ORIGINAL RESEARCH

The beneficial effect of *Adansonia digitata* products success to modulate lipid profiles and inhibit LDL oxidation *in-vitro*: An associational study

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Abstract

Background

There is a growing interest in medicinal plants in recent years due to their many therapeutic benefits and low side effects. Among the medicinal plants is the African *Adansonia digitata* (baobab) that has edible fruit. In the current study, the effect of *A. digitata* juice consumption on the lipid profile was investigated. In addition, inhibition of the oxidation of low-density lipoprotein cholesterol (LDL-C) *in-vitro* by *A. digitata* essential oil (EO) was also investigated.

Methods

In this cohort study, a total of 70 subjects of *A. digitata* users (AD group, 42 male and 28 female) and 70 non *A. digitata* users (Non-AD group, 44 male and 26 female) were recruited to participate in this study. We evaluated lipid profile, HbA1c, liver/kidney functions, and phytosterol contents in fasting blood samples of all participants.

Results

The present findings illustrated significantly lower levels of total cholesterol, triglycerides, and LDL in the AD group compared to Non-AD ($p < 0.01$). In addition, essential oil of *A. digitata* inhibited LDL oxidation *in-vitro* as shown by the significant decreases in the formation of malonaldehyde (MDA), protein carbonyl (PC), and lipid hydroperoxide (LHP) ($P<0.05$). No significant changes in fasting blood glucose, HbA1c, HDL, kidney function, and liver function enzymes between the two groups were detected ($P>0.05$).

Conclusion

The juice of *A. digitata* has hypolipidemic and antioxidative effects and might be beneficial for the management of lipid levels in the body.

Keywords: *Adansonia digitata*, Baobab, lipid profile, lipid oxidation, essential oil

Introduction

Adansonia digitata L. also known as Baobab is a huge tree that grows in many countries in sub-Saharan Africa[1]. The *A. digitata* tree is used for food and in the management of many conditions such as fever, malaria, diarrhea, skin wounds, and microbial infections[2]. In Sudan, the population commonly consumes a juice prepared from *A. digitata* fruit (locally known as Gongulaze). The juice is rich in nutrients such as vitamins, minerals, fructose, glucose, and crude proteins[3-5]. In addition, the fruit of *A. digitata* is rich in antioxidants such as procyanidins and flavonol glycosides[6-8].

Oxidative modification of LDL plays a role in the initiation and progression of atherosclerosis[9,10]. High levels of total cholesterol and LDL in the circulation enhance monocyte adhesion to arterial walls and the subsequent accumulation of LDL in the intima[11]. Inside the intima, accumulated LDL can undergo oxidative modification in the arterial lesions by several types of cells such as muscle, macrophages, and endothelial cells, resulting in plaque formation[12]. Individuals with hyperlipidemia have a higher risk of cardiovascular disease (CVD) compared to healthy ones[13]. Therefore, the prevention of LDL oxidation is considered a front line against the development of atherosclerosis and cardiovascular diseases[14]. Nutrients rich in antioxidants have been shown to delay or block the oxidation of LDL and to prevent/delay atherosclerosis[15,16]. Since *A. digitata* fruit is rich in antioxidants, consumption of the plant fruit is expected to modulate LDL oxidation in users.

The beneficial health effects of A. digitata were investigated in several previous studies. However, the effect of *A. digitata* consumption on the blood biochemical profile and LDL oxidation remains to be investigated. Therefore, in the current study, the effect of *A. digitata* juice consumption on the lipid profile was investigated. In addition, inhibition of the oxidation of low-density lipoprotein cholesterol (LDL-C) in-vitro by *A. digitata* essential oil (EO) was also investigated.

Methods

Subjects and Design

The study is cohort design and included adult Sudanese nationals (>18 years old) living in Saudi Arabia.

