



Acute Toxicity, Antidiarrhoeal and Antioxidant Activities of Methanolic Leaf Extract of *Baphia macrocalyx* in Mice

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Abstract

The aim of the current study was to assess the acute toxicity, antidiarrhoeal and antioxidant potentials of methanolic leaf extract of *Baphia macrocalyx* (BM) in mice. Acute toxicity test of BM extract was performed based on OECD guideline 423. Results revealed that the extract did not produce any changes in general behaviour, body weight or mortality of the tested mice even at the highest oral administered dose (2000 mg/kg body weight). The antidiarrhoeal effect was examined using castor oil induced diarrhoea test. Results showed that BM extract delayed the onset of diarrhoea, decreased frequency of defaecation and reduced the severity of diarrhoeal drops in a dose dependent manner at doses of (100, 500 and 1000 mg/kg), respectively. The group that received the dose of 1000 mg/kg showed a significant difference ($p < 0.001$) in inhibition of diarrhoeal drops compared to the control group. For the determination of antioxidant activity, lipid peroxidation assay was used and BM extract significantly ($p < 0.05$) counteracted rotenone induced oxidative stress in brain tissues in a graded dose. Taken together, this study showed the potential of BM in inhibition of diarrhoea and oxidative stress with no toxic effects on tested mice, suggesting the use of this plant as an antioxidant and for ethno-medical management of diarrhoea.

Keywords: *Baphia macrocalyx*; Antidiarrhoeal; Antioxidant; Acute toxicity.

Introduction

Baphia macrocalyx (Harms) is a native leguminous plant in the family Fabaceae. It is locally distributed in coastal woodland or dry forest patches in northern Mozambique and southern Tanzania (Kilwa, Lindi and Mtwara). *Baphia macrocalyx* (BM) is a small tree plant of about 10 m high and is commonly known as “mkoromombe” (Makonde). There is no available information in the literature on the use of BM in traditional medicine in East Africa. However, a recent study on phytochemical and bioactivity study of BM revealed the presence of high phenolic compounds especially those

of c-methylated flavones possessing high antioxidant and antileishmania activities in *in vitro* studies (Bwire 2015). In West Africa, two species of *Baphia* namely *Baphia nitida* and *Baphia bancoensis* are used for treatments of several ailments such as skin, gastrointestinal, inflammatory and venereal diseases (Adeyemi et al. 2006, Yao-Kouassi et al. 2008). Phytochemical constituents of *Baphia* species have been identified to be saponins, tannins, flavonoids, isoflavonoids, isoflavones, and alkaloids (Adeyemi and Akindele 2008, Kapingu et al. 2008, Ogunwa 2016).

The main groups of polyphenols are phenolic acids, flavonoids, anthocyanins that together with vitamin E, C and carotenoids are referred to as antioxidants. Such compounds have shown to display a wide spectrum of antioxidant activities in *in vitro* and *in vivo* studies (Djeridane et al. 2006, Choudhary and Swarnkar 2011, Bursal and Gülçin 2011, Saeed et al. 2012, Bwire 2015, Aronsson et al. 2016) and are believed to exert protective effects against major non-communicable diseases such as cancer, cardiac and neurodegenerative diseases (Tapiero et al. 2002, Essa et al. 2016).

The existing theory of free radicals in medicine and biology explains that reactive oxygen species (ROS) are involved in promoting aging and non-communicable diseases (Maestri et al. 2006, Fusco et al. 2007, Hwang 2013). The excessive production of ROS against endogenous antioxidants defence contributes to oxidative stress that brings damage at cellular and tissue levels thereby affecting biological molecules; lipids, proteins and nucleic acids (Fusco et al. 2007). The use of synthetic antioxidants in foods and oils has been an attempt of preventing oxidative damage. However, synthetic compounds have been associated with adverse side effects (Maestri et al. 2006). In recent years, the natural antioxidants are receiving attention since they have been refined from the natural products that have been utilized for many years in different societies (Lee et al. 2005, Nakamura et al. 2006, Kolosava et al. 2006, Gao et al. 2012, Solanki et al. 2015, Ali et al. 2019).

