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Evaluation of Nutritional Properties, Antioxidant and Antihyperlipidemic Activity of Sudanese *Annona senegalensis* Fruits

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ABSTRACT

This study aimed to evaluate the nutritional properties, antioxidant activity, and antihyperlipidemic effects of Sudanese *Annona senegalensis* fruits. The proximate composition and mineral content of the pulp, seeds, and external crust were analyzed, while the antihyperlipidemic activity was specifically evaluated using ethanolic extracts from the pulp and external crust (seeds were not used in the *in vivo* study). Thirty hypercholesterolemic Wistar rats were divided into six groups: normal control (A), high-cholesterol diet (B), atorvastatin treatment (C), pulp extract (D), crust extract (E), and mixed extract (F). Results revealed distinct nutritional profiles: pulp showed high carbohydrate content (77.37%), seeds contained substantial oil (24.09%) and fiber (49.01%), while the crust demonstrated the highest antioxidant activity (71.91%). The high-cholesterol diet (Group B) significantly increased total cholesterol (from 50.60 to 67.60 mg/dL) and HDL (from 46.00 to 61.00 mg/dL), while decreasing LDL (from 48.20 to 49.20 mg/dL, non-significant) and triglycerides (from 35.00 to 24.00 mg/dL) compared to normal controls. Treatment with mixed extract (Group F) showed the most potent hypocholesterolemic effect, significantly reducing TC (41.80 to 30.75 mg/dL) and TG (60.40 to 45.25 mg/dL). The crust extract (Group E) significantly increased HDL levels (40.50 to 65.00 mg/dL). These findings demonstrate that *A. senegalensis* fruits, particularly the crust, possess strong antioxidant and antihyperlipidemic properties, with potential applications in managing hyperlipidemia.

القيمة الغذائية ، مضادات الأكسدة والنشاط الخافض للدهون من الفواكه السودانية *Annona senegalensis*

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الخلاصة

هدفت هذه الدراسة إلى تقييم الخصائص الغذائية والنشاط المضاد للأكسدة والتأثيرات المضادة antihyperlipidemic الدم لثمار ***Annona senegalensis*** السودانية. تم تحليل التركيب التقريبي والمحتوى المعدني للب والبذور والقشرة الخارجية ، بينما تم تقييم النشاط antihyperlipidemic على وجه التحديد باستخدام مستخلصات إيثانوليك من اللب والقشرة الخارجية (لم يتم استخدام البذور في الدراسة في الجسم الحي). تم تقسيم ثلاثين من الفئران المصابة بفرط الكوليسترول في الدم إلى ست مجموعات: normal control (A), high-cholesterol diet (B), atorvastatin treatment (C), pulp extract (D), crust extract (E), and mixed extract (F). كشفت النتائج عن ملامح ان اللب أظهر نسبة عالية من الكربوهيدرات (77.37٪) ، واحتوت البذور على زيت كبير (24.09٪) وألياف (49.01٪) ، بينما أظهرت القشرة أعلى نشاط مضاد للأكسدة (71.91٪). أدى النظام الغذائي عالي الكوليسترول (المجموعة ب) إلى زيادة كبيرة في الكوليسترول الكلي (من 50.60 إلى 67.60 مجم/ديسيلتر) وهدل (من 46.00 إلى 61.00 مجم/ديسيلتر) ، مع تقليل البروتين الدهني منخفض الكثافة (من 48.20 إلى 49.20 مجم/ديسيلتر ، غير مهم) والدهون الثلاثية (من 35.00 إلى 24.00 مجم/ديسيلتر) مقارنة بالضوابط العادية. أظهر العلاج بمستخلص مختلط (المجموعة و) أقوى تأثير لنقص الكوليسترول في الدم ، مما قلل بشكل كبير من تك (41.80 إلى 30.75 مجم/ديسيلتر) و تغ (60.40 إلى 45.25 مجم/ديسيلتر). زاد مستخلص القشرة (المجموعة هـ) بشكل كبير من مستويات البروتين الدهني عالي الكثافة (40.50 إلى 65.00 مجم/ديسيلتر). تظهر هذه النتائج أن أ. تمتلك ثمار سينغالينسيس ، وخاصة القشرة ، خصائص قوية مضادة للأكسدة antihyperlipidemic ، مع تطبيقات محتملة في إدارة مستوى الدهون في الدم.

INTRODUCTION

Annona senegalensis, frequently recognized in Africa as wild custard apple, and wild soursop *It belongs to the Annonaceae family*. It is a fruit tree native to Senegal and establish in semi- dry to subhumid of African areas (Elegbeleye *et al*., 2022; Okhale *et al*., 2016). *Annona senegalensis* is a botanic that is communal and mostly establish in some regions of africa. It is citing to as the wild soursop in English. Essentially, the genus forename 'annona' is derivative from a Latin term anon that mean to 'annual production'. However, limited studies have been conducted on the dehydrated fruit, while numerous investigations have focused on other parts of the plant such as leaf (Onwusonye *et al*., 2014), stalk, bay, roots to expose approximately of its possibilities (Theophine C Okoye *et al*., 2012).

The flesh of Annonas contains high number of micronutrients (Donhouedé *et al*., 2023; Gyamfi *et al*., 2011) and also a possible basis of carbohydrate. The dietary and health benefits of the fruits are largely attributed to their high content of phytonutrients**.** (Shukry *et al*., 2019). Annonas seeds comprise respectable quantity of oil (Mariod *et al*., 2010) that can be utilized for manufacturing usage. In addition, the leaves, roots, berries, fruits, and seeds of *Annona* species have been identified as potential sources of therapeutically significant compounds. (Mariod *et al*., 2011). The leaves, stems, and roots of *Annona senegalensis* are commonly utilized in traditional medicine to alleviate ailments like diarrhea, dysentery, stomach aches, and headaches. Moreover, this plant is readily available (Diallo *et al*., 2024; Diallo *et al*., 2022)

The objective of this research is to evaluate nutritional Properties, Antioxidant , Anti-hyperlipidemic Activity of Sudanese *Annona senegalensis* fruits in order to determine its nutritious appropriateness and healthy factors related to its usage as portion of the dietary humane.

