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CHOLESTEROLEMIC AND ANTIOXIDANT EFFECTS OF EXTRACTS OF SOME LOCALLY CONSUMED FRUITS IN MAIDUGURI

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ABSTRACT

The study assessed the *in vitro* antioxidant potential of *Cucumis sativus, Solanum macrocarpon and Solanum aethiopicum* and cholesterolemic effect of *Solanum macrocarpon* ethanolic extract in *in vivo* models. The antioxidant activity of the fruit was determined by the DPPH radical scavenging method. CCl_4 was used to induce hepatotoxicity, catalase and superoxide dismutase was used to study the antioxidant potential of the ethanolic extract of *Solanum macrocarpon.* Lipid profile- HDL, LDL, TGs, VLDL and Cholesterol were used to study the effect of the ethanolic extract of *Solanum macrocarpon* on the lipid panel during hepatotoxicity. The *in vitro* antioxidant screening showed *Cucumis sativus*, *Solanum macrocarpon* and *Solanum aethiopicum* all had antioxidant activities but *Solanum macrocarpon* had the highest antioxidant activity (286.52% RSA), and this, informed its use for further *in vivo* antioxidant and Lipid profile studies. CCl_4 treated groups had significantly elevated concentrations of LDL 0.80mmol/l and significantly decreased concentration of cholesterol 1.76mmol/l. The extract fed groups showed significant decrease in HDL and cholesterol concentrations respectively (HDL-1.74mmol/l, Chol-1.73mmol/l and 1.46mmol/l). CCl_4 had no significant effect on the antioxidant enzymes. Thus, *Solanum macrocarpon* may be said to possess plasma lipid lowering potential whose mechanism may not be associated with free radical scavenging.

INTRODUCTION

Antioxidants are compounds with the characteristic ability to trap free radicals which are produced by oxidation of other compounds. They are capable of inhibiting the oxidation of other molecules which produce free radicals; these free radicals can in turn start chain

reactions in cells causing cell death/damage. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables (Prior et. al, 1998).

Many degenerative human diseases have been recognized as being a consequence of free radical damage, there have been many studies undertaken on how to delay or prevent the onset of these diseases. The most likely and practical way to fight against degenerative diseases is to improve body antioxidant status, which could be achieved by higher consumption of vegetables and fruits. Foods of plant origin usually contain natural antioxidants that can scavenge free radicals (Alia et. al., 2003).

Oxidative stress is considered to play a prominent role in the causation of many diseases, e.g., inflammation, aging, cancer, etc. Lipid profile or *lipid panel* is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids, such as cholesterol and triglycerides. They can identify certain genetic diseases and can determine approximate risks for cardiovascular disease, certain forms of pancreatitis, and other diseases. This test is used to identify dyslipideamia (various disturbances of cholesterol and triglyceride levels), many forms of which are recognized risk factors for cardiovascular disease and pancreatitis.

A total cholesterol reading can be used to assess an individual's risk for heart disease; however, it should not be relied upon as the only indicator. The individual components that make up total cholesterol reading-LDL, HDL, and VLDL are also important in measuring risk.

Fruits have been used over the years as traditional medicine and contain a number of antioxidants like polyphenolic flavonoids, vitamins, and anthocyanin. These compounds help protect the system from oxidative stress and boost immunity levels.

MATERIALS AND METHODS

Sample Collection and Processing: Fresh *Cucumis sativus*, *Solanum aethiopicum* and *Solanum macrocarpon* fruits were purchased at the Baga road market, Maiduguri, Borno state, Nigeria. These were authenticated by a plant taxonomist, Prof. Sanusi in Biological Science Department, University of Maiduguri, Borno state, Nigeria. The voucher number was obtained and deposited in the herbarium. The fruits were washed and shade dried to a

constant weight. The dried fruits were ground to fine powder with mortar and pestle and stored in a dried container.

Chemicals: All the chemicals used in this study were of analytical grade and purchased from various sources.

Ethanolic Extract Preparation: This was carried out by soaking 200g of the fruit powder in 300ml 70% ethanol for 24 hrs after which the mixture was filtered, this was repeated 3 times and the filtrate was evaporated at 45^{o}C to dryness using a water bath.