On the other hand, diarrhoea is one of the most health threats to societies in poor tropical countries with the estimation of 2 billion cases per year, of which 1.7 billion cases are in children of less than five years old (WHO 2019). Diarrhoea is a frequently passage of loose or watery stool mainly due to unbalanced water and electrolytes within the intestinal lumen. Diarrhoea disease is characterized by decreased electrolyte absorption, increased intestinal motility,

increased luminal osmolarity and increased electrolytes secretions (Sweetser 2012). Several studies have examined and reported the antidiarrhoeal properties of different tropical medicinal plants including *Baphia* species (Adeyemi and Akindele 2008 Mbagwu and Adeyemi 2008, Onoja et al. 2018, Mekonnen et al. 2018, Naher et al. 2019). Thus, the present study sought to investigate toxicity and efficacy of methanolic leaf extract of *B. macrocalyx* against diarrhoea and oxidative stress in mice.

Materials and Methods

Chemicals and reagents

All reagents were of analytical grade. Methanol (Scharlau, Barcelona–Spain), dimethyl sulfoxide (DMSO) (Fisher Scientific, Leicestershire–UK), acetonitrile (Carlo Erba, Barcelona–Spain), 1,1,3,3-tetramethoxypropane (Sigma–Aldrich–Darmstadt–Germany), rotenone (Sigma - Aldrich- Darmstadt–Germany), 1-methyl-2-phenylindole (Wako Chemical Ltd- Osaka - Japan), loperamide hydrochloride (Bafna Pharmaceutical, Chennai–India) and tris hydrochloride (2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride) (Amresco, Ohio–USA) were purchased and used in the study.

Collection of plant material

Fresh individual leaves of *B. macrocalyx* were collected in August 2016 from Lindi southeastern Tanzania and were identified by Mr. F. Mbago a botanist from University of Dar es Salaam (UDSM). A voucher specimen (FMM 3579) is preserved in the herbarium, Department of Botany, UDSM.

Preparation of extract

Leaves were dried under shade and then grounded to powder. A weight of 500 g was soaked in 1000 ml methanol for 2 days at room temperature with occasional agitation. A filtrate was obtained through a Whatman No. 1 filter paper and the re-extraction was repeated three times using 500 ml, 250 ml and

150 ml of methanol consecutively with an interval of one day each. The pooled filtrate was kept in the oven at 40 °C for seven days to allow the evaporation of methanol. The obtained extract was weighed and transferred in the glass vial for storage in the fridge at 4 °C under darkness until required.

Experimental animals

Swiss albino mice (30-35 g) aged 6 to 8 weeks were used for the experiments. These animals were maintained at standard laboratory environmental conditions in animal house in the department of Zoology and Wildlife Conservation, University of Dar es Salaam. They were bred in aluminium cages in a room with temperature of 22 ± 3 °C and 12:12 hours light-dark light cycle. Animals had free access to water and standard commercial mashed grower feed (Riyami Miller Ltd, Dar es Salaam, Tanzania). The experimental protocol was approved by the Committee for Higher Degrees, Research and Publication, College of Natural and Applied Sciences, University of Dar es Salaam.

Acute toxicity test

Acute oral toxicity of methanolic leaf extract of BM was performed to male mice following the Organization of Economic Co-operation and Development (OECD) guidelines protocol number 423 (OECD 2001). Mice were randomly divided into seven groups, five mice each. Each mouse was weighed before subjected to the experiment and subsequently body weights were measured weekly during the experimental study. The doses of BM were 100, 250, 500, 750, 1000 and 2000 mg/kg. The volume administered was 1 ml/100 g body weight. One group served as a control in which the animals received 1% DMSO as a vehicle orally. Different markings were put on different parts of the body like head, tail, leg and neck for easy identification during the study.