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Table 1: Clinical and biochemical characteristics of the study group participants

Character	A. digitata (AD) n=70	Non-AD n=70	P-value
Age (years ± SD)	46±5.3	45±3.7	0.23
Gender (male: female)	(42: 28)	(44: 26)	0.95[a]
BMI (kg/m^2)	24.1±2.1	24.6±4.4	0.36
HbA_1c (%)	5.3±0.8	5.6±1.7	0.16
Blood pressure (mm/Hg)			
Systolic	120.4±5.9	122.2±7.1	0.15
Diastolic	78.6±4.8	79.3±6.1	0.51
Duration of JD (year)	6.2±1.2	-	-
Lipid profiles and CK enzyme:			
TC (mmol/L)	4.8±0.7	5.3±1.1	0.001*
TG (mmol/L)	2.13±0.3	2.30±0.4	0.002*
LDL (mmol/L)	4.0±0.6	4.5±1.1	0.001*
HDL (mmol/L)	1.41±0.3	1.32±0.4	0.10
CK (U/L)	68.2±14.8	72.1±16.7	0.11
Liver profiles:			
Albumin (g/dl)	4.4±1.2	4.3±0.8	0.61
Total protein (g/dl)	7.2±0.9	7.1±1.1	0.61
ALT (U/L)	28.3±7.8	26.8±5.4	0.15
AST (U/L)	24.5±5.5	26.0±8.2	0.17
Kidney profiles:			
Urea (mg/dL)	19.3±3.7	18.7±2.2	0.19
Creatinine (mg/dL)	0.82±0.7	0.86±0.2	0.60

AD: Adansonia digitata. n: number. BMI: body mass index. HbA_1c: hemoglobin A1 C. JD: juice drinker. TC: Total cholesterol. TG: Triglycerides. LDL: low-density lipoprotein cholesterol. HDL: high-density lipoprotein cholesterol. CK: Creatine Kinase. ALT: Alanine Transaminase. AST: Aspartate Transaminase.

*: Significant p<0.01 (unpaired t-test). [a:] (Chi-square p values).

Table 2: Antioxidant effect of essential oil of A. digitata and vitamin E on LDL oxidation, TPC, and FC.

	DPPH test µg/ml	Lag time (min)	Oxidation ratio (nmol/min/mg LDL protein)	Maximum CD (nmol /mg LDL protein)	TPF (mg GAE/100g DW)	FC (mg RE/g DW)
A. digitata oil	5.6±1.1	226.1±22.8* (8.5)	3.6±1.3*	231.5±22.7*	520.4±67.7	36.1±12.1
Vitamin E	5.8±1.2	219.8±20.2* (8.3)	3.9±1.1*	237.1±19.8*	509.2±72.3	32.4±9.2
Control		26.6±4.5 (1.0)	11.4±2.8	389.9±34.2	-	-

DPPH: 1,1-diphenyl-2-picrylhydrazyl, CD: conjugated diene. TPC: Total phenol content. FC: Flavonoid content GAE: Gallic Acid Equivalent. RE: Runtin Equivalent. DW: Dry Weight.

*: Significant in comparison with control level (p<0.001).

