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Phytochemical and Hypolipidemic Effects of Methanolic Extract of *Aframomum melegueta* Seed

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Authors' contributions

This work was carried out in collaboration between all authors. Author SOO designed the study, wrote the protocol and first draft of the manuscript. Author MIE performed the statistical analysis, and wrote part of the manuscript. Author YNO did the literature search and also wrote part of the manuscript. Author ECE managed the animals, analyses of the study and collected all data. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study was aimed at establishing the phytochemical contents and hypolipidemic potentials of the methanolic extract of *Aframomum melegueta* seed in rats.
Study Design: Phytochemical constituent, serum lipid profile and relative organ weight.
Place and Duration of Study: Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike Abia State, between July 2013 and November 2013.
Methodology: Dried and pulverized seeds of *Aframomum melegueta* were extracted by cold maceration method for 48 hours at room temperature using absolute methanol in a Winchester bottle. The extract (100, 200 and 400 mg/kg) was dosed to albino Wistar rats orally for 21 consecutive days by gastric gavage. Twenty four hours later, blood was collected from the rats

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through direct cardiac puncture. The separated serum was used to determine the serum lipid profile. Phytochemical analysis of the extract was also done using standard method.
Results: The extract produced significant ($P < 0.05$) dose-dependent decrease in the serum level of cholesterol, triglyceride, Low density lipoprotein cholesterol (LDL-C), very Low density lipoprotein cholesterol (VLDL-C) and increase in serum level of high density lipoprotein cholesterol (HDL-C). Phytochemical analysis of the extract revealed the presence of saponins, tannins, terpenes/sterols, glycosides, alkaloids, flavonoids.
Conclusion: The methanolic extract of *A. melegueta* seed demonstrated hypolipidemic effects which may help in the prevention of development of cardiovascular diseases in patients and may be due to its phytochemical constituents.

Keywords: Hypolipidemic; Aframomum melegueta; phytochemistry; cardiovascular disease.

1. INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of death and disability worldwide with 447,000 hospitalizations and 3.9 million patient days in the hospital each year, contributing to an overall yearly cost of $18.5 billion [1]. The prevalence of CVD is predicted to double by the year 2018 [2]. The CVD of clinical importance include coronary artery disease, peripheral vascular disease, stroke/transient ischemic attack, chronic kidney disease, hypertension, etc. The predisposing factors to CVD include diabetes, dyslipidemia, cigarette smoking and Obesity [1]. Dyslipidemia is one of the key factors that are amenable to intervention. Studies have shown that lowering low-density lipoprotein (LDL) cholesterol can reduce cardiovascular events and the lower the LDL-cholesterol achieved the lower the cardiovascular risk. Individuals who achieved an LDL-cholesterol of < 50 mg/dl had a lower cardiovascular risk than those with higher levels of LDL-cholesterol [3,4]. The use of orthodox hypolipidemic agents is expensive and associated with some side effects like flatulence, constipation and dyspepsia [5]. The use of herbs as medicines has played an important role in nearly every culture on earth, including Asia, Africa, Europe and America [6,7]. Several herbs help to reduce blood sugar and high blood cholesterol concentrations, provide some protection against cancer and stimulate the immune system [7]. Recently there has been an increased interest in the therapeutic potential of plants as lipid lowering agent.

Aframomum melegueta Schum also known as alligator pepper (indigenous names include: Atare in Yoruba, Ose-oji in Igbo and Citta in Hausa) is a herbaceous perennial plant native to swampy habitats along the West African coast. It is a tropical perennial with tufted leafy stems to 1½ m high. Its trumpet-shaped, purple flowers develop into 5 to 7 cm long pods containing numerous small seeds. The pods are almost oval in shape, hard, shiny, and have a reddish-brown color. It has lanceolate leaves up to 30cm long. In Nigeria and some other parts of West Africa, the seeds are used as a spice during entertainment and have a wide range of folkloric uses in traditional medicine. They are used as a remedy for treating stomach ache, diarrhoea and snakebite. Previous studies have established the anti-ulcer, anti-microbial, anti-inflammatory and sexual performance enhancing effects of the seed extract [8-11]. The seeds are very rich in e non volatile pungent compounds gingerol, shogaols, paradol and related compounds [10]. The chemical constituents of the essential oil from various parts of the *A. melegueta* have been reported [12]. The present study was aimed at establishing the hypolipidemic potentials and the phytochemical contents of the methanolic extract of *Aframomum melegueta* seed.

2. MATERIALS AND METHODS

2.1 Plant

The freshly harvested fruit of *Aframomum melegueta* (Alligator pepper) were bought from Ndoro market, Oboro in Ikwuano LGA of Abia State in the month of July 2013 and were authenticated by Dr. I. C. Okwulehie of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike and the voucher specimen catalogued MOUAU/CVM/VPP/2013/03 was kept for reference purposes in the departmental herbarium.