Prior the test substance administration, animals were fasted for 3 hrs. Following the

fasting time, the test substance was administered in a single dose by an oral gavage to individual mouse. After the dose administration, the food was not given to them for a period of 2 hrs. The individual animal was closely observed for any signs of toxicity within 30 minutes after dose administration, every 4 hours for the first 12 hours and at least twice per day daily. Any changes and symptoms that were shown by the mice were recorded daily for 14 days. The observed parameters were tremors, alertness, urination, skin colour, grooming, pinna reflex, lacrimation, touch response, salivation, diarrhoea, sleep and hyper activity. At the end of the experiment, the median lethal dose (LD₅₀) for toxicity was calculated arithmetically by the Karber's formula:

$$LD_{50} = LD_{100} - \sum \left(\frac{a \times b}{n} \right)$$

Where: n = total number of animals in a group;

a = difference between two successive doses;

b = average number of dead animals in two successive doses and

LD₁₀₀ = lethal dose causing the 100% death to all animals, as was described by Ahmed (2015).

Antidiarrhoeal study

Castor oil induced diarrhoea test

Diarrhoea was induced using castor oil according to Awouters et al. (1978). Thirty non-pregnant female mice were randomly divided into five groups of six mice each. They were fasted overnight with free access to water before drug administration. The first group was given distilled water (2 ml/100 g) serving as negative control, while the second group received loperamide, 3 mg/kg body weight as a positive control. Groups 3, 4 and 5 received different doses of the BM extract (100, 500 and 1000 mg/kg), respectively. The selected doses were based on results of acute toxicity tests. Thirty minutes later, all individual animals were given 1 ml of castor

oil orally. A blotting paper of known weight was placed in each cage to collect faeces. Then, the severity of diarrhoea was assessed each hour for 4 hours. The mean total number of dry and wet faeces was determined from each group in relation to negative control group. The total score of diarrhoeal faeces for the control group was considered as 100%. The results were expressed as a percentage of inhibition of diarrhoea relative to the negative control group. The parameters that were assessed included; onset of diarrhoea that was useful in calculation of the delay of diarrhoea response, rate of purging (number of wet stools), and total number of faecal output and total weight of faecal output. The numerical scores based on stool consistency assigned were; normal stool, semi-solid stool and watery stool. The percent of inhibition of defaecation and diarrhoea were calculated using the following formulas:

$$\% \text{ Inhibition of defaecation} = \frac{(\text{Total number of faeces in negative control} - \text{total number of faeces in treated groups})}{\text{Total number of faeces in negative control}} * 100$$

$$\% \text{ Inhibition of diarrhoeal drops} = \frac{(\text{Total number of diarrhoeal drops in negative control} - \text{total number diarrhoeal drops in treated groups})}{\text{Total number of diarrhoeal drops in negative control}} * 100$$

Antioxidant study

Rotenone and *Baphia macrocalyx* extracts administration

One cohort of mice was used; the mice were randomly divided into five groups, with six mice in each group. Group 1 received 1% DMSO orally as a vehicle control; group 2 received a subcutaneous injection of rotenone 1.5 mg/kg three times per week; groups 3, 4, and 5 received rotenone 1.5 mg/kg subcutaneously three times per week in combination with BM (100, 750 and 2000 mg/kg, respectively). BM was administered orally. All animals received the treatments for 2 weeks and then were euthanized by

chloroform for tissues collection. Brains were quickly dissected out on an ice-cold plate and specific areas (striatum and midbrain) were chopped, washed with ice-cold phosphate-buffered saline (pH 7.4), weighed, and stored at -20°C for not more than a week for biochemical analysis. At the end of the experiment, all dead animal bodies were wrapped into plastic bags and properly buried underground.