MATERIAL AND METHODS

The fruits of *Annona senegalensis* were purchased from the local market of Khartoum city **during the 2015-2016 harvesting season. Although the experimental data were collected during the 2015-2016 season, they remain highly relevant as this study provides the first comprehensive baseline on the nutritional and bioactive properties of** *Annona senegalensis* **fruits from Sudan, representing a valuable contribution to the characterization of understudied indigenous plant species.** The fruits were identified by a taxonomist medicinal, aromatic plant national center for research Institute (MAPRI) Khartoum Sudan, washed, dehydrated, weighed, skinned and pulped. The separated flesh of the fruit were standardized and filtered, then was wrapped in plastic bags, store at 2 °C until farther examination. Upon accomplishment of the in vitro examination, the extract with the highest antioxidant potential will be choosed for the experimental studies.

Ethanolic extraction of *Annona senegalensis*

The Soxhlet method of ethanol extraction was done according by (M.D Luque de Castro & L.E García-Ayuso, 1998), (Petrović *et al*., 2010).

Experimental animals:

Thirty albino rats of both sex (albino rats at 21 days were used in this study), their weight (100-220) g. They were kept under standard situations, in Medical and Aromatic Plants Institute (MAPI). They had unrestricted access to water and standard feed and were maintained under these conditions for a 15-day adaptation period.. The rats were allowed to adapt for a week, where they were located in cages and in groups, receipt water and feed Afterwards, the investigation was started, using a one month management interval

The basic dietary average: The experimental animals were feed basic dietary that achieved their prerequisite. The diet consisted of 692 g of wheat flour, 165 g of dried beef, and 3 g of sodium chloride.

Investigational method: Six groups of five rats each were created from the rats, in well ventilated cage with facility for food and water. Feed and water were given to the rats for the course of the 30-day duration. The names of these groupings were A, B, C, D, E, and F. As a negative control, Group **A** received the baseline diet (negative control). Group **B** received basal diet supplemented 1% egg yolk and 1% groundnuts oil and acted as a constructive check.(positive control)

Group **C** received basal diet supplemented 1% egg yolk and 1% groundnuts oil with Atorvastatin 0.4 mg/kg p.o. (per oral).

Group **D** received basal diet supplemented 1% egg yolk and 1% groundnuts oil, rats received a single oral dose of the ethanolic extract of *Annona senegalensis* pulp every day at a rate of 200 mg/kg for body weight,

Group **E** received basal diet supplemented 1% egg yolk and 1% groundnuts oil,rats were treated daily with a single oral doses (200 mg/kg for body weight) of the ethanolic extract of the crust of *Annona senegalensis* for each,

Whereas group **F** received basal diet supplemented 1% egg yolk and 1% groundnuts oil,rats were treated daily with a single oral doses (200 mg/kg for body weight) of the ethanolic extract of mixed portion of crust and pulp of *Annona senegalensis*for each,

Blood sampling:

Following an overnight fast, 1.5 ml of blood was drawn from the experimental animals using vessel tubes and placed in bottles. The blood was centrifuged for 10 minutes at 5000 rpm, and the plasma was then transferred to plane vessels and stored at -20 °C until analysis.

Determination of *Annona senegalensis* fruits pulp, seed and External crust chemical component:-

Moisture content: The AOAC (AOAC, 2005) method was used to measure the moisture content.

Ash content: The ash content of the samples was was assessed using the (AOAC, 2005).

Oil content %: The AOAC (AOAC, 2005) was used to measure the crude fat.

Protein content: The protein content of the samples was measured by the macro-kjedahl technique using the method of (AOAC, 2005).

Carbohydrates content %: The total carbohydrates was calculated by difference according to (AOAC, 2005).

Minerals content: Calcium, magnesium, sodium and potassium were measured using (AOAC, 2005), using a (Perkin Elmer, Model 2330) Atomic Absorption Spectrophotometer.

Crude fiber content (%): (AOAC, 2005) was used to measure the crude fiber content.

Antioxidant activity (DPPH radical-scavenging test):

The antioxidant capacity of the extracts was assessed by means of in vitro 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging radical method (Susanti *et al*., 2017).

Blood lipid profile:

All of the various lipid fractions were identified using Roche Diagnostics.

The process of the analyzer: Each parameter was measured using Hitachi sample cups filled with 0.5 cc of plasma. Following the determination of the parameters, the sample cups were placed in a precise location on the analyzer. were picked out and shown on the screen. The machine was then turned on, and within ten minutes, the findings were printed.

Statistical analyses:-

The analysis of variance (ANOVA; $P < 0.05$) and the SPSS statistical program for Windows (Release 8.0) were used to complete two statistical analysis methods.

RESULTS AND DISCUSSION

This comprehensive study provides valuable insights into the nutritional value, antioxidant potential, and antihyperlipidemic properties of different parts of *Annona senegalensis* fruit from Sudan. The findings demonstrate significant variation in bioactive components and biological activities between the pulp, seeds, and external crust, highlighting the importance of analyzing fruit components separately.

Figure 1 shows the chemical composition of *Annona senegalensis* fruits pulp, seed and external crust. The average value of moisture content 60.70 %, oil content 0.82%, ash content 3.56%, crude fiber 5.36%, crude protein; 6.196 % and carbohydrate 77.37 %, for

Annona senegalensis pulp. Higher and lower values for chemical composition of different varieties of Annona squamosa fruit pulp were reported by (Hassan *et al*., 2008), the pulp moisture content was 70 %, fat content 11.50 %, protein content 4.40%, fiber content 46.30% and carbohydrate content 30.33%.

