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Antidiarrheal and Lipid Profile Ameliorative Activity of a Bi-Herbal Aqueous Extract on Some Biochemical Parameters in Male Wistar Rats and Mice



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Abstract

Natural remedies play a notable role in managing and treating numerous diseases. The growing interest in medicinal plants for managing a range of diseases has prompted investigations into their potential to treat diarrhoea and lower cholesterol levels. This work evaluated the effects of a bi-herbal extract from *Ocimum gratissimum* (scent leaf) and *Psidium guajava* (guava) on diarrhoea, lipid profile and acute toxicity response in adult male Wistar rats and mice. The tested animals were randomly assigned to five groups (n = 3). The bi-herbal extract was administered at 25 mg/kg (Group 3), 50 mg/kg (Group 4), and 100 mg/kg (Group 5), with 2 mg/kg loperamide (Group 2) and 0.5 ml/kg distilled water (Group 1) as controls. Diarrhoea was induced using castor oil and magnesium sulphate, and lipid parameters and anti-diarrhoeal activities were evaluated. The results indicated that the bi-herbal extract, particularly at 50 mg/kg, significantly reduced ($p < 0.05$) Total cholesterol and low-density lipoprotein levels compared to the controls. The acute toxicity study revealed no death or untoward effects up to 100 mg/kg. The effect of the bi-herbal extract on high-density lipoprotein levels (28.00 ± 1.00 mg/dL to 30.50 ± 5.50 mg/dL) did not differ significantly across groups ($p > 0.05$). The study also indicates that the bi-herbal aqueous extract displayed significant antidiarrheal activity, effectively reducing stool frequency and water content in both magnesium sulphate ($MgSO_4$) and castor oil-induced diarrhea. In conclusion, it can be inferred that the bi-herbal extract possesses significant anti-diarrheal and hypolipidemic properties, effectively lowering key atherogenic lipids without toxicity.

Keywords: Bi-herbal extract, diarrhea, lipid profile, acute toxicity, phytochemistry and medicinal plants.

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INTRODUCTION

The use of medicinal plants and their secondary metabolites in treating a wide range of illnesses is growing, often as an adjunct to conventional medicine (Hamilton, 2004). The World Health Organisation (WHO) defines traditional medicinal plants as natural plant materials used, at least in the absence of industrial processing, for treating diseases at a local or regional scale (Tilburt and Kaptchuk, 2008). Since the dawn of civilisation, human communities have maintained a strong relationship with their surroundings and used environmental resources to produce food and medicine. In primary healthcare, between 75 and 80 per cent of the world's population still turns to herbal medicine, primarily in impoverished nations (Kamboj, 2000). This is due mainly to the widespread perception that herbal medications are safe, affordable, and readily available without adverse side effects. Much of modern medicine has its roots in the ancient practice of using plants for medicinal purposes. Plants are the source of many traditional medications; just a century ago, the majority of the few effective medications came from plants. Examples include morphine (from the opium poppy), quinine (from cinchona bark), dioxin (from foxglove), and aspirin (from willow bark) (Tirtha, 1998). *Ocimum gratissimum* (scent leaf) and *Psidium guajava* (guava) are two medicinal plants with extensive traditional uses and scientifically validated biological activities (Ezekwe et al., 2020; Barbalho et al., 2021). *Ocimum gratissimum* (Lamiaceae) is prevalent in tropical regions and is widely used as a spice and in folk medicine for treating conditions like diarrhoea, malaria, and coughs (Olowofela et al., 2020). Similarly, *Psidium guajava*, a tree in the Myrtaceae family, is cultivated for its fruit, and its leaves are used in traditional medicine systems, particularly for gastrointestinal issues such as diarrhoea and dysentery (Díaz-De-Cerio et al., 2017). Guava leaves are a source of various bioactive compounds, which contribute to their documented antidiarrheal, antimicrobial, antioxidant, and anti-hyperglycaemic properties (Shabbir et al., 2021).