2.2 Preparation of the Extract

Dried and pulverized seeds of *Aframomum melegueta* were extracted by cold maceration

method for 48 hours at room temperature using absolute methanol in a Winchester bottle. The *Aframomum melegueta* extract (AME) was filtered with Whatman No 1 filter paper. The filtrate was concentrated *in-vacuo* using vacuum rotary evaporator at 40°C and was later concentrated to dryness in a hot air oven at 40°C. The extract was stored in a refrigerator at 4°C throughout the duration of this study.

2.3 Determination of the Yield of the AME

An empty clean and dry beaker was weighed and later the extract was poured into it. The beaker was weighed after the extract has been concentrated to constant weight. The weight of the extract was calculated as follows:

The percentage yield of extract (%) w/w =

$$\frac{weight\ of\ beaker\ and\ extract - wieght\ of\ empty\ beaker}{weight\ of\ plant\ material} \times \frac{100}{1}$$

2.4 Animals

Twenty four male Wistar albino rats weighing between 120 - 170 g were obtained from Department of Zoology, University of Nigeria, Nsukka and kept in the Animal house of Department of Biochemistry, Micheal Okpara University of Agriculture, Umudike. The animals were allowed access to feed and water *ad libitum* and were allowed two weeks to acclimatize before the commencement of the experiment. The animals were kept in well ventilated aluminium cages at room temperature and under natural light/darkness cycles. They were maintained in accordance with the recommendation of the *Guide for the care and use of laboratory animals* [13]. The experiment was approved by the University Animal Ethics Committee with ref. MOUAU/CVM/EAEC/2013/201.

2.5 Phytochemical Spot Test

The AME was tested for the presence of alkaloids, flavonoids, tannins, glycosides, saponins, terpenes/sterols, carbohydrates, and starch using the standard procedures as described by Trease and Evans [14].

2.6 Experimental Design

Twenty four male albino wistar rats were randomly divided into four groups of six animals each. Group 1 served as the control and received 10 ml/kg of distilled water. Group 2 received 100 mg/kg of the AME. Group 3 received 200 m/kg of the AME while group 4 received 400 mg/kg of the AME. The animals were dosed daily for 21 days and were observed daily for behavioural changes and other signs of toxicity and death throughout the period of study. Twenty four hours after the last treatment blood obtained through direct cardiac puncture was used to assay for effects of AME on serum lipid profile. The animals were weighed, sacrificed through cervical dislocation and organs (spleen, heart, liver and kidney) were collected for the determination of the relative organ weight using the formula below:

Relative weight = (Organ weight / Body weight) × 100.

2.7 Biochemical Analysis

2.7.1 Serum preparation

The blood used for serum preparation was collected via direct heart puncture with 21 G needle attached to 5 ml syringe, following mild chloroform anaesthesia of the rats. The serum was prepared using standard method as described by Yesufu et al. [15]. Briefly, blood was allowed to clot for 30 minutes and then centrifuged at 2500 rpm for 15 minutes and serum harvested.

2.7.2 Serum lipid profile studies

Serum total cholesterol was determined according to Allain et al. [16]. Serum high density lipoprotein cholesterol (HDL-c) was determined according to Bergmenyer [17]. Serum total triglycerides were determined according to Footsati and Principe [18]. Serum low density lipoprotein cholesterol (LDL-c) was calculated according to Friedewald et al. [19] with the following equation;

LDL= Total cholesterol -HDL-Triglycerides/5.

The above mentioned serum biochemical parameters were assayed using reagent kits supplied by Randox Laboratories incorporated UK.

2.8 Statistical Analysis

Data obtained were analyzed using one-way analysis of variance (ANOVA) and the variant means were separated by least significant difference (LSD) of the different groups.

Significance was accepted at the level of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Extraction

The methanolic extraction of the dried seed of *A. melegueta* yielded 8.34 % w/w dry extract which was oily and dark-brown in colour.

3.1.2 Phytochemical spot test of AME

The phytochemical spot test showed that the extract contained saponins, tannins, terpenes/sterols, glycosides, alkaloids, flavonoids.

3.1.3 The effect of AME on the serum lipid profile of treated rat

The result of the effects of AME on the serum lipid profile of treated rats is presented in Table 1. The extract (100, 200 and 400 mg/kg) produced a significant ($p < 0.05$) dose dependent decrease in serum cholesterol level (79.19, 75.78 and 67.27, respectively) in treated rats when compared to serum cholesterol level (95.36) of the negative control rat. The extract also, produced a significant ($p < 0.05$) dose dependent reduction in the serum triglyceride, LDL-C and VLDL-C levels of the treated rats when compared to the negative control group of rats. The result of the effects of the extract on the serum HDL-C showed that it caused a significant ($p < 0.05$) dose dependent increase in the serum HDL-C level in the treated rats when compared to the distilled water treated rats.

3.1.4 Effect of AME on relative organ weight of treated rat

The result of the effects of AME on relative organ weight is presented in Table 2. The extract did not produce any significant ($p > 0.05$) difference in the relative organ weight of the treated rats when compared to the negative control rats.