Determination of Total Flavonoid and Phenolic Contents: The total flavonoid content in each fruit extract was determined using the method described by Park et al.(1995). Briefly, 1 ml of fruit extract was mixed with 4 ml distilled water after which, 0.3 ml of 5% $NaNO_2$ was added at the baseline. After 5 mins, 0.3 ml of 10% $AlCl_3$ was added followed by the addition of 2 ml 1M NaOH 6 mins later. The volume was then adjusted to 10 ml by the addition of distilled water (2.4 ml). The mixture was vigorously shaken to ensure adequate mixing, and the absorbance was read at 510nm. A calibration curve was prepared using a standard solution of catechins (20, 40, 60, 80 and 100 µg/ml). The results were expressed as milligram catechins equivalents (CEQ) per kg of fruit sample.

The concentrations of phenolics in the fruit extracts were estimated using a modified spectrophotometric Folin-Ciocalteau method (Singleton et al, 1999). Briefly, 1 ml of the fruit extract was mixed with 1 ml of Folin-Ciocalteau phenol reagent. After 3 mins, 1 ml of 10% Na_2CO_3 solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 mins, after which the absorbance was read at 725nm using a UV/VIS spectrophotometer. Tannic acid was used to calculate a standard curve (20, 40, 60, 80 and 100 µg/ml). The concentration of phenolic compounds was measured in triplicate. The results were reported as the mean ± standard deviation and expressed as mg of tannic acid equivalents (TAEs) per kg fruit extract.

***In Vitro* Screening for Free Radical Scavenging Activity of Ethanolic fruit extracts using DPPH**: The free radical scavenging activity of ethanolic fruit extracts was determined using DPPH, based on the method proposed by Ferreira et al. (2009). 0.5 ml of the ethanolic fruit extract was mixed with methanolic solution containing DPPH radicals (0.024 mg/mL, 2.7 mL). The mixture was vigorously shaken and left to stand for 15 minutes in the dark. The

absorbance was measured at 517nm wavelength. The radical scavenging activity (RSA) was calculated as the percentage of DPPH discoloration using the following equation: % RSA = $([A_{DPPH} - A_S]/A_{DPPH}) \times 100$, where A_S is the absorbance of the solution when the ethanolic fruit extract was added at a particular level and A_{DPPH} is the absorbance of the DPPH solution**.**

Laboratory Animals: Forty male Albino rats (Wister strain) with average weight of 100-150g were used for this study. The rats were bought from the Biochemistry Department, University of Maiduguri Animal house. They were fed with commercial feed and had free access to clean water. They were kept in controlled breeding room (temperature of 25 ± 2^oC, humidity $60\pm5\%$, and 12 hour dark/light cycle).

Experimental Design: The experimental rats were divided into eight groups of 5 rats each. Groups 1, 2 and 8 served as normal, negative and positive control groups respectively. Groups 3 and 4 were CCl_4 administered groups respectively treated with 100 and 400mg/kg ethanolic extract of *Solanum macrocarpon* while groups 5 and 6 were the extract control groups. Group 7 served as ascorbic acid control group.

Hepatotoxicity was induced as the CCl4 was administered to groups 2,3,4,7 and 8 via intraperitoneal administration of 1:1 dilution of CCl_4 in olive oil at a dose of 2ml/kg body weight for two days (once daily). The ethanolic extract was administered orally to the rats using a feeding tube (BMI feeding tube size 8) for 21days. Normal and negative controls were administered with water for the duration of the study while group 7 was administered with 100mg/kg ascorbic acid and 35mg/kg Silymarin was administered to group 8 rats.

Daily food and water intake and weekly body weights were monitored throughout the experimental period.

The rats were sacrificed 24hours after the last treatment. Sera was harvested from the blood and used to assay for lipid profile, catalase and superoxide dismutase using standard described methods.

Statistical Analysis: The results are presented as mean $\pm$ SEM. Student's t-Test was used to compare test and control groups. The results were considered statistically significant at $p< 0.05$.

RESULTS

Table 1.1: Qualitative Test for the Presence of Polyphenols and Flavonoids from Aqueous and Ethanolic Extracts of Solanum macrocarpon, Solanum aethiopicum and Cucumis sativus.