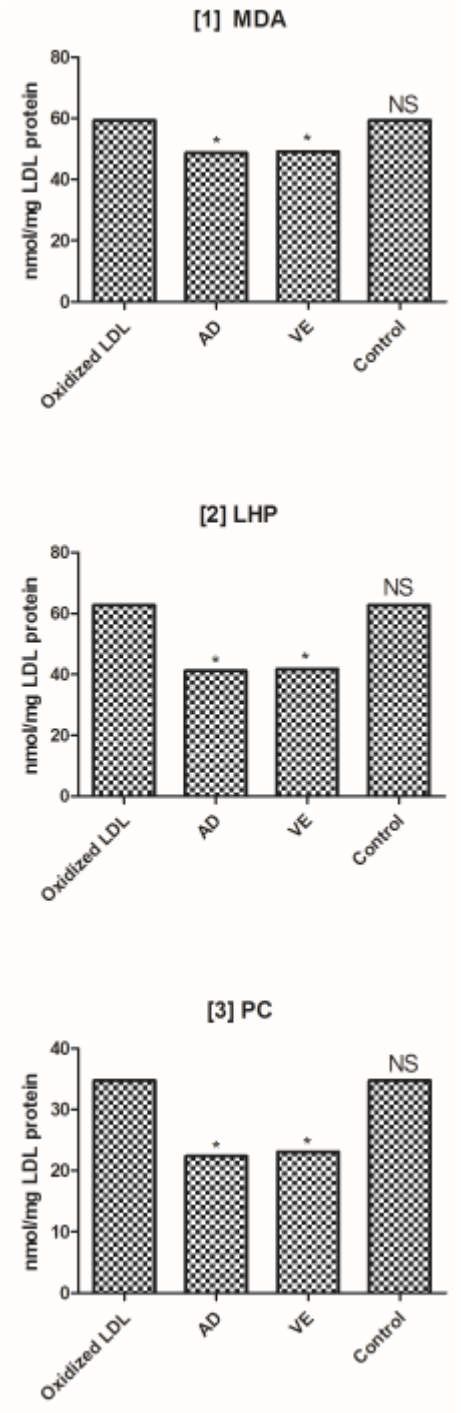

Figure 1: Effect of A. digitata essential oil versus vitamin E on the end products of LDL oxidation: (1) Malonaldehyde (MDA). (2) Lipid hydroperoxide (LHB). (3) Protein carbonyl (PC) in CuSO4 enhanced LDL oxidation.

***: Indicates significant difference (p<0.01).**

NS: not significant.

Participants were divided into two groups. Group 1 (AD group, n=70, 42 male and 28 female, mean age = 46±5.3 years) were subjects who use AD fruit juice on daily basis (at least 1 cup: 200 mL juice per day) for the last 3 months prior to blood sampling. Group 2 (Non-AD, n=70, 44 male and 26 female, mean age = 45±3.7 years) were subjects who did not use AD fruit in the past 3 months. Subjects were recruited from Madinah city, Saudi Arabia during March-May of 2020 via advertisements placed in living complexes dominated by Sudanese nationals. Participants who met the inclusion criteria were given appointments at Taibah University Health Center to complete the study procedures. All subjects were

Table 3: Phytosterols contents and their derivatives in EO of AD.

Phytosterols Content (mg/100 g)	
B-Sitosterol	512.2
Campesterol	171
Campestanol	59.7
Stigmasterol	39.8
Cycloartenol	12.4
Cycloartanol	1.2
Egrosterol	0.23
Total Plant Sterols	796.53

healthy and were not taking any medications for at least 3 months prior to blood sample collection. Subjects with diabetes, hypertension or chronic illness were excluded from the study. Blood samples were collected from each participant after 12h fasting in EDTA and plain tubes. All subjects gave written informed consent as required by IRB (No. CLS 201978) of Taibah University, Saudi Arabia.

Anthropometric parameters and blood pressure

Systolic/diastolic blood pressure and body mass index (BMI) kg/cm^2 were measured as previously described[17].

Extraction of essential oil from A. digitata fruit

Fruit pulps (dried) of *A. digitata* were obtained from Khartoum city, Sudan from different grain markets. The essential oil was extracted from the powder using a Clevenger apparatus and hydro-distillation process according to the British Pharmacopoeia Specifications[18]. In brief, fruit dried materials (2000 g) were properly washed, chopped, and mixed with 5 liter of distilled water (DW) inside the distillation flask. Therefore, the fluid mixture was heated until it steamed. Then, the steam was cooled down and condensed. Then the essential oil (EO) was collected over hexane in a collection container for the Clevenger apparatus.

LDL isolation

LDL cholesterol was prepared as previously described19. In brief, a blood sample (5 ml from each donor, n=10) was collected in an EDTA tube and the plasma was separated and centrifuged at 200xg for 40 min at 4°C using an instrument obtained from Beckman (Glenrothes, UK). LDL was separated with a concentrated LDL protein and its concentration was determined using a commercially available kit (Pierce Laboratories, Rockford, IL). Phosphate buffered saline was used to dilute LDL samples to a final concentration of 70 µg/mL LDL protein. The samples were stored until used in LDL oxidation experiments.

