CARDIOVASCULAR RISK INDICES IN APPARENTLY HEALTHY INDIVIDUALS AFTER 14 DAYS OF CONSUMPTION OF *Costus afer* STEM EXTRACT.

ABSTRACT

Costus afer has been used for both medicinal and nutritional purpose ~~use~~ without adequate knowledge of its systemic effect. This study therefore, sought to determine the cardiovascular risk indices in fifteen (15) apparently healthy individuals aged between 20-35years after 14days of consumption of 1.28g/180ml of *Costus afer* stem extract by estimating High density lipoprotein cholesterol (HDLC), Total Cholesterol, Triglycerides, low density lipoprotein-cholesterol(LDL-C), Atherogenic index of Plasma (AIP), Castelli Risk Index I and II, Total Protein and Albumin. Samples taken before the commencement of the study served as control samples while samples collected on day one (1), seven (7), and fourteen (14) days served as test samples.Total cholesterol, Triglyceride, High density lipoprotein-cholesterol were estimated using colorimetric methods. Total Protein was estimate using Biuret method while Albumin was estimated using Bromocresol green method.The results showed that the mean ± Standard deviation of Total cholesterol (3.38± 0.68mmol/l), Triglycerides (0.74 ±0.16mmol/l), High density lipoprotein cholesterol (0.95 ± 0.19mmol/l), low density lipoprotein-cholesterol (1.92 ± 0.90mmol/l), Atherogenic index of plasma (AIP) (-0.11 ± 0.06) Castelli risk index I (3.68 +1.15), castelli risk index II (2.15 ±1.29) Total protein (74.80 ±5.17mmol/l) and albumin (39.40 ± 3.21mmol/l) did not show any significant difference (P>0.05) after 14days of consumption when compared to control Total cholesterol (4.01 ±0.30mmol/l), Triglycerides (0.97 ± 0.51mmol/l), High density lipoprotein-cholesterol (HDL-C) (0.97 ±0.25mmol/l), Low density lipoprotein cholesterol (LDL) (2.59 ± 0.55mmol/l),Atherogenic index of plasma (AIP) (-0.03 ± 0.23), Castelli risk index I (4.34 ± 1.09), Castelli risk index II (2.86 ±1.09), Total protein (77.00 ± 7.21mmol/l) and Albumin (38.80± 1.50mmol/l).Consumption of *Costus afer* did not affect High density lipoprotein Cholesterol,low density Lipoprotein Cholesterol,Triglyceride and Total Cholesterol hence ,does not affect cardiovascular risk indices.

Keywords: Lipids, Cardiovascular, Castelli risk indices, Atherogenic index of plasma ,*Costus afer*, Herbal plants.

1.INTRODUCTION

Costus afer is a rhizomatous herb that belongs to the family Zingiberacea, *Costus afer* is a perennial plant which can grow as tall as 4m[1]. Costus afer is a tropical monocot plant with creeping rhizome [2][3].

Costus afer is found in the forest belt from Senegal to Ethiopia and in the East of Tanzania in Africa. *Costus afer* is also found in the rain forest and river banks of Tropical west African countries such as Ghana, Sierra Leone, Senegal, Guinea, Togo, Cameroun and Nigeria [4][1].

Different African countries and tribes call *Costus afer* different names. Ghana calls it 'Osommbaa' [5]. It is called 'Mwandando' in Cameroun [6], it is called 'Jofa' in Sierra Leone [7] and in Nigeria different tribe calls it different names, the Yorubas call it 'Irekeomed' [8]. 'Opete' or 'Okpete' in Igbo [8][9], 'Kakizawo' or 'Dudun Kodi' in Hausa and 'Mbititem' in Efik [10].

In West Africa, the suckled stem is chewed to quench thirst and also to treat cough and its accompanying sore throat [4][11]. Different parts of the plant is used to treat different ailments such as the stem is used to treat inflammation [4][12] and Gonorrhea [13] and measles [4]. The rhizome is used to treat malaria [14], gastric ulcer [15]. Apart from the medicinal properties of *Costus afer*, it is used due to its nutritional properties in preparation of food [16][14].