Extracts	Polyphenols	Flavonoids
	Aqueous extracts	
Solanum macrocarpon	+++	+++
Cucumis sativus	+	+
Solanum aethiopicum	++	++
	Ethanolic extracts	
Solanum macrocarpon	+++	+++
Cucumis sativus	+	++
Solanum aethiopicum	++	+

Results are expressed as (+). The number of (+) represents color intensity observed, which translates to the quantity of phytonutrient in the extract sample.

Table 1.2. Free Radical Scavenging Activity of Aqueous and Ethanolic Fruit Extracts (% RSA) of Solanum macrocarpon, Solanum aethiopicum and Cucumis sativus.

Extracts	%RSA
	Aqueous extracts
Solanum macrocarpon	68.86 ± 1.67
Cucumis sativus	54.29 ± 1.18
Solanum aethiopicum	67.14 ± 2.10
	Ethanolic extracts
Solanum macrocarpon	286.52 ± 17.64
Cucumis sativus	188.17 ± 8.48
Solanum aethiopicum	155.41 ± 5.88

DPPH scavenging activities of aqueous and ethanolic extracts of *Solanum macrocarpon, Solanum aethiopicum and Cucumis sativus* (Mean ± SEM) DPPH = 1, 1-diphenyl-2-picrylhydrazyl.

Table 1.3: Total Phenolic Content, Total Flavonoid Content and Total Ascorbic acid Content of Ethanolic Extracts of Solanum macrocarpon, Solanum aethiopicum and Cucumis sativus.

Ethanolic extracts	Total phenol content TAE/g	Total flavonoid content CEQ/g	Total ascorbic acid content AA/g
Solanum macrocarpon	132.00 ± 1.21	237.50 ± 6.70	336.23 ± 1.29
Cucumis sativus	118.13 ± 1.18	105.00 ± 1.20	366. 53 ± 3.20
Solanum aethiopicum	102.23 ± 1.21	300.00 ± 5.42	440.98 ± 3.20

Data represent means of triplicate determinations of total phenolic content, total flavonoid content and total ascorbic acid content of fruit extracts (Mean ± SEM).

Table 1.4: The Effect of Oral Administration of *Solanum macrocarpon* Ethanolic Extract on Lipid Profile of CCl_4 Treated Albino Rats.

Groups	HDL(mmol/l)	LDL(mmol/l)	Chol(mmol/l)	TG(mmol/l)	VLDL(mmol/l)
Normal control	2.19 ± 0.14	0.60 ± 0.07	2.04 ± 0.13	0.62 ± 0.07	0.38 ± 0.07
CCl_4 control	1.77 ± 0.16*	0.61 ± 0.09	2.26 ± 0.30	1.41 ± 0.36*	0.72 ± 0.16*
CCl_4 + 100mg/kg extract	1.87 ± 0.24	0.66 ± 0.08	2.55 ± 0.38	1.13 ± 0.23	0.50 ± 0.11
CCl_4 + 400mg/kg extract	1.79 ± 0.11	0.80 ± 0.04*	1.76 ± 0.22*	1.41 ± 0.39	0.40 ± 0.04*
100mg/kg extract	1.74 ± 0.10*	0.59 ± 0.02	1.73 ± 0.11*	0.57 ± 0.05	0.26 ± 0.02
400mg/kg extract	2.16 ± 0.08	0.54 ± 0.02	1.46 ± 0.09*	0.78 ± 0.10	0.40 ± 0.05
CCl_4 + 100mg/kg ascorbic acid	1.82 ± 0.02	0.76 ± 0.06	2.75 ± 0.20	1.10 ± 0.12	0.53 ± 0.04
CCl_4 + 35mg/kg Silymarin	2.32 ± 0.36*	0.79 ± 0.12	3.02 ± 0.63*	1.37 ± 0.23	0.64 ± 0.12

Results are expressed as Mean ± SEM. CCl_4 control group was compared with the normal control group, CCl_4 experimental groups were compared with the CCl_4 control group while non CCl_4 groups were compared with the normal control group. (*) indicates significantly different with its respective control group at $p< 0.05$.

Table 1.5: The Effect of Oral Administration of *Solanum macrocarpon* Ethanolic Extract on Antioxidant Enzymes in CCl_4 Treated Albino Rats.

