**EFFECT OF *Bryophyllumpinnatum*LEAF EXTRACT CONSUMPTION ON LIPID CONCENTRATIONS AND CARDIOVASCULAR RISK INDICES IN APPARENTLY HEALTHY INDIVIDUALS.**


## ABSTRACT

*Bryophyllumpinnatum* has been consumed locally for various ailments without proper documentation of it's effect on Lipid concentrations and cardiovascular risk indices in humans. This study sought to determine the effect of *Bryophyllumpinnatum*leaf extract consumption on lipid concentrations and cardiovascular risk indices in apparently healthy individuals by estimating Total cholesterol, Triglycerides, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Atherogenic Index of Plasma (AIP), Castelli Risk Index I and II, Total protein and Albumin in 15 apparently healthy individuals after consumption of 1.32g/180ml of *Bryophyllumpinnatum* leaf extract for 14 days. Samples taken before the commencement of the study served as control samples, while samples collected on the 1st,7th and 14thday after consumption of 1.32g/180ml of *Bryophyllumpinnatum* leaf extractserved as test samples. Total Cholesterol, Triglycerides, High Density Lipoprotein were estimated using Colorimetric methods.Total protein was estimated using Biuret method while Albumin was estimated using Bromocresol green method. The results showed that levels of LDL(1.04±0.44mmol/l),AIP(-0.34±0.09),Castelli index I (2.16±0.53) and Castelli index II(0.94±0.47) were significantly reduced ($p<0.05$) after 7 days of consumption when compared to control LDL(2.65±0.18mmol/l),AIP(-0.15±0.18),Castelli index I (3.84±0.32) and Castelli index II(2.44±0.33).Further comparism of levels of LDL(2.22±0.74mmol/l),AIP((-0.15±0.18),Castellic index I ((3.74±1.80) and Castellic index II (2.42±0.98) after 14 days of consumption with levels after 7 daysLDL(1.04±0.44mmol/l),AIP(-0.34±0.09),Castelli index I (2.16±0.53) and Castelli index II(0.94±0.47) showed significant increase ( $p< 0.05$). Results obtained suggest that short term consumption of *Bryophyllumpinnatum* leaf extract would reduce cardiovascular risk indices in humans but continuous long term use can predispose consumers to increased risk of developing cardiovascular pathological consequences.



**KEY WORDS*:*** *Bryophyllumpinnatum*,Cardiovascular risk indices,Lipids,Traditionalmedicine,Miracleplant,Castelli index .


## INTRODUCTION

*Bryophyllumpinnatum* also known as *kalanchoepinnata* of the familycrassulaceae.It is also known as air plant, cathedral bells, life plants, miracle leaf, resurrection plant.*Bryophyllumpinnatum* is a leafy plant commonly found in Madagascar and other parts of the world such as Pakistan, Nepal, Brazil and United States (Yadav *et al*,.2013, Smith*et al.*,2019). The plant is locally called "Never Die" in Nigeria. It is a plant that is classified as weed.*Bryophyllumpinnatum*grows widely throughout the southern part of Nigeria (Gill, 2017). Itis a tall,erect and succulent perennial herb measuring up to 2meter in height( Silva *et al.*, 2015).Its leaves are oval in shape with rounded tips measuring 5-25cm long and 2-12cm broad(Dalzeil,2017).*Bryophyllumpinnatum* has been reported to contain a wide range of active compounds such as alkaloids, glycosides, flavonoids, steroids,lipids and organic solvents(Naude,2017).The plant is widely used in traditional medicine for the treatment of variety of ailments hence the name 'miracle plant' as it is known forit'shaemostatic and wound healing properties (Araújo*et al.*, 2023,Naude, 2017).