The phytochemical analysis of the leaves, stem and rhizome of the *Costus afer* in different solvents shows the presence chemical compounds such as alkaloids, phenols, Saponins, triterpene, tannis and glycocides [9],[14][16]. These phytochemicals and nutrients may justify the belief that the plant can be use for protection against chronic diseases which affect several body organs. However the need for authentication of such claim is necessary [17][18][10].

Regulation of concentrations of substances such as lipids are important in avoiding abnormal retention of fats within the cell which are associated with several disorders such as cardiovascular diseases. Triglyceride, total cholesterol (TC), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) are lipids whose concentrations can be regulated through dietary intake to avoid the risk of developing cardiovascular problems.

Cardiovascular disease is a class of disease that involves the heart or blood vessels which also includes coronary artery disease (CAD) such as angina and myocardial infarction commonly known as heart attack. cardiovascular disease is a broad umbrella term used to describe all conditions affecting the heart and circulatory system including coronary heart disease, stroke, heart attacks and aortic disease [19].
Risk factors of cardiovascular disease are particular habits, behaviours, circumstances or conditions that increases a person's risk of developing cardiovascular diseases. Risk factors for cardiovascular disease can be split into two categories; modifiable and non-modifiable [20]. Non modifiable cardiovascular risk factors are those that cannot be changed such as age, ethnicity and family history. Modifiable cardiovascular disease risk factors are those that can be reduced or controlled with altered behaviour. By making certain lifestyle changes such as not smoking, good diet and regular exercise to reduce the chance of developing cardiovascular disease.
Possessing one or more risk factors increase the risk of developing cardiovascular diseases [21]. Lipid such as cholesterol when elevated is called hpercholesterolaemia which is found in nephritic syndrome, diabetes mellitus, coronary thrombosis and angina pectoris. Decreased level of cholesterol is called hypocholseterolaemia which is characterized by thyrotoxicosis, haemolytic jaundice.
Low level of triglyceride is called hypotriglyceamia which causes intravascular lipolysis which is the formation of high density lipoprotein. When triglyceride is elevated it is called hyper triglyceamia and it is associated with an increased atherogenic risks [22].
Atherogenic risk index of plasma, castelli risk 1 and castelli risk 2 are all predictors of infarction. It is a diagnostic tool apart from the routine lipid profile. They are used in monitoring cardiovascular risk and effectiveness of therapy [23]. This study therefore sought to determine the cardiovascular risk indices in apparently healthy individuals after 14 days of consumption of *Cosus afer* stem extract.

2.MATERIALS AND METHODS

2.1 Study Area

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

2.2 Study Population

A total of fifteen healthy individuals aged between 20-35 years and weighing between 44-102kg were recruited for the study. All participants were volunteers who were well informed about the study before giving their written consent to participate.

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2.3 Ethical Approval

Ethical approval was gotten from Rivers State College of Health Science and Management Technology Board.

2.4 Plant Purchase and Identification

The plant parts used for the study were fresh stem of *Costus afer* obtained from a botanical garden in Elele district of Rivers State, Nigeria. The stem of *Costus afer* was identified by a pharmacist in Rivers State College of Health Science and Management Technology, Port Harcourt Rivers State.

2.5 Plant Preparation

Freshly cut stem from a botanical garden in Elele , Rivers State,Nigeria were used for the study.Contaminants such as sand, dirts, and dry matters were removed.The cut stems were weighed using a digital weighing balance (DW 1100) made in China. The weight was noted. The stem were then washed and allowed to dry and then reweighed before grinding into a fine paste. The paste was mascerated in a clean jar with 17.7 litres of water. It was allowed to stand for an hour and then filtered to remove the shaft leaving the concentrate in a liquid form.