Groups	Catalase (U/L)	SOD (U/L)
Normal control	121.00 ± 0.63	0.90 ± 0.01
CCl_4 control	122.40 ± 1.84	0.90 ± 0.01
CCl_4 + 100mg/kg extract	125.20 ± 2.73	0.88 ± 0.01
CCl_4 + 400mg/kg extract	113.10 ± 1.10	0.88 ± 0.01
100mg/kg extract	117.40 ± 0.40	0.88 ± 0.01
400mg/kg extract	128.00 ± 0.32	0.86 ± 0.01
CCl_4 + 100mg/kg ascorbic acid	128.50 ± 2.84	0.89 ± 0.01
CCl_4 + 35mg/kg Silymarin	120.80 ± 3.93	0.88 ± 0.01

Results are expressed as Mean ± SEM. CCl_4 control group was compared with the normal control group, CCl_4 experimental groups were compared with the CCl_4 control group while non CCl_4 groups were compared with the normal control group. (*) indicates significantly different with its respective control group at $p< 0.05$.

DISCUSSION

The *in vitro* screening for free radical scavenging activity using DPPH revealed that *Solanum macrocarpon* ethanolic extract showed a concentration dependent activity and it can be deduced that the fruits of *S. macrocarpon* might offer protection against increased blood lipid concentrations. Studies have also shown that lipid-associated non-communicable diseases like diabetes and obesity are on the increase in the developing world and a continuous study is required to identify indigenous plant materials that can mitigate against, or be useful in management of lipid related diseases (Dalal et al., 2011; Kapiga, 2011; Negin et al., 2011).

Hence, the investigation of the influence of the consumption of *S. macrocarpon* fruit on plasma lipid profile is of interest.

Lipids are a heterogeneous group containing active metabolic substances that play an important role in the pathogenesis of chronic diseases e.g. liver diseases. As a major organ of the antioxidant defense system, the liver plays a pivotal role in the regulation of lipoprotein transport in plasma and cholesterol biosynthesis. During lipoprotein transport LDL and HDL appear to be particularly important. LDL is a converted form of VLDL, rich in cholesterol and cholesterol esters, and is regarded as bad cholesterol, whereas, HDL contains relatively little cholesterol, as high levels are associated with hyperlipedemia.

Dyslipideamia is known to be indicated by elevated plasma triacylglycerols, total cholesterol, LDL-cholesterol and VLDL-cholesterol with a concomitant reduced level of HDL-cholesterol.

Dietary management with fruits has long been recommended as part of the scrupulous controls necessary to prevent and/or manage dyslipideamia (Broekmans *et al*., 2001; Mirmiran *et al*., 2009). CCl_4 was shown to cause hyperlipedemia in this study as the concentration of HDL was significantly reduced; VLDL and triglycerides concentrations were significantly elevated, this agrees with finding by Vadivel et al., 2010. The extract treated groups showed reduction in hyperlipedemia as the concentrations of VLDL and cholesterol were significantly reduced this agrees with findings by Edijala *et al*., 2005. Fatty liver results mainly from the accumulation of TG (Vadivel et al., 2010). Increased TG levels after ethanol ingestion may be due to the increased availability of FFA, glycerophosphates, decreased TG lipase activity, and decreased fatty oxidation. These increased TG levels may lead to increased availability of FFA for esterification. Free fatty acids are the principal components present in most lipids of biological importance. The observed increase in the levels of FFA may be directly due to lipid breakdown.

As presented in table 1.5, it was observed that the hepatotoxicity induced using CCl_4 and *Solanum macrocarpon* ethanolic extract administration had no significant effect ($p<0.05$) on the concentration of the antioxidant enzymes in the experimental rats. Catalase is a key component of the antioxidant defense system. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical induced cellular damage. Excessive production of free radicals may result in alterations in the biological activity of cellular macromolecules

(Iweala *et al*., 2005). Therefore, the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of *Solanum* species had no effect on the activity of catalase in CCl_4 induced liver damaged rats to prevent the accumulation of excessive free radicals and protects the liver from CCl_4 intoxication. The concentration of SOD was not significantly affected ($p<0.05$) by the administration of *Solanum macrocarpon* extract. Decrease in enzyme activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury.

