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HYPOLIPIDEMIC POTENTIAL OF RAW SEEDS OF *DACRYODES EDULIS* ON WISTAR RATS

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ABSTRACT
The phytochemical screening of ethanol extract of *Dacryodes edulis* seeds and its effect on lipid profile of albino Wistar rats was investigated. Fresh seeds were obtained, air-dried at room temperature and pulverized. The powder was macerated in 80% ethanol and the filtrate was concentrated to obtain crude extract. The crude extract was subjected to phytochemical screening and also administered to albino Wistar rats. Twenty-four (24) albino Wistar rats weighing between 150 – 180g were obtained and randomly selected into 4 groups with 6 animals in each group. Group I served as control while Groups II, III and IV served as test groups and were administered 100, 200 and 300 mg/kg body weight of the extract respectively, daily for 14 days by use of cannula attached to a syringe. All the animals were allowed access to rat feed and clean water *ad libitum*. Result show that the seed extract has a wide array of phytochemicals which include saponins, flavonoids, terpenes, alkaloids, tannins, cardiac glycosides and phlobatannins. Triglyceride, total cholesterol, LDL-cholesterol and VLDL-cholesterol concentrations were significantly reduced following the administration of the extract to Wistar rats while a dose dependent increase was observed in HDL-cholesterol concentrations. The ethanol extract of seeds of *Dacryodes edulis* is rich in phytochemicals and have a positive lipid modulatory effects.

KEYWORDS: *Decryodes edulis* seeds, cholesterol, triglyceride, saponins, flavonoids.

INTRODUCTION

Plants are very important sources of diverse products for human benefits. Different parts of plant materials – leaves, stems, roots, barks, fruits and seeds are exploited by man for several purposes. For example, plant parts are subjected to intense scientific investigation to proffer solutions to satisfy current or anticipated medicinal needs. Nigeria is blessed with many trees and shrubs which have been reported to have potent medicinal and good nutritional values.[1] Several of these plants have seeds that possess both nutritional and medicinal importance.[2,3]

In recent times, African health care problems have been identified to emanate from toxicity of Western drugs, mutation of target organs, protracted treatment protocol and inadequate repertoire of compound/s from which the novel drugs can be made.[4] Unavailability and unaffordability have also been identified as the hindrance in assessing orthodox drugs.[5] There is serious focus on the potential use of medicinal plants as possible solution to African health care problems, one which is cardiovascular problem associated with blood lipids. Blood lipids concentration like many other essential compounds of the body attracts clinical attention when present in abnormal concentration.[6] Increased or decreased concentrations of plasma lipids usually occurs because of abnormalities in synthesis, degradation and transports of the associated lipoprotein particles.[7]

Dacryodes edulis Lam is a well-known seasonal indigenous African tree that belongs to the family Burseraceae, classified as pome fruit and characterized by central core surrounded by edible flesh layer.[8] It is commonly known as native (local) pear and is called ‘eben’ by the Ibibios, ‘ube’ by the Igbos and ‘elemi’ by the Yorubas of Southern, Eastern and Western Nigeria respectively.[9] The fruits are either put in hot water to soften or roasted in dry heat for consumption. The fruit is known to be rich in edible oil, vitamins and a wide range of amino acids.[10] Its mature fruit pulp is eaten with roasted or boiled yam, eaten along with corn (maize) or used in sipping garri.[4]

Dacryodes edulis has many trado-therapeutic claims, the seeds are used traditionally as a remedy for stomach problems like diarrhea, dysentery etc.[11] The decoction of the plant bark has been reported to be used in the management of wounds, leprosy, tonsillitis and as mouth wash. The leave decoction has been documented to be used for treatment of headache and fever and is chewed for its anti-emetic potential.[12] The leaves have been

used locally in the management of skin diseases such as ringworm, scabies rashes etc while the stem twigs are used as chewing sticks for oral hygiene.[13,14,15] Phytochemical analysis and antimicrobial activity of the seeds have been reported.[16,14]

The leaves of *D. edulis* have been reported to normalize sickle cell blood erythrocytes and compounds such as quercitrin and ethyl gallate have been isolated from the leaves.[13,17] Good antioxidant activity has also been reported of the leaves and essential oil of the plants.[14,18] The exudates of the plant have also been studied and were reported to be a potential source of feedstock for the pharmaceutical industry.[19] A study by Uhegbu *et al*.,[20] on the effect of aqueous extract of pear seeds on some biochemical parameters in albino rats reported that the pear seed is not toxic to the liver. Furthermore, antimalarial activity of *Dacryodes edulis* have been studied.[21] Traditionally, the seeds are discarded after consumption of the fruits. Though discarded by humans, the seeds are commonly used as feeds for domestic livestock such as sheep and goats. [22]

There is dearth of information on the *D. edulis* raw seeds on lipid profile. The present study is aimed at investigating the lipidemic effect *D. edulis* raw seeds on experimental animals.

