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Antihypercholesterolemic, Cardioprotective and Vitamins E and C Sparing Properties of *Bryophyllum pinnatum* in Rabbits

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Authors' contributions

This work was carried out in collaboration between all authors. Author ASA designed the study wrote the protocol, author TIA performed the statistical analysis, authors ASA, TIA and JPK wrote the first draft of the manuscript. Authors ASA, TIA, EBO, JPK and BBA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Potential of *Bryophyllum pinnatum* in preventing excessive lipaemia and oxidation, hence reducing predisposition to chronic diseases was assessed. *B. pinnatum* supplement was prepared by macerating a known weight of the plant in a known volume of distilled water. Ten rabbits were used for the study and were equally grouped into 2. Rabbits in group 1 were given 0.1 ml of distilled water once daily for 60 days and they served as control animals. A dose of 0.1 ml containing 0.2 g/ml of *B. pinnatum* supplement was given to each of the rabbits in group 2 once daily for 60 days. *Bryophyllum pinnatum* caused reduction in cholesterol concentration in serum, kidney, heart, intestine, brain and liver, while increased total lipids concentrations were observed in serum and kidney. *B. pinnatum* supplement reduced lipid peroxidation in heart, brain and liver. Administration

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of *B. pinnatum* caused increased glutathione reductase activity as shown by increased GSH concentration. *Bryophyllum pinnatum* demonstrated vitamins C and E sparing effects. Conclusively, *B. pinnatum* may be a good candidate as a nutraceutical.

Keywords: Antihypercholesterolemia; cardioprotective; antioxidant; lipids; medicinal plants; nutraceutical.

1. INTRODUCTION

In recent times, there has been increase in observation of side effects due to administration of synthetic/orthodox drugs [1,2]. In addition, development of resistance to some of these drugs and their high cost which has taken them out of reach of common men, particularly those living in economically disadvantaged nations have resulted in increase in morbidity and mortality. Therefore, there is a need to search for substances from other sources with proven pharmacological activity. Consequently, this has led to search for more effective pharmacological agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new drugs.

Hypercholesterolemia is a complex disorder characterized by high level of serum cholesterol and it represents a major risk factor for coronary heart disease [3,4] and atherosclerosis [5,6], where oxidative stress has been reported to play major role [7]. Of particular pathophysiological significance, 8 pointed increased lipid peroxidation as a key mechanism for the development of atherosclerosis. Accordingly, natural products from plant origin with antioxidant activity that can prevent against hypercholestero-lemia are of utmost importance.

Bryophyllum pinnatum (Lam. Kurz (Crassulaceae) is a perennial herb growing widely and used in folkloric medicine in tropical Africa, tropical America, India, China and Australia [8,9]. In Nigeria, the plant flourishes throughout the Southern part [10]. The leaves of the plant have been extensively applied externally and taken internally for all types of pains and inflammations, various bacterial, viral and fungal infections, leishmaniasis, earaches, upper respiratory infections, stomach ulcers, flu and fever [11]. In Peru, indigenous tribes mix the leaf with aguardiente (sugar cane rum) and apply the mixture to the temples for headaches; they soak the leaves and stems overnight in cold water and then drink it for heartburn, urethritis, fever and for all sorts of respiratory conditions.

Substantial evidence from the literature revealed that *B. pinnatum* exhibits a variety of pharmacological activities including antihelmintic [12], hepatoprotective [13], anti-inflammatory [14,15], antidiabetic [16] and cardioprotective [17] activities. In addition, *B. pinnatum* was shown to have the potential to inhibit oxytocin-induced increase of the intracellular concentration in human myometrial cells [18].

Several chemical constituents that may account for the therapeutic potential of *B. pinnatum* have been isolated from different plant parts and preparations. This include alkaloids and triterpenes [19], flavonoids [20], bufadienolides [8], glycosides [21], organic [22] and phenolic acids [23].

Although numerous studies have investigated the different health benefits of this plant, scanty information is available as regards its effects on lipid profiles particularly in important organs including brain, liver, kidney and intestine. This study was undertaken to investigate possible lipid lowering and cardio-protective properties, as well as antioxidative characteristics via vitamins C and E sparing effects of *B. pinnatum* supplementation in an experimental model.

2. MATERIALS AND METHODOLOGY

2.1 Materials

2.1.1 *B. pinnatum*

The leaves of *Bryophyllum pinnatum* was collected from its natural habitat and authenticated by Dr Oyekunle of Department of Pure and Applied Biology at Ladoke Akintola University of Technology, Ogbomoso.

