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The Effect of Methanol Seed Kernel Extract of *Mangifera Indica* on Guinea Pig Tracheal Smooth Muscles

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Abstract

Mangifera indica, commonly known as mango, holds a revered position in traditional medicine for the numerous health benefits derived from different parts of the plant. The purpose of this study was to investigate the effect of methanol seed kernel extract of *Magnifera indica* on isolated guinea pig trachea. The acute toxicity profile was first evaluated as well as its phytochemical screening before exposing various concentrations of the methanol seed kernel extract to acetylcholine (Ach)-precontracted guinea pig tracheal rings. From the results, a dose of 5000mg/kg of the extract did not produce any form of toxicity. The presence of tannins, flavonoids and cardiac glycosides was revealed which probably fuels the medicinal properties of the seed kernel. Exposure of acetylcholine pre-contracted tracheal rings to different concentrations of the methanol kernel extract, resulted in a shift of the Ach dose response curve to the right but without achieving any maximum relaxation. There was no observed IC_{50} but rather an IC_{30} concentration was observed around 0.0013M. However, the observed minimal relaxation of Ach pre-contracted rings was not statistically significant ($p \geq 0.5$). It is possible that the observed minimal relaxation potential of the kernel extract coupled with its reported anti-inflammatory properties boosts its application in the relief of bronchial asthma claimed in ethnomedicine.

Keywords: Asthma; *Mangifera indica*; Guinea pig trachea; Acetylcholine.

INTRODUCTION

Bronchial asthma is a chronic airway disease characterised by a significant morbidity and mortality and imposes a daunting socioeconomic burden globally (Nunes et al., 2017); (Ge et al., 2013). According to the World Health Organization, an estimated 262 million individuals were affected globally with about 455,000 deaths in 2019. In Nigeria, the prevalence ranges from 5.1% to 14.3% (Musa et al., 2014) with studies indicating that approximately 15% of adults over 45 years of age experience wheezing episodes annually (GBD 2019 Diseases and Injuries Collaborators, 2020). Essentially, asthma is marked by airway inflammation and hyper⁼responsiveness (Bradding et al., 2024) which leads to wheezing, coughing and shortness of breath. This inflammatory process can manifest in acute, sub-acute, or chronic forms, resulting in alterations in airway tone, increased vascular permeability, neural activation, enhanced mucus secretion, and structural changes in the airways, which may be reversible or permanent (Tirgar et al., 2012).

The pharmacological management of asthma typically involves two primary approaches: the inhibition of inflammation, often achieved through corticosteroids such as prednisolone, and the alleviation of bronchospasms using bronchodilators like salbutamol (Kondo & Tamaoki, 2018). These pharmacologic interventions are effective in minimizing or eliminating symptoms, stabilising pulmonary function, and enabling patients to maintain an active lifestyle without significant limitations, including exercise (Kondo & Tamaoki, 2018). Unfortunately, unlike natural products (Etti et al., 2016); (Etti et al., 2020), these treatments are accompanied by adverse events, particularly in patients with comorbid conditions. Challenges such as drug resistance, the high cost of medications coupled with dwindling economies especially in low-income countries and the inevitable side effects of synthetic drugs have spurred interest in alternative treatments derived from natural sources, which are perceived as more accessible, affordable, and less toxic. Consequently, a substantial proportion of the population, especially in developing countries, utilizes herbal remedies alongside conventional medicine (Adefolaju, 2014); (Singh et al., 2004).

Mangifera indica L., commonly known as mango, is a succulent drupe from the family of Anacardiaceae

within the Sapindales order. Mangoes are esteemed not only for their palatable fruit but also for their rich content of micronutrients, vitamins, and diverse phytochemicals (Jahurul et al., 2015). In addition to their nutritional value, various components of the mango tree, notably the seed kernel, have been utilized in traditional medicine for their purported therapeutic benefits. The seed kernel has been reported to be a repository of bioactive compounds. Traditional medicine practices have claimed the efficacy of mango seed kernel extracts in alleviating respiratory conditions, such as asthma. the scientific validation of such claims remains limited, in spite of these assertions. Thus, this work aims to investigate the smooth muscle relaxant effects of mango seed kernel extract, to validate the ethnobotanical claims.