Kinetic oxidation of LDL

Oxidation of LDL was done as previously reported[14]. In brief, a total of 100 µg/mL LDL protein was mixed with 20 µL of A. digitata essential oil or vitamin E at 37°C for 15 minutes. Then 10 µl of 0.167 mM copper sulfate ($CuSO_4$) freshly prepared was added to stimulate the process of oxidation. Oxidation kinetic was calculated by monitoring changes in OD (using Hitachi U2000 spectrophotometer at 234 nm) every 10 minutes for up to 200 minutes. The rate of oxidation was calculated as described previously[14]. A total of

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10 samples were used in the assay and in duplicates.

Scavenging assay

For scavenging of free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA) was used. In brief, aliquot amounts of essential oil of A. digitata or vitamin E were mixed with the addition of 2 ml of DPPH solution (0.1 mM DPPH that was dissolved in ethanol). Then incubated for 30 minutes at 37°C. Changes in OD were determined at 516nm wavelength using a Hitachi U2000 spectrophotometer. Scavenging activity was calculated as previously described[20]. A total of 10 samples were used in the assay and in duplicates.

Assessment of phenol content

Phenolic content was assessed using Folin-Ciocalteu reagent (FCR) as previously described 21. In brief, 125 µl of FCR (10%) was mixed with 125 µl of the essential oil, and the mixture was incubated for six minutes. A total of 125 µl of 7% $Na2CO_3$ was mixed with the mixture then incubated at room temperature (RT) further to 30 min. The product reaction (blue-colored solution) was read at 760nm using a spectrophotometer. Measurements were calibrated with the gallic acid standard curve. Phenolic content was expressed as mgGAE/g dry weight.

Assessment of flavonoid content

A total of 250 µL of *A. digitata* was mixed with 780 µL of DW. Then, 60 µL of 5% $NaNO_2$ was added to the solution and incubated for six minutes. 60 µL of 10% aluminum trichloride solution was added to the solution and incubated for six minutes. A total of 400 µl of 1 Molar NaOH was also mixed. The OD was read immediately at 510nm. Flavonoid content was expressed as mgRE /g dry weight[22].

Estimation of the end product of LDL oxidation

A total of 100 µg/mL LDL protein was mixed with 20 µL of A. digitata essential oil or vitamin E at 37 °C for 15 minutes. Then 10 µl of 0.167 mM copper sulfate ($CuSO_4$) freshly prepared was added to stimulate the process of oxidation. The reaction was continued for 24 hours. Then to stop the reaction, 10 mM of EDTA was added. MDA, LHP, and PC were quantified as previously described[23].

Biochemical parameters

Total cholesterol (TC), triglyceride (TG), LDL, HDL, albumin, total protein, urea, and creatinine were determined based on enzymatic reaction methods using full autoanalyzer 704 (Hoffman-La Roche Ltd., Basel, Switzerland). In addition, ALT, AST and CK were measured by kinetic method with the same analyzer. The total of phytosterol contents was measured in the samples as previously described[24].

Data analysis

Data were analyzed using Prism software (GraphPad Company, CA, USA) version 5. Biochemical parameters were compared between the two groups using an unpaired t-test. Multiple comparisons were performed using the ANOVA test. Data were expressed as mean ± standard deviation (SD). A p-value of ≤ 0.05 was considered significant.

Results

The biochemical characteristics of the A. digitata fruit juice users (AD group, n = 70) and the non-users (Non-AD) are shown in Table 1. The AD group reported drinking the *A. digitata* fruit juice for 8.2 ± 1.2 years period. There was no statistical difference in age, gender, and blood pressure between the AD group and Non-AD ($P > 0.05$, Table 1). The AD group have a significant lower plasma total cholesterol ($p < 0.001$), triglyceride ($p < 0.005$) and LDL ($p < 0.001$). With respect to HDL, it was higher in AD group than the Non-AD, but it did not show a significant difference ($p = 0.1$). However, there were no differences in plasma creatinine level ($p = 0.11$), liver function measures (albumin, total protein, and transaminase enzymes) or kidney function measures (urea and creatinine) between the two groups ($P > 0.05$).