2.6 Calculation of Plant Concentration

Weight of stem of *Costus afer* before grinding. =83.8g
Weight of stem after grinding and removal of shaft. =126g
Total volume if water used to mascerate stems. =17.7L (17700ml)
Concentration of stem extract in 17.7liters of water. =126g/17.7litres
Each cup used by each participants can contain 180ml by volume
17.7Litres contains 126g of the stem extract

180ml will contain. $\frac{126g}{17700ml} \times 180ml = 1.28g$

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Concentration of stem extract consumed daily by each participants was 1.28g/180ml daily for 14days

2.7 Sample Collection

Blood samples were collected from each participants before commencement of the study as control samples while other samples were collected on Day 1, Day 7 and Day 14 after consumption of 1.28g/180ml stem extract daily for 14days as test samples. Fasting samples were collected by vein puncture and dispensed into well labelled lithium heparin bottles. Plasma samples gotten were analysed immediately in the laboratory for fasting lipid profile, Total Protein and Albumin.

2.8 Determination of parameters

Triglyceride,Total cholesterol and High Density lipoprotein were estimated using colorimetric method [24].

Low density lipoprotein cholesterol(LDL) was calculated using the formular as shown below.

LDL cholesterol (mmol/l) = T.C – (HDL + 0.46 x T-G)

where T/C = Total cholesterol,T.G. = Triglycides, HDL= High Density Lipoprotein.

Castellic risk indice 1 was calculated using the formular below [25].

CRI – I = $\frac{\text{TC}}{\text{HDL}}$

Castellic risk indice 11 was calculated using the formular below [25].

CRI – II = $\frac{LDL-C}{HDL-C}$

Atherogenic Index of Plasma(AIP) was calculated using the formular below [25].

ALP = Log $\left[\frac{TG}{HDL-C}\right]$

Total protein was estimated using Biuret method while Albumin was estimated using Bromocresol green method [26]. Samples were analysed using Graph pad prism and expressed as Mean ± Standard deviation. P<0.05 was considered as significant.

3. RESULTS

Table 1: Cardiovascular Risk Indices in Apparently Healthy Individuals after one day of consumption of *Costus afer* and control n=15

Parameters	Control Mean ± S.D n=15	Test after Day 1 Mean ± S.D n=15	t-Test	p-Value
Total Cholesterol (mmol/l)	4.01± 0.30	3.84±0.66	2.34	0.08
Triglycerides (mmol/l)	0.97 ± 0.51	0.67 ± 0.23	1.24	0.28
HDL(mmol/l)	0.97 ± 0.25	0.89±0.11	0.59	0.59
LDL(mmol/l)	2.59 ± 0.55	1.98±0.62	1.69	0.17
AIP	-0.03± 0.23	-0.14±0.16	1.13	0.32
CRI I	4.34 ± 1.09	3.63±0.87	1.26	0.28
CRI II	2.86 ± 1.09	2.27±0.77	1.05	0.35
Total Protein(mmol/l)	77.00±7.21	79.40±1.34	0.75	0.49
Albumin(mmol/l)	38.80±1.50	36.20±5.31	0.93	0.38

p<0.05 Significant

HDL – High Density Lipoprotein-Cholesterol LDL – Low Density Lipoprotein-Cholesterol

AIP – Atherogenic Index of Plasma CRI-I – Castellic Risk Index I

CRI-II - Castellic Risk index II

Table 1 shows that total cholesterol, Triglycerides, High density Lipoprotein-Cholesterol, Low Density, Lipoprotein-Cholesterol, Atherogenic Index of Plasma, Castellic Risk Index I, Castellic Risk Index II, Total Protein and Albumin did not show any significant difference when the control was compared to the test result after one day of consumption of *Costus afer* ($p > 0.05$).

Table 2: Cardiovascular Risk Indices in Apparently Healthy Individuals after seven days of consumption of *Costus afer* and control.