MATERIALS AND METHODS

Extraction

An 88.60g weight of powdered seed sample was macerated using an extraction tank and extracted using 3.5 L of 70% ethanol. This was done by adding solvent to the seed sample in the extraction tank and allowing the mixture to stand for 72 hours with frequent agitation by stirring twice a day. After 72 hours, the extract was filtered and the filtrate obtained was concentrated to dryness using a water bath at a temperature of 49^{O}C. The obtained extract of 11.55g was stored in a sealed container at 4 ^{O}C in a refrigerator within the experimental period. The percentage yield of the plant extract was calculated**.**

Laboratory Animals

Guinea pigs of either sex weighing 450–500 g and Swiss albino mice of either sex weighing between 20–30 g were used for the experiments. The guinea pigs were purchased from an animal vendor from Ikot-Ekpene, Akwa Ibom state, Nigeria and were used for anti-asthmatic evaluations. The mice were purchased from the animal house, Department of Pharmacology and Toxicology, University of Uyo. All animals were kept in plastic cages; males were separated from females and allowed two weeks of acclimatization. The animals had free access to dry rodent pellets and tap water and were exposed to natural light/dark cycle and room temperature. They were handled according to standard protocols for the use of laboratory animals (National Institute of Health, 2002). The experimental protocol was approved by the Faculty of Pharmacy Institutional Animal Care and Use Committee of the University of Uyo.

Qualitative Analysis of Phytoconstituents

The methanol seed extract of *M. indica* was subjected to qualitative chemical screening for secondary plant metabolites using conventional methods (Sofowora, 1993).

Test for Saponins

Frothing Test: The seed powder extract (0.5 g) was shaken vigorously with 5 mL of distilled water in a test tube for 1 minute. Frothing, which persisted during warming indicated the presence of saponins (Sofowora, 1993).

Test for Tannins

Ferric Chloride Test: The seed powder extract (0.5 g) was mixed with 5 mL of distilled water and filtered. 2 drops of 5% Ferric Chloride were added to the filtrate. A blue-black. precipitate was seen as evidence for the presence of tannins (Evans, 2009).

Test for Flavonoids

Magnesium Metal Test: A few pieces of magnesium metal were added to 5 mL of seed powder root extract solution and 1 mL of concentrated hydrochloric acid was added. The presence of orange colour was taken as an indicator of flavonoids (Evans, 2009).

Sodium Hydroxide Test: The seed powder extract (0.5 g) was dissolved in 3 mL of distilled water. Few drops of 5% sodium hydroxide were added. A yellow colouration indicated the presence of flavonoids (Evans, 2009).

Test for Cardiac Glycosides

Salkowski's Test: The seed powder extract (0.5 g) was dissolved in 4 ml of chloroform. Concentrated sulphuric acid was gently added by running it down the side of the test tube to form a distinct lower layer. A reddish-brown colouration at the interphase indicated the presence of a steroidal cardiac glycoside (Sofowora, 1993).

killer-killianı Test: The seed powder extract (0.5 g) was dissolved in 5 mL of glacial acetic acid containing one drop of 5% ferric chloride solution. The solution was then underlay with 1 mL concentrated sulphuric acid by slowly running it down the test tube side to form a distinct lower layer. A brown ring at the interphase indicated the presence of glycoside (Evans, 2009)

Lieberman's Test: The seed powder extract (0.5 g) was dissolved in 4 mL acetic anhydride and cooled sufficiently in ice. Concentrated tetraoxosulphate (V1) acid was carefully added to form the lower layer. A colour change from violet to blue to green was taken as evidence for the presence of a steroidal nucleus; the aglycone portion of the cardiac glycoside (Evans, 2009).