These findings were comparable to results of (Villela *et al*., 2013) who stated that the average value of moisture content 78.92%, oil content 1.39%, ash content 1.59%, crude fiber 3.50%, crude protein 1.38 and carbohydrate 13.22, for pulp content of Annona crassi flora pulp and (Mohammed Adam Yahya Abdualrahman *et al*., 2019) stated that the Annona squamosal fruit pulp varieties grown in Sudan, average value of moisture content was 78.54%, ash content 2.84%, crude fiber5.90%, crude protein1.13%, fat content0.79% and carbohydrate 10.80 %.

As shown in figure 1the average value of *Annona senegalensis* seed content were 4.64% moisture content, 24.09% oil content, 1.30% ash content, 49.01% crude fiber, 6.19% crude protein and 0.5 % for carbohydrate content. These findings were comparable in some point to (Mohammed Adam Yahya Abdualrahman *et al*., 2019) who reported that the composition of Annona crassi floraseed were 6.65 % for moisture content, 5.24 % for ash content, 18.34 % for crude protein, 30.41%for crude fat, 17.56 % for crude fiber and 50.0 – 21.80 % for total carbohydrate. These outcomes also is similar to (Hassan *et al*., 2008), results who stated that (the composition of Annona squamosa seed of moisture content, ash content, crude fiber, crude protein, fat content and carbohydrate were 42.51%; 2.78%; 36.33%; 44.00 % and 12.45% respectively).As shown in figure 1 the average value of *Annona senegalensis* external crust or peel content were 6.49% moisture content, 2.21% oil content, 4.15% ash content, 36.61% crude fiber, 5.26% crude protein and 45.28% for carbohydrate content.

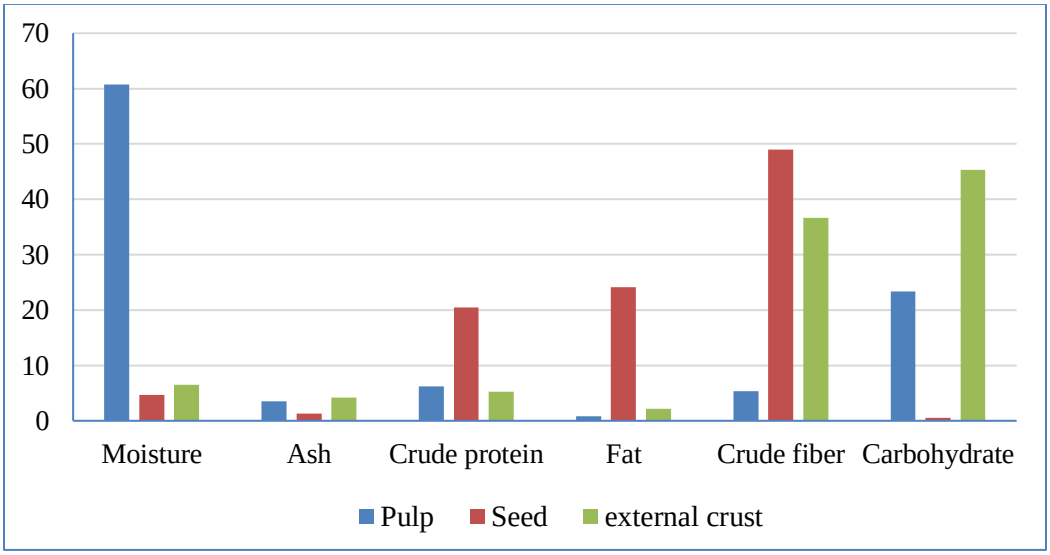

Figure 1 Chemical component of Annona senegalensis fruits pulp, seed and external crust

As shown in figure.2 the average value of minerals content of *Annona senegalensis* fruit pulp content was content of sodium (28.00 ppm), calcium (44.95 ppm), phosphorous (16.50 ppm), potassium (597.67ppm) and magnesium (459.83ppm). these findings were lower to those obtained by (Mohammed Adam Yahya Abdualrahman *et al*., 2019) who reported that the annona squamosa fruit pulp varieties grown in Sudan has content of sodium

(122.7 ppm), calcium (338.5 ppm), phosphorous (408.0 ppm), iron (7.70 ppm), copper (2.9ppm), zinc (3.7 ppm), potassium (3714.5 ppm) and magnesium (223.7 ppm). These results are also lower to those obtained by (Hassan *et al*., 2008), who reported that the Annona squamosal fruit pulp varieties of calcium (4500 ppm), phosphorous (268.8ppm), iron (17 ppm), copper (0.2ppm) , zinc (3ppm), potassium (450.0ppm) and magnesium (4000 ppm) as dry weight.

As shown in figure 2 the average value of minerals content of *Annona senegalensis* fruit seed content was content of sodium (29.63 ppm), calcium (73.70 ppm), phosphorous (21.50 ppm), potassium (181.00 ppm) and magnesium (467.83 ppm). These findings were to some extent similar with (Mohammed Adam Yahya Abdualrahman *et al*., 2019) who reported that the Annona squamosal fruit seed varieties grown in Sudan has content of sodium (282.7 ppm), calcium (1871.2ppm), phosphorous (327.5 ppm), iron (208.4ppm), copper (239.1ppm), zinc (221.7ppm), potassium (3558.4ppm) and magnesium (162.2ppm). These outcomes are also in agree with the values achieved by (Hassan *et al*., 2008), who reported that the Annona squamosal fruit pulp varieties of calcium (6500 ppm), phosphorous (210ppm), iron (20.5 ppm), copper (0.3ppm) , zinc (3.2ppm), potassium (220ppm) and magnesium (500 ppm) as dry weight.

As shown in figure 2 the average value of minerals content of external crust or peel content was content of sodium (39.58 ppm), calcium (67.40 ppm), phosphorous (19.00 ppm), potassium (758.83 ppm), magnesium (474.33ppm).