Parameters	Control Mean±S.D n=15	Test after Day 7 Mean ± S.D n=15	t-Test	p-Value

TotalCholesterol (mmol/l)	4.01± 0.30	3.66±1.17	0.84	0.45
Triglycerides (mmol/l)	0.97 ± 0.51	0.61±0.13	1.36	0.25
HDL (mmol/l)	0.97 ± 0.25	0.92±0.07	0.42	0.70
LDL (mmol/l)	2.59 ± 0.55	2.45±1.10	0.53	0.62
AIP	-0.03± 0.23	-0.19±0.08	1.25	0.28
CRI I	4.34 ± 1.09	3.93±1.12	1.55	0.20
CRI II	2.86 ± 1.09	2.62±0.50	1.33	0.26
Total Protein(mmol/l)	77.00±7.21	76.00±5.24	0.29	0.79
Albumin(mmol/l)	38.80±1.50	37.80±3.63	0.45	0.68

p<0.05 Significant

Table 2 shows that Total Cholesterol, Triglycerides, High Density Lipoprotein, Low Density Lipoprotein, Antherogenic Index of Plasma, Castelli risk index I, Castelli Risk index II, Total Protein, Albumin did not show any significant difference when the control was compared to the test group after seven days.

Table 3: Cardiovascular risk indices in apparently healthy individuals after fourteen days of consumption of *Costus afer* and control.

Parameters	**Control Mean ± S.D n=15**	**Test after Day 14 Mean ± S.D n=15**	**t-Test**	**p-Value**
Total Cholesterol (mmol/l)	4.01± 0.30	3.38±0.68	2.30	0.08
Triglycerides (mmol/l)	0.97 ± 0.51	0.74±0.16	1.26	0.28
HDL (mmol/l)	0.97 ± 0.25	0.95±0.19	0.09	0.93
LDL (mmol/l)	2.59 ± 0.55	1.92±0.90	1.66	0.17
AIP	-0.03± 0.23	-0.11±0.06	1.01	0.37
CRI I	4.34 ± 1.09	3.6/8±1.15	0.84	0.45
CRI II	2.86 ± 1.09	2.15±1.29	0.91	0.42
Total Protein(mmol/l)	77.00±7.21	74.80±5.17	1.22	0.29
Albumin (mmol/l)	38.80±1.50	39.40±3.21	0.34	0.75

p < 0.05 Significant

Table 3 shows that Total Cholesterol, Triglycerides, High Density Lipoprotein, Low Density Lipoprotein, Atherogenic Index of Plasma, Castelli Risk Index I, Castelli Risk index 2. Total Protein and Albumin did not show any significant difference when the control was compared to the test group after fourteen days of consumption of *Costus afer*. (p >0.05).

Table 4: Cardiovascular Risk Indices apparently healthy individuals after seven days and fourteen days of consumption of *Costus afer*.

Parameters	**Day 7 Mean ± S.D n=15**	**Test after Day 14 Mean ± S.D n=15**	**t-Test**	**p-Value**
Total Cholesterol (mmol/l)	3.66±1.17	3.38±0.68	0.06	0.58
Triglycerides (mmol/l)	0.61±0.13	0.74±0.16	1.11	0.33
HDL(mmol/l)	0.92±0.07	0.95±0.19	0.32	0.76
LDL (mmol/l)	2.45±1.10	1.92±0.09	0.95	0.40
AIP	-0.21±0.07	-0.11±0.06	2.52	0.09
CRI I	3.93±1.12	3.68±1.45	0.37	0.73
CRI II	2.62±.11	2.15±1.29	0.62	0.57
Total Protein(mmol/l)	76±5.24	74.80±5.17	0.48	0.66
Albumin(mmol/l)	37.80±3.63	39.40±3.21	0.57	0.60

p< 0.05 Significant

Table 4 there was no significant difference in Total Cholesterol Triglycerides, High Density Lipoprotein-Cholesterol, Low Density Lipoprotein-Cholseterol, Atherogenic Index Plasma, Castelli Risk Index I, Castelli Risk Index II. Total protein and Albumin when the seven days of consumption was compared with the test fourteen days after consumption of *Costus afer* stem extract. (p>0.05)

Table 5: Cardiovascular risk indices in apparently healthy individuals after day one of consumption of *Costus afer* and the fourteenth day of consumption.