4. DISCUSSION

The increased levels of total cholesterol in blood could induce arterial endothelial dysfunction, and vascular endothelial injury is the initial factor in atherosclerosis. Therefore, lowering lipid and protecting vascular endothelium play significant roles in preventing atherosclerosis, which is a major contributor to the pathogenesis of cardiovascular diseases. Elevated blood concentration of cholesterol, especially in LDL-C, constitutes the key risk factor for atherosclerosis [20]. Atherogenic index indicates the deposition of foam cells or plaque or fatty infiltration or lipids in heart, coronary vessel, aorta, liver, and kidneys. The higher the atherogenic index the higher the risk of the above organs for developing oxidative damage [21].

Table 1. The effect of AME on the serum lipid profile of treated rat

Group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Distilled water 10 ml/kg	95.36±6.63	50.96±5.60	48.19±0.76	10.19±1.12	36.99±1.61
AME 100 mg/kg	79.19±0.49*	43.77±5.08	51.71±2.43	8.75±1.01	15.49±2.78*
AME 200 mg/kg	75.78±0.74*	38.22±3.96	53.61±0.20*	7.20±4.00	13.43±3.13*
AME 400 mg/kg	67.27±0.49*	34.30±7.36	54.46±1.71*	8.36±1.49	12.75±5.05*

**P < 0.05 when compared to the negative control*

Table 2. Effect of AME on relative organ weight of treated rat

	Relative organ weight (%)			
Group	Spleen	Heart	Liver	Kidney
Distilled water 10 ml/kg	0.36±0.03	0.21±0.01	2.26±0.09	0.36±0.02
AME 100 mg/kg	0.29±0.04	0.21±0.01	2.31±0.28	0.36±0.02
AME 200 mg/kg	0.33±0.06	0.21±0.01	2.47±0.16	0.45±0.06
AME 400 mg/kg	0.27±0.06	0.20±0.01	2.68±0.15	0.45±0.06

No significant difference (p > 0.05)

The present study evaluated the hypolipidemic effects of AME on a normal rat. The choice of the doses used in this study was based on the report of previous studies [8,22]. The extract which was oily and dark brown in colour demonstrated a potent dose dependent hypolipidemic effect in the treated rats. The mechanism of lipid lowering effect is not known but could be either through reduction in absorption of cholesterol from the gut or by reduction in the biosynthesis of cholesterol [23]. Sitosterol lowers the serum lipid level by reducing the absorption of cholesterol from the gut [24]. The decreased absorption of exogenous cholesterol and possibly increased metabolism of endogenous cholesterol into bile acids in the liver leads to increased expression of LDL receptor on hepatocytes, and hence increased clearance of LDL-C from the blood and a reduced concentration of LDL-C in plasma [23].

Another possible mechanism through which lipid lowering drugs (colesevelam) can act is by binding to bile acid in intestine, which will impede their reabsorption. As the bile acid pool becomes depleted, the hepatic enzyme, cholesterol 7-α-hydroxylase, is upregulated, increases the conversion of cholesterol to bile acids. This causes an increased demand for cholesterol in the liver cells, resulting in the dual effect of increasing transcription and activity of the cholesterol biosynthetic enzyme, 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase, and increasing the number of hepatic LDL receptors. These compensatory effects result in increased clearance of LDL-C from the blood, resulting in decreased serum LDL-C levels. Serum TG levels may increase or remain unchanged [25].

Furthermore, another possible mechanism of lipid lowering effects is the inhibition of rate limiting enzyme, HMG-CoA reductase, in the biosynthesis of cholesterol. HMG-CoA reductase catalyse the conversion of HMG-CoA to mevalonic acid [23]. Statin derivatives of hypolipidemic agents act by reversible and competitive inhibition of HMG-CoA reductase which leads to decreased hepatic cholesterol synthesis, up regulation of LDL receptor synthesis and increased LDL-C clearance from the plasma into liver cells [23]. AME may have worked through one or a combination of the above mechanisms

The extract produced increased serum level of HDL-C, which suggest that it may enhance reverse cholesterol transport. High density lipoprotein transport cholesterol from blood to liver or steroidogenic organs such as adrenals, ovary and testes by both direct and indirect pathways [26]. This leads to reduced serum cholesterol level and reduced risk of CVD development.

Some of the phytochemical constituents of *A. melegueta* like 6-gingerol, sterols, shogaol and paradol have demonstrated some lipid lowering properties [9,27,28]. This suggests that the lipid lowering effects of AME may be mediated by some of its phytochemical compositions.

In conclusion, the demonstrated hypolipidemic effects of the extract of *A. melegueta* seed suggest that the extract may help in the prevention of development of cardiovascular diseases in patients. The authors recommend that further work should be done to isolate the active compound responsible for its hypolipidemic effect and to determine its possible mechanisms of action.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and therefore have been performed in accordance with the ethical standards laid down in the 1964 Declarations of Helsinki and Michael Okpara University of Agriculture, Umudike, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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