Test for Alkaloids

The seed powder extract (0.5 g) was heated with 10 mL of 5% HCL in a test tube filled with boiling water. The mixture was allowed to cool and then filtered. A few drops of Dragendorff's precipitation reagent were added and observed. A yellow or red precipitate was taken as indication of the presence of alkaloids (Evans, 2009).

Oral Acute Toxicity of Methanol Seed Extract of Magnifera Indica

The median lethal dose (LD50) of the seed extract of *Magnifera indica* was estimated in mice using a modified Lorke's method (Lorke, 1983); (Etti et al., 2024). The animals were randomly allotted to five groups of three animals each. Group I was orally administered 2 ml/kg of distilled water and groups II–V were orally administered 2000, 3000, 4000, and 5000 mg/kg of *M. indica* extract concentrations respectively. The animals were deprived of feed 24 hours prior to treatment but had free access to water before administration of single doses of the seed extract of *M. indica*. The general behaviors of mice were observed continuously for 1 hour after treatment and intermittently for hours and thereafter over a few days for any sign of toxicity and death.

Isolated Guinea Pig Tracheal Ring Experiments

Seed extract of *M. indica* was tested on isolated tracheal rings obtained from guinea pigs (Ozolua et al., 2011). The guinea pig was mercifully sacrificed, the trachea was dissected out quickly and placed in a Petri dish containing physiological salt solution (PSS). The trachea was cleaned of adherent connective tissues as much as possible and cut into rings of 2.5 mm in length. The rings were suspended in L-shaped wire loops in 50 ml organ baths containing the PSS and thereafter sensitized**.** The composition of the PSS (Krebs) was (g/l): NaCl 6.9, KCl 0.353, $MgSO4.7H_20$ 0.2, glucose 2.0, $CaCl_2$ 0.28, $NaHCO_3$ 2.10**.** The PSS was bubbled throughout the experiment with a 95% O_2 and 5% CO_2 gas mixture (BOC Gases, Nigeria Plc) and the temperature was maintained at 37 °C. Responses were measured with a force displacement transducer (FT 302) which was connected to a Grass 7D polygraph (Grass instrument Co, Quincy, MA, USA).

The rings were given a resting force of 1 g and allowed an equilibration period of 45 min during which the PSS was changed four times. After equilibration, the rings were depolarized with 80 mMKCl before experimental protocols. Anti-asthmatic effect was evaluated by first applying cumulative concentrations of histamine (1×10^{-5} to 1×10^{-3} M) to the organ baths. Then in the presence of different concentrations (5×10^{-6} mg/ml, 5×10^{-5} mg/ ml, 5×10^{-4} mg/ml and 5×10^{-3} mg/ml) of the extract, cumulative concentrations of histamine were applied.

The concentrations of histamine producing 50% of maximum response (EC50) and maximum response (Emax) by the tracheal rings in each experiment were calculated. The spasmolytic effect was evaluated by pre-contracting tracheal rings with the EC50 of histamine (2 × 10−4 M). At maximum contractile response, cumulative concentrations of *M. indica* seed extract (5×10^{-5} - 5×10^{-3} mg/ml) were injected into the organ bath. Similar concentration-response relationships were obtained of Acetylcholine (1×10^{-5} to 1×10^{-1} M) in rings pre-contracted with the EC50 of histamine. The Emax was compared across the three concentrations of the extract.

Statistical Analysis

The statistical evaluation was performed using non-linear regression in GraphPad Prism software (GraphPad Software Inc., San Diego, USA). All the experimental groups were compared appropriately to assess the significance using Student's t-test. Data are presented as mean ± SEM (standard error of the mean) IC50 (concentration producing 50% inhibition of maximum contractile response after pre-contraction) and Emax (maximum relaxant response) values were estimated graphically. Statistical significance was set at $p< 0.05$.