Parameters	Day 1 Mean± S.D n=15	Test after Day 14 Mean ± S.D n=15	T. Test	P. Value
Total Cholesterol (mmol/l)	3.18±0.66	3.38±0.68	0.59	0.59
Triglycerides (mmol/l)	0.97±0.51	0.74±0.16	1.26	0.23
HDL (mmol/l)	0.87±0.11	0.95±0.19	1.09	0.34
LDL (mmol/l)	1.98 ±0.62	1.92±0.90	0.21	0.84
AIP	-0.14±0.16	-0.11±0.06	0.45	0.68
CRI I	3.63±0.87	3.68±1.15	0.10	0.93
CRI II	2.27±0.77	2.15±1.29	0.25	0.81
Total Protein(mmol/l)	79.40±1.34	74.80±5.17	1.76	0.15
Albumin(mmol/l)	36.20±5.31	39.40±3.21	1.22	0.29

p< 0.05 Significant

Table 5 shows that there was no significant difference in Total cholesterol, Triglycerides, High Density Lipoprotein-Cholesterol, Low Density Lipoprotein-Cholesterol, Atherogenic Index of Plasma, Castelli Risk Index 1 Castelli Risk Index 2, Total Protein and Albumin when the test group was compared to the first day after consumption of *Costus afer*. (p>0.05).

4. DISCUSSION

This study assessed the cardiovascular risk indices in apparently healthy individuals after fourteen (14) days of consumption of *Costus afer* stem extract. In this study, cardiovascular risk indices: Castelli Risk Index 1, Castelli Risk Index 2 and Atherogenic Index of Plasma did not show any significant difference in individuals after fourteen (14) days of consumption of *Costus afer* stem extract when compared to control,day 1 and day 7 (P>0.05). This study indicates that consumption of *Costus afer* did not increase the risk of developing cardiovascular problems. Castelli risk indices are also called cardiac risk ratio.They are ratios that have been studied as markers of lipid atherogenic risk. These are the calculated fractions which are used in clinical setting for assessing the risk of cardiovascular disease beyond the routinely done lipid profile. Castelli risk index 1 is the ratio of Total Cholesterol to the High Density Lipoprotein-Cholesterol while Castelli Risk Index 2 is the ratio of the Low Density Lipoprotein-Cholesterol to the High Density Lipoprotein-Cholesterol [23].Atherogenic index of plasma is a strong predictor of infarction. It is used by some practioners as a significant predictor of atherosclerosis [27].

Furthermore, this study shows that lipids such as Total Cholesterol, Triglycerides, High Density Lipoprotein-cholesterol and Low Density Lipoprotein- cholesterol did not show any significant difference after fourteen (14) days of consumption of *Costus afer* stem extract when compared to control,day 1 and day 7 (P>0.05). Cholesterol is a fatty substance that is carried around the body by proteins. Low Density Lipoprotein- cholesterol and High Density Lipoprotein- cholesterol are two types of cholesterol. Low Density Lipoprotein- cholesterol are the bad cholesterol which can lead to complications when it is increased in blood. High levels of Low Density Lipoprotein- cholesterol is often caused by unhealthy diet, smoking, kidney and liver disease [28]. High Density Lipoprotein-Cholesterol are the good cholesterol. High level of High Density Lipoprotein-Cholesterol helps in lowering cardiovascular risk [28]. Results obtain in this study are similar to findings [12] in male and female rats which demonstrated no sign of acute toxicity after oral consumption of *costus afer*. Conclusively, this study suggest that consumption of *Costus afer* does not predispose consumers to cardiovascular risk as it does not cause any significant changes in cardiovascular risk indices such Castelli Risk Index 1, Castelli Risk Index 2 and Atherogenic Index of Plasma. Lipid parameters such as Total Cholesterol, Triglycerides, High Density Lipoprotein-Cholesterol, Low Density Lipoprotein-Cholesterol were also not affected by consumption of *Costus after* stem extract.

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Comment [S3]: Write conclusion for your work before references

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