Lipid peroxidation (LPO) measurements in brain tissues

Lipid peroxidation was determined by measuring the levels of malondialdehyde (MDA) in brain homogenate tissues according to the procedure previously described by Siddique et al. (2012). In brief, reagent one (R1) was prepared by dissolving 1-methyl-2-phenylindole (64 mg) in acetonitrile (30 ml) to which methanol was added (10 ml) to bring the volume to 40 ml. A preparation of 37% HCl was served as the reagent R2. Mice brains were dissected and sections from striatum and midbrain regions were isolated under microscope in ice-cold Tris hydrochloride (20 mmol/L). Homogenate was prepared from 100 mg wet weight/group; three replicates/group in Tris hydrochloride and centrifuged at 3000 X g for 20 minutes. Thereafter, the supernatant from each tube was collected and transferred into fresh tubes. Subsequently, a volume of 100 μl of supernatant and R2 (300 ml) was added in a tube containing 1.3 ml of R1 and the mixture was vortexed and incubated at 45°C for 40 minutes. After incubation, the tubes were cooled on ice and centrifuged at 15000 X g for 10 minutes at 4°C . Note: One molecule of MDA reacts with two molecules of 1-methyl-2-phenylindole to form a stable chromophore with maximal absorbance at 586 nm. All samples were read on TOMOS UV-1600 spectrophotometer at 586 nm.

Data analysis

Data were analysed using Graph Pad Prism version 8. The data were first tested for

normality using the Shapiro–Wilk test and found to be not normally distributed ($p < 0.05$) and thus Friedman or Kruskal Wallis followed by Dunn’s multiple comparisons tests were used to test for the significance in differences among the treated groups.

Results

Toxicological study of *Baphia macrocalyx* in mice

The current study was conducted as per OECD guideline 423 and found that there was no mortality or any physical signs that resulted from the methanolic BM leaf extract of any dose administered to the animals (even up to 2000 mg/kg). The oral LD₅₀ was indefinable showing to be above 2000 mg/kg body weight (Table 1). Thus, it did not warrant assessing the extract at higher doses, as the extract was reasonably non-toxic even after 14 days.

Table 1: Effects of methanolic leaf extract of *Baphia macrocalyx* on acute oral toxicity test in mice

Group	Dose/Day	Mortality x/N
I	DMSO	0/5
II	100 mg/kg	0/5
III	250 mg/kg	0/5
IV	500 mg/kg	0/5
V	750 mg/kg	0/5
VI	1000 mg/kg	0/5
VII	2000 mg/kg	0/5

Table 2 signifies the parameters monitored before and after the test substance administration. All the parameters witnessed after giving the test substances were normal from the control to the group that was given the highest dose. These results continue to substantiate that the LD₅₀ of the methanolic

BM leaf extract is greater than 2000 mg/kg body weight in a single oral dose administration. In addition, BM extract did not affect body weight of treated groups in the entire study period of 14 days when compared to control group (Figure 1).

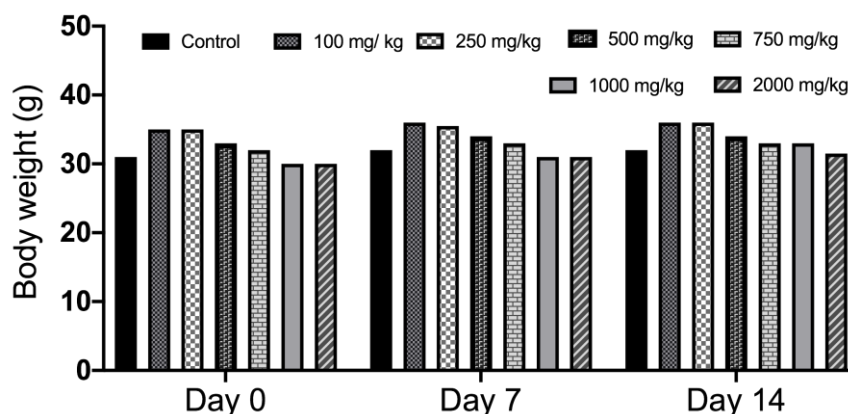


Figure 1: The effects of different doses of methanolic leaf extract of *Baphia macrocalyx* on body weights (g). Results are single measurement, n = 5 per group.