The rationale for combining these plants in a bi-herbal extract for this study is strongly supported by their shared and complementary activities, especially against diarrhoea and related metabolic disturbances. Both plants have demonstrated significant antidiarrheal effects by reducing intestinal motility and fluid secretion. Furthermore, their broad-spectrum antimicrobial properties target common enteric pathogens, while their antioxidant and anti-inflammatory activities can mitigate associated oxidative stress and inflammation. Crucially, both *Ocimum gratissimum* and *Psidium guajava* have shown modulatory effects on lipid metabolism (Prabhu et al., 2020; Oluwole et al., 2022). For instance, *Ocimum gratissimum* has shown enzyme-inhibitory activity against pancreatic lipase (Udo et al., 2020), and *Psidium guajava* has demonstrated anti-hyperlipidaemic effects by improving lipid profiles. This

suggests that their combined application could not only address the primary infection but also positively influence the host's metabolic response. Therefore, this study aims to determine the result of administering a bi-herbal aqueous extract from *Ocimum gratissimum* and *Psidium guajava* on the lipid profile of Wistar rats infected with diarrhea. Lipid profiling measures key parameters, including Total Cholesterol, Triglycerides, High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL).

Materials and Methods

Plant material collection

Fresh leaves of *Ocimum gratissimum* and *Psidium guajava* (Voucher numbers UBH-0333 and UBH-P378, respectively) were obtained from Ugbowo, Benin City, Nigeria. Prof H. O. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Nigeria authenticated the plants.

Preparation of the bi-herbal extract

The collected fresh leaves were rinsed with running tap water and dried in the shade for two weeks. The dried, crunchy samples were separately pulverised using a grinding machine. The pulverized samples of *O. gratissimum* and *P. guajava* were weighed for extraction at a 1:1 ratio (60 g respectively), then macerated for 48 hours in 900 mL of distilled water. The mixture was manually vortexed intermittently during maceration. The sieved macerated mixture and the resultant filtrate were concentrated to a powder. The samples were then stored in a sterilised vessel and refrigerated (4 °C) for future use.

Experimental animals

Fifteen (15) male Wistar rats (140 – 245 g) and mice (19-27 g) were obtained from the Department of Pharmacology, Faculty of Pharmacy, University of Benin. They were randomly divided into five groups (n = 3). The animals were kept in a plastic, clean, and conducive cage in the Phytomedicine Unit animal house of the Department of Plant Biology and Biotechnology at the University of Benin. The animals were acclimatised for one week and provided with adequate pelleted food and water. The animals were handled according to standard protocols for the use of laboratory animals (Adegboye et al., 2008) and the internationally accepted guiding principles for biomedical research involving animals (CIOMS & ICLAS, 2012)

Assessment of antidiarrheal activity via castor oil-induced diarrhea

The method described by Shoba & Thomas (2001) was followed in this study. The animals were administered the aqueous bi-herbal extract before inducing diarrhea with oral castor oil at varying doses, based on the animal's weight. Distilled water served as the negative control, and loperamide

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as the positive control. The positive control was administered to the rats at different doses based on their weight. All the administrations were administered orally. Each animal was placed in a separate cage, with the floor covered with blotting paper. The blotting paper was changed every hour. Diarrhea was induced by oral administration of 0.5 mL of castor oil to the animal 30 minutes after the above treatments. During a 4-hour observation period, the total number of faeces and the number of diarrheic faeces defaecated by the rats were noted. A numerical score based on stool consistency was assigned as follows: normal stool = 1, semisolid stool = 2 and watery stool = 3.

Determination of antidiarrheal activity via magnesium sulfate-induced diarrhea

This study's procedure was similar to that of the earlier study on castor oil-induced diarrhea. Diarrhea was induced in the animals by administration of magnesium sulphate (2 mg/kg) orally, 30 minutes after pre-administration of the extract, distilled water (negative control), and Loperamide (positive control drug) to the respective test groups. All administrations were carried out orally (Doherty, 1981; Uddin et al., 2005). Briefly, the mice (19- 27 g) were selected randomly into five (5) groups of 3 animals each. The Groups (I, II, and III) were orally pre-treated with 25, 50, and 100 mg/kg of the bi-herbal aqueous extract. **Group IV** (negative control) received 0.5 mL/kg of distilled water, and **Group V** (positive control) received loperamide (2 mg/kg) orally. Stool type (dry or diarrheal stools)/characteristics were observed. Dry stools are formed stools that lack water, while diarrheal stools are watery stools. The total number of stools is the combined count of both dry and diarrheal stools excreted during the experimental period, and stool weight was expressed in grams.