RESULTS AND DISCUSSION

Figure 1. *Magnifera indica* seed kernel extraction process.

Percentage Yield of *Magnifera indica* Seed Extract

The percentage yield of methanol *Magnifera indica* seed extract was calculated to be 13.0% from an initial weight of 88.6g using the formula below (equation 1).

$$\% \text{ Yield} = \frac{We}{Ws} \text{ X } 100 \quad (1)$$

Where: *We* if Weight of dried extract obtained (g) and *Ws*is the Initial weight seed sample (g)

Phytochemical Constituents of *Magnifera indica* Seed Extract

The preliminary phytochemical screening of the methanol seed extract of *Magnifera indica*revealed the

presence of various secondary metabolites such as tannins, flavonoids, cardiac glycosides as shown in the table below.

Table 1. Phytochemical constituents of methanol seed extract of *Magnifera indica.*

S/N	Test	Observation	Inference
1.	Alkaloid	Yellow solution with no precipitate was observed	–
2.	Saponin	There was no preserve of frothing while warming	–
3.	Tannins	Blue-black coloration was observed	+ + +
4.	Flavonoids	An orange coloration was observed	+ +
5.	Salkowskis(cardiacglycosides)	A reddish-brown color was observed at the interphase	+ +
6.	Keller	Brown ring at the interphase was observed	+ +
7.	Leibermann	Slight yellow solution was observed with no reddish brown at interphase	–

Acute Toxicity Studies of *Magnifera indica* Seed Extract

Magnifera indica seed extract did not cause any sign of toxicity after the oral administration of any of the oral doses (2000mg/kg, 3000mg/kg, 4000mg/kg, 5000mg/kg). The median lethal dose for the extract was thus concluded to be greater than 5000mg/kg. No notable gross toxicological symptom was seen within the period of observation.

Effect of Acetylcholine and Histamine on Contraction of Guinea Pig Tracheal Rings

The two contractile agents, acetylcholine and histamine were shown to produce a dose-dependent increase in contraction of the tracheal rings. From the EC50 values, Histamine produced more contractility when compared with acetylcholine (Figure 2). Both contractile agents produced a ceiling effect and had the same efficacy, indicated by their maximal attainable response.

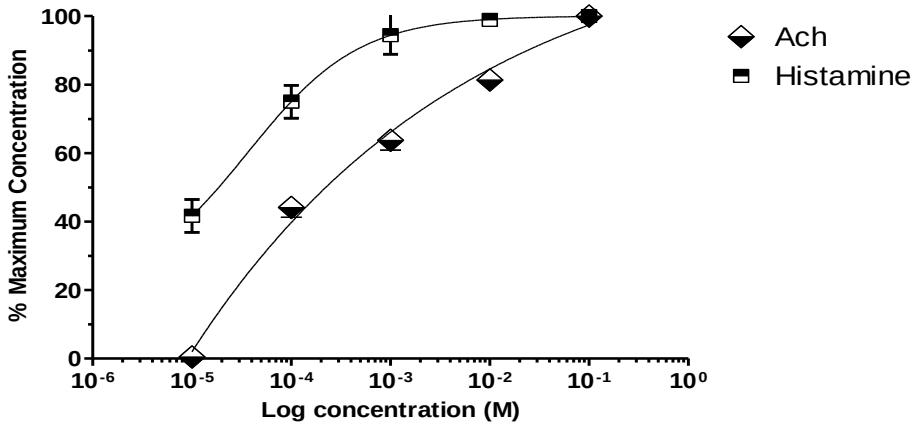

Figure 2. Effect of acetylcholine on contraction of Guinea Pig Tracheal rings. Values are expressed as mean ± SEM for acetylcholine and histamine concentration.