In traditional medicine, the leaves of the plant have been used for antimicrobial (Oliver – Bever, 2013) anti-ulcer, anti -inflammatory (Pal *et al*., 2015),antihypertensive (Ojewole, 2015) uses. The leaves and bark are used as bitter tonic, useful in treating diarrhea and vomiting (Kirtika*et al.*,2015). It is applied externally and taken internally for all types of pains and inflammations,bacterial, viral and fungal infections, earaches, upper respiratory infection, stomach ulcers and fever (Silva *et al.*, 2015). *Bryophyllumpinnatum* acts as a herbal remedy for an array of human disorders, including hypertension,diabetes mellitus, bruises,wound and boils(Araújo*et al.*,2023,Saluja,2017), abscesses, insect bites, arthritis, rheumatism, joint pain, headaches and body pain(Lin and Rong,2014). Reports indicate

that the leaves of the plant (*Bryophyllumpinnatum*) have strong medicinal value and are used both externally and internally (Dalzeil,2017).*Bryophyllumpinnatum* is highly recommended intreatment of nocturia(Mirzayeva*et al*., 2023), bleeding disorders, piles and is suggested to be a good source of vitamin (Okwu&Nnamdi, 2014).It is consumed with the believe that the plant helps to clean the bladder and also serves as a toxin remover in the intestines ( Chennkappan and Kadarkarai,2019).Reports suggest that it helps in weight loss (Nwali,*et al*., 2016),treatment of inflammation (Nayak,*et al* ., 2017) and bronchial condition (Ojewole,2015). Study by Raymond et al.,( 2010) suggest possible safety of the aqueous extract of *Bryophyllumpinnatum* in Sprague-Dawley rats.

Lipid concentrations can be used to determine "Good cholesterol" and "Bad cholesterol and serve as initial screening tool for abnormalities in lipids. The result of lipid test can identify certain genetic diseases, certain forms of pancreatitis and cardiovascular disorders. Fasting lipid test includes four (4) basic parameters; Total cholesterol (T.C), Triglycerides (T.G), High density lipoprotein (HDL) cholesterol, Low density lipoprotein (LDL) cholesterol (Pagana*et al*., 2019).

High density lipoprotein (HDL) cholesterol, also known as the 'good cholesterol' absorbs cholesterol and carries it back to the liver where it flushes it from the body. High levels of high density lipoprotein can lower the risk for heart diseases and stroke while High levels of low density lipoprotein (LDL) cholesterol known as 'bad cholesterol' leads to a buildup of cholesterol in the arteries where it forms plaque. The plaque builds up in the arteries leading to Atherosclerosis (Catapano*et al.*, 2019). This study therefore sought to determine theeffect of *Bryophyllumpinnatum*leaf extract consumption on lipid concentrations and cardiovascular risk indices in apparently healthy individuals.

## MATERIAL AND METHODS

### Study area

The study was conducted in Rivers State College of Health Science and Management Technology Port Harcourt, Rivers state,Nigeria.

### Study population

A total of fifteen (15) apparently healthy individuals aged between 20-25 years and weighing between 55-75kg were recruited for the study. All participates were volunteers who were well informed about the study before giving their written consent to participate.

### Plant purchase and identification

The plant parts used for the study were fresh leaves of *Bryophyllumpinnatum* obtained from a botanical garden in Iwofe district in Port Harcourt, Rivers state in Nigeria. The leave of *Brophyllumpinnatum* was identified by a pharmacist in the department of Pharmacy in Rivers State College of Health Science and Management Technology.

### Plant preparation

Freshly cut leave from a botanical garden in Iwofe district in Port Harcourt,RiversState,Nigeria were carefully pluggedand sorted out to remove contaminated matters. The leaved were weighed using a digital weighing balance (dw100) made in china. The weight was noted.

The leaves were then washed and allowed to dry and reweighed in a clear jar with 13.3 liters of water. It was allowed to stand for an hour than filtered to remove the shaft leaving the concentrate.

### Calculation of plant concentration

Weight of leaves before grinding = 450g
Weight of leaves after grinding and removal of shaft = 97.4g
Total volume of water used to macerate leaves = 13.3 liters (13,300ml)
Concentration of leaf extract in 13.3 liters of water = 97.4g/13.3 liters
Each cup used by participant can contain 180ml by volume

13.3 liters (13300ml) leave extract contains 97.4g.

180ml will contain $97.4g \times \frac{180ml}{13300ml} = 1.318g$

Concentration of leaf extract consumed daily by each participant = 1.32g/180ml daily for 14 days.