Lipid profile evaluation

The lipid profile was evaluated in serum. The serum was prepared using an earlier procedure described by Yakubu et al. (2005) and Erhabor & Idu (2022). Serum was obtained from blood collected from the sacrificed animals after the castor oil-induced diarrhoea study. The reagents/diagnostics kits were of Randox Laboratories Limited, VIS-7220G Biotech Engineering Management CO. LTD.(UK). Total cholesterol was determined enzymatically using the method described by Udenze et al. (2012). Briefly, 1000 µL of the reagent was pipetted into three test tubes: A, B, and C. Subsequently, 10 µL of distilled water, a standard solution, and serum were pipetted into the test tubes, respectively, according to the manufacturer's instructions. The test tubes were vortexed and incubated at room temperature (37 °C) for 10 minutes, and the absorbance was measured at 500 nm against the blank using a UV spectrophotometer (Model M501, UK). Serum biochemical parameters were determined using an enzymatic colourimetric method with commercially available wet reagent diagnostic kits, following a modified protocol based on Udenze et al. (2012). Briefly, 1000 µL of

the working reagent was dispensed into three labelled test tubes: reagent blank (A), standard (B), and sample (C). Subsequently, 10 µL of the standard solution was added to tube B, while 10 µL of the serum sample was added to tube C. Tube A contained only the reagent and served as the blank. The contents of each tube were gently mixed and incubated at room temperature (25°C) for 10 minutes to allow complete color development. Thereafter, the absorbance of the blank, standard, and sample was measured at 500 nm using a UV-visible spectrophotometer. All measurements were performed according to the manufacturer's instructions, and the procedure was repeated for all samples. High-density lipoprotein cholesterol (HDL-C) was determined using an enzymatic colourimetric method as described by Assem and et al. (2012), with slight modifications. A precipitating reagent containing phosphotungstic acid (0.55 mmol/L) and magnesium chloride (25 mmol/L) was used to selectively precipitate non-HDL lipoproteins (very low-density lipoproteins and low-density lipoproteins), leaving HDL in the supernatant. The precipitating reagent was diluted with distilled water at a 4:1 (v/v) ratio. Thereafter, 500 µL of the reagent was dispensed into two labelled test tubes: standard (A) and sample (B). Subsequently, 200 µL of the HDL standard solution was added to tube A, while 200 µL of the serum sample was added to tube B. The mixtures were gently vortexed and allowed to stand at room temperature (25°C) for 10 minutes to ensure complete precipitation and color development. Following incubation, the absorbance of the standard and sample was measured at 500 nm using a UV-visible spectrophotometer against an appropriate reagent blank. HDL-C concentration was calculated from the sample absorbance relative to the standard. Low-density lipoprotein cholesterol (LDL-C) was determined using an enzymatic colourimetric method as described by Rifai et al. (1999). Briefly, serum samples were first treated with a precipitating reagent to remove interfering lipoproteins, including very low-density lipoproteins (VLDL) and chylomicrons, thereby isolating LDL fractions. The resulting preparation was then subjected to enzymatic reactions involving cholesterol esterase and cholesterol oxidase. Cholesterol esterase hydrolyses cholesterol esters present in LDL to free cholesterol, which is subsequently oxidized by cholesterol oxidase to produce hydrogen peroxide. The generated hydrogen peroxide reacted with a chromogenic substrate in the presence of peroxidase, forming a coloured quinoneimine complex. The intensity of the color formed was directly proportional to the LDL cholesterol concentration (Friedewald et al., 1972). After adding the reagents, the reaction mixture was incubated at room temperature (25°C) for 10 minutes to allow complete colour development. The absorbance of both the standard and sample was measured at 500 nm using a UV-visible spectrophotometer. LDL-C concentration was then calculated from the absorbance values relative to the standard.