Antioxidant activity of the A. digitata essential oil was measured by scavenging free radicals of DPPH, subsequent inhibition of LDL oxidation, and concentration of phenols and flavonoids in A. digitata essential oil (Table 2). Vitamin E was used as control positive. The DPPH scavenging activity was 50% (inhibitory concentration, IC50) at concentrations of 5.6 ± 1.1 and 5.8 ± 1.2 µg/ml of *A. digitata* essential oil and vitamin E respectively. In addition, *A. digitata* essential oil and vitamin E showed similar high reducing activity against $CuSO_4$-induced LDL oxidation (3.6 and 3.9 nmol/min/mg LDL protein, respectively). Moreover, the lag time was significantly greater by approximately 8.5- and 8.3-fold, in essential oil and vitamin E respectively when compared with the control untreated group ($p > 0.01$). Furthermore, the formation of conjugated diene was reduced by the addition of essential oil and vitamin E (231.5 ± 22.7 and 237.1 ± 19.8 nmol/mg LDL protein, respectively) significantly compared to the untreated control group (389.9 ± 34.2 nmol/mg LDL protein). Additionally, the results showed a high quantity of phenols (520.4 ± 67.7 mgGAE/g dry weight) and flavonoids (36.1±12.1 mg RE/g dry weight) in the oil.

Figures 1 shows CuSO4-induced LDL oxidation, lipid peroxidation were increased levels of MDA, LHP, and PC. The addition of *A. digitata* essential oil or vitamin E to the LDL oxidation reaction resulted in a similar and significant decrease in the production of MDA, LHP, and PC ($P<0.05$).

Table 3 shows the contents of AD EO of phytosterol profiles, in which, EO has total sterols equal 796.53 mg/100g.

Discussion

In the current research, the effects of *A. digitata* on biochemical profile and LDL oxidation in vitro were examined. Total cholesterol, triglycerides, and LDL were significantly lower in *A. digitata* fruit juice users. In addition, LDL oxidation and lipid and protein peroxidation were significantly reduced in vitro by treatment with essential oil of *A. digitata* fruit.

The observed effects of *A. digitata* consumption on lipid profile are supported by three previous studies performed using animal models[25-27]. Hypolipidemic impact of *A. digitata* is attributed to the phytochemical contents of the plant. According to previous reports, the *A. digitata* fruit is rich in phytosterols[1], saponin[25], tannin[28], pectin[29], gallic acid, and epicatechin[30] that might modulate lipid profile. For example, the phytosterol content of the *A. digitata* fruit pulp can reduce total cholesterol and thus inhibit the absorption of biliary and dietary cholestero[l31]. The saponin and tannins continent of *A. digitata* have been shown to de-conjugate cholesterol from bile acids[32,33], whereas the pectin component has hypolipidemic activity[34]. Moreover, gallic acid and epicatechin can inhibit the action of cholesterol esterase enzyme and the subsequent elevate of the excretion of primary bile acids[29] and decrease in the buildup of cholesterol micelles[35]. Thus, several mechanisms through which *A. digitata* might impact

lipid profile[36]. The plant contents of total phytosterols were high and comparable with that of other edible plants oil[24].

It is well known that LDL oxidative modification has a significant role in atherosclerosis progression[37]. In the present study, we reported that essential oil of *A. digitata* possessed a strong antioxidant ability that significantly inhibited the formation of the free radical of DPPH, a finding supported by previous work[38], It is believed that phenol and flavonoid rich plants such as *A. digitata* have high antioxidant capabilities because of the redox activity of these compounds[39-41]. The high content of *A. digitata* fruit in phenol and flavonoid reported in the current investigation highlights the beneficial effects of this plant for cardiovascular health[38,42]. Moreover, *A. digitata* is very rich in Vitamin C (>100mg/100g)[43,44] which is a powerful antioxidant known to boost immunity and reduce blood pressure[29].