MATERIALS AND METHODS

Plant Material and Extraction: Mature fruits of *Dacryodes edulis* were collected from Afaha Etok in Ibesikpo- Asutan Local Government Area, Akwa Ibom State, Nigeria and identified by a taxonomist in the Department of Botany, University of Uyo, Nigeria. They were washed with tap water to remove dirt, cut opened and the seeds removed. They were chopped into very tiny pieces and pulverized using an electric blender. The seed powder was macerated in 80% ethanol and allowed to stand for 72 hours for the solvent to solubilize the active ingredients with continuous stirring. The filtrate was obtained by carefully siphoning the supernatant using a syringe and needle. The supernatant was concentrated in a water bath at 45^{o}C.

Phytochemical Screening: The concentrated extract of the seed was screen for the presence of phytochemicals using standard procedures.[23] The presence of saponins, tannins, phlobatannins, anthraquinones, cardiac glycosides, flavonoids, terpenes and alkaloids were tested.

Experimental Animals and Design: Twenty-four (24) albino wistar rats weighing between 150 – 180g, obtained from the Animal House of college of Health Sciences, University of Uyo, were used for the study. The animals were randomly grouped into 4 groups with 6 animals in each group. They were kept under standard laboratory condition and allowed access to rat feed and clean drinking water ad libitum. Group I animals served as control and were administered 1ml distilled water while Groups II, III and IV were administered orally, 100 mg/kg, 200 mg/kg and 400 mg/kg of the concentrated seed extract of *Dacryodes edulis* respectively daily for 14 days.

At the end of administration of the extract, the animals were fasted for 12 hours, anaesthetized using chloroform and sacrificed. Blood sample were obtained through cardiac puncture using sterile needles and syringes into well labelled plain sample tubes. Sera was obtained by centrifugation of the whole blood at 3000 rpm for 10 minutes and used for analysis.

Lipid Profile Assay: All the kits used for the assay of lipid profile in the study were obtained from Randox Laboratory, UK. Cholesterol concentration was assayed using the enzymatic endpoint method of Richmond[24] and Allain.[25] The method described by Fossati and Principe[26] was used to determine the concentration of triglyceride in the serum. HDL-cholesterol precipitant method according to Burstein *et al*.,[27] and Lopez-Virella *et al*.,[28] was employed to estimate HDL-C concentration. LDL-C and VLDL-C concentrations were calculated using Friedwald's equation.[29]

Statistical Analysis: Statistical analysis was performed using Windows SPSS 20.0. One-way ANOVA was carried out as well as post hoc multiple comparison test. Results are presented as Mean ± Standard Deviation. Values were considered statistically significant at $P < 0.05$.

RESULTS

The result of the phytochemical screening of the raw seeds of D. edulis is presented in Table I and the lipid profile of rats administered with ethanol extract of *D. edulis* raw seeds are presented in Table II.

Table I: Phytochemical screening of *D. edulis* raw seeds

Compounds	Inference
Saponins	+++
Tannins	++
Phlobatannins	++
Anthraquinones	-
Cardiac Glycosides	+
Flavonoids	++
Terpenes	+
Alkaloids	++

Key: +++ = Strongly present; ++ = Present; + = Trace; - = Absent

Table II: Lipid profile of albino Wistar rats administered with ethanol extract of *D. edulis* raw seeds.

Groups	Triglyceride (mg/dl)	Total Cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	VLDL-cholesterol (mg/dl)
Control	86.97 ± 1.97	64.46 ± 1.57	17.52 ± 0.83	29.94 ± 2.01	17.40 ± 0.35
Group II (100mg/kg)	62.73 ± 1.83*	60.59 ± 1.26*	18.14 ± 1.15	29.90 ± 1.63	12.55 ± 0.36*
Group III (200mg/kg)	70.43 ± 2.56*	58.57 ± .16*	20.48 ± 0.38*	24.00 ± 1.38*	14.09 ± 0.51*
Group IV (400mg/kg)	81.54 ± 0.51*	55.14 ± 0.93*	25.87± 0.77*	12.97 ± 0.06*	16.31 ± 0.01*

Data presented as Mean ± Standard Deviation

*Represents significant difference when compared to the control at $P < 0.05$.