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$$LDL \text{ (mg/dl)} = \frac{\text{Total cholesterol} - \text{Triglycerides}}{5} \dots\dots\dots(1)$$

Acute toxicity study

Acute toxicity was conducted in accordance with the modified protocol outlined earlier by Erhabor & Idu (2017). Fifteen rats were randomly divided into five (5) groups. The rats in Group I served as the negative control and received distilled water daily. Loperamide (2mg/kg) was given to Group II rats, while rats in Groups III, IV, and V received 25, 50, and 100 mg/kg of the bi-herbal extract, respectively, by intragastric gavage. Within this period, the animals were daily assessed for toxicity signs. Thereafter, the animals were left under similar conditions and observed for general behavioural changes for 14 days. Observations focused on locomotion, aggressiveness, faecal appearance, urination, and death. The median Lethal Dose (LD₅₀) was determined using the modified Lorke method (Lorke, 1983).

The LD₅₀ was determined using the formula.
 LD₅₀= √(D0×D100); D₀=Highest dose with no mortality;
 D₁₀₀= Lowest dose with mortality

Statistical analysis

The data collected were presented as the mean ± standard error of the mean (SEM). A one-way analysis of variance was used to compare group means. The GraphPad Prism (version 7, USA) software package was used to analyse the data. Statistical significance was determined at the *p* < 0.05 level.

Results

Acute toxicity effect of the bi-herbal extract

The results of the acute effect of the bi-herbal extract are displayed in Table 1. The acute toxicity showed no mortality or untoward effects on the male Wistar rats.

Table 1: Acute Toxicity Effects of the Bi-herbal Leaf Extract on Male Wistar Rats

Parameters	Control	Bi-herbal extract(mg/kg)		
		25	50	100
Number of mortality	0	0	0	0
% mortality	0	0	0	0
Adverse effects	Nil	Nil	Nil	Nil

Control- distilled water

Effect of bi-herbal aqueous extract on Lipid Profiles

The results from Table 2 showed that across the graded doses of the bi-herbal extract, there was no significant difference in the levels of total cholesterol, except at 50 mg/kg, where a slight but significant decrease was noted compared to the control. Figure 1 indicates that there was no significant effect

on triglyceride levels across the graded doses of the bi-herbal extract. At 25 mg/kg and 100 mg/kg, a slight but significant decrease was observed compared to the control. The data in Table 3 show that the bi-herbal extract doses did not significantly change high-density lipoprotein (HDL) levels compared with the control group, suggesting a consistent response across all doses. Figure 2 shows that across the graded doses of the bi-herbal extract, there was no significant effect on low-density lipoprotein (LDL) levels, except at 50 mg/kg of the aqueous extract, where a notable difference was observed compared to the control.

Table 2: Effects of the bi-herbal leaf aqueous extract on total cholesterol level in rats

Groups	Doses (mg/kg)	Cholesterol (mg/dl)
1(Untreated)	0.5 ml	100.00 ± 2.00 ^a
2 (Loperamide)	2	108.00 ± 1.90 ^a
3 (Bi-herbal extract)	25	108.00 ± 4.00 ^a
4 (Bi-herbal extract)	50	96.00 ± 4.00 ^b
5 (Bi-herbal extract)	100	100.00 ± 1.00 ^a

Values are stated as mean ± SEM, n=3. Values having similar alphabets in a column are not significantly different at (*p* > 0.05), while values with dissimilar superscripts are significantly different (*p* < 0.05). *Untreated (Negative control) = 0.5 ml/kg of distilled water; Loperamide (Positive control) = 2 mg/kg.

Table 3: Effects of the bi-herbal leaf aqueous extract on high-density lipoprotein level in rats

Groups	Doses (mg/kg)	High-Density Lipoprotein (mg/dl)
Untreated control	0.5 ml	30.00 ± 1.00 ^a
Loperamide	2	30.50 ± 5.50 ^a
Bi-herbal extract	25	30.50 ± 2.50 ^a
Bi-herbal extract	50	28.00 ± 1.00 ^a
Bi-herbal extract	100	29.00 ± 1.00 ^a

Values are mean ± SEM, n=3. Values with similar alphabets in a column are not significantly different at (*p* > 0.05), while values with dissimilar superscripts are significantly different (*p* < 0.05). Untreated control (Negative control): 0.5 ml/kg of Distilled water; Loperamide (Positive control): 2 mg/kg.