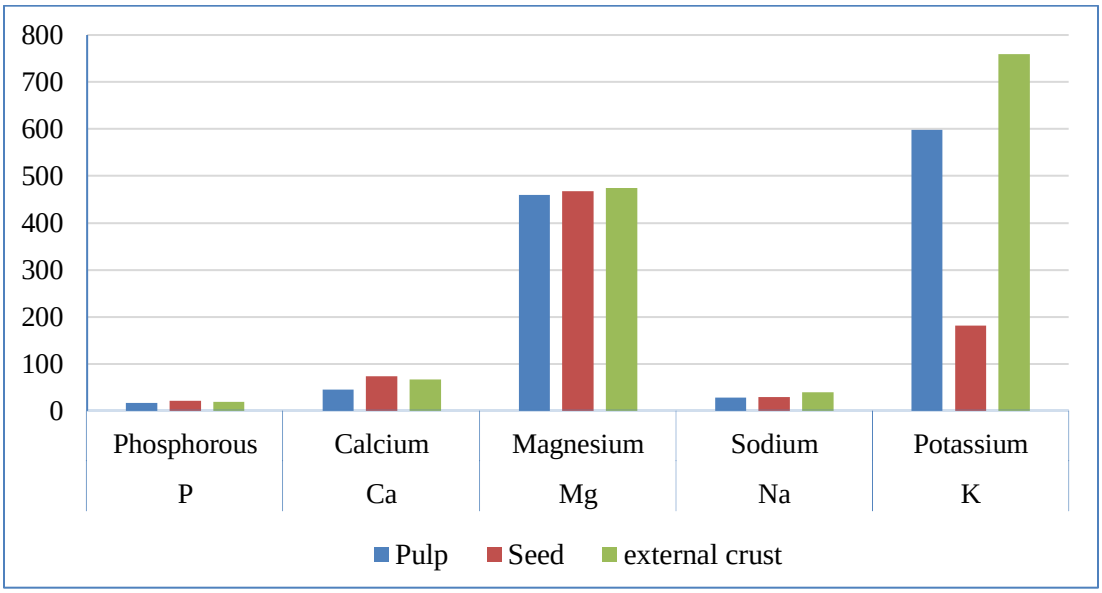

Figure 2 Mineral content of Annona senegalensis fruits pulp, seed and external crust (ppm)

Ethanol extracts of Pulp and external crust of *Annona senegalensis* were assessed for antioxidant activity 2,2-diphenyl-1-Picrylhydrazyl (DPPH)radicals were used. The obtained outcomes were compared with standard antioxidants agent propyl gallate (PG). DPPH test is based upon the capability of DPPH stable free radicals to be decolored from purple in the existence of antioxidants. It is lineal and dependable means of measurement of antioxidant activity. The DPPH radical comprises an odd electron, which is accountable for the absorbance at 517nm and for a visible deep purple color. Results are presented in figure (3). The remarkably high antioxidant activity observed in the external crust extract

(71.91%) compared to the pulp extract (22.89%) aligns with findings from other *Annona* species. For instance, (Agu & Okolie, 2017). reported similar patterns in *Annona muricata*, where the peel exhibited superior free radical scavenging capacity compared to the pulp, attributed to higher concentrations of phenolic compounds and flavonoids. This phenomenon can be explained by the protective function of fruit peels against environmental stressors, leading to accumulation of secondary metabolites with antioxidant properties (Hassan *et al*., 2008). The crust's robust antioxidant activity suggests its potential as a natural source of antioxidants for food preservation or nutraceutical application.

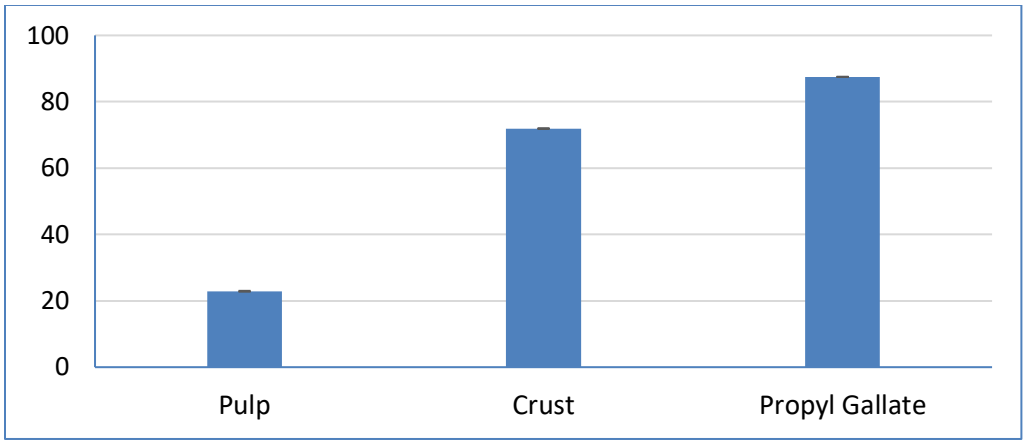

Figure 3 DPPH scavenging activity of ethanol extracts of Annona senegalensis Pulp and Crust.