Effect of Methanol Seed Kernel Extract of *Magnifera indica* on the Dose Response Curve of Acetylcholine

The *Magnifera indica* seed kernel extract was seen to minimally reduce the tracheal contraction elicited by acetylcholine (Figure 3)

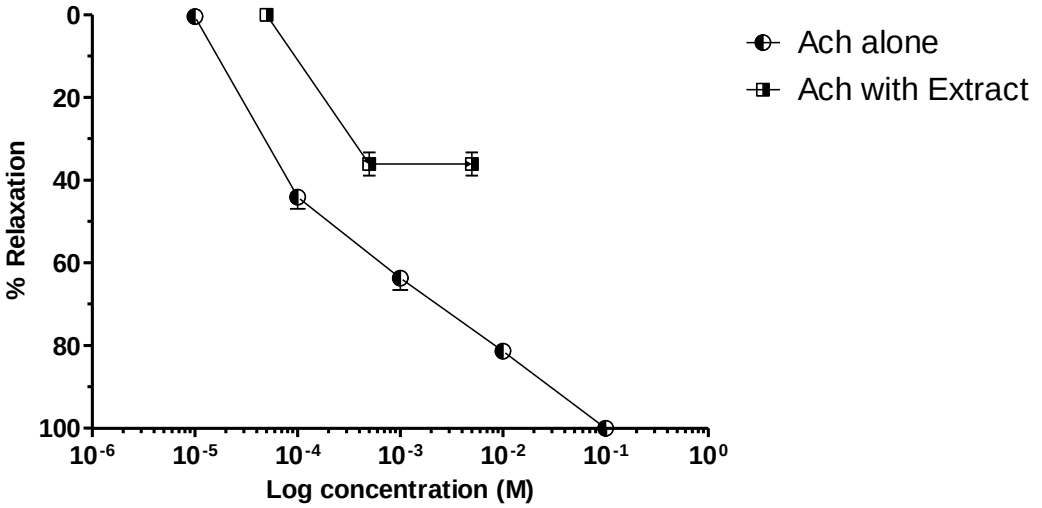

Figure 3. Effect of *Magnifera indica* seed kernel extract on guinea pig tracheal rings. Values are expressed as mean ± SEM.

DISCUSSION

Plants have long been observed and reported to contain an array of bioactive compounds that not only benefit the plants but also offer diverse pharmacological activities to humans. *Mangifera indica L.*, a tropical fruit widely consumed for its nutritional value is no exception to the rule. It has been recognized to possess abundant bioactive compounds. While much attention has been devoted to the fruit pulp and peel, the seed kernel—a by-product of mango processing—remains underutilized, despite its reported medicinal properties. Studies have highlighted the presence of numerous phytochemicals in the mango seed kernel, including phenolic acids, flavonoids, tannins, and saponins, which are associated with numerous activities like: antimicrobial, anti-inflammatory, antioxidant, antidiabetic, and hepatoprotective activities. This study aims to validate the assertion made by traditional practices on the relevance of the mango seed kernel extract in airway respiratory disease by performing a phytochemical screening of the mango seed kernel extract and evaluating its possible tracheal smooth muscle relaxant effect.