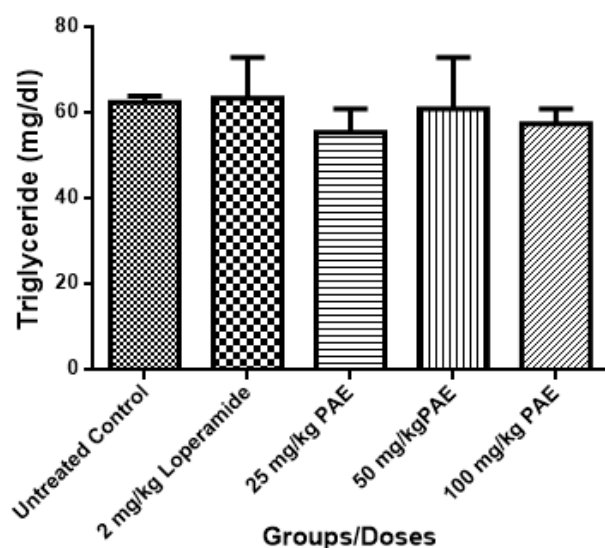


Figure 1: Effects of the bi-herbal leaf aqueous extract on triglyceride level in Rats. Values are expressed as mean \pm SEM, n=3. Bars with similar alphabets are not significantly different at ($p > 0.05$), while bars with dissimilar superscripts are significantly different ($p < 0.05$). Untreated control (Negative control): 0.5 ml/kg of Distilled water (Group 1); Loperamide (Positive control): 2 mg/kg Group 2), 25 mg/kg (Group 3), 50 mg/kg (Group 4), 100 mg/kg (Group 5).

Effect of the bi-herbal extract on magnesium sulphate and castor oil-induced diarrhoea in mice

Table 4 shows the results of magnesium sulphate-induced diarrhoea in mice and the effects of the crude extract on stool characteristics. The results showed that the extract reduced the severity of diarrhoea and the total number of stools in the test groups, and these differences were statistically significant compared to the negative control. Table 5 shows the effects of the bi-herbal aqueous extract on diarrhoea induced by castor oil in the experimental animals. The control group (distilled water) exhibited a high frequency of diarrhoeal stools, total stool output, and stool weight,

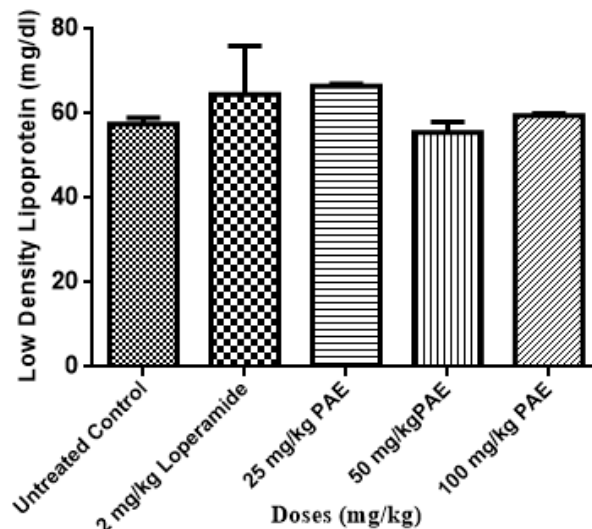


Figure 2: Effects of the bi-herbal leaf aqueous extract on low-density lipoprotein level in Rats. Values are expressed as mean \pm SEM, n=3. Bars with similar alphabets are not significantly different at ($p > 0.05$), while bars with dissimilar superscripts are significantly different ($p < 0.05$). Untreated control (Negative control): 0.5 ml/kg of Distilled water; Loperamide (Positive control): 2 mg/kg.

affirming successful induction of diarrhoea. Treatment with the antidiarrhoeal drug loperamide (2 mg/kg) completely suppressed diarrhoea, resulting in zero diarrhoeal stools, total stools, and stool weight, with 100% inhibition. The bi-herbal extract produced a dose-dependent antidiarrheal effect, with the 25 mg/kg dose showing a marked reduction in the number of diarrheal stools, total stool number, and stool weight, resulting in a high percentage inhibition (89%) comparable to that of the standard drug (loperamide). At higher doses of 50 and 100 mg/kg, the extract significantly reduced diarrhoeal parameters relative to the control, although the inhibitory effect (67%) was lower than that observed at 25 mg/k.