The distinct nutritional profiles of different fruit parts offer insights for potential applications. The pulp's high carbohydrate content suggests its value as an energy source, while the seed's high oil and fiber content indicates potential for edible oil extraction or as a dietary fiber source. The crust, with its exceptional antioxidant activity, represents a promising ingredient for functional foods or nutraceuticals aimed at managing oxidative stress-related disorders

The impact of a high-cholesterol diet (Group B) for one month on the plasma lipid profile compared to the normal control group (Group A) is presented in Table 1. Values are expressed as mean ± standard deviation (SD; n=5). Mean LDL cholesterol levels in Group A showed no significant change ($P \geq 0.05$) from 47.20 ± 0.84 mg/dL at zero time to 46.60 ± 0.89 mg/dL after one month. Conversely, in Group B, LDL levels increased from 48.20 ± 1.30 mg/dL to 49.20 ± 2.86 mg/dL, although this change was not statistically significant ($P \geq 0.05$), as indicated by the shared superscript letters 'a' within the row for Group B in Table 1. There was a significant reduction ($P \leq 0.05$) in the high-density lipoprotein (HDL) in plasma of group (A) rats, from 39.00 to 36.60 mg/dl, therefore there were significant escalations ($P \leq 0.05$) of (HDL) in plasma of group B rats, from 46.00 to 61.20 mg/dl. There was a non-significant reduction ($P \leq 0.05$) in the total cholesterol in plasma of group (A) rats, from 47.20 to 46.60 mg/dl, therefore there was significant increase ($P \leq 0.05$) of total cholesterol in plasma of group B rats, from 50.60 to 67.60 mg/dl. There was a non-significant increase ($P \leq 0.05$) in the Triglyceride in plasma of group (A) rats, from 80.00 to 84.40 mg/dl, therefore there were significant decrease ($P \leq 0.05$) of Triglyceride in group B rats, from 35.00 to 24.00 mg/dl. The outcomes presented the impact of dietary intake with high cholesterol level (Group B) for one month on lipid

parameters compared with basal diet (Group A) is presented in Table 1. Group B showed a significant increase ($P < 0.05$) in total cholesterol (from 50.60 to 67.60 mg/dL) and HDL (from 46.00 to 61.00 mg/dL), while triglycerides significantly decreased (from 35.00 to 24.00 mg/dL). The LDL level showed a non-significant increase from 48.20 to 49.20 mg/dL. In contrast, Group A maintained relatively stable lipid levels throughout the study period, with no significant changes observed in any lipid parameters. These results indicate successful induction of hypercholesterolemia in Group B and are consistent with previous reports that high-fat diets increase total cholesterol and HDL while decreasing triglycerides. These outcomes were in in agree with the reports from other investigates that the management of high fat diets rise total cholesterol, LDL and HDL, but decrease Triglyceride (Hwang *et al*., 2001).

Table 1 The levels of triglyceride and total cholesterol, and its fraction, low density lipoprotein and high-density lipoprotein in serum group B compared to group Ain zero time and after one month after the induction of hypercholesterolemia.

Sample	LDL (mg/dL)		HDL(mg/dL)		Total Cholesterol		Triglyceride	
	Zero time	After one month	Zero time	After one month	Zero time	After one month	Zero time	After one month
A	47.20 ±0.84 [a]	46.60 ±0.89 [b]	39.00 ±13.29 [a]	36.60 ±13.59 [b]	47.20 ±10.28 [a]	46.60 ±10.74 [a]	80.00 ±14.78 [a]	84.40 ±23.59 [a]
B	48.20 ±1.30 [b]	49.20 ±2.86 [a]	46.00 ±12.10 [b]	61.00 ±25.37 [a]	50.60 ±14.47 [b]	67.60 ±50.30 [a]	35.00 ±13.84 [a]	24.00 ±11.36 [b]

*Means within the same row for each parameter bearing different superscript letters (a, b) are significantly different ($P \leq 0.05$) according to least significant test (LSD)
*Each value in the table is a mean ± SD (n=5)
Where
Group A = Negative control (Received the basal diet and served as control).
Group B = Positive control (Received supplemented 1% egg yolk and 1% groundnuts oil and served as positive control.).

Cholesterol is an amphipathic lipid present in tissues and plasma.Cholesterol levels play a central role in the genesis of atherosclerosis and coronary heart disease (Tanwi Priya *et al*., 2013). The results of plasma total cholesterol levels of group A, B, C, D, E and F rats were presented in Table 2. There was no significant difference ($P \leq 0.05$) in total cholesterol Mg/dl of the plasma of rat at binging of the study (zero time) from 38.40 to 50.60 Mg/dl.

There was significant variance ($P \leq 0.05$) in total cholesterol Mg/dl of the plasma of albino rat after month from the beginning of the study, level ranging from 67.60 to 30.75 Mg/dl. The level of plasma total cholesterol in group B (positive control) is significantly ($P< 0.05$) higher than the control group A (negative control). In group C (drug treated) the level of plasma total cholesterol is non-significantly lesser than group A, whereas it is significantly ($P<0.05$) higher associated to group D (pulp diet). Though, in group D (pulp diet) the level of plasma total cholesterol is non-significantly ($P<0.05$) less associated to group B, while group E (crust diet) and F (mixed diet) were non-significant with group A. There was non-significant difference ($P \leq 0.05$) in total cholesterol Mg/dl of the plasma of rat at binging of the study to the end after one month for groups A,C and D, (47.20 - 46.60), (38.40 - 38.20) and (44.20 - 50.60) respectively, though there were important variance ($P \leq 0.05$) in group B, E and F (50.60 to 67.60), (49.75 to 47.50) and (41.80 to 30.75) respectively, these difference may be due to change in pattern of feeding according to research plan.The outcomes presented that the plasma total cholesterol level decreasedin

group A, C, E and F from zero time to the end of study. Group F plasma cholesterol showed the highest significant (P< 0.05) decrease, from 41.80 in zero time to 30.75 mg/dl after one month, rats in this group treated orally with mixture of ethanolic extract of pulp and crust. Follow by group E, total plasma cholesterol of these rats show significant (P< 0.05) decrease from 49.75 in zero time to 47.50 mg/dl after one month, in the last came group D by significant (P< 0.05) increase from 44.20in zero time to 50.60mg/dl after one month. These result revel that the Mixture of ethanolic extract of pulp and crust group F has highest hypo cholesterolemic effect among other group, these outcomes similar to (Adarsh M Verma *et al*., 2011) who stated that methanolic extract of Annona cherimolafruitswas evaluated for its anti-hyper lipid emicpotenti allowered total cholesterol, triglyceride and LDL-cholesterol levels.
However cholesterol level in group D and E shows significant decrease (P< 0.05)44.20 to 50.60 and 49.75 to 47.50 respectively.