This study revealed the large safety margin (OECD/OCDE 423 2001) of the extract, which is similar to that of previously reported studies (Okoro et al., 2016). The phytochemical screening of *Mangifera indica* seed kernel extract revealed the presence of various bioactive compounds, including alkaloids, gums, flavonoids, phenols, saponins, steroids, tannins, and xanthoproteins. These phytochemicals are known for their diverse pharmacological properties and their ability to confer health benefits, many of which may have been implicated in respiratory health. Tannins, a prominent component identified in the extract, are polyphenolic compounds known for their astringent properties. They can bind and also contribute to a plant's defense mechanism (McGarvey et al., 2006). Medicinally, tannins exhibit anti-inflammatory and antioxidant properties (He et al., 2015) which could play a role in mitigating airway inflammation, a key factor in bronchial asthma (Okuda & Ito, 2011). Flavonoids, another important class of phytochemicals identified in the extract, have been well-documented for their potent antioxidant, bronchodilatory, anti-inflammatory (Riella et al., 2012) and immune-modulating effects as well as anti-cancer properties (Etti, et al., 2017). These compounds can inhibit the release of inflammatory mediators such as histamine and leukotrienes, thereby reducing airway hyperresponsiveness and promoting bronchodilation (Middleton et al., 2000). Quercetin, a representative flavonoid, has been specifically highlighted for its capacity to relax tracheal smooth muscle and suppress allergic airway inflammation (Carrillo-Martinez et al., 2024). This glucocorticoid-like effect may be helpful for prophylaxis and long-term treatment of asthma (D'Urzo et al., 2015). Flavonoids inhibit antigen-induced release of histamine from mast cells (Park et al., 2008) and also inhibit contractions induced by broncho-constrictors such as histamine among many other anti-asthmatic properties (Tanaka & Takahashi, 2013). Many plants with similar phytoconstituents as *Magnifera indica* have also exhibited anti-asthmatic properties (Nagore et al., 2009).

Spasmogens such as histamine and Acetylcholine activate calcium release and utilization processes that accentuate airway smooth muscle contraction (Panula P. . et al., 2015). Histamine produced more contractility when compared with acetylcholine (Figure 1). Effect of histamine (1×10^{-5}) as shown on the graph, showing some level of contractile effect on the tracheal musculature used with cumulative dosing shows greater more significant effect. Furthermore, the result of the effect of the histamine (1×10^{-4}mg/ml) shows an

increasing contractile effect on the tracheal ring.When the extract (5 x 10^{-4}mg/ml) was used to challenge the contractile effect of histamine (1 x 10^{-4}mg/ml), there was no marked difference in the contraction seen, that is, the extract did not significantly affect the normal contractile responses induced by histamine. The log concentration versus relaxation curves for Acetylcholine alone, and Acetylcholine in the presence of the extract as shown in Fig. 2, indicate that the methanol seed extract of *Magnifera indica* did not significantly relax the tracheal contraction elicited by acetylcholine in guinea pigs. No IC_{50} was observed in the pre-contracted tracheal rings when exposed to the seed kernel extract. There was no statistical difference between the Ach-pre-contracted tracheal rings and rings exposed to the plant extract ($p \geq 0.5$). However, an inhibitory concentration of 20% was observed at about 25 x 10^{-5} M concentration of the extract. Salbutamol gave a percentage maximum relaxation of 100% at just a single dose of 1 x 10^{-4} (data was not included in the graph). The minimal relaxation to the Acetylcholine pre-contracted guinea pig tracheal ring is an indication of the anti-inflammatory action of substances to promote the relief of bronchospasm induced by spasmogens such as histamine and acetylcholine (Goldie et al., 1986).The minimal or negligible bronchodilatory effect observed in this study is an indication that the claims made by traditional practitioners are likely due to the collective anti-inflammatory and anti-oxidant effect of the seed kernel extract as mediated by its bioactive phytochemicals.

CONCLUSION

This study investigated the phytochemical composition and the effect of methanol seed kernel extract of *Mangifera indica* on guinea pig tracheal smooth muscles. The phytochemical screening revealed the presence of abundant flavonoids and tannins, cardiac glycosides, along with other bioactive components known for their anti-inflammatory and bronchodilatory properties. These findings support the potential of mango seed kernel extract as a source of pharmacologically active compounds with relevance to respiratory health. Despite the promising phytochemical profile, the extract's effect on tracheal smooth muscle relaxation was relatively modest, achieving an IC20 value but not reaching an IC50 threshold. This suggests limited direct efficacy in inducing bronchodilation under the experimental conditions used. While the observed effects may point to subtle pharmacodynamic activity, they also highlight the need for further investigation into the extract's mechanism of action and potential synergistic effects *in vivo.*

Competing Interests: The authors declare that there are no competing interests.

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