The current findings showed significant decreases in lipid (MDA and LHP) and protein (PC) peroxidation following treatment of LDL with essential oil of *A. digitata in vitro*. The magnitude of decreases in lipid and protein peroxidation is equivalent to that observed with the strong antioxidant vitamin E. These findings are in agreement with a previous report from Sudan[45].

The present study showed no effect of *A. digitata* on fasting blood glucose. This is in disagreement with a previous report from Sudan[25]. This is could be due to the current sample, which included only healthy subjects. In the current research, *A. digitata* juice showed no harmful effects on renal or liver health, indicating consumption of the *A. digitata* plant has few or no side effects. The *A. digitata* plant is rich in many beneficial active substances that have important biological activities such as minerals, vitamin C, proteins, and others that extend the medicinal uses of this plant.

Among the limitations, the relatively small sample size, so other studies are needed for further confirmation of the present findings. In addition, identification of the active compounds in *A. digitata* fruit that modulate lipid profile is strongly recommended in future investigations. Finally, data on the participants' physical activity status was not collected. In addition, due to the small sample size, it is not possible to apply stratification of the sample according to gender. Therefore, taking these confounding factors into account can provide a better insight of the medicinal benefits of *A. digitata* fruit.

Conclusion

Consumption of *A. digitata* fruit juice lowers total cholesterol, triglycerides, and LDL. In addition, the essential oil of *A. digitata* reduced LDL oxidation in vitro. The beneficial effects of *A. digitata* fruit could be attributed to its high contents of phytosterols, phenols, and flavonoids.

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Conflict of interest

None.

References:

1.Rahul J, Jain MK, Singh SP, et al. Adansonia digitata L.(baobab): a review of traditional information and taxonomic description. Asian Pacific Journal of Tropical Biomedicine. 2015;5(1):79-84.

2.Kamatou G, Vermaak I, Viljoen A. An updated review of Adansonia digitata: A commercially important African tree. South African Journal of Botany. 2011;77(4):908-919.

3.Gurashi[1] NA, KordofaniP MA, Abdelgadir KA, AM A. Variation in chemical composition of Baobab (Adansonia digitata L). 2016.

4.Gebauer J, Luedeling E. A note on baobab (Adansonia digitata L.) in Kordofan, Sudan. Genetic resources and crop evolution. 2013;60(4):1587-1596.

5.Gebauer J, El-Siddig K, Ebert G. Baobab (Adansonia digitata L.): a review on a multipurpose tree with promising future in the Sudan. Gartenbauwissenschaft. 2002;67(4):155-160.

6.Braca A, Sinisgalli C, De Leo M, et al. Phytochemical Profile, Antioxidant and Antidiabetic Activities of Adansonia digitata L. (Baobab) from Mali, as a Source of Health-Promoting Compounds. Molecules. Nov 27 2018;23(12).

7.Ismail BB, Pu Y, Fan L, Dandago MA, Guo M, Liu D. Characterizing the phenolic constituents of baobab (Adansonia digitata) fruit shell by LC-MS/QTOF and their in vitro biological activities. Sci Total Environ. Dec 1 2019;694:133387.

8.Ismail BB, Pu Y, Guo M, Ma X, Liu D. LC-MS/QTOF identification of phytochemicals and the effects of solvents on phenolic constituents and antioxidant activity of baobab (Adansonia digitata) fruit pulp. Food Chem. Mar 30 2019;277:279-288.

9.Aviram M, Kaplan M, Rosenblat M, Fuhrman B. Dietary antioxidants and paraoxonases against LDL oxidation and atherosclerosis development. Handb Exp Pharmacol. 2005(170):263-300.