Table 2 The effect of feeding ethanol extracts of Annona senegalensis pulp and external crust on the Total cholesterol level in serumofan induced hypercholesterolemia albino rats

Sample	Total Cholesterol	
	Zero time	After one month
A	47.20 ± 10.28^{aa}	46.60 ± 10.74^{ba}
B	50.60 ± 14.47^{ab}	67.60 ± 50.30^{aa}
C	38.40 ± 5.32^{aa}	38.20 ± 19.77^{ba}
D	44.20 ± 8.14^{aa}	50.60 ± 16.02^{ab}
E	49.75 ± 7.63^{aa}	47.50 ± 14.99^{bb}
F	41.80 ± 6.98^{aa}	30.75 ± 5.54^{bb}
$Lsd_{0.05}$	23.40*	
SE±	8.231	

*Means within the same row for each parameter bearing different superscript letters (a, b) are significantly different ($P \leq 0.05$) according to least significant test (LSD)
*Where the first letter for the feeding rats differences diets effect in the same column, and the second letter for the period of study (one month) effect in the same row.
*Each value in the table is a mean ± SD (n=5)
Where
Group A = Negative control (Receipt the basal diet and aided as control).
Group B = Positive control (Receipt supplemented 1% egg yolk and 1% groundnuts oil).
Group C = Positive control + Drug (Atorvastatin0.4 mg/kg p.o.)
Group D = Positive control + Ethanolic extract of the pulp.
Group E = Positive control + Ethanolic extract of the external crust
Group F = Positive control + Mixture of Ethanolic extract of pulp and crust.

LDL is an important groups of lipoproteins which are important physiologically and in medical finding. (Vasudevan *et al*., 2013). The results of serum low density lipoprotein cholesterol (LDL) levels of group A, B, C, D, E and F were obtainable in Table 3. There was important difference ($P \leq 0.05$) in LDL Mg/dl of the plasma of rats at binging of the study (zero time) were level ranging from 47.20 to 53.80 Mg/dl. There significant increase level of plasma of LDL in group C, E and F from another group. There was significant variance ($P \leq 0.05$) in plasma LDL Mg/dl of the plasma of albino rat after month from the beginning of the study, level ranging from 45.95 to 49.20Mg/dl. The level of plasma LDL in group E (crust diet) is significantly (P< 0.05) lower than the other groups.

There was non-significance reduction ($P \leq 0.05$) in plasma LDL Mg/dl of the plasma of albino rat at binging of the study to the end after one month for groups A and D,

(47.20 - 46.60) and (49.40 - 49.00) respectively, however there were vital variance ($P \leq 0.05$) in group B, C, E and F (48.20 to 49.20), (53.80 to 48.00) and (50.50 to 45.95) and (51.80 to 48.00) respectively, these difference may be due to change in pattern of feeding according to research plan.In this studythe levels of LDL shadowed the similar style (significant decrease) of the serum totalcholesterol levels in these treated groups except group D (pulp diet), which lead to a significant ($P<0.05$) decrease at the end of the study (one month) from the control group (positive control) who show significant increase ($P<0.05$), while in the group A (negative control) there was a significant decrease.

This outcome presented that, the raise of serum LDL cholesterolconcentration has a optimistic association with the raise of serum totalcholesterol attentiveness this propose that, the rise of serum totalcholesterol, is payable to rise of the serum LDL cholesterol, these result in agreement with Giscuolo (1994) who reported that the Dietary cholesterolelevates serum cholesterol and LDL cholesterol levels, but the extent ofincrease is extremely adjustable.In the current study feeding orally of Ethanolic extract of annonasenegalensis pulp (D), external crust (E) and mixture of both (F) and significant reduction ($P \leq 0.05$) LDL levels in these groups (49.49 - 49.00), (50.50 - 45.50) and (51.80 - 48.00) Mg/dl respectively.These result revel that theethanolic extract of crust, group E has highest hypocholesterolemic effect among other group, these results is similar to (Adarsh M Verma *et al*., 2011) who state that the methanolic extract of Annona cherimola have pointedly reduced the lipid points excepting HDL-cholesterolm that may be due to themethanolic extract may affect any phase in the cholesterol biogenesis.

(Miettinen, 2001) Reported that, dietary control,especially with plant stanol ester, which inhibits cholesterolabsorption, improves the fatty acid pattern, lower LDL–cholesterolsufficiently in many hypercholesterolemic patients, and is a usefuladjunct to pharmacological therapy.

Table 3 The effect of feeding ethanol extracts of Annona senegalensis pulp and external crust on the low-density lipoprotein cholesterol (LDL) level in serum of a stimulated hypercholesterolemia albino rats

Sample	LDL	
	Zero time	**After one month**
A	47.20 ± 0.84^{ba}	46.60 ± 0.89^{aa}
B	48.20 ± 1.30^{bb}	49.20 ± 2.86^{aa}
C	$53.80^{aa} \pm 3.77^{aa}$	48.00 ± 1.00^{ab}
D	49.40 ± 2.88^{ba}	49.00 ± 2.12^{aa}
E	50.50 ± 2.69^{aa}	45.95 ± 2.05^{bb}
F	51.80 ± 5.36^{aa}	48.00 ± 5.05^{ab}
$Lsd_{0.05}$	3.762^{*}	
SE±	1.323	

*Means within the same row for each parameter bearing different superscript letters (a, b) are significantly different ($P \leq 0.05$) according to least significant test (LSD)

*Where the first letter for the feeding rats differences diets effect in the same column, and the second letter for the period of study (one month) effect in the same row.

*Each value in the table is a mean ± SD (n=5)

Where

Group A = Negative control (Receiving the basic dietary and provide as control).