Table 4: Effects of bi-herbal aqueous extract in magnesium sulphate-induced diarrhoea

GROUPS	Doses (mg/kg)	Number of Diarrheal (Wet stool)	Number of Dry Stools	Total Number of Stools	Weight of Stool (g)	Percentage Inhibition (%)
Control	0.5 ml	5.33 \pm 1.45 ^a	0.00 \pm 0.00 ^a	5.33 \pm 1.45 ^a	1.33 \pm 0.03 ^a	0
Loperamide	2	0.00 \pm 0.00 ^b	0.33 \pm 0.00 ^a	0.33 \pm 0.00 ^b	0.03 \pm 0.00 ^b	100
Bi-herbal Extract	25	0.67 \pm 0.07 ^c	0.00 \pm 0.00 ^a	0.67 \pm 0.00 ^b	0.17 \pm 0.01 ^c	87
Bi-herbal Extract	50	2.00 \pm 0.00 ^d	0.00 \pm 0.00 ^a	2.00 \pm 0.00 ^c	0.20 \pm 0.06 ^c	63
Bi-herbal Extract	100	1.00 \pm 0.58 ^d	2.00 \pm 0.00 ^b	3.00 \pm 0.25 ^c	0.53 \pm 0.04 ^c	81

Values are Mean \pm SEM, n=3; values with different alphabets were statistically significant at a $p < 0.05$ level of significance. Control-distilled water

Table 5: Effects of bi-herbal aqueous extract in Castor oil-induced diarrhoea

Groups	Doses (mg/Kg)	Number of Diarrheal (wet stool)	Number of Dry Stools	Total Number of Stools	Weight of Stool (G)	Percentage Inhibition
Control	0.5 ml	6.00±2.52 ^a	3.33±0.00 ^a	9.33±2.19 ^a	5.00±0.10 ^a	0
Loperamide	2	0.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b	100
Bi-herbal Extract	25	0.67±0.07 ^b	0.00±0.00 ^b	0.67±0.07 ^b	0.17±0.01 ^b	89
Bi-herbal Extract	50	2.00±0.00 ^c	1.33±0.00 ^c	3.33±0.30 ^c	1.67±0.17 ^c	67
Bi-herbal Extract	100	2.00±0.58 ^c	1.67±0.00 ^c	2.67±0.88 ^c	1.33±0.03 ^c	67

Results were expressed as Mean ± SEM; values with different alphabets were statistically significant at the $p < 0.05$ level of significance across the column. Control-distilled water

Discussion

Herbal treatments are frequently used to treat and prevent various illnesses due to their potent pharmacological properties. With the growing popularity of alternative and herbal medicine for treating multiple conditions, concerns over the safety of medicinal plants are also increasing (Zhou et al., 2013). This is mainly because of their propensity to be toxic and to have adverse effects that are both general and herb-specific. Poor pharmaceutical quality control during harvesting and preparation, as well as the widespread belief that herbal remedies are inherently safe, can result in unintentional herbal poisoning. Complex combinations of active ingredients, interactions with other herbs and drugs, contamination, adulteration, or intrinsic poisonous properties can all contribute to the toxicity of therapeutic plants (Rodrigues & Barnes, 2013). For herbal medicines to be used safely in both traditional and clinical contexts, these problems must be resolved. The current study utilised a combination of *Psidium guajava* and *Ocimum gratissimum* to investigate the anti-diarrhoeal potential and the impact of the bi-herbal extract on lipid profiles in experimental rats. In addition to demonstrating the bi-herbal aqueous extracts' ability to modulate lipid profiles, the study's biosafety evaluation provided critical new insights into their potential medical applications. A significant risk factor for cardiovascular disorders like atherosclerosis and coronary artery disease is hyperlipidaemia, a condition characterised by elevated blood lipid levels. It is characterised by a sharp increase in blood lipid indicators, including low-density lipoproteins, triglycerides, and cholesterol, which certain plant-based compounds can occasionally trigger. This investigation demonstrated that lipid-related characteristics, such as triglycerides, total cholesterol, high-density lipoproteins, and low-density lipoproteins, were associated with the parameters used to evaluate toxicity (Magili & Bwatanglang, 2018). The investigation showed that the bi-herbal extract selectively modulated the lipid parameters. Compared with the control group, the bi-herbal aqueous extract at graded

