ± 0.38	0.87 ± 0.84 ^{ns}	21.21

Table 5 Effects of extracts/fraction on egg-albumin induced paw edema

		Edema					
Group	**0 hour**	**1 hour**	**2 hours**	**3 hours**	**4 hours**	**5 hours**	**6 hours**
Methanol (500mg/kg)	4.74 ± 0.55	3.67 ± 0.90^{ns} (19.38%)	3.23 ± 0.53* (26.88%)	3.05 ± 0.54* (27.46%)	2.62 ± 0.58* (32.65%)	2.22 ± 0.69* (36.14%)	1.91 ± 0.67^{ns} (37.92%)
Methanol (250mg/kg)	4.08 ± 0.61	3.67 ± 1.16^{ns} (19.30%)	3.22 ± 0.95* (27.10%)	3.01 ± 0.95* (28.55%)	2.46 ± 0.92* (36.76%)	2.04 ± 0.86* (41.21%)	1.79 ± 0.89* (41.82%)
Butanol (500 mg/kg)	4.24 ± 0.15	4.67 ± 0.81^{ns} (-2.73%)	4.04 ± 0.64^{ns} (8.64%)	3.76 ± 0.61 (10.64%)	3.22 ± 0.94^{ns} (17.33%)	2.76 ± 0.84^{ns} (20.58%)	2.35 ± 0.91^{ns} (23.57%)
Butanol (250mg/kg)	4.42 ± 0.29	3.34 ± 0.96^{ns} (26.59%)	3.01 ± 0.71* (31.95%)	2.64 ± 0.83* (37.29%)	2.25 ± 0.61* (42.26%)	1.96 ± 0.57* (43.63%)	1.67 ± 0.52* (45.78%)
(Positive Control-Piroxicam)	4.22 ± 0.26	4.09 ± 0.81^{ns} (10.02%)	3.43 ± 0.48^{ns} (22.44%)	2.86 ± 0.36* (31.97%)	2.35 ± 0.26* (39.69%)	2.14 ± 0.41* (38.39%)	1.81 ± 0.34^{ns} (41.10%)
(Water – Negative Control)	4.14 ± 0.25	4.55 ± 0.39	4.42 ± 0.41	4.21 ± 0.42	3.89 ± 0.41	3.47 ± 0.60	3.08 ± 0.98

4. Discussion

The results of topical anti-inflammatory effect represent and the system anti-inflammatory effect are shown in Tables 4 and 5. Topical administration of the extract and fractions to the mouse's ear resulted in a potent inhibition or suppression of acute edema induced by xylene, particularly for the n-butanol fraction at 250µg/ear f.250µg/ear of n-butanol fraction exhibited good anti-inflammatory activity with inhibition of 21.21% when compared with piroxicam (32.73%). Xylene irritates the mouse ear instantly leading to fluid buildup and edema, which are symptoms of an acute inflammatory response; the inhibition of this reaction is a plausible indicator of an anti-phlogistic effect (Atta and Alkohafi, 1998; Okoli *et al.*, 2006). In the egg albumin-induced paw edema model, both the methanol extract and the n-butanol fraction at 250mg/kg and 500mg/kg exhibited good anti-inflammatory activity from 2 hours post administration with maximum effect at 6 hours. Specifically, at 6 hours the methanol extract at 250 mg/kg exhibited 41.21% inhibition; n-butanol fraction at the same dose exhibited 43.63% inhibition when compared with piroxicam (500 mg/kg) with 38.39% inhibition. The n-butanol fraction possesses a better anti-inflammatory activity in both the topical and systemic models used in the study. The constituents of this fraction may have blocked the release or effects of numerous inflammatory mediators that mediate acute inflammation generated by phlogistic agents, such as histamine, serotonin, kinins, and prostanoids (Damas *et al*., 1990; Utsunomiya and Ohishi, 1991; Lalenti *et al*., 1992; Osadebe and Okoye, 2003; Okoli *et al*., 2006).The HPLC results revealed that there many phytocompounds in the various fractions and the crude extract, namely catechin and septicine from the n-hexane fraction, brodiphether from the ethyl acetate fraction, Para hydroxybenzoic acid, cyclo(prolyvalyl), septicine from the butanol fraction, and cyclo (prolyvalyl) and septicine from the methanol extract (Hassan *et al*., 2004; Gosslau *et al*., 2011; Lee *et al*., 2009). The qualitative comparative analysis of the UV data of these identified compounds, their retention time and biological activities in relation to the UV data in the library hit with the correlative resolution as shown in Tables 2,3 and 4.Some of the biological activities of the detected compound include anti-inflammatory, anti-microbial and anti-convulsant activities as reported by Shayong, 2018; Ndjaten, *et al.*, 2014; Hassan, *et al.*, 2004; Lee, *et al.*, 2011; Haq, 2006; Sticker, 2007; Ong, 2008 and Vazquez-G, *et al.*, 2020. Previous report (Venkataraman, 2001) stated that some phenolic compounds isolated from A. heterophyllus had potent anti-inflammatory effect by inhibiting the production of pro-inflammatory mediators in lipopolysaccharide (LPS)-activated murine macrophage cells and this substantiates the result of the experiment.

5. Conclusion

From this study, we discovered that the extract and fractions of the leaves of *Artocarpus heterophyllus* elicited anti-inflammatory activities due to their bioactive secondary metabolites. This study revealed that the isolated compounds in the crude extract and butanol fraction could be contributing to the anti-inflammatory effects of the plant which justifies its importance in traditional medicines in the management of inflammatory conditions. Further work aimed towards employing other phlogistic agents to outline the appropriate mechanism of action of the extract and fraction leading to purifying and elucidating the structures of the unknown compounds using both the GC-MS and NMR which was not within the scope of the current inquiry are thereby recommended.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The author declares no conflict of interest.

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