10.Bucholtz I. Cellophane Cover Slips and a Method for Mounting. Science. Nov 8 1940;92(2393):436.

11.Rosenfeld ME, Khoo JC, Miller E, Parthasarathy S, Palinski W, Witztum JL. Macrophage-derived foam cells freshly isolated from rabbit atherosclerotic lesions degrade modified lipoproteins, promote oxidation of low-density lipoproteins, and contain oxidation-specific lipid-protein adducts. The Journal of clinical investigation. 1991;87(1):90-99.

12.Regnstrom J, Nilsson J, Tornvall P, Hamsten A, Landou C. Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man. The Lancet. 1992;339(8803):1183-1186.

13.Karr S. Epidemiology and management of hyperlipidemia. The American journal of managed care. 2017;23(9 Suppl):S139-S148.

14.Steffen Y, Jung T, Klotz L-O, Schewe T, Grune T, Sies H. Protein modification elicited by oxidized low-density lipoprotein (LDL) in endothelial cells: Protection by (–)-epicatechin. Free Radical Biology and Medicine. 2007;42(7):955-970.

15.Hartley A, Haskard D, Khamis R. Oxidized LDL and anti-oxidized LDL antibodies in atherosclerosis - Novel insights and future directions in diagnosis and therapy<sup/>. Trends Cardiovasc Med. Jan 2019;29(1):22-26.

16.Kattoor AJ, Pothineni NVK, Palagiri D, Mehta JL. Oxidative Stress in Atherosclerosis. Curr Atheroscler Rep. Sep 18 2017;19(11):42.

17.Ahmed AM, Elabid BEH, Elhassan KEH, Waggiallah HA. Metabolic syndrome among undergraduate students attending medical clinics for obligatory medical screening. Tropical Journal of Pharmaceutical Research. 2015;14(2):317-321.

18.Kayode RM, Azubuike CU, Laba SA, Dauda AO, Balogun MA, Ajala SA. Chemical composition and anti-microbial activities of the essential oil of Adansonia digitata stem-bark and leaf on post-harvest control of tomato spoilage. Lwt. 2018;93:58-63.

19.Ahmed AM, Ozbak HA, Hemeg HA. Effect of essential oil of traditional two Saudi mint types and its possible role in cardiovascular and throat health. International journal of clinical and experimental medicine. 2015;8(5):8060.

20.Esterbauer H, Striegl G, Puhl H, Rotheneder M. Continuous monitoring of in vztro oxidation of human low density lipoprotein. Free

radical research communications. 1989;6(1):67-75.

21.Singleton VL, Orthofer R, Lamuela-Raventós RM. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in enzymology. Vol 299: Elsevier; 1999:152-178.

22.Eberhardt MV, Lee CY, Liu RH. Nutrition: Antioxidant activity of fresh apples. Nature. 2000;405(6789):903.

23.Nourooz-Zadeh J, Tajaddini-Sarmadi J, Ling KL, Wolff SP. Low-density lipoprotein is the major carrier of lipid hydroperoxides in plasma. Relevance to determination of total plasma lipid hydroperoxide concentrations. Biochem J. Feb 1 1996;313 (Pt 3):781-786.

24.Yang R, Xue L, Zhang L, et al. Phytosterol contents of edible oils and their contributions to estimated phytosterol intake in the Chinese diet. Foods. 2019;8(8):334.

25.Ebaid H, Bashandy SA, Alhazza IM, Hassan I, Al-Tamimi J. Efficacy of a Methanolic Extract of Adansonia digitata Leaf in Alleviating Hyperglycemia, Hyperlipidemia, and Oxidative Stress of Diabetic Rats. BioMed research international. 2019;2019.

26.Muhammad I, Wudil A. Evaluation Of Anti-Hyperlipidemic Potentials Of Aqueous Fruit Pulp Extract Of Adensonia Digitata In Experimental Rats. European Scientific Journal. 2016;12(12).