2.1.2 Experimental animals

Eight rabbits with an average weight of 1.03 kg were used for the study. They were kept in a well ventilated cage in the animal house with conducive atmospheric pressure and temperature range between 25ºC-30ºC. The animals did not suffer from any observable disorder and had unrestricted access to clean water and feed. The study conformed to the laid down regulation of the Ethics on animal experiment by the Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

2.1.3 Chemicals and reagent kits

All chemicals used including solvents, were of analytical grade. Folin Ciocalteu´s phenol reagent, malondialdehyde (MDA),bis-(dimethyl acetal) (MDA), Thiobarbituric acid (TBA), vitamin E standard, ascorbic acid standard, trichloroacetic acid (TCA), dinirophenyl hydrazine (DNPH), throurea, ferric chloride, $\propto - \propto$ -dipyridyl, 5, 5-dithiobisnitro benzoic acid (DTBV), sodium nitrate, disodium hydrogen phosphate (Na_2HPO_4) and monosodium dihydrogen phosphate (NaH_2PO_4) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Kits used for the measurement of total cholesterol; triglyceride and total proteins were of LABKIT manufactured by CHEMLEX SA, Barcelona, Spain.

2.2 Methods

2.2.1 Preparation of *B. pinnatum* supplement

After authentication, fresh leaves were collected in bulk, washed, and blended into paste without adding water. Thereafter, 4 g of the paste was dissolved in 20 ml of distilled water to give *B. pinnatum* supplement with 0.2 mg/ml concentration. This preparation mimicked one of the ways this plant is being prepared for human consumption in this environment.

2.2.2 Handling of experimental animals

The rabbits were separated into two groups, each group consisting of four rabbits. Group one consisted of rabbits given 0.1 ml (0.2 mg/ml) of *B. pinnatum* supplement; while group two consisted of rabbits that were given only 0.1 ml of distilled water in lieu of *B. pinnatum* supplement (control).

2.2.3 Sample collection

The rabbits were sacrificed on the 25th day i.e. a day after the last administration of the *B. pinnatum* and after 14 hours overnight fast, by cervical dislocation. Each rabbit was opened up and 10 ml of blood was collected directly from the heart into appropriately labeled sample bottles and centrifuged at 4000 rpm for 10 minutes. The supernatant was decanted into plain bottles and kept in freezer for analysis of biochemical parameters.

Organs such as the liver, intestine, brain and heart were quickly excised and washed with appropriate washing buffer (9% normal saline). The excised tissues were homogenized in phosphate buffer using ratio 1:4 (tissue: phosphate buffer). The homogenates were centrifuged and the supernatants were decanted into appropriately labeled sample bottles. All samples were kept in a refrigerator at temperature range of 2-6ºC, for analysis of biochemical parameters. The biochemical parameters assayed in the serum included total cholesterol, triglyceride, total lipids, total protein, GSH, vitamins C and E. In the tissues, biochemical parameters assayed for included total cholesterol, triglyceride, total lipids, total protein, MDA and GSH. Total phenolics, vitamins C and E were also quantified in the plant.

2.2.4 Analysis of lipids

Total cholesterol was determined using enzymatic method of [24]. Triglyceride was determined using method of [25].

2.2.5 Determination of serum concentration of vitamin C

Vitamin C was estimated using method described by [26]. Briefly, 0.5 ml of 6% TCA was added to 0.5 ml of serum and this was centrifuged at 3500 g for 2 minutes. From the supernatant, 0.5 ml was pipetted into test tube and 1 ml of 2% dinitrophenyl hydrazine (2% DNPH) and 4% thiourea in 9 N sulphuric acid were added. The mixture was incubated for 3 hours at room temperature. After incubation, 2.5 ml of 85% sulphuric acid was added and colour developed was read at 530 nm after 30 mins.

2.2.6 Determination of serum concentration of vitamin E

Vitamin E was estimated using method described by mordified method of [27]. Briefly, 1 ml of serum was mixed with 0.32 ml of ethanol and 0.4 ml of petroleum ether and the mixture was centrifuged at 4000 rpm for 15 minutes. The supernatant was decanted into separate test tubes and this was placed in the water bath at 85ºC to evaporate the petroleum ether. Thereafter, 0.2 ml of 5% ferric chloride and 0.2 ml of 2% $\propto - \propto$ - dipyridyl were added. This was allowed to incubate for 5 minutes at room temperature. After incubation, 2 ml of butanol was added and absorbance read at 520 nm.

2.2.7 Determination of reduced glutathione

The reduced glutathione (GSH) in both the serum and tissues was determined by method described by [28]. Briefly, to 0.3 ml of sample, 1.2 ml of 5% TCA was added and then centrifuged at 4000 rpm for 5 minutes. The supernatant was decanted. To 1 ml of the supernatant, 0.5 ml of Ellman's reagent (19.8 mg of 5, 5-dithiobisnitro benzoic acid (DTBV) in 100 ml of 0.1% sodium nitrate) and 3 ml of phosphate buffer were added to the solution. The absorbance was read at 412 nm.

2.2.8 Determination of Malondialdehyde (MDA) in liver tissue

Lipidperoxidation was assessed by measuring the formation of thiobarbituric acid reactive substances (TBARS) using the procedure of [29]. The assay was based on the reaction of chromogenic reagent (2 – TBA) with MDA (end product of lipid peroxidation) under acidic condition to yield a stable pink chromophone with maximum absorbance at 532 nm. Briefly, to 0.3 ml of tissue homogenate, 1.2 ml of 5% TCA was added and the mixture was centrifuged at 4000 rpm for 15 minutes. The supernatant was decanted. To 1 ml of supernatant, 2 ml of TBA solution was added and this was boiled for 10 minutes and cool at room temperature. Absorbance was read at 532 nm.

2.3 Statistical Analysis

Data were analysed using SPSS software and results presented as mean ±SD. Two-way analysis of variance was used to compare values among groups. Comparison between groups was carried out using the Bonferoni multiple comparison method. Significance was determined at $p \leq 0.05$.

3. RESULTS

Concentration of total phenolics contents in the *B. pinnatum* was 2.41 mg/g of garlic equivalent. Results also showed the influence of *B. pinnatum* on serum and tissue concentrations of the different biochemical parameters. Although the reductions were not statistically significant ($p \geq 0.05$), supplementation with *B. pinnatum* caused reduction in the cholesterol concentrations in the serum and all the tissues (kidney, heart, intestine, brain and the liver) (Fig. 1).

B. pinnatum supplementation reduced concentrations of triglyceride in serum and liver, however elevated concentrations were observed in kidney, heart, intestine and brain. However, statistical analyses showed that those differences were not significant ($p \geq 0.05$) (Fig. 2).

The influence of *B. pinnatum* administration on total protein concentration in the serum and the different tissues varied. While it caused elevated concentrations in the serum, kidney and intestine, it reduced the concentrations in the liver, heart and brain. However, these differences did not reach level of statistical significance ($p \geq 0.05$) (Fig. 4).

Lipid peroxidation was reduced in heart, brain and liver of rabbits given *B. pinnatum* supplement as seen by reduction in concentrations of malondialdehyde. *B. pinnatum* supplementation did not reduce oxidation in the kidney and intestine (Fig. 5).

Administration of *B. pinnatum* increased conjugation of glutathione in serum, kidney, intestine and brain as seen in the concentrations of the reduced glutathione. In liver and heart, there were significant reductions ($p \leq 0.05$) in the concentrations of reduced glutathione (Fig. 6).

Significant increase ($p \leq 0.05$) in vitamin C concentration was observed in serum of rabbits given *B. pinnatum* (Fig. 7). The increased concentration in vitamin E observed in rabbits given *B. pinnatum* was not statistically significant ($p \geq 0.05$) (Fig. 8).

The levels of the estimated 4 trace elements in the leaves of *B. pinnatum* showed that Fe had the the highest concentration (1.335±0.0013), followed by Cu (0.031±0.0003), then Cr (0.029±0.0002), while Zn had the least (0.019±0.0014) (Table 1). The results of the heavy metals contents of *Bryophyllum pinnatum* leaves showed that Cd level was the highest (0.023±0.003), followed by Pb (0.016±0.0003), while As had the least (0.012±0.0002) (Table 2).

4. DISCUSSION

The conditions encircling hypercholesterolemia have been reported umpteen times to include increase serum LDL cholesterol levels, which leads to the accumulation of cholesterol in tissues, followed by the prevalence of atherosclerosis and coronary heart disease [30]. This premature coronary heart disease is promoted by xanthomas (caused by LDL-cholesterol deposit) and peripheral cholesterol deposit, while the resulting arterial wall atherosclerosis worsens the situation [31]. Although several articles strictly emphasize the sole involvement of cholesterol deposits as the major cause of coronary artery disease, hypertriglyceridemia has recently been seen to increase the risk for cardiovascular diseases (CAD) because some clinical trials found high serum triglycerides as an independent risk factor.

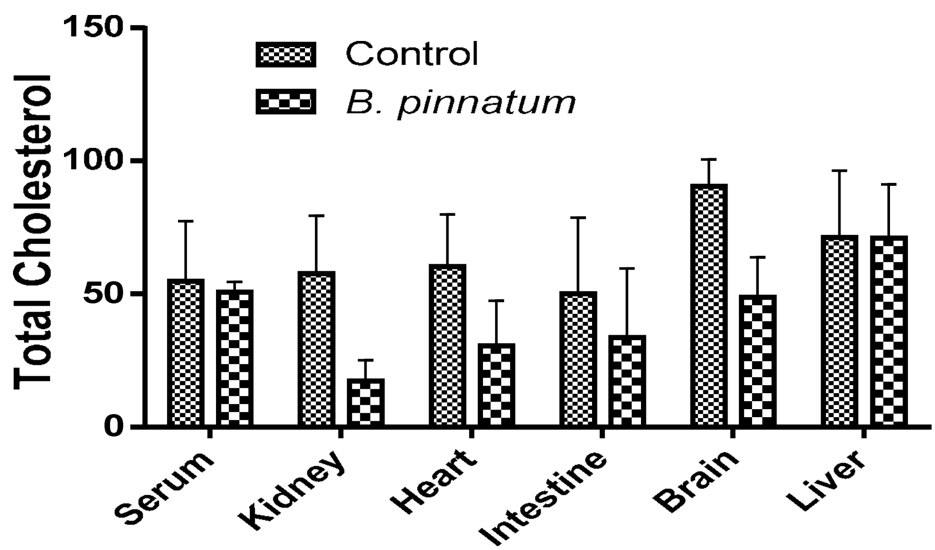

Fig. 1. Effects of *B. pinnatum* on total cholesterol in serum and the different tissues

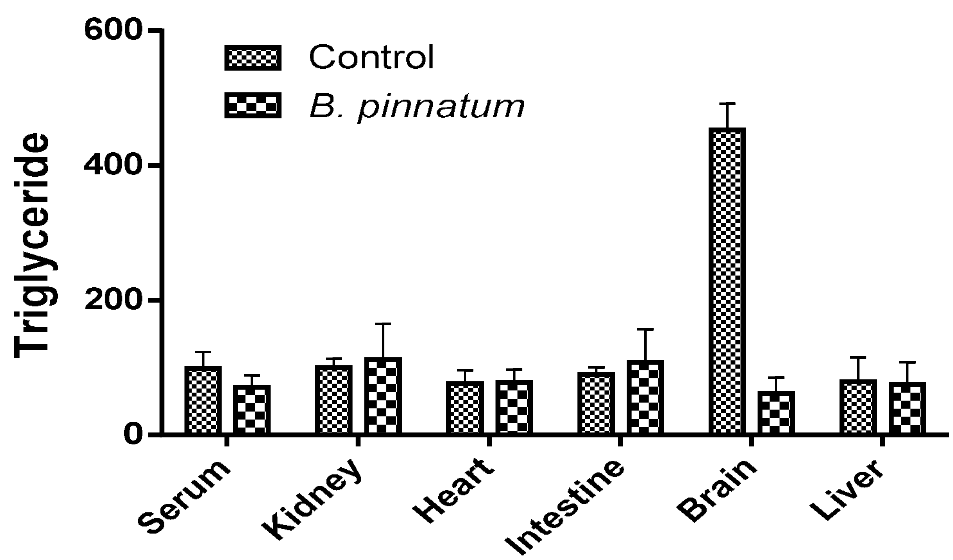

Fig. 2. Effects of *B. pinnatum* on triglyceride in serum and other tissues

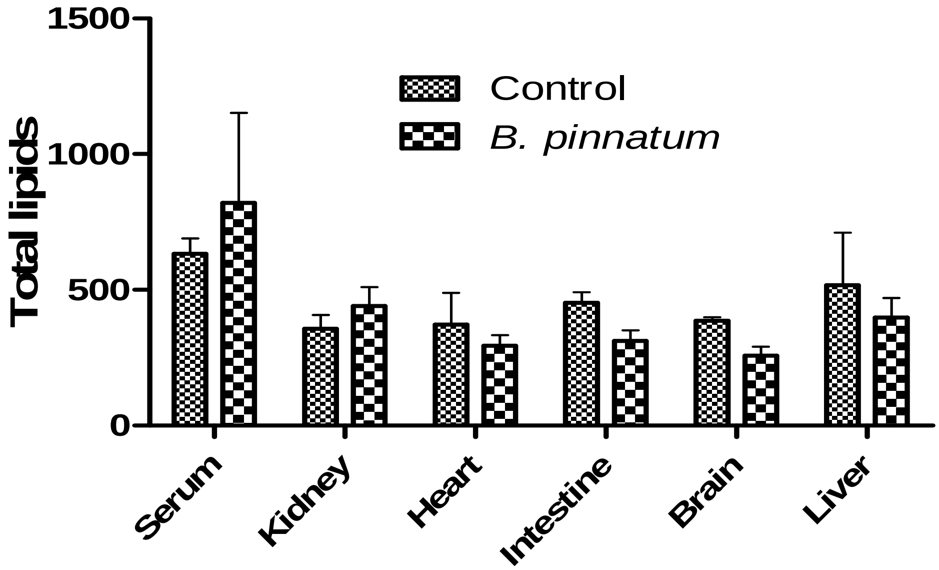

Fig. 3. Effects of *B. pinnatum* on total lipids in serum and the different tissues

