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Research Article

ANTIOXIDANT AND ANTI-LIPIDEMIC EFFECT OF CITRULLUS LANATUS PULP ON CALCIUM CARBIDE INDUCE TOXICITY IN MALE WISTAR RAT MODEL

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Abstract

Calcium carbide, a fruit-ripening agent most used in this part of the globe, is made available because of its low cost and usage, which is more convenient to vendors. It is known that cell death results from an imbalance between oxidant and antioxidant defense system, which links with lipid peroxidation and leads to dyslipidemia. Twenty-(20) male Wistar rats weighing 140-200g were used for the study and subdivided into four groups of five animals each. Group A received feed and distilled water only, group B received 100mg/kg of Calcium carbide only and left untreated, group C received 100mg/kg and treated with the 100mg/kg of ECLS. Group D received 100mg/kg and treated with 500mg/kg of ECLS through oral gavage for 21 days. Data were obtained for total cholesterol, Triglyceride, High-density lipoprotein, low-density lipoprotein, Superoxide-dismutase, Catalase, and glutathione reductase, Glutathione Peroxidase, and Total antioxidant capacity were subjected to SPSS version 25 (IBM, USA, 2018). ANOVA was used to analyzed the data followed by post HOC Turkey HSD, and values were considered significant at $p<0.05$. The results showed that Calcium carbide caused a significant ($p<0.05$) decrease in Superoxide-dismutase, Catalase, Glutathione reductase, Glutathione Peroxidase, and Total antioxidant capacity compared to group A, while treatment with ECLS raised the antioxidants levels significantly as observed in group C and D. Calcium carbide had a non-significant ($p>0.05$) decrease in total cholesterol, Triglyceride, High-density lipoprotein, and Low-density lipoprotein; treatments with the ECLS revealed a significant ($p<0.05$) higher level of triglyceride at group D, High-density lipoprotein had a significant ($p<0.05$) higher level at group C, and low-density lipoprotein showed a significant ($p<0.05$) higher level at group C and D. In conclusion, the ethanolic extract of *Citrullus lanatus* possesses antioxidant activities and anti-lipidemic activity based on dose-dependent.

Keywords: Calcium carbide, *Citrullus lanatus*, Anti-oxidants, oxidative stress, Dyslipidemia.

INTRODUCTION

Calcium carbide (CaC2) is a common hazardous chemical often used by local fruit vendors globally and in Nigeria to artificially stimulate the fruit ripening process (1,2). This chemical may pose a great deal of risk to human health when consumed, according to health organization (3). However, despite this warning, this chemical is continuously utilized partly due to its cheap nature, availability and continuous demand for fruits in the market (4). The CaC2 production is in different sizes and can result in physiological and biochemical dysfunction of human health if ingested (5,6). Its colourless, whitish-grey to black powder or crystal react with water to form acetylene gas (7). The CaC2 is commonly used in enhancement fruit ripening, desulfurization of hot metal and welding (8). Calcium carbide reacts with water to form acetylene gas (9,10). Acetylene gas is an analogue of ethylene (C2H2), a natural plant hormone that triggers the fruit ripening process (11). Dyslipidemia and oxidative interruption of calcium carbide may lead to serious health hazards (12). It ranges from headache, nausea, vomiting, dizziness, mood disturbances, insomnia (13), mental confusion, memory loss, cerebral oedema and seizures (14). A report revealed that Calcium carbide exposure is linked with severe diseases, and its usage in fruit ripening should be prohibited(3). Lipid peroxidation is associated with oxidative stress resulting in the excess production of reactive oxygen species (ROS) and their reactive intermediates (15).

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However, its onset has led to metabolic dysfunction, which causes increase oxidation of Low-density lipoprotein and inactivation of nitric oxide in the arterial wall leading to atherosclerosis and other cardiovascular risks (16). Oxidative stress is involved in the progress of metabolic syndromes, obesity, and vascular diseases resulting from reactive oxygen species generation from environmental toxicant (17). Antioxidants are substances at low concentrations that bring about a delay in the oxidation of a substrate. It acts through numerous chemical signalling processes to elicit their diverse actions; such process includes hydrogen atom transfer, single electron transfer, and the ability to chelate transition metals (18). Antioxidant capacity measures the amount of a certain free radical captured by an antioxidant sample (19); however, it is a significant scavenger present in food contributes to health promotion by numerous dietary supplements, nutraceuticals and functional food ingredients. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are endogenous antioxidant systems with the potency of regulating the damage of enzymatic and non-enzymatic natures that allow ROS to be inactivated (15,20). Watermelon (*Citrullus lanatus*) is a member of the Cucurbitaceae family (21). It is a juicy fruit with green bark and red to pink flesh with numerous seeds embedded. It has 92% of water constituents (22) and is a rich source of vitamins, antioxidants and essential minerals (23). Some of these bioactive constituents include vitamin B6, niacin, folate, riboflavin, thiamin, pantothenic acid, lycopene, carotene and β-carotene, choline, magnesium, selenium, phosphorus, copper, zinc, manganese (24,25). Watermelons intake reduces the risk of

certain disorders like obesity due to low calories, heart diseases and diabetes (26), which has a link with dyslipidemia and the generation of reactive oxygen species. These protective effects may be due to its vasodilatory, antioxidant, analgesic and anti-inflammatory properties (27,28). *Citrullus lanatus* is a cheap natural source of high lycopene and citrulline, essential phytonutrients for cardiovascular health (29). Citrulline is a vital non-protein amino acid in the biosynthesis of arginine and nitric oxide (30); clear evidence exists on the increased synthesis of nitric oxide following acute ingestion of synthetic L-Citrulline or natural from watermelon (31,32). The significant lycopene inducer of the antioxidant enzymes; glutathione peroxidase (GSH-Px), SOD, reduced glutathione (GSH), thereby reducing the levels of lipid peroxidation in disease cases (33). However, an antioxidant-rich beverage like watermelon is essential in protecting the body against oxidative stress (34). Further, there is a limited study revealing the antioxidant and anti-lipidemic activity of *C. lanatus* on calcium carbide induced toxicity; therefore, this study tends to investigate the antioxidant and anti-lipidemic activity of *C. lanatus* in an experimental model.