**Sample Collection**

Blood samples were collected from each participant before commencement of the study as control samples while test samples were collected on day 1, day 7 and day 14 from each participant.All samples were collected by veinpuntureand dispense into well labeled lithium heparin bottle. The samples were centrifuged at 1500 rotations per minute for 5minutes to obtain plasma samples which were analysed immediately forfasting lipid parameters.

**Determination of Test parameters:** Total cholesterol,Triglycerides,High density lipoproteins were determined using Colometric methods as described by Ochei. and Kolhatkar (2017).Total protein was determined using Biuret method while Albumin was estimated using Bromocresol green method (Ochei. and Kolhatkar (2017)).

Atherogenic index of plasma(AIP) was calculated using the formular $\log_{10}$ (TG/HDL-C) as described by Kanthe, *et al*., (2012) and Nyat*et al*., (2018).

**Castelli Risk Index I and II**

Castelli Risk Index I and II were calculated using the formula as described by Oguejifor*et al.*, (2012).

$$\text{CAST I} = \frac{\text{TC}}{\text{HDL-C}}$$

$$\text{CAST II} = \frac{\text{LDL-C}}{\text{HDL-C}}$$

**Statistical Analysis**

Data were analyzed using Microsoft excel version 2007 and Graph pad prism version 5 to determine the statistical significance.$P < 0.05$ was considered to be statistically significant



**RESULTS**

The study was conducted to determine Total cholesterol, Triglycerides, High Density Lipoprotein, Low Density Lipoprotein, Atherogenic Index of Plasma, Castelli Risk Index I and II, Total Protein and Albumin) in Apparently 15 healthy Individuals after the Consumption of *Bryophyllumpinnatum*for 14 days.
Results are summarized in the table 1 to table 5.

**Table 1: Lipid Concentrations,AtherogenicIndex,Castelli Risk Index I and II in Apparently Healthy Individuals after one day of Consumption of *Bryophyllumpinnatum* and control n=15**

| **Parameter** | **Control Mean±SD n =15** | **Test (After day one) Mean±SD n= 15** | **T-test** | **P-value** |
|---|---|---|---|---|
| Total cholesterol(mmol/L) | 4.13± 0.08 | 3.10± 1.07 | 2.13 | 0.10 |
| Triglycerides (mmol/L) | 0.87±0.33 | 0.65±0.18 | 1.83 | 0.14 |
| HDL (mmol/L) | 1.08±0.09 | 1.02±0.16 | 0.83 | 017 |
| LDL (mmol/L) | 2.65±0.18 | 1.78±1.11 | 1.67 | 0.17 |
| AIP | -0.15±0.18 | -0.20±0.13 | 0.85 | 0.45 |
| CAST I | 3.84±0.32 | 3.13±1.35 | 0.99 | 0.38 |
| CAST II | 2.44±0.33 | 1.83±1.32 | 0.94 | 0.40 |
| Total Protein(mmol/L) | 72.20±5.36 | 74.80±5.07 | 0.64 | 0.56 |
| Albumin(mmol/L) | 42.00±1.87 | 40.20±1.48 | 1.45 | 0.23 |

HDL – High Density LipoproteinLDL – Low Density Lipoprotein
AIP – Atherogenic Index of PlasmaCast I – Castelli Risk Index I
Cast II – Castelli Risk Index IIP<0.05 – Significant

**Table 1** shows that**,** Total cholesterol, Triglycerides, High Density Lipoprotein, Low Density Lipoprotein, Atherogenic Index of Plasma, Castelli Risk Index I and II, Total Protein and Albumin did not show any significant difference when the test group was compared to the control after one day of consumption of *Bryophyllumpinnatum*.P>0.05.