Group B = Positive control (Receiving supplemented 1% egg yolk and 1% groundnuts oil).

Group C = Optimistic control + Drug (Atorvastatin0.4 mg/kg p.o.)

Group D = Optimistic control + Ethanolic extract of the pulp.

Group E = Optimistic control + Ethanolic extract of the external crust

Group F = Optimistic control + Mixture of Ethanolic extract of pulp and crust.

HDL is the main transference form of cholesterol from tissue to liver which is later defaecated throughout the bile. The level of HDL in serum is inversely associated to the prevalence of coronary infarction HDL is known as (good cholesterol), (Tanwi Priya *et al*., 2013).

The outcomes of serum High Density Lipoprotein cholesterol (HDL) levels of group A, B, C, D, E and F were obtainable in Table 4.There was no significant difference ($P \leq 0.05$) in high density lipoprotein (HDL) Mg/dl of the plasma of albino rat at binging of the study (zero time) from 35.00 to 46.00 Mg/dl. There was significant difference ($P \leq 0.05$) in plasma HDL Mg/dl of the albino rat after month from the beginning of the study, level ranging from 61.00 to 36.60Mg/dl. The level of plasma HDL in group B (positive control) and E(crust diet) is significantly ($P< 0.05$) increased than the other groups, 61.00, 65.00Mg/dl respectively, andthe group F (mixed diet) record the lowest reading36.75 Mg/dl.There was non-significant difference ($P \leq 0.05$) in plasma HDL Mg/dl of the plasma of albino rat at binging of the study to the end after one month in groups A, C, D and F (39.00- 36.60), (42.20 - 49.40) (39.20- 44.80) and (35.00 - 36.75) respectively, however there were significant hifher ($P \leq 0.05$) in group B (positive control) and E (crust diet), (46.00 – 61.00) and (40.50 to 65.00) respectively, these difference may be due to change in pattern of feeding according to research plan.

In the current study feeding orally of Ethanolic extract of *Annona senegalensis* pulp (D), external crust (E) and mixture of both (F) toan induced hyper cholesterolemic albino rats caused asignificant increase ($P \leq 0.05$) in HDL levels (39 - 44.80), (40.50 - 65.00) and (35.00 - 36.75) Mg/dl respectively.These finding were comparable to results of (Adarsh M Verma *et al*., 2011) who state that the methanolic extract of Annona cherimola have significantly reduced the lipid levels excepting HDL-cholesterol levels by 9% and 23.20% at 6 and 24 hours conduct. In the present study, feeding of Ethanolic extract of *Annona senegalensis* resulted in obvious increase of HDL and decease of LDL, there is clear reversible relation between the level of HDL and LDL, these fact was reporting in many studies,(Stark *et al*., 2000) proposed that nourishing of 5% fish oil to rats lead to in higher HDL levels payable to the shyness of apo D action which is accountable for the transporting of cholesteryl ester (CE) into VLDL.(Murugaiah *et al*., 1999) Recommended that mingling of 35 and 70 mg of ginger with rats high dietary lipids rising HDL level significantly, this is may be due to the reduced production of VLDL. (Ballard *et al*., 2004; Trandafir *et al*., 2023; Yance, 2004) proposed that nourishing of 5 % flax seeds to rats raised HDL levels and reduced the production of VLDL.

There is a reverse association among plasma HDL-cholesterol level and cardio vascular disease (Jahromi *et al*., 1993). It was also stated that HDL is the key constituent in reverse cholesterol transportation, which eliminates surplus cholesterol from exterior tissues for secretion through the liver (Vedhachalam *et al*., 2007). At the same time, rise in LDL-cholesterol and raised hazard of the development of coronary disease. Thus the required therapeutic nutrition plan to cured hyperlipidemia must raise HDL-cholesterol at the same time should reduction LDL-cholesterol levels concurrently.

Table 4 The effect of feeding ethanol extracts of Annona senegalensis pulp and external crust on the HDL level in a serum of induced hypercholesterolemia albino rats

Sample	HDL	
	Zero time	After one month
A	39.00 ± 13.29 ^{aa}	36.60 ± 13.59 ^{ba}
B	46.00 ± 12.10 ^{ab}	61.00 ± 25.37 ^{aa}
C	42.20 ± 10.92 ^{aa}	49.40 ± 14.48 ^{ba}
D	39.20 ± 7.66 ^{aa}	44.80 ± 13.52 ^{ba}
E	40.50 ± 13.44 ^{ab}	65.00 ± 6.44 ^{aa}
F	35.00 ± 4.64 ^{aa}	36.75 ± 9.01 ^{ca}
$Lsd_{0.05}$	16.16*	
SE±	5.84	

*Means within the same row for each parameter bearing different superscript letters (a, b) are significantly different ($P \leq 0.05$) according to least significant test (LSD)
*Where the first letter for the feeding rats differences diets effect in the same column, and the second letter for the period of study (one month) effect in the same row.
*Each value in the table is a mean ± SD (n=5)
Where
Group A = Negative control (Received the basal diet and served as control).
Group B = Positive control (Received supplemented 1% egg yolk and 1% groundnuts oil).
Group C = Positive control + Drug (Atorvastatin0.4 mg/kg p.o.)
Group D = Positive control + Ethanolic extract of the pulp.
Group E = Positive control + Ethanolic extract of the external crust
Group F = Positive control + Mixture of Ethanolic extract of pulp and crust.

Triglycerides are the main formula of lipid in the animal body, and has a position in lipid transference and storing body fat, and in many illnesses such as overweightness and diabetes mellitus, but it is reduced associated to cardiovascular disease than cholesterol (Murray *et al*., 2003).The results of serum triglyceride levels of group A, B, C, D, E and F were obtainable in Table 5.There was no significant difference ($P \leq 0.05$) in triglyceride Mg/dl of the plasma of albino rat at binging of the study (zero time) from 80.00 to 35.00 Mg/dl.There was important difference ($P \leq 0.05$) in plasma triglyceride Mg/dl of the albino rat after month from the beginning of the study, level ranging from 84.40 to 24.00Mg/dl. The level of plasma triglyceridein group A (negative control), C(drug treated) and D (pulp diet) is significantly ($P< 0.05$) raised than in other groups, 84.40, 51.80 and 61.20 Mg/dl respectively, and the group B (positive control) record the lowest reading24.00Mg/dl.