DISCUSSION

Medicinal plants are used in the treatment of various ailments throughout the world especially in the developing and underdeveloped countries of Africa, Asia and South America. The efficacy of medicinal plants in the treatment of diseases is a function of their numerous bioactive phytochemicals such as flavonoids, saponins, tannins, cardiac glycosides, alkaloids etc.[30] These agents may act collectively or independently to bring about the expected effects. Effects of medicinal plants have been reported to be dependent on whether they contain potent drug-base bioactive ingredient with proven scientific efficacy.[31]

The result of the phytochemical screening of the raw seeds of *D. edulis* showed the presence of appreciable content of secondary metabolites namely; saponins, terpenes, cardiac glycosides, flavonoids, alkaloids, tannins and phlabotannins. Our results agree with reports cited on phytochemical content of *D. edulis* raw seeds by Nwokonkwo.[16] Furthermore, boiling has been reported to potentiate the phytochemicals present in the seeds.[18] The presence of these phytochemicals has been associated with antimicrobial, analgesic, haemolytic and purgative potentials.[32,33] The raw seeds of *D. edulis* are particularly rich in saponins, flavonoids, terpenes, alkaloids and tannins.

Alkaloids of plant origin have been reported to possess emetic, antipyretic, anesthetic, analgesic and anti-helminthic properties.[23] Cardiac glycosides are said to be used in the treatment of disease of heart muscles.[34] Saponins have the general characteristics of foam formation in aqueous solutions.[35] They serve as natural antibiotics, helping the body to fight infections and microbial invasions.[36,35] Saponins have also been known to lower blood cholesterol thereby reducing deleterious cardiovascular events while tannins have been reported to be astringent and has wound healing properties justifying the use of this plant in treatment of wounds.[37] Flavonoids have been known popularly for its antioxidant potentials hence its wide usage in the management of conditions where free radical generation is implicated.[38]

Serum concentrations of total cholesterol, triglyceride and lipoprotein cholesterols have been considerably reported by epidemiological and laboratory studies to be related to the incidence of coronary heart disease, particularly atherosclerosis.[40] Plants have been shown to be a reliable source of lipid lowering agents and several studies have authenticated the lipid lowering potential of several plants.[41,42] The administration of varying doses of ethanol extract of *D. edulis* raw seeds showed significant decreases in the concentrations of triglyceride, total cholesterol, LDL-C and VLDL-C with increase concentration of HDL-C in a dose dependent manner. Cholesterol synthesis and utilization must be tightly regulated in order to prevent over accumulation and deposition within the body.

A measurement of serum cholesterol concentrations is important as an indicator of liver function, intestinal absorption of bile and in diagnosis and classification of hyper-lipoproteinamias. Of particular importance, the abnormal deposition of cholesterol and cholesterol-lipoprotein (LDL-C) in the coronary arteries is of clinical significance. Such deposition eventually leads to atherosclerosis.[40] The dose dependent significant lowering of cholesterol in this study underlines the beneficiary effect of this seeds and its utilization in traditional medicine as hypocholesterolemic agent. The lipid lowering effect observed in this study corroborates reports of other scientific investigations into lipid lowering potential of some medicinal plants. *Citrullus colocynthis* seed extracts have been reported to have cholesterol lowering effect when administered to New Zealand rabbits.[43]

Evidence has suggested that the combination of high plasma levels of LDL-C with low levels of HDL-C predisposes atherosclerosis.[44] In our study, administration of ethanol raw seed extract of *D. edulis* showed decreased in LDL-C with increased concentration of HDL-C in dose dependent manner. Deposition of LDL-C in arterial membranes have been implicated in the pathogenesis of atherosclerosis hence is often regarded as 'bad cholesterol'. Endogenous cholesterols are often packaged and transported to the liver as HDL-C where they are being metabolized.[45] The present study showed a dose dependent reduction in the concentration of serum LDL-C in the treated groups when compared with the control while the HDL-C concentration was decreased significantly at medium and high dose of the extract.

A combination of reduced LDL-C and increased HDL-C concentrations following the administration of the extract is a positive indication of the anti-atherogenic potential of the extract. Plants have been reported to be a good

source of medicinal agents for the management of lipid profile abnormalities particularly raised cholesterol and LDL-C concentrations. Aqueous extract of *Ocimum sanctum* and Venonia amygdalina have been reported to have lipid lowering potentials just like the herb in the present study.[46,42] Very low density lipoprotein cholesterol (VLDL-C) concentration have also been showed to be significantly reduced in the test groups when compared to the control group in this study.

The triglyceride concentration of the test animals in this study decreased significantly when compared to the control and corroborates the changes observed following the administration of *trigonella foenugraecunn* Linn seed powder to experimental animals.[47] The lipid modulatory effect of the seed extract in this study may be attributed to the phytochemicals present in the extract particularly saponins and cardiac glycosides which have been known to exert such effects.

CONCLUSION

The ethanol extract of the seeds of *Dacryodes edulis* have been shown to contain phytochemicals such as flavonoids, tannins, phlobatannins, cardiac glycosides, terpenes and alkaloids. The ethanol extract of the seed has shown positive lipid modulatory potential following the administration of 100, 200 and 300 mg/kg body weight to albino Wistar rats.

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