From this research, *in vitro* studies carried out showed *Solanum macrocarpon* to possess blood lipid lowering potential that may not be related to antioxidant effects *in vivo*.

REFERENCES

1. Akindahunsi A. A., Salawu S. O. Antioxidant indices of some green leafy vegetables. *Tropical Science*, 2005a; 45: 35-35.
2. Akindahunsi A. A., Salawu S. O. Phytochemical screening and nutrient-antinutrient composition of selected tropical green leafy vegetables. *African Journal of Biotechnology*, 2005b; 46: 497-501.
3. Alia M., Horcajo C., Bravo L., Goya L. Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats. *Nutritional Research*, 2003; 23: 1251–1267.
4. Amic D., Davidovic-Amic D., Beslo D., and Trinajstic N. Structure-radical scavenging activity relationship of flavonoids. *Croat Chemistry Annals*, 2003; 76: 55–61.
5. Ferreira I. C. F. R., Aires E., Barreira J. C. M and Estevinho L. M. Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry*, 2009; 1(114): 1438–1443.
6. Park Y. K., Koo M. H., Sato H. H. and Contado J. L. Survey of some components of propolis which were collected by *Apis mellifera* in Brazil, Curitiba, 1995; 38(1): 1253-1259.
7. Prior R.L., Cao G., Martin A., Sofic E., McEwen J., O'Brien C., Lischner N., Ehlenfeldt M., Kalt W., Krewer G., and Mainland C. M. *Journal of Agriculture and Food Chemistry*, 1998; 46(11): 2686-2693.
8. Recknagel R. O., Glende E. A., Dolak J. A., Waller R. L. Mechanism of carbon-tetrachloride toxicity. *Pharmacology Therapeutics*, 1989; 43(43): 139-154.

9. Sodipo O. A., Abdulrahman F. I., Alemika T. E., and Gulani I. A. Chemical composition and biological properties of the petroleum ether extract of *Solanum macrocarpon* L. (Local Name: Gorongo). *British Journal of Pharmaceutical Research*, 2012; 2(2): 108-128.
10. Sodipo O. A., Abdulrahman F. I., Akan J. C., and Akinniyi J. A. Phytochemical screening and elemental constituents of the fruit of *Solanum macrocarpon* L. *Continental Journal of Applied Sciences*, 2008; 3: 85-94.
11. Singleton V. L., Orthofer R. and Lamuela-Raventos R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. *Methods of Enzymology*, 1999; 299(4): 152–178.
12. Dalal S., Beunza J. J., Volmink J., Adebamowo C., Bajunirwe F., Njelekela M., Mozaffarian D., Fawzi W., Willett W., Adami H.O. and Holmes M.D. Non-communicable diseases in sub-Saharan Africa: what we know now. *International Journal of Epidemiology*, 2011; 40: 885-901.
13. Kapiga S. Commentary: Non-communicable diseases in sub- Saharan Africa: a new global health priority and opportunity. *International Journal of Epidemiology*, 2011; 40: 902- 903.
14. Negin J., Cumming R., de Ramirez S. S., Abimbola S. and Sachs S. E. Risk factors for non-communicable diseases among older adults in rural Africa. Tropical Medicine for Internatonal Health, 2011; 16: 640-646.
15. Vadivel A., Mani K., Kandhan K., Ganesan D., and Sankaran M. Antioxidant and antihyperlipidemic effect of *Solanum nigrum* fruit extract on the experimental model against chronic ethanol toxicity. Pharmacognsy Magazine, 2010; 6(21): 42–50.
16. Broekmans W. M., Klopping-Ketelaars W. A., Kluft C., van den Berg H., Kok F. J. and van Poppel G. Fruit and vegetables and cardiovascular risk profile: a diet controlled intervention study. European Journal of Clinical Nutrition, 2001; 55: 636-642.
17. Mirmiran P., Noori N., Zavareh M. B. and Azizi F. Fruit and vegetable consumption and risk factors for cardiovascular disease. Metabolism, 2009; 58: 460-468.
18. Edijala J. K., Asagba S. O., Eriyamremu G. E. and Atomatofa U. Comparative Effect of Garden Egg Fruit, Oat and Apple on Serum Lipid Profile in Rats Fed a High Cholesterol Diet. Pakistani Journal of Nutrition, 2005; 4(4): 245-249.