**Table 2: Lipid Concentrations,Atherogenic Index,Castelli Risk Index I and II in Apparently Healthy Individuals after Seven days of Consumption of *Bryophyllumpinnatum* and control n=15**

| Parameter | Control Mean±SD n =15 | Test (after 7days) Mean±SD n = 15 | T-test | P-value |
|---|---|---|---|---|
| Total Cholesterol(mmo/L) | 4.13± 0.08 | 2.43±0.57 | 6.66 | 0.00 |
| Triglycerides (mmol/L) | 0.87±0.33 | 0.52±0.09 | 2.59 | 0.06 |
| HDL (mmol/L) | 1.08±0.09 | 1.05±0.09 | 0.56 | 0.58 |
| LDL (mmol/L) | 2.65±0.18 | 1.04±0.44 | 7.52 | 0.00 |
| AIP | -0.15±0.18 | -0.34±0.09 | 2.71 | 0.05 |
| CAST 1 | 3.84+0.32 | 2.16±0.53 | 5.27 | 0.00 |
| CAST II | 2.44±0.33 | 0.94±0.47 | 5.01 | 0.00 |
| Total Protein (mmol/L) | 72.20±5.36 | 75.4±9.07 | 0.67 | 0.51 |
| Albumin (mmol/L) | 42.00±1.87 | 41.4±5.32 | 0.24 | 0.81 |

HDL – High Density LipoproteinLDL – Low Density Lipoprotein
AIP – Atherogenic Index of PlasmaCAST I – Castelli Risk Index I
CAST II – Castelli Risk Index IIP<0.05 – Significant

Table 2 shows that Total cholesterol, Low Density Lipoprotein, Atherogenic index of plasma,CAST I and II were significantly reduced (P<0.05) when the test group was compared to the control after 7 days of consumption of *Bryophyllumpinnatum*.Triglycerides, High Density Lipoprotein, Total protein and Albumin did not show any significant difference(P>0.05) when test group was compared to the control after 7 days of consumption of *Bryophyllumpinnatum*.

**Table 3:Lipid Concentrations,AtherogenicIndex,Castelli Risk Index I and II in Apparently Healthy Individuals after 14 days of Consumption of *Bryophyllumpinnatum* and Control n=15.**

| Parameter | Control Mean±SD n=15 | Test (after14 days) mean± SD n = 15 | T-test | P-value |
|---|---|---|---|---|
| Total cholesterol(mmol/L) | 4.13±0.08 | 3.47±0.75 | 1.92 | 0.09 |
| Triglycerides(mmol/L) | 0.87±0.33 | 0.69±0.20 | 0.99 | 0.35 |
| HDL (mmol/L) | 1.08±0.09 | 0.95±0.14 | 1.52 | 0.16 |
| LDL (mmol/L) | 2.65+018 | 2.22±0.74 | 1.14 | 0.31 |
| AIP | -0.15±0.18 | -0.15±0.18 | 0.28 | 0.78 |
| CAST I | 3.84+0.32 | 3.74±1.8 | 0.19 | 0.85 |
| CAST II | 2.44±0.33 | 2.42±0.98 | 0.10 | 0.91 |
| Total Protein (mmol/L) | 72.20±5.36 | 70.80±3.03 | 0.51 | 0.63 |
| Albumin (mmol/L) | 42.00±1.87 | 42.40±3.65 | 0.21 | 0.83 |

HDL – High Density LipoproteinLDL– Low Density Lipoprotein
AIP– Atherogenic Index of PlasmaCAST I – Castelli Risk Index 1
CAST II – Castelli Risk Index 2P<0.05 – Significant

Table 3 shows that**,** Total cholesterol, Triglycerides, High Density Lipoprotein, Low Density Lipoprotein, Atherogenic Index of Plasma, Castelli I and II, Total protein and Albumin did not show any significant difference when the test group were compared to the control group after 14 days of consumption of *Bryophyllumpinnatum. P*>0.05