27.Mohammed NA, Elmula TMF, Mohammed AA. THE EFFECT OF ETHANOLIC PULP EXTRACT OF ADANSONIA DIGITATA IN HYPERLIPIDEMIC EXPERIMENTAL ALBINO RATS. 2017.

28.Nkafamiya I, Osemeahon S, Dahiru D, Umaru H. Studies on the chemical composition and physicochemical properties of the seeds of baobab (Adasonia digitata). African Journal of Biotechnology. 2007;6(6).

29.De Caluwé E, Halamová K, Van Damme P. Adansonia digitata L.–A review of traditional uses, phytochemistry and pharmacology. Afrika focus. 2010;23(1).

30.Tembo DT, Holmes MJ, Marshall LJ. Effect of thermal treatment and storage on bioactive compounds, organic acids and antioxidant activity of baobab fruit (Adansonia digitata) pulp from Malawi. Journal of Food Composition and Analysis. 2017;58:40-51.

31. Ntanios FY, Jones PJ. Dietary sitostanol reciprocally influences cholesterol absorption and biosynthesis in hamsters and rabbits. Atherosclerosis. 1999;143(2):341-351.

32.Matsuura H. Saponins in garlic as modifiers of the risk of cardiovascular disease. The Journal of nutrition. 2001;131(3):1000S-1005S.

33.Park S-Y, Bok S-H, Jeon S-M, et al. Effect of rutin and tannic acid supplements on cholesterol metabolism in rats. Nutrition Research. 2002;22(3):283-295.

34.Lattimer J, Haub M. Effects of dietary fiber and its components on metabolic health. Nutrients 2: 1266–1289. 2010.

35.Ngamukote S, Mäkynen K, Thilawech T, Adisakwattana S. Cholesterol-lowering activity of the major polyphenols in grape seed. Molecules. 2011;16(6):5054-5061.

36.Brown L, Rosner B, Willett WW, Sacks FM. Cholesterol-lowering effects of dietary fiber: a meta-analysis. The American journal of clinical nutrition. 1999;69(1):30-42.

37.Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. Journal of Biological Chemistry. 1997;272(34):20963-20966.

38.Braca A, Sinisgalli C, De Leo M, et al. Phytochemical profile, antioxidant and antidiabetic activities of Adansonia digitata L.(baobab) from Mali, as a source of Health-Promoting compounds. Molecules. 2018;23(12):3104.

39.Ohmori R, Iwamoto T, Tago M, et al. Antioxidant activity of various teas against free radicals and LDL oxidation. Lipids. 2005;40(8):849-853.

40.Thounaojam MC, Jadeja RN, Devkar RV, Ramachandran A. In Vitro Evidence for the Protective role of Sida rhomboidea. Roxb Extract Against LDL Oxidation and Oxidized LDL-Induced Apoptosis in Human Monocyte–Derived Macrophages. Cardiovascular toxicology. 2011;11(2):168-179.

41.Gajaria TK, Patel DK, Devkar RV, Ramachandran A. Flavonoid rich extract of Murraya Koenigii alleviates in-vitro LDL oxidation and oxidized LDL induced apoptosis in raw 264.7 Murine macrophage cells. Journal of food science and technology. 2015;52(6):3367-3375.

42.Vertuani S, Braccioli E, Buzzoni V, Manfredini S. Antioxidant capacity of Adansonia digitata fruit pulp and leaves. Acta phytotherapeutica. 2002;2(5):2-7.

43.Muthai KU, Karori MS, Muchugi A, et al. Nutritional variation in baobab (Adansonia digitata L.) fruit pulp and seeds based on Africa geographical regions. Food science & nutrition. 2017;5(6):1116-1129.

44.Osman MA. Chemical and nutrient analysis of baobab (Adansonia digitata) fruit and seed protein solubility. Plant foods for human nutrition. 2004;59(1):29-33.

45.El-Arab NB. Evaluation of antioxidant capacity and physicochemical properties of Sudanese baobab (Adansonia digitata) seed-oil. International Food Research Journal. 2017;24:S441-S445.