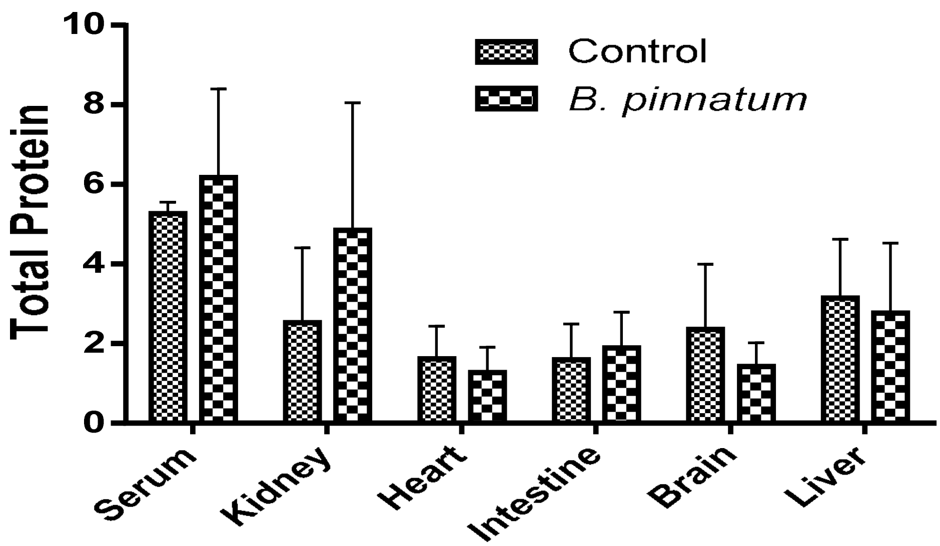

Fig. 4. Effects of *B. pinnatum* on total protein in serum and other tissues

Irrespective of its effect on the risk of CAD, severe hypertriglyceridemia is linked with acute pancreatitis [32-34]. While triglyceride-rich lipoproteins and their remnants may directly facilitate the arterial wall foam cells formation, the complexity surrounding the association between triglyceride and artheroschelosis focus on proartherogenic biochemical abnormalities. These abnormalities include obesity, type-2 diabetes, decreased HDL-C, increased LDL-C, increased free fatty acids, dysglycemia, hyperinsulinemia, increased plasma viscocity, increased inflammatory molecules, impaired fibrinolysis and prothrombosis [35]. In this study, due to the traditional usage of *Bryophyllum pinnatum* in the management of cardiovascular related complications, we find it expedient to explore its antihyperglyceridemc, antihypercholesterolemic and antioxidant properties. Our result demonstrated that *Bryophyllum pinnatum* supplementation ameliorated the raised total cholesterol level in all tissues with liver standing out in exception. The triglyceride levels of serum, kidney, heart, intestine, brain and liver were also reduced by this plant extract. Total lipids were reduced in the heart, intestine, brain and liver; but, such effect was not observed in serum and kidney lipid. It is well established that hyperglyceridemia flocks together with some "bad friends" thereby, promoting the occurrence of some risk factors including obesity, metabolic syndrome, pro-inflammation and type 2 diabetes mellitus [36].