**Table 4: Lipid Concentrations,AtherogenicIndex,Castelli Risk Index I and II in Apparently Healthy Individuals after 1 day of Consumption of *Bryophyllumpinnatum* and 7 days. n= 15.**

| Parameter | After 1 day Mean±SD n=15 | After 7days mean ±SD n = 15 | T-test | P-value |
|---|---|---|---|---|
| Total cholesterol(mmol/L) | 3.10±1.07 | 2.43±0.57 | 1.64 | 0.17 |
| Triglycerides (mmol/L) | 0.65±0.18 | 0.52±0.09 | 1.49 | 0.13 |
| HDL (mmol/L) | 1.02±0.16 | 1.05±0.09 | 0.95 | 0.13 |
| LDL (mmol/L) | 1.78±1.11 | 1.04±0.44 | 1.84 | 0.13 |
| AIP | -0.20±0.13 | -0.34±0.09 | 1.94 | 0.08 |
| CAST I | 3.13±1.35 | 2.16±0.53 | 1.40 | 0.17 |
| CAST II | 1.83±1.32 | 0.94±0.47 | 1.41 | 0.19 |
| Total Protein (mmol/L) | 74.80±5.07 | 75.40±9.07 | 0.15 | 0.88 |
| Albumin (mmol/L) | 40.20±1.48 | 41.40±5.32 | 0.42 | 0.69 |

HDL – High Density LipoproteinLDL – Low Density Lipoprotein
AIP – Atherogenic Index of PlasmaCAST 1 – Castelli Risk Index 1
CAST II – Castelli Risk Index IIP<0.05 – Significant

Table 4 shows that**,** Total cholesterol, Triglycerides, High Density Lipoprotein, Low Density Lipoprotein, Atherogenic Index of Plasma, Castelli I and II, Total protein and Albumin did not show any significant difference after one day of consumption was compared to 7 days of consumption of *Bryophyllumpinnatum. P*>0.05

**Table 5:Lipid Concentrations,AtherogenicIndex,Castelli Risk Index I and II in Apparently Healthy Individuals after 7 Days of Consumption of *Bryophyllumpinnatum* and 14 days. n= 15.**

| Parameter | After 7 days Mean±SD n=15 | After 14days mean+ SD n= 15 | T-test | P-value |
|---|---|---|---|---|
| Total cholesterol(mmol/L) | 2.43± 0.57 | 3.47± 0.75 | 0.97 | 0.38 |
| Triglycerides (mmol/L) | 0.52± 0.09 | 0.69± 0.20 | 1.72 | 0.16 |
| HDL (mmol/L) | 1.05± 0.09 | 0.95 ±0.16 | 1.59 | 0.18 |
| LDL (mmol/L) | 1.04 ±0.44 | 2.22+0.4 | 3.42 | 0.02 |
| AIP | -0.34± 0.09 | -0.15 ±0.18 | 2.37 | 0.05 |
| CAST 1 | 2.16 ±0.53 | 3.74 ±1.8 | 3.94 | 0.01 |
| CAST II | 0.94± 0.47 | 2.42± 0.98 | 5.99 | 0.00 |
| Total Protein(mmol/L) | 75.40± 9.07 | 70.80± 3.03 | 1.19 | 0.29 |
| Albumin (mmol/L) | 41.40 ± 5.32 | 42.40 ±3.65 | 0.33 | 0.75 |

HDL – High Density LipoproteinLDL – Low Density Lipoprotein
AIP – Atherogenic Index of PlasmaCAST I– Castelli Risk Index I
CAST II – Castelli Risk Index IIP<0.05 – Significant

Table 5 shows that,Low density lipoprotein(LDL) was significantly increased in test group after 14 days of consumption of *Bryophyllumpinnatum*was compared to 7 days of consumption.Atherogenic index of plasma ,Castelli I and II(CAST I and II) were also significantly increased in the test group after 14days of consumption of *Bryophyllumpinnatum*was compared to 7 days of consumption.Total cholesterol(T.C),Triglycerides(T.G),High density lipoprotein (HDL),Total protein(T/P)and Albumin did not shows any significant difference when the test group after 14 days of consumption of *Bryophyllumpinnatum*were compared to 7 days of consumption.

**Discussion**

This study assessed lipid concentrations,AtherogenicIndex,Castelli Risk Index I and II in apparently healthy individuals after consumption of 1.32g/180ml of*Bryophyllumpinnatum*leaf extract.Results of this study showed no significant difference in levels of lipid parameters,Atherogenic Index of Plasma , Castelli Risk Index I and II ,Total protein and Albumin after one day of consumption of*Bryophyllumpinnatum*leaf extract was compared to results before consumption. This could explain why the leave have been used as food source as it is shown to possess some levels of carbohydrate,proteins,fats and oil( Nwali*et al*., 2014).