MATERIALS AND METHODS

Location of the Study: This study was carried out in the Department of Physiology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. Ethical approval consent was obtained for the progress of this study from the Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. Rats handling and treatments conform to guidelines of the National Institute of Health (NIH publication 85-23, (1985) for laboratory animal care and use.

Materials: Twenty-(20) inbreed male Wistar rats, *Citrullus lanatus* (Watermelon pulp), Absolute Ethanol (J.H.D. Chemicals, Guangdong China), distilled water, Whatman qualitative filter paper no. 1, oral cannula, Automatic Water distiller (SZ-1 Search Tech Instrument), Randox reagent kit (Sigma Aldrich, U.S.A.), (Olympus XSZ-107BN), Rotary evaporator (Digital) TT-52 (Techmel & Techmel, U.S.A.), Thermostat Oven (DHG-9023A, PEC MEDICAL USA), UV-VIS 752N Spectrophotometer (Shanghai, Yoke Instrument Co., Ltd. China), chloroform (Guangdong Guandgua Chemical Factory Co. Ltd. Shatou, Guondghuo, China), non-heparinized capillary tube, Electronic weighing balance (M-Metallar M311), 2ml hypodermic sterile syringe, animal weighing balance (Camry LB11), Normal laboratory chow (Standard Pellet), and standard cage.

Plant Identification of *Citrullus lanatus* pulp: Samples of watermelon bought from Nnwko market, Nnewi, Anambra State. It was identified at the Department of Botany, with the herbarium NAU/1025 deposited in their herbarium catalogue.

Preparation of ethanolic extract of *Citrullus lanatus* pulp: The watermelon (*Citrullus lanatus* pulp) were bought from Nnwko market, Nnewi, Anambra State, and was washed in running tap water to remove dirt and air-dried under ambient temperature. The dried *Citrullus lanatus* pulp was milled into coarse form using a local grinder. 250g of the dried coarse form of *Citrullus lanatus* pulp was macerated in 1000mls of 95% absolute ethanol for 48hours. It was then filtered using a clean handkerchief and further filtration using Whatman No 1 filter paper. The filtrate was concentrated using a rotatory evaporator (TT-52 Techmel & Techmel U.S.A.) and was further dried using a Thermostat oven (DHG-9023A Pec Medicals U.S.A.) at 45oc into a gel-like form. The extract was preserved in a refrigerator for further usage. The extract was done as described by Al-Attar and Abu-Zeid(35)with modifications.

Experimental Animals: Twenty-(20) inbreed male Wistar rats weighing 140-200 gram were used for the study. The experimental animals were housed in the animal house Department of Physiology, College of Health Sciences, N.A.U. The animals were maintained in standard cages at ambient temperature with standard pellet and distilled water *ad libitum*, and acclimatization was done for two weeks before commencement of administration of the extract. Animals were maintained under 12 hours' light and dark cycles.

Experimental Design: Group A received feed and distilled water only, group B received 100mg/kg Calcium carbide only, Group C (received 100mg/kg Calcium Carbide for seven days and treated with 100mg/kg of E.C.L.S.), and group D received (received 100mg/kg Calcium Carbide for seven days and treated with 500mg/kg of E.C.L.S.). The administration of the extract (E.C.L.S.) lasted for 21 days and was given through oral gavage.

Acute Toxicity of *Citrullus lanatus* pulp: The Ld50 of ethanolic extract of *Citrullus lanatus* pulp were done according to the method described by Dietrich Lorke (1983)(36), and it is divided into two-phase consisting of 13 animals. Phase 1 had three animals in three groups, and phase two had one animal each into four groups. The result of the Ld50 revealed that the ethanolic extract of *Citrullus lanatus* pulp was higher than 5000mg/kg, which safe for consumption.

Sample collection: Animals were anaesthetized with chloroform in an enclosed container 24 hours after the last administered dose of the E.C.L.S. Blood was collected using a heparinized capillary tube and put in both plain and EDTA tubes (Parasuraman, Raveendran, & Kesavan, 2010)(37), and centrifuge using a centrifuge (England) for 10minutes at 3000 RPM. The retrieved serum was used to assayed Lipid profile (Total cholesterol, Triglyceride, High-density lipoprotein, and Low-density lipoprotein) and serum antioxidants (S.O.D., C.A.T., G.S.H., GPx, and T.A.C.).

Catalase (C.A.T.) activity was assayed by measuring the degradation rate of H2O2 using Beutler's method. The rate of disappearance of H2O2 was monitored spectrophotometrically at 230 nm. The assay medium consisted of 50 µl 1 M Tris HCI buffer (pH 8), 930 µl 10 mM H2O2, 930 µl deionized water, and 20 µl serum sample. One unit of C.A.T. activity is defined as the amount of enzyme causing about 90% destruction of the substrate in 1 minute in a volume of 1 ml. C.A.T. activity in the serