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Phytochemical and Acute Toxicity Screening, *In vivo* Antipyretic and Anti-inflammatory Activity of *Morinda citrifolia* Seeds Extracts

***[1]Imieje, Vincent O., [1]Akahomhen, Lucky I., [1]Unegbu, Nelson N. and [2]Obarisiagbon, Philip A.**

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, P.M.B 1154, Ugbowo, Benin City, Nigeria,

[2]Department of Pharmacology &Toxicology Faculty of Pharmacy, University of Benin, P.M.B 1154, Ugbowo, Benin City, Nigeria

***Correspondence Email:** vincent.imieje@uniben.edu

ABSTRACT

This study aimed to evaluate the antipyretic and anti-inflammatory activities of *Morinda citrifolia* (Noni) seed extracts in animal models alongside phytochemical and acute toxicity assessments. The aqueous extract was subjected to phytochemical screening using established methods, and the acute toxicity was determined using an adaption of Lorke's method. The powdered sample was successively extracted to obtain MC-HEX, MC-DCM, MC-DCM/ME, and MC-Met crude extracts. The antipyretic and anti-inflammatory activity of the extracts were evaluated *in vivo* in albino rats. The *in vivo* antipyretic activity was assessed using a brewer's yeast-induced pyrexia model. Data were expressed as the mean ± S.E.M, and ANOVA was used to compare differences between the treatment groups and negative control. Phytochemical tests revealed the presence of alkaloids, flavonoids, tannins, and saponins in plant extract. Acute toxicity tests showed no mortality or observable toxic effects at doses up to 5000 mg/kg. The results demonstrated that MC-DCM and MC-ME extracts at 200 mg/kg bw showed significant antipyretic activities compared to the negative control ($p<0.05$), and also results showed that MC-HEX and MC-ME at 200 mg/kg caused a significant ($p < 0.05$) percentage inhibition of inflammation in the xylene-induced ear oedema compared to the negative control. Results of the study showed that Noni seed extract possesses significant antipyretic and anti-inflammatory activity. These findings highlight that Noni seed extract holds the potential for developing antipyretic and anti-inflammatory agents and alternatives to NSAIDS and steroids. Further studies are warranted to isolate and characterise the active compounds for drug development.

Keywords: Acute toxicity, Anti-inflammation, Antipyretic, *Morinda citrifolia*

INTRODUCTION

Medicinal plants continued to gain global attention due to their therapeutic potential in managing various diseases, including fevers, inflammatory disorders, cancers, and diabetes (Ekor, 2014; Petrovska, 2012). Inflammatory diseases and pyrexia (fever) are common health concerns that contribute significantly to global morbidity and mortality, particularly in low-resource settings where access to conventional therapies may be limited (Ghosh et al., 2011). The search for safer, affordable, and efficacious alternatives has driven extensive research into the pharmacological properties of medicinal plants.

Morinda citrifolia (commonly known as Noni), a member of the Rubiaceae family, has a long history of use in ethnomedicine across Polynesia, Southeast Asia, and other tropical regions. Traditionally, its fruits, leaves, roots, and seeds have been utilised to treat various ailments, including pain, inflammation, infections, and fever (McClatchey, 2002). Pharmacological studies have validated its therapeutic properties, demonstrating antioxidant, anti-inflammatory, anticancer, and antimicrobial activities. These effects are attributed to bioactive phytochemicals, including flavonoids, alkaloids, tannins, and saponins (Wink, 2015; Pawlus & Kinghorn, 2007).

Despite the extensive studies on Noni, research on the seeds remains limited. Preliminary phytochemical analyses suggest that the seeds contain unique compounds with significant pharmacological potential, yet their antipyretic and anti-inflammatory properties have not been systematically investigated. This study aims to bridge this gap by evaluating the phytochemical composition, acute toxicity, and *in vivo* antipyretic and anti-inflammatory activities of Noni seed extracts.

The relevance of this work lies in its potential contribution to drug discovery, particularly in identifying novel bioactive compounds for managing fever and inflammatory conditions. By elucidating the therapeutic

properties of Noni seeds, this study provides scientific validation for their traditional use and lays the groundwork for further pharmacological exploration.

MATERIALS AND METHODS

Reagents and Solvents

Analytical grade Methanol (80%), dichloromethane, and n-hexane were sourced from Sigma Aldrich (Germany), Ferric chloride, sodium hydroxide, Folin-Ciocalteu, sulphuric acid, Dragendorff, and Mayers' reagents (Pyrex, UK). Others include distilled water, acacia gum, orogastric feeding tube, rotary evaporator (Stuart, US), digital thermometer, Metler weighing balance, scissors, Plastic cages, and rodents chow (Flower Mill, Nigeria).

Collection and Preparation of Plant Material

Ripe, fresh fruits of *Morinda citrifolia* (Noni) used in this experiment were collected from a garden at Edjeba Town (coordinates: 5:5421ºN, 5.7369ºE) in Warri South Local Government Area, Delta State, Nigeria, courtesy of Mr. Uche, in June 2021. The botanical identity was authenticated at the Department of Plant Biology and Biotechnology Herbarium Unit, Faculty of Life Sciences University of Benin Benin City, Edo State, Nigeria, where a voucher specimen (UBH-M427) was assigned. The fruit was soaked in water for about 24 hours, after which the seeds were separated from the fruit. The seeds were dried on a flat surface and were ground to powder with a milling machine.

Extraction of Plant Material

A total of 400 g of the powdered seed was subjected to successive maceration in different solvents of 700 mL each in increasing order of polarity, nHexane (MC-HEX), dichloromethane (MC-DCM), dichloromethane:methanol (MC-DCM:MET), and methanol (MC-MET) with intermittent agitations for 72 h. Consequently, the different solvent extracts were filtered and concentrated to dryness *in vacuo* at 40ºC with a rotary evaporator. The dried extracts were stored in an air-tight container and kept in the refrigerator at 4ºC until further use.

Phytochemical Screening

A total of 20 g of the powdered seed of the sample was weighed into a beaker to which 100 mL of distilled water was added and heated to boil for 3 min. This was filtered using a Whatman No 1 filter paper, and the filtrate collected in a conical flask was used to conduct qualitative phytochemical analysis using standard protocols (Sofowora, 1993; Harborne, 1998) to detect the presence of steroid, phenol, tannin, terpenoid, alkaloids, carbohydrate, flavonoids, reducing sugar, saponin, and proteins.

Ethical Approval

Before the commencement of the study, ethical (EC/FP/018/33) approval was obtained from the Faculty of Pharmacy, University of Benin, Benin City ethical committee on the use of experimental animals.

Acute Toxicity Testing

An adaptation of the Lorke's method (Bulus *et al*., 2011) was used in carrying out acute toxicity studies. This method has two phases, phase 1 and 2, respectively.

Phase I: Swiss albino mice weighing 17-23 g of either sex, fasted overnight, were used for this experiment. Three mice each were allocated to three groups: A-C. The weight of each mouse was measured and recorded. Groups A to C received 10, 100, and 1000 mg/kg body weight of the extracts. The extract was administered orally with the aid of an orogastric tube. General symptoms of toxicity and mortality of the mice in each group were observed within 24 hours.

Phase II: Three mice were distributed into three groups, with one mouse in each group. The weight of each mouse was measured and recorded. The mice were administered higher doses (1600, 2900, and 5000 mg/kg body weight) of pure MC-MET and then observed for 24 h for behaviour and mortality. The median lethal dose (LD_{50}) was calculated using equation (1):

$$LD_{50} = \sqrt{(D_0\, x\, D_{100})} \qquad (1)$$

Where;
D_0 = Highest dose that gave no mortality
D_{100} = Lowest dose that produced mortality

Antipyretic Activity
Test animals

Albino rats of either sex and weighing 120 - 150 g were used for the antipyretic test. The animals were kept under controlled conditions (temperature, 25 ± 2℃; 12hr light/dark cycle) and fed with a standard rodent pellet diet and water *ad libitum*. The animals were handled according to the protocol approved by the Faculty of Pharmacy ethical committee and by the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA).

Preparation of extract for animal studies

The crude extracts (250 mg) were mixed with the equivalent weight of acacia gum and dissolved in 5 mL of distilled water. The control treatment was distilled water, while the reference standard was paracetamol (250 mg) suspended in 5 mL of distilled water.

Induction of pyrexia

The antipyretic activity was measured using a modified method described by Dewan *et al*. (2000). The rats were divided into ten groups of four animals each. Rectal temperature was measured by introducing a 3 cm digital thermometer (Model MT-101, N & B Medical, Delhi) coated with glycerin (lubricant) into the rectum. Pyrexia was induced by intra-subcutaneous injection of 20%Baker's yeast suspended in 0.9% saline.

Drug administration

After 4 h post-yeast injection, the animal groups received the extracts (100 mg/kg or 200 mg/kg), paracetamol (reference standard) 150 mg/kg, and distilled water (10 mL/kg) subcutaneously. Body temperature was measured via the rectum hourly from 0 to 8 h.

Xylene-induced ear oedema

A total of 40 Swiss male albino mice were used for this experiment. The mice were divided into 10 groups of four animals in each group. The group and its corresponding treatment are as follows:

Group 1 - 100 mg/kg of Mc-MET
Group 2 - 200 mg/kg of Mc-MET
Group 3 - 100 mg/kg of Mc-HEX
Group 4 - 200 mg/kg of Mc-HEX
Group 5 - 100 mg/kg of Mc-DCM
Group 6 - 200 mg/kg of Mc-DCM
Group 7 - 100 mg/kg of Mc-DCM:MET
Group 8 - 100 mg/k of Mc-DCM:MET
Group 9 – 1 mg/kg bw dexamethasone (positive control)
Group 10 – 5 mL/kg bw distilled water (negative control)

The extracts and standard drugs were administered using an orogastric tube. After 30 min, oedema was induced in each mouse group by applying a drop of xylene to the inner surface of the right ear. After 15 min, the animals were sacrificed under chloroform anaesthesia, and both ears were cut off with a cork borer, sized, and weighed. The anti-inflammatory activity was expressed as the percentage inhibition of oedema in the treated mice compared to the control mice. The indication of inflammation (the thickness of the ears) was used to calculate the percentage inhibition of oedema from equation (2).

$$100 - (\frac{R-L}{L}\ x\ 100) \qquad (2)$$

R = weight of the right ear, and L = Weight of the left ear.

Statistical Analysis

Data were expressed as the mean ± standard error of the mean (S.E.M). Comparison between the treatment groups and negative control was done using a one-way variance analysis (ANOVA) followed by the Dunnet post hoc test. Analysis and data presentation was done using GraphPad Prism version 8.0.2. Results were considered significant when $*p<0.05$, $**p<0.01$, $***p<0.001$.

RESULTS

Extraction yield and organoleptic properties of plant extracts

The results of the extraction, percentage yield, and organoleptic properties of the extracts are presented in Table 1.

Table 1: Extracts, percentage yield, and organoleptic properties

Extracts	Amount (g)	% yield	Organoleptic properties
MC-HEX	15	3.75	Light brown oily liquid, Characteristic smell, greasy to touch
MC-DCM	28	7.00	Brown semi-solid, pungent smell and greasy
MC-DCM/ME	79	19.75	Brown solid, pungent smell, smooth texture
MC-ME	86	21.50	Brown solid, pungent smell, smooth texture

NOTE: MC-HEX = n-hexane extract, MC-DCM = dichloromethane extract, MC-DCM/ME = dichloromethane-methanol extract (1:1), MC-ME = methanol extract

Phytochemical Screening

The aqueous extract of the powdered sample of the seed of Noni contained phenols, flavonoids, reducing sugars, saponins, steroids, acidic components, tannins, carbohydrates, lipids and fats, alkaloids, and proteins, as shown in Table 2.

Table 2: Results of phytochemical screening of seed extract of Noni

Phytochemicals	Inference
Steroids	+
Phenols	+
Alkaloids	+
Tannins	+
Carbohydrates	+
Saponins	+
Protein	+
Lipids and fats	+
Acidic components	+
Reducing sugars	+
Flavonoids	+

Key: + = Present

Acute Toxicity Testing

In the acute toxicity studies, no death was recorded in all doses of the crude Noni seed extract administered during treatment. The animals were healthy with no sign of toxicity up to 5000 mg/kg. Thus, LD_{50} was more than 5,000 mg/kg, as shown in Table 3.

Table 3: Phase I and II results of the acute toxicity study of Noni seeds extract in mice

Group	Dose (mg/kg)	Animal(s) showing signs of toxicity	Animal(s) showing signs of mortality
Phase I			
Group I	10	0/3	0/3
Group II	100	0/3	0/3
Group III	1000	0/3	0/3
Phase II			
Group I	1600	0/1	0/1
Group II	2900	0/1	0/1
Group III	5000	0/1	0/1

Antipyretic Activity

The results and statistical analysis of the antipyretic activity of different solvent extracts of Noni seed are shown in Figures 1 – 4.

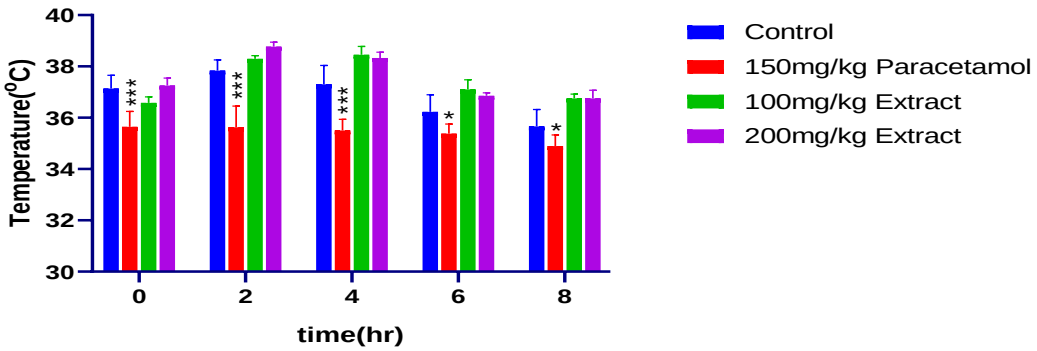

Figure 1: Antipyretic effects of the MC-HEX extract of Noni seeds
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

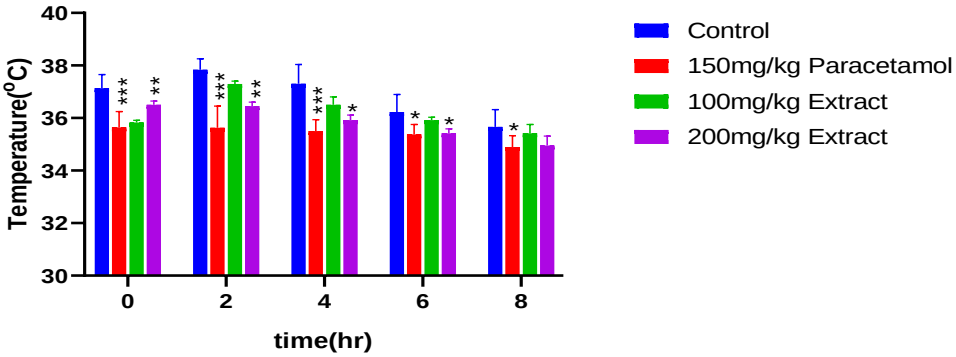

Figure 2: Antipyretic effects of the MC-DCM extract of Noni seeds
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

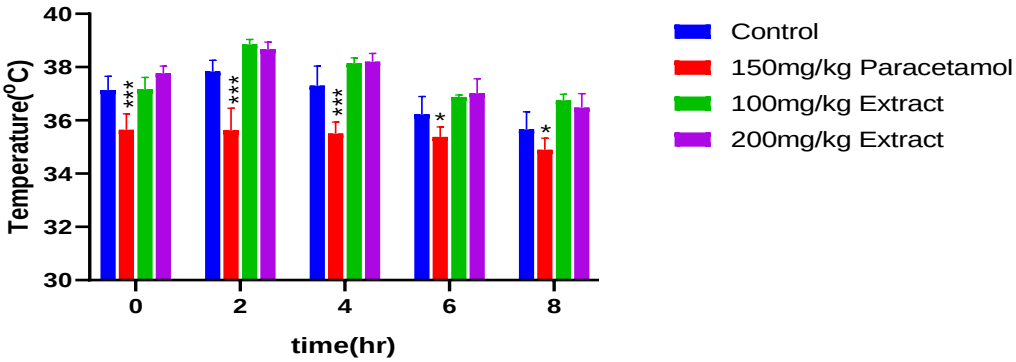

Figure 3: Antipyretic effects of the MC-DCM:MET extract of Noni seeds
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

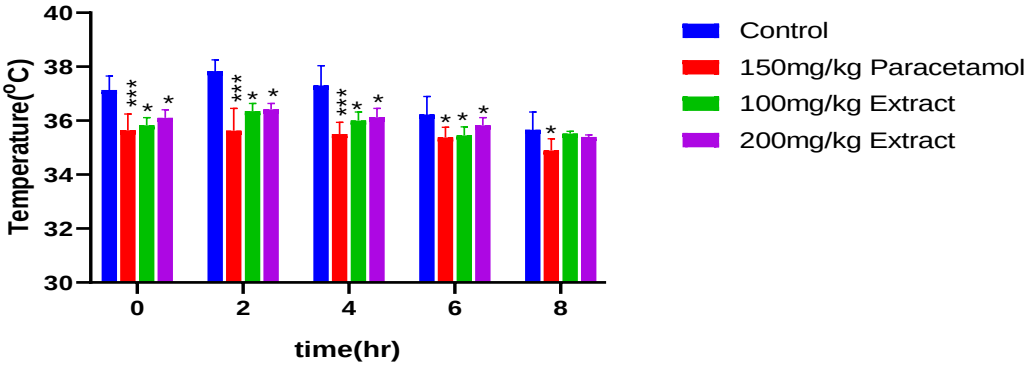

Figure 4: Antipyretic effects of the MC-MET extract of Noni seeds
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Anti-inflammatory Activity

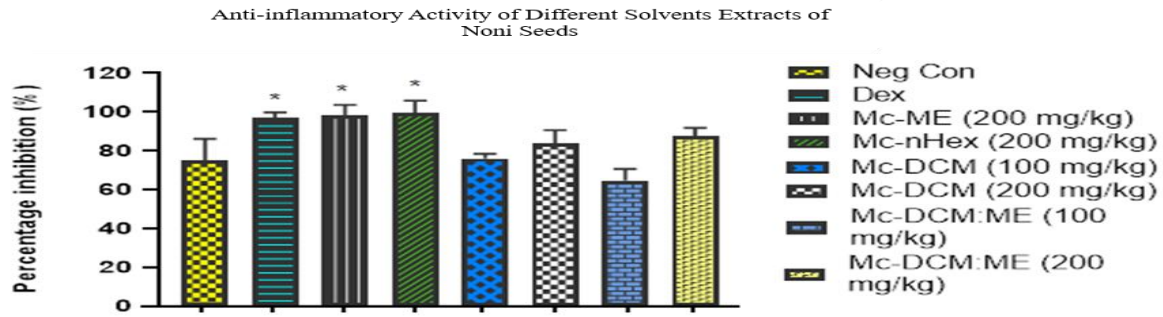

Figure 5: Percentage inhibition of inflammation of the different extracts of Noni seeds extract
Values are expressed as Mean ± S.E.M (n = 4). Treatment/standard group Vs. Negative control group: *$p < 0.05$ is considered significant.
Dex=Dexamethasone, Mc-ME=Methanol, Mc-DCM=Dichloromethane, Mc-nHex= n-Hexane