However,comparism after seven days consumption showed significant reduction in lipid parameters, Atherogenic Index of Plasma , Castelli Risk Index I and II. This would suggest that short term consumption of *Bryophyllumpinnatum*leaf extract would reduce cardiovascular risk indices inhumans.Studies by Kalio *et al*., 2022 on herbal plant suggest results contrary to the findings of this study and concluded that herbal plants such as *Costusafer* when consumed do not affect lipid parameters and does not predispose consumers to cardiovascular risk. Reduced Low density lipoprotein observedin the study after 7 days consumption suggest that short term consumption of bryophyllumpinnatum lowers bad cholesterol level (LDL) in the body, Low density lipoprotein cholesterol is an important indicator of plaque buildup in the arteries which predicts atherosclerosis and cardiovascular disease,henceshort term consumption *Bryophyllumpinnatum*leaf extract can aid in reducing the risk of cardiovascular disease.*Bryophyllumpinnatum*leaf extract has been shown to possess anti-inflammatory properties ( Ojewole,2005) and contains flavonoids andAscorbic acid which are good antioxidants (Raymond *et al*., 2010) capable of preventing pathological consequences through their neuroprotective and anticonvulsant properties.This could also explain the ability of the leave extract in reducing low density lipoprotein levels.Also,the reduce Atherogenic Index of Plasma level observed after seven days consumption is also suggestive of the ability of the leave extract in reducing cardiovascular disease. Atherogenic Index of Plasma is a marker of future cardiovascular disease and a predictor of anterogenicity( Dobiasova*et al*., 2011,Manoj *et a*l., 2019). Atherogenic Index of Plasma reflect the lipoprotein composition in the plasma and is an independent risk factor for coronary disease.

Furthermore, results of 14 days of consumption of *Bryophyllumpinnatum* when compared to 7 days of consumption of *Bryophyllumpinnatum*showed increased low density lipoprotein cholesterol,AIP and CAST I and II after 14 days consumption . This would suggest that continuous long term consumption would further predispose consumers to increase concentrations of cardiovascular risk indices in humans.Antherogenic Risk Index (AIP) is a strong prediction of infarction and can be used alone forcardiac risk estimation. It is used by some practitioners as a significant predictor of Atherosclerosis (Dabiaswu, 2011). Castelli Risk Index which is of two types, Castelli risk index I and II. Castelli Risk Index is also known as cardiac risk ratio (CRI). The ratios have been studied as markers of Lipid Atherogenic. These are calculated fractions which can be used in clinical setting to assess the risk of cardiovascular disease beyond the routinely done Lipid profile (Oguejifo*et al.*,2012).

This study also showed that Low Density Lipoprotein was significantly higher after 14days of consumption of *Bryophyllumpinnatum*was compared to 7 days of consumption. Increased levels of Low Density Lipoprotein is linked to an increased risk of heart and blood vessel disease including coronary artery disease, heart attack and death. Low Density Lipoprotein is a combination of fat and protein found in the blood. It is called bad cholesterol because it picks cholesterol from the blood and take it to the cells and build up walls in the arteries. These fatty deposits form plague that lines up in the arteries can cause blockage. Thus build up is known as Atherosclerosis (Catapano*et al*., 2019).LDL moves cholesterol to vascular cells and can be use to determine deposition of intracellular lipids (Venugopal and Jialal,2019). Increase Low density lipoprotein observed in this study suggest after 14 days consumption suggest that *Bryophyllumpinnatum* consumption may predispose consumers to increase risk of heart and blood vessel disease.

## Conclusion

This study showed that long term consumption of *Bryophyllumpinnatum*could predispose consumers to increase levels of cardiovascular risk indices.Consequently,continuous long term consumption should be discouraged to prevent risk of developing cardiovascular problems.

## Consent

As per international standard or university standard, Participants' written consent has been collected and preserved by the author(s).

## Ethical Approval

Ethical approval was gotten from Rivers State college of Health Science and Management Technology research board.

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