doses reduced triglyceride concentrations, particularly total cholesterol at 50 mg/kg, and low-density lipoprotein at 50 mg/kg. Compared to the control, there was a negligible difference in the high-density lipoproteins. These decreases at specific dose(s) in triglycerides, total cholesterol, and low-density lipoprotein levels may demonstrate the bi-herbal extract's potential hypolipidemic capacity. These actions could be brought about by increased insulin sensitivity, interference with lipoprotein synthesis, antioxidant and anti-inflammatory effects, or suppression of cholesterol production. These decreases in the parameters (triglyceride, total cholesterol, and low-density lipoprotein levels) are consistent with the findings of Magili & Bwatanglang (2018) and Murphy et al. (2007). At 50 mg/kg, the bi-herbal extract produced a significant reduction ($p < 0.05$) in low-density lipoprotein (LDL) levels, alongside marked decreases in total cholesterol and triglycerides. These findings indicate a hypolipidemic effect of the extract, particularly in modulating atherogenic lipid fractions. Elevated LDL is a well-established risk factor for atherosclerosis due to its central role in cholesterol deposition and plaque formation within arterial walls (FERENCE et al., 2017). This finding is consistent with research by Castilla et al. (2006) and Odetola et al. (2006), which demonstrated the hypolipidemic effects of red grape juice and the aqueous extract of fermented *Parkia biglobosa* in rats with alloxan-induced diabetes. In contrast, high-density lipoprotein (HDL) levels remained relatively unchanged across all treatment groups, suggesting that the extract exerts a more pronounced effect on lipid reduction rather than on HDL-mediated reverse cholesterol transport. This selective modulation of lipid fractions is consistent with previous reports on plant-derived hypolipidemic agents, which often demonstrate stronger effects on LDL and triglycerides than on HDL (Rosenson et al., 2020). Overall, the significant reductions in total cholesterol, triglycerides, and LDL levels indicate that the bi-herbal extract may confer cardioprotective benefits by reducing the atherogenic lipid burden, likely through phytochemical-mediated regulation of lipid metabolism and

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oxidative pathways. Castor oil-induced diarrhoea is a secretory diarrhoea, as ricinoleic acid, the active ingredient of castor oil, induces diarrhoea through a hypersecretory response (Stewart et al., 1975). Since the aqueous extract of the bi-herbal sample (*Ocimum gratissimum* and *Psidium guajava*) effectively inhibited the castor oil-induced diarrhoea, it is probably expected that the antidiarrheal action was mediated via an antisecretory mechanism. This was also obvious in the reduction in the total number of wet faeces in the respective test groups during the experiment. Magnesium sulphate has been described to induce diarrhoea by increasing the volume of intestinal contents by preventing the reabsorption of water. It has also been demonstrated that it promotes the release of cholecystokinin from the duodenal mucosa, thereby increasing small intestinal secretion and motility and preventing the reabsorption of sodium chloride and water. (Galvez et al., 1993). The bi-herbal aqueous extract also reduced diarrheal symptoms in this model. The aqueous extract increased the absorption of water and electrolytes from the gastrointestinal tract. The administration of the bi-herbal extract at doses of 25, 50, and 100 mg/kg had no toxic effects on the animals compared with the control (distilled water). This current work aligns with the study by Sekhar et al. (2014) on the aqueous extract of *Psidium guajava* bark, which did not cause mortality up to 5000 mg/kg and the animals were found to be normal after continuous observation for 10 days. Additionally, no mortality was noted at the highest dose of 100 mg/kg after fourteen days of observation. This result is also similar to the report by Hermione et al. (2019), which evaluated the acute and sub-acute toxicities of *Psidium guajava* methanolic bark extract, in which the extract was not toxic.

Conclusion

The study concluded that administering graded doses of the bi-herbal extract from *Psidium guajava* and *Ocimum gratissimum* reduced levels of low-density lipoprotein, total cholesterol, and triglycerides, but had no apparent effect on high-density lipoprotein. The hypolipidemic properties of the bi-herbal extract demonstrated its ability to reduce poor lipid levels, which can help prevent cardiovascular ailments and other health threats. The study also indicates that the bi-herbal extract is likely helpful in the treatment of diarrhoea and is acutely safe at the tested doses.

Conflict of Interest

The authors declare that there are no existing competing interests.

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