Table 2: Oral toxicity effects of methanolic leaf extract of *Baphia macrocalyx* on general behaviour of tested mice

S/ N	Response	Group I		Group II		Group III		Group IV		Group V		Group VI		Group VII	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	Alertness	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	Grooming	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	Touch	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
4	Skin colour	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	Tremors	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
6	Lacrimation	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N	N
8	Pinna reflux	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9	Salivation	N	N	N	N	N	N	N	N	N	N	N	N	N	N
10	Urination	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11	Diarrhoea	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
12	Hyper activity	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs

Note: N = Normal; Abs = Absent

Group I = DMSO only, Group II = 100 mg/kg, Group III = 250 mg/kg, Group IV = 500 mg/kg, Group V = 750 mg/kg, Group VI = 1000 mg/kg, Group VII = 2000 mg/kg

Effects of *Baphia macrocalyx* extract on castor oil-induced diarrhoea

Castor oil induced diarrhoea in all treated mice. Early onset was observed in a negative control group that received water only. The mean number of diarrheal drops was 10.67 ± 0.17 , while in the extract-treated groups (100, 500 and 1000 mg/kg), the mean values decreased with dose. There were no diarrhoeal drops, which were observed in the highest extract dose of 1000 mg/kg in which a significant inhibition of diarrhoea against negative control was noted ($p < 0.001$). In the positive control group that received loperamide, the mean value was 0.5 ± 0.17 ,

that was significantly different in comparison to the negative control group ($p < 0.01$, Table 3). The results showed that the total number of faeces and the weight of fresh faecal output were dose dependent and there were significant differences between negative control group in comparison with the highest tested dose of the extract (1000 mg/kg) and loperamide treated group ($p < 0.05$, Table 3). Interestingly, 1000 mg/kg BM extract inhibited diarrhoea by 100%, which showed a higher rate compared to 95% of loperamide the standard used antidiarrhoeal drug (Table 3).

Table 3: Effects of the *Baphia macrocalyx* extract on castor oil-induced diarrhoea test

Groups	Dose mg/kg	Onset of diarrhoea (minutes)	Total number of faeces	Total number of diarrhoeal drops	Weight of fresh faecal output (g)	Inhibition of defaecation (%)	Inhibition of diarrhoea (%)
Group 1 (water)	–	45.89 ± 6.12	11.67 ± 0.06	10.67 ± 0.17	2.9 ± 0.12	0	0
Group 2 (loperamide)	3	57.92 ± 8.35	7.5 ± 0.17*	0.5 ± 0.17**	1.25 ± 0.1	35.73	95.31
Group (extract)	3 100	55.92 ± 3.23	9.33 ± 0.08	6.67 ± 0.24	2.83 ± 0.12	20.05	37
Group (extract)	4 500	61.72 ± 5.86	9.33 ± 0.08	4.33 ± 0.24	1.3 ± 0.06	37.19	59.42
Group (extract)	5 1000	51.17 ± 5.17	6 ± 0.11*	0***	0.8 ± 0.15	48.58	100

Values are expressed as mean ± SEM (n = 6); * p < 0.05; ** p < 0.01; *** p < 0.001 compared to the negative control determined by Kruskal-Wallis followed by Dunn's multiple comparisons tests.