There was non-significant difference ($P \leq 0.05$) in plasma triglycerideMg/dl of the plasma of albino rat at binging of the study to the end after one month in groups A, D and E (80.00 - 84.40), (64.20 - 61.20) and (58.50 - 54.25) respectively, however there were significant decrease ($P \leq 0.05$) in group B, C and F (35.00 - 24.00), (64.60- 51.80) and (60.40 - 45.25) respectively, these difference may be due to change in pattern of feeding according to research plan.In the current research feeding orally of Ethanolic extract of *Annona senegalensis* pulp (D), external crust (E) and mixture of both (F) to an persuaded hyper cholesterolemic albino rats produced non-significant decrease ($P \leq 0.05$) in triglyceride levels (64.20 – 61.20), (58.50 – 54.25) and (60.40 – 45.25) Mg/dl respectively, While in group A (positive control) there were a non-significant increase (80.00 -84.40) Mg/dl. These outcomes are reliable with those of (Adarsh M Verma *et al*., 2011) who reported that methanolic extract reduced total cholesterol, triglyceride and LDL-cholesterol levels.

The relationship between the Triglycerides and HDL-Cholesterol in the simple percentage termed Atherogenic Index of Plasma (AIP) theoretically reflects the balance between hazard and protecting lipoprotein forces. It was already stated that AIP was a strong forecaster of myocardial infarction (Gaziano *et al*., 1997).

Table 5 The effect of feeding ethanol extracts of Annona senegalensis pulp and external crust on the triglyceride level in serum of an induced hypercholesterolemia albino rats

Sample	Triglyceride	
	Zero time	After one month
A	80.00 ± 14.78^{aa}	84.40 ± 23.59^{aa}
B	35.00 ± 13.84^{aa}	24.00 ± 11.36^{bb}
C	64.60 ± 21.54^{aa}	51.80 ± 29.37^{ab}
D	64.20 ± 20.46^{aa}	61.20 ± 29.76^{aa}
E	58.50 ± 30.84^{aa}	54.25 ± 24.96^{ba}
F	60.40 ± 7.50^{aa}	45.25 ± 9.55^{bb}
$Lsd_{0.05}$	27.10^{**}	
SE±	9.529	

*Means within the same row for each parameter bearing different superscript letters (a, b) are significantly different ($P \leq 0.05$) according to least significant test (LSD)
*Where the first letter for the feeding rats differences diets effect in the same column, and the second letter for the period of study (one month) effect in the same row.
*Each value in the table is a mean ± SD (n=5)
Where
Group A = Negative control (Receiving the basic dietary and served as control).
Group B = Positive control (Receiving supplemented 1% egg yolk and 1% groundnuts oil).
Group C = Positive control + Drug (Atorvastatin0.4 mg/kg p.o.)
Group D = Positive control + Ethanolic extract of the pulp.
Group E = Positive control + Ethanolic extract of the external crust
Group F = Positive control + Mixture of Ethanolic extract of pulp and crust.

The significant modulation of lipid parameters observed in hypercholesterolemic rats treated with *A. senegalensis* extracts, particularly the mixed pulp-crust extract (Group F) and crust extract (Group E), represents a noteworthy finding. The mixed extract's potent hypocholesterolemic effect, reducing TC and TG levels more effectively than the standard drug atorvastatin in some parameters, suggests a synergistic interaction between compounds present in both pulp and crust. These lipid-lowering effects may be mediated through multiple mechanisms. The high antioxidant capacity likely reduces oxidative stress, which is known to exacerbate hyperlipidemia and atherosclerosis (Jahromi *et al*., 1993)Furthermore, bioactive compounds in *Annona* species, particularly acetogenins and alkaloids, have been reported to inhibit HMG-CoA reductase activity (the rate-limiting enzyme in cholesterol biosynthesis) and interfere with cholesterol absorption in the intestine (Adarsh M Verma *et al*., 2011). The significant increase in HDL levels induced by the crust extract is particularly promising, as HDL plays crucial roles in reverse cholesterol transport and cardiovascular protection (Vedhachalam *et al*., 2007). Our results corroborate findings from other studies on *Annona* species. (Adarsh M Verma *et al*., 2011) demonstrated that methanolic extract of *Annona cherimola* significantly reduced total cholesterol, triglyceride, and LDL-cholesterol levels in hyperlipidemic models. Similarly, (Stark *et al*., 2000) reported that natural antioxidants from plant sources can effectively improve lipid profiles through reduction of oxidative stress.

CONCLUSION

This study demonstrates that Sudanese *A. senegalensis* fruits, was found rich in carbohydrate, and mineral. Particularly the external crust, possess substantial antioxidant and antihyperlipidemic properties. Administration of *Annona senegalensis* extract orally to an induced hyper cholesterolemic rat's decrease total cholesterol numerically. Administration of *Annona senegalensis* extracts orally to a persuaded hyper cholesterolemic rat had important rise effect in HDL cholesterol level. *Annona senegalensis* extracts caused numerical decrease in plasma LDL-cholesterol levels. Administration of *Annona senegalensis* extracts orally to an induce hyper cholesterolemic rat had significant decrease effect in triglycerides. The findings support the traditional use of this plant and suggest its potential as a source of nutraceuticals for managing hyperlipidemia and related metabolic disorders. The variation in bioactivity between different fruit parts underscores the importance of utilizing specific components for targeted applications in functional food and pharmaceutical development.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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