DISCUSSIONS

The phytochemical screening of the aqueous extract of *Noni* seeds revealed the presence of phenols, flavonoids, tannins, saponins, reducing sugars, alkaloids, and sterols, among other compounds. These bioactive constituents provide a basis for the plant's pharmacological properties and therapeutic applications. **Phenolic compounds** are key antioxidants known to reduce oxidative stress and combat chronic diseases such as cancer and heart disease (Wang *et al.*, 2016). The phenols in *Noni* likely contribute to its antioxidant and anti-inflammatory activities, supporting its traditional medicinal use. **Flavonoids** are another class of antioxidants with broad pharmacological properties, including anti-inflammatory, anticancer, and neuroprotective effects. Their ability to modulate cellular enzyme functions, such as xanthine oxidase and cyclooxygenase, enhances their role in managing conditions like Alzheimer's disease and atherosclerosis (Feliciano *et al*., 2015). **Tannins,** polyphenolic compounds, have antioxidant, antimicrobial, and anti-carcinogenic effects. Their interaction with microbial proteins and oxidative pathways underscores their potential therapeutic relevance (Andrade *et al*., 2005). **Saponins** and **steroids** reduce blood cholesterol, enhance immune function, and manage hyperglycemia. Saponins may stimulate insulin release and slow gastric emptying, supporting their hypoglycemic effects (Metwally *et al*., 2012). Sterols, in addition to lowering cholesterol, exhibit antiviral and anticancer activities, further highlighting the potential of *Noni* in metabolic and immune-related disorders (Bouic, 2001). **Alkaloids,** known for their broad biological activity, including analgesic and anti-inflammatory effects, also contribute to the plant's pharmacological profile. Commonly used alkaloids like morphine and quinine have well-established roles in medicine, emphasizing the therapeutic promise of these compounds in *Noni* (Kurek *et al*., 2019). The presence of **reducing sugars** highlights their role as metabolic precursors in synthesising bioactive secondary metabolites. These sugars are vital in stress responses and disease resistance, enhancing the plant's medicinal potential (Rolland *et al*., 2002). The acute toxicity evaluation of the crude methanol extract of Noni seeds revealed a high safety margin, with no mortality or significant adverse effects observed at a single oral dose of 5,000 mg/kg body weight (Table 3). The LD_{50} value was established as greater than 5,000 mg/kg, classifying the extract as practically non-toxic according to standard toxicity guidelines (Kennedy *et al*., 1986). This aligns with the World Health Organization's acknowledgment of the reliance on traditional medicine by 80% of populations in remote areas, emphasizing the safety and accessibility of plant-based therapeutics (Shi et al., 2010). However, a study by Elizabeth *et al*. (2011) reported an isolated case of acute hepatotoxicity in a 14-year-old boy after a drink of *Morinda citrifolia* (Noni Berry) juice. Another study on the chronic toxicity of fruit juice and leaf extract revealed that chronic intake of the juice induced liver toxicity in mice, but the leaf extract showed no toxicity.

The growing preference for herbal remedies over synthetic drugs is driven by their perceived safety, lower toxicity, and therapeutic efficacy, particularly in developing countries with limited access to modern healthcare (Engebretson, 2002; Qi *et al*., 2013). With its rich biodiversity, Malaysia has long recognised the value of medicinal plants, including Noni, in addressing chronic diseases and maintaining health (Sultana *et al*., 2014). This study underscores the practical safety of Noni seed extract, supporting its extensive use in traditional medicine. However, the findings also highlight the need for further investigations into its comprehensive toxicity profile. Evaluating serum biochemical parameters, haematological indices, and histopathological effects on vital organs will provide a complete understanding of the extract's safety for therapeutic applications. The non-toxic nature of Noni seed extract at high doses paves the way for its potential use in future preclinical and clinical studies, validating its role as a safe and effective component of traditional and modern herbal medicine systems.

The antipyretic effects of various doses of Noni extracts were evaluated using a yeast-induced pyrexia rat model, which simulates pathogenic fever caused by prostaglandin (PGE2) production (Aman *et al*., 2011). The MC-MET and MC-DCM extracts demonstrated significant antipyretic activity at doses of 100 mg/kg and 200 mg/kg, with MC-DCM (200 mg/kg) showing effects comparable to the standard drug, paracetamol (150 mg/kg) (Figure 2), particularly at the 6th-hour post-administration. Statistical significance for the extracts was observed at varying levels: paracetamol ($p<0.001$), MC-DCM 200 mg/kg ($p<0.01$), and MC-MET 100 mg/kg and 200 mg/kg ($p<0.05$). In contrast, the MC-HEX and lower doses of MC-DCM and MC-MET did not exhibit significant temperature reduction compared to the negative control. These findings suggest a dose-dependent antipyretic effect, potentially attributed to the bioactive compounds present in the extracts.

The antipyretic mechanism is likely linked to inhibiting cyclooxygenase (COX) enzymes, specifically COX-3, which reduces prostaglandin synthesis in the hypothalamus, lowering fever (Okokon *et al*., 2010). Flavonoids, prominent in Noni, have been reported to inhibit TNF-α and arachidonic acid peroxidation, reducing prostaglandin levels and associated fever (Adesokan *et al*., 2008; Germain *et al*., 2011). Additionally, saponins and alkaloids may contribute synergistically to this effect by

modulating inflammatory mediator pathways (Zakaria *et al*., 2007). The results align with previous studies on the pharmacological properties of Noni. Tanikawa *et al*. (2021) reported that the seed extract reduces inflammatory mediator expression, supporting its therapeutic potential. Similarly, Younos *et al*. (1990) identified Noni as a selective COX-2 inhibitor with analgesic efficacy comparable to 75% of morphine but without addictive properties or adverse effects. The Mc-MET and Mc-DCM extracts of Noni exhibit significant antipyretic activity, likely mediated by the inhibition of prostaglandin synthesis through the action of flavonoids, saponins, and alkaloids. These findings reinforce the therapeutic potential of Noni in managing pyrexia and inflammatory conditions. Further studies are recommended to explore the molecular mechanisms and optimize dosage for clinical applications.