Effects of *Baphia macrocalyx* extract on rotenone induced oxidative stress

The efficacy of methanolic leaf extract of BM in reducing oxidative stress in brain tissues was tested in rotenone induced mice model of Parkinson's disease. Three doses were tested; 100 mg/kg, 750 mg/kg and 2000 mg/kg. Figure 2A shows the MDA standard curve that was used to estimate the levels of MDA in the tissues using LPO assay. Results revealed that BM extract reduces the level of oxidative stress induced by rotenone in brain tissues in a dose dependent manner. The levels of MDA in mice treated with rotenone and co-treated with the lowest dose of BM

(100 mg/kg) were less compared to that of mice treatment with rotenone alone, although it was not statistically different. However, using Friedman followed by Dunn's multiple comparisons tests showed that there were significant differences between animals treated with rotenone alone in comparison with groups co-treated with rotenone and BM extracts of 750 mg/kg and 2000 mg/kg, respectively (p < 0.05) (Figure 2B). The control group that was neither treated with rotenone nor *Baphia*, showed lower levels of MDA compared to rotenone treated animals, although they were not statistically different (p > 0.05).

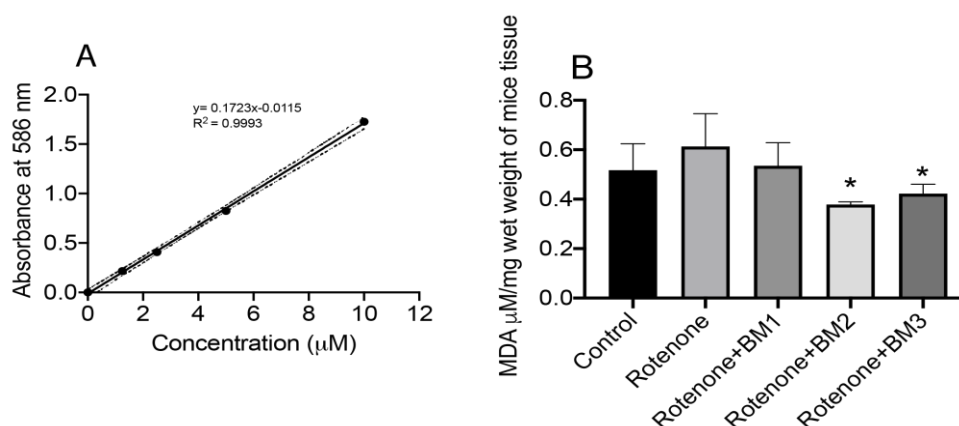


Figure 2: Estimation of lipid peroxidation in brain tissues. A: MDA standard curve; B: Estimation of MDA in brain tissues (striatum and midbrain); MDA = malondialdehyde; BM1 = 100 mg/kg; BM2 = 750 mg/kg; BM3 = 2000 mg/kg. Values are expressed as mean \pm SEM (n = 3), * p < 0.05 denotes significant difference from rotenone determined by Friedman followed by Dunn's multiple comparisons tests.

Discussion

Some *Baphia* species are used for treatment of several ailments in Africa. The present study sought to evaluate the acute toxicity of BM in mice. The non-toxic nature of methanolic leaf extract of BM was evident by the lack of mortality or visible toxic signs in the oral administration of BM of up to 2000 mg/kg body weight. One will argue the lack of toxicity could be probably due to low gastrointestinal absorption of the extract. This can be explained with the fact that BM leaf extract showed the antidiarrhoeal and antioxidant properties in the present study in which the mode of administration was through the oral route. Intraperitoneal injection would be the option but was avoided because of the crude nature of the extract. On the other hand, lack of mortality or observable toxic signs can be accompanied by high metabolic rate of liver and kidney in detoxification and excretion of toxic substances. Liver and kidney are known to be target organs for chemicals due to their primary body functions (Bello et al. 2016). Furthermore, the body weights did not vary significantly among the tested groups and control group. The loss of body weight that is

an important marker of gross toxicity did not appear in the present study and further substantiate that the BM extract did not cause acute toxicity in tested mammals. To ensure the safety of the natural compounds is a common practice in medicine and drug discovery. A key step is acute toxicity test in suitable animal models. A number of toxicity studies in mice and rat testing different plant extracts exist (Lalitha et al. 2012, Banerjee et al. 2013, Nyigo et al. 2015, Bello et al. 2016, Ali et al. 2019).