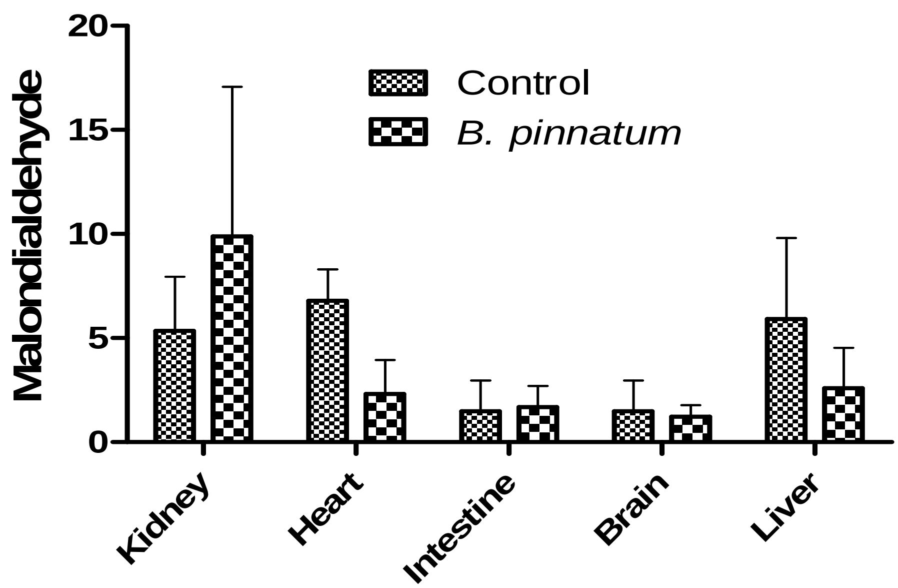

Fig. 5. Effects of *B. pinnatum* on lipid peroxidation in the different tissues

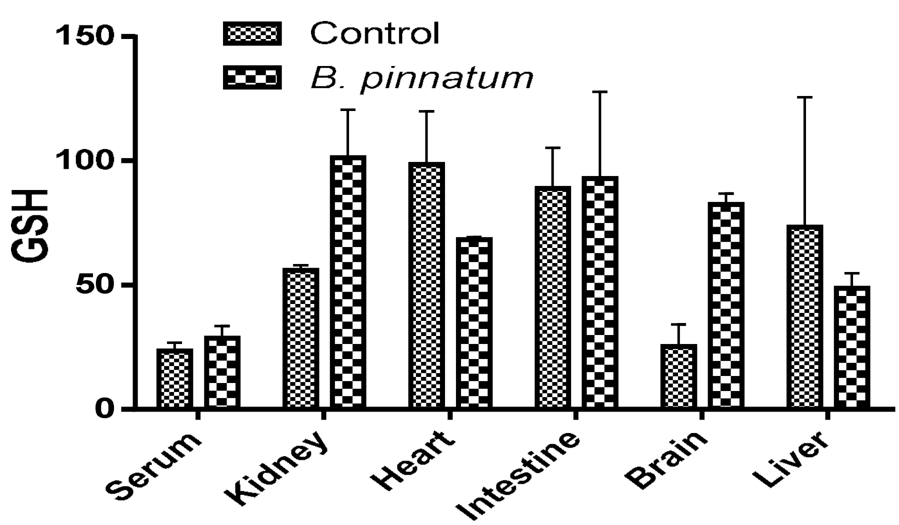

Fig. 6. Effects of *B. pinnatum* on conjugation of glutathione in serum and different tissues

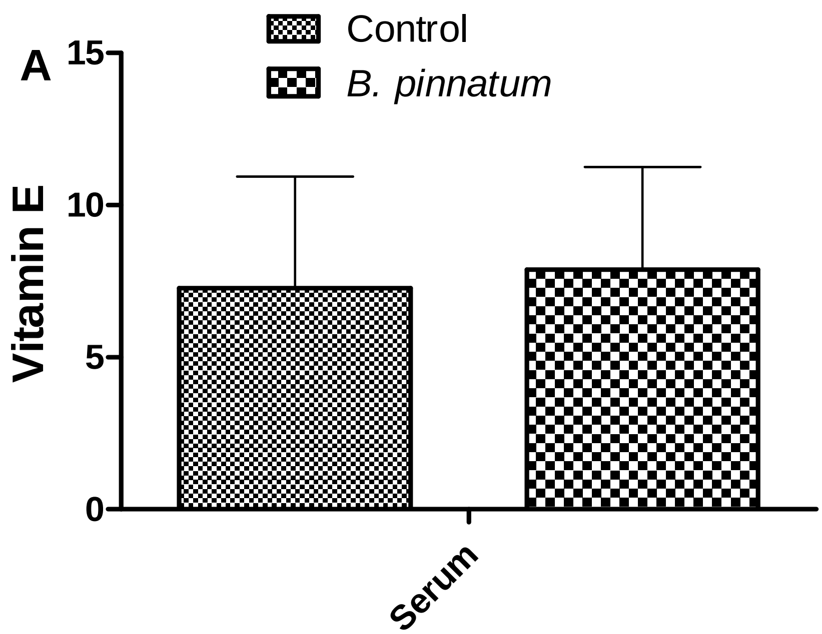

Fig. 7. *B. pinnatum* increased serum concentration of vitamin C

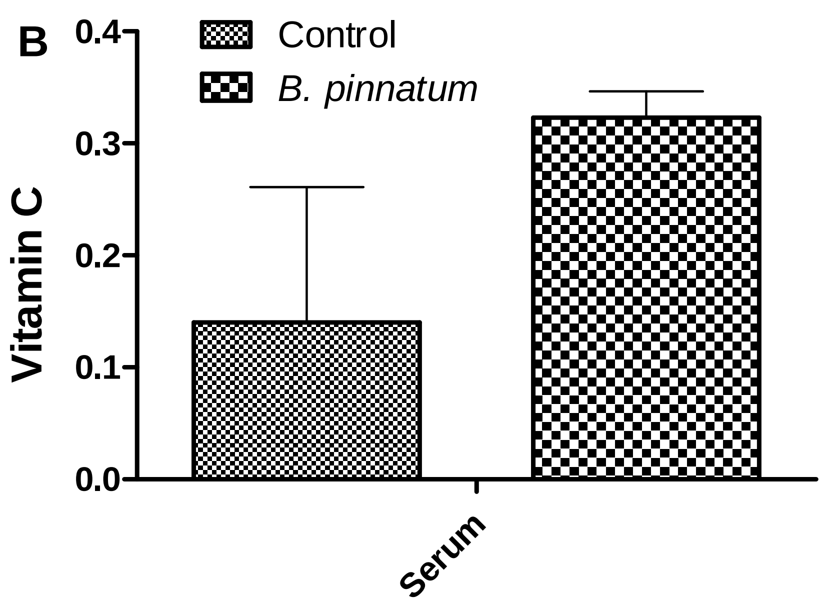

Fig. 8. *B. pinnatum* increased serum concentration of vitamin E

Numerous natural plant products have been traditionally endorsed for the treatment of a wide range of diseases. *Buchholzia coriacea* seeds have been shown to contain some potent antihypercholesterolemic agent which contributes to their effectiveness in the management/treatment of hypercholesteromia and other cardiovascular relatives [37]. Piper beetle extract possesses antihypercholesterolemic properties in experimental hypercholesterolemic wistar rats [38]. Antioxidant activities have been shown to be expressed by *Wasabia japonica* extract while its diet expresses anti-hypercholesterolemic action in high cholesterol diet fed rats [39]. It then infers that the ability of *Bryophyllum pinnatum* extract to ameliorate total lipid content, triglyceride and cholesterol in tissues might go a long way to prevent other cardiovascular complications thereby, justifying its traditional usage for disease management/treatment.

Natural plant products are mixture of compounds in which one or more can be responsible for the beneficial effects of the plant. As, a rule, they contain flavonoids and phenolic compounds that can account for their health promoting effects. Generally, the mechanisms exploited by these bioactive compounds include their ability to scavenge free radicals, chelate metal catalysts, activate *in vivo* antioxidant enzymes, reduce tocopherol radicals, and inhibit oxidases [40,41]. The redox summation of their hydroxyl groups contribute to their potent antioxidant prowess [42,43]. Besides this individual antioxidant potential, synergistic strength of some antioxidants like vitamin C and vitamin E have been reported, although in vivo evidences of these facts have not been thoroughly investigated. Although we have not characterized the extract of *Bryophyllum pinnatum* which can constitute a limitation of this study, we can extrapolate that its components can be at least, in part, responsible for its antioxidant action observed in this study. Our data revealed that *B. pinnatum* supplement increased vitamin C and E content in the serum of rabbits when compared with the control.

Table 1. Concentration of trace elements in *Bryophyllum pinnatum*

Trace elements	Concentrations in ppm
Iron(Fe)	1.335±0.0013
Zinc(Zn)	0.019±0.0014
Copper(Cu)	0.031±0.0003
Chromium(Cr)	0.029±0.0002

Values represent mean ± standard deviation. ppm= part per million

Table 2. Concentration of heavy metals in *Bryophyllum pinnatum*

Heavy metals	Concentrations in ppm
Cadmium(Cd)	0.023±0.0003
Lead(Pb)	0.016±0.0003
Arsenic(As)	0.012±0.0002

Values represent mean ± standard deviation. ppm= part per million

The magnitude of injury mediated free radical lipid peroxidation can be measured by biological markers like conjugated dienes, 4-hydroxynonenal and malondialdehyde (MDA) [44]. Naturally, lipid hydroperoxides and aldehydes present in biological system promote oxidative stress through the increase of 2-thiobarbituric acid reactive substances (TBARS) [45]. TBARS assay has been the prominent method used for estimating MDA in tissues. At 532nm maximum absorbance, 2-thiobarbituric acid formed from the reaction between one molecule of MDA and 2 molecules of 2-thiobarbituric acid gives a chromophore (TBARS) that could be used to estimate MDA concentration [46]. We found that *Bryophyllum pinnatum* supplementation ameliorated the raised MDA content in the heart, brain and liver, possibly suggesting its cardioprotective effect. The potential of *Bryophyllum pinnatum* to attenuate lipid peroxidation could be one the most potent mechanism through which it exerts its antioxidant and cardioprotective activities as this could be the reason behind its folklore usage for the treatment/management of cardiovascular related diseases.

The tripeptide glutathione (GSH) is an antioxidant which has a critical role in the protection of cells against oxidative stress. Glutathione oxidase and glutathione peroxidase catalyze the oxidation of GSH to GSSG while glutathione reductase regenerates GSH from GSSH in the presence of NADPH. This cycle has been implicated in many oxidative stress related diseases. Here, *Bryophyllum pinnatum* extract was able to increase GSH level in the kidney, intestine, serum and brain tissues of rabbits while that of liver and heart were lowered.

The reduced concentrations of reduced glutathione in both liver and heart can be linked with lower malondialdehyde concentrations recorded in both organs after administration of *B. pinnatum*. Formation of malondialdehyde is due to presence of free radicals causing oxidation of lipids. The higher the rate of generation of free radicals and their capacity to overwhelm the tissue antioxidant system, the more the products of lipid peroxidation. On the other hand, in protecting the tissues from toxins, intracellular glutathione (including GSH), in a reaction catalyzed by glutathione-s-transferase (GST), form conjugates with variety of chemicals posing electrophilic center. The final GSH-conjugates have increased hydrophilicity, which facilitates their further metabolism and elimination [47]. The low GSH concentration observed in both liver and heart may be due to either reduced generation of free radicals in these two tissues, or due to availability of other non-enzymic antioxidants such as vitamins C and E, or other phytochemicals present in the *B. pinnatum* which are able to detoxify toxins by preventing generation or quenching already formed free radicals. Hence, reduced formation of malondialdehyde.

It has been reported that many plants and vegetables contain vitamin C and E [48-50] which are powerful antioxidants and are healthy and beneficial to the functioning of our body. For instance, consumption of plants or plant-derived products rich in vitamin E has been linked to the prevention of cardiovascular conditions and certain cancers. Suggestions have come from different reports of *in vivo* studies that antioxidant vitamins like vitamin C and vitamin E can prevent lipid peroxidation and their ability to do this in a more effective fashion had been attributed to their synergistic strength. It is therefore possible to presume that the increased serum concentrations of vitamin C and E observed in the group given *B. pinnatum,* could be due to presence of the two vitamins in the plant and this certainly, might have contributed to the reduced lipid peroxidation and cholesterolemia observed in this study. This hypothesis corroborates with the results of [51] showing that vitamin E can increase activity of antioxidant enzymes and prevent against hypercholesterolemia.

Furthermore, presence of zinc, copper, chromium and iron in the plant confers nutritive and pharmacological properties on *B. pinnatum*. Apart from their numerous roles as part of metalloenzymes, these elements have been reported to have antioxidative and lipid lowering properties. For instance, oral zinc supplementation decreased total and LDL-cholesterol, whereas HDL-cholesterol increased in both normal and diabetic humans [52,53]. Antioxidative and anti-inflammatory properties of zinc have been reported [54,55] and its deficiency have been reported to cause increased plasma lipids atherosclerotic markers in LDL- $R^{-/-}$ mice [56].

Also, various studies have suggested that copper supplementation may decrease the cholesterol concentration in Longissimus dorsi, in addition to improving the fatty acid profile [57]. Copper have been reported to alter adipose tissue metabolism and decreases cholesterol synthesis, therefore deficiency of copper causes hypercholesterolemia [58].

Evidence of a role for chromium in lipid metabolism and chromium deficiency in the development of atherosclerosis is accumulating from animal and human studies [59]. Chromium has been reported to have lipid lowering effects [60] and antioxidative properties *in vivo* [61]. Its deficiency have been associated with elevated serum cholesterol triglycerides and increased incidence of aortic plaques [62].

Furthermore, according to our results, the concentration of heavy metals Lead (Pb), Arsenic (As) and Cadmium (Cd) in the leaves of the plant are 0.016 ppm, 0.012 ppm and 0.023 ppm respectively. These concentrations are far below the World Health organization [63] limit for herbal medicine which is 10 ppm, 0.3 ppm and 1 ppm for Lead, Arsenic and Cadmium respectively. The low concentrations of these three metals may be indicative of relative safety of B. Pinnatum as a nutraceutic.

5. CONCLUSION

Collectively, our data explains why *Bryophyllum pinnatum* could be used as nutraceuticals in the management/treatment of hypercholesterolemic, hypertriglyceridemic and cardiovascular related disorders.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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