Similarly, the result of the anti-inflammatory effect of the different doses of the test extracts ME (200 mg/kg), nHex (200 mg/kg), DCM (100 mg/kg, 200 mg/kg), and MET:DCM (100 mg/kg, 200 mg/kg), is shown in Figure 5. Mc-ME (200 mg/kg) and n-HEX extract (200 mg/kg) showed a significant increase in percentage inhibition when compared to the negative control group, at $p < 0.05$. Mc-DCM (100 mg/kg, 200 mg/kg) and Mc-DCM:MET (100 mg/kg, 200 mg/kg) did not show any significant increase ($p > 0.05$) in percentage inhibition when compared to the negative control group. Animal experiments and studies of the anti-inflammatory effects of *Morinda citrifolia* extract on xylene-induced ear oedema in mice indicated that the extract possesses anti-inflammatory activity by reducing ear oedema. Methanol extract at 200mg/kg and n-hexane extract at 200 mg/kg showed the highest percentage inhibition. This may be due to the solvents' ability to extract phytochemicals from the plant seed through pharmacological activities. The observed anti-inflammatory effects of Noni extracts align with the acute inflammatory model induced by xylene, which partially involves substance P (SP) as a mediator of increased capillary permeability and leukocyte infiltration (Milenkovic *et al*., 2019). The methanol and n-hexane extract likely modulate neurogenic inflammation by inhibiting the release or action of substance P, a neurotransmitter involved in the initial neurogenic phase of inflammation. During this phase, substance P and bradykinin induce vasodilation, plasma exudation, and the release of nitric oxide, which contributes to the inflammatory response (Cutolo *et al*., 2019; Needham *et al*., 2019). The extracts may counteract these processes by reducing ear oedema, highlighting their potential as therapeutic agents for acute inflammation. Another study (Huang *et al*., 2014) highlighted the anti-inflammatory potential of noni fruit extracts (ethanol and ethyl acetate extract) in C57BL/6 mice. The extracts reduced both neutrophil chemotaxis and production of inducible nitric oxide (iNOS) and COX-2 at 500 mg/kg daily oral dose. The findings suggest that methanol and n-hexane solvents facilitate the extraction of phytochemicals with anti-inflammatory properties, possibly explaining their superior activity compared to the dichloromethane-based extracts.

This current study underscores the role of innate immune cells in the initial stages of inflammation, as these cells regulate vascular responses and leukocyte migration into affected tissues. The xylene-induced ear oedema model provided insights into the acute anti-inflammatory activity of Noni, supporting its potential application in inflammatory conditions (Yousuf *et al*., 2019; Almeida-Souza *et al*., 2016). Future research would focus on isolating the active compounds within these extracts and understanding their specific mechanisms of action to validate their clinical relevance.

CONCLUSION

This study highlights the antipyretic and anti-inflammatory potential of Noni seed extracts, underscoring their therapeutic promise. The dichloromethane and methanol extracts demonstrated potent antipyretic activity, likely mediated through the suppression of inflammatory mediators, attributed to the bioactive secondary metabolites identified in the extracts. Similarly, the methanol and hexane extracts exhibited significant anti-inflammatory effects, effectively reducing ear oedema in mice at an oral dose of 200 mg/kg bw. Additionally, the aqueous extract showed an $LD_{50} >$ 5000 mg/kg bw, indicating its safety for potential human applications. These findings provide compelling evidence for the medicinal value of *Noni* seeds and suggest that further isolation and characterization of their bioactive compounds could pave the way for developing novel and targeted antipyretic and anti-inflammatory therapies. This research contributes to the growing knowledge of natural remedies and their role in addressing inflammation and fever-related disorders.

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