The present study also shows that methanolic leaf extract of BM has antidiarrhoeal effects in castor oil induced diarrhoea mice model. Some studies have shown the use of castor oil as an inducer of diarrhoea (Adeyemi and Akindele 2008, Rahman et al. 2015, Degu et al. 2016, Naher et al. 2019). Ricinoleic acid is an active metabolite of castor oil that causes irritation of mucosal wall of the intestine that stimulates the release of inflammatory mediators such as histamine, nitric oxide and prostaglandins. Mechanisms of prostaglandins as diarrhoeagenic agents, particularly of E series have been documented both in experimental animals

and human subjects (Robert et al. 1976, Tunaru et al. 2012, Fujii et al. 2016). In this context, the current study investigated the potential of BM on counteracting the effects of castor oil induced diarrhoea in mice. BM inhibited diarrhoea induced by castor oil in a dose dependent manner giving 100% diarrhoeal drops inhibition by a dose of 1000 mg/kg. Adeyemi and Akindele (2008) demonstrated the positive dose graded effects of *Baphia nitida* in castor oil induced diarrhoea. A recent phytochemical analysis of BM revealed the presence of high polyphenol compounds particularly flavonoids of class flavones (Bwire 2015). Previous studies have shown the ability of flavonoids to inhibit water and electrolytes secretions, intestinal motility, and to inhibit biosynthesis and release of prostaglandins (Carlo et al. 1993, Dosso et al. 2012, Rahman et al. 2015). In addition, other components of plants including tannins and alkaloids have been reported to be responsible for treating diarrhoea (Mbagwu and Adeyemi 2008). Taken together, the phytochemical constituents of BM leaf extract attributed to its antidiarrhoeal ability in tested mice probably via protecting gastric mucosa against irritation and inflammation caused by prostaglandins and other diarrhoeagenic mediators. These findings are supported by similar study in which leaf extract of *Baphia nitida* demonstrated to have antidiarrhoeal effects via interference of L-arginine nitric oxide pathway (Adeyemi and Akindele 2008).

The present study also sought to investigate the antioxidant activity of BM in mice brain tissues. The findings showed a significant potential of BM in reducing rotenone induced oxidative stress in the brain tissues of mice. Rotenone is a neurotoxic substance that inhibits complex I of mitochondria electron transport chain thereby increasing ROS production that in-turn leads to oxidative stress (Sherer et al. 2003). In the present study, BM showed the potential of counteracting the increase in levels of MDA

in rotenone treated mice in a dose dependent manner evidencing the potential of capturing free radicals generated by rotenone probably due to the presence of phenolic compounds such as flavonoids that have been shown to have antioxidants properties facilitated by their hydroxyl groups (Maestri et al. 2006). Thus, comparable with our findings, other studies have demonstrated the antioxidant efficacy of phenolic compounds in *in vitro*, *in vivo* studies and clinical trials (Fusco et al. 2007, Sahreen et al. 2011, Choudhary and Swarnkar 2011, Saeed et al. 2012, Gao et al. 2012, Sokolov et al. 2013, Bwire 2015, Solank et al. 2015).

Thus, future studies focusing on isolating the bioactive compounds from *Baphia* species for drug formulation and antioxidant supplements are recommended.

Conclusions

The findings obtained from the current study indicate that BM extract possesses antidiarrhoeal activities as revealed by inhibition of diarrhoeal drops and reduction of faecal outputs. BM also possesses high potential of antioxidant activities by reduction of MDA production in brain tissues. The absence of acute toxicity signs demonstrated the non-toxic effects of BM extract. Thus, this study supports the use of *Baphia* species in traditional settings; however, the desire for isolating the bioactive compounds for antioxidant supplements and drug formulation for antidiarrhoeal is recommended.

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Declaration of Interest

Authors declare that they have no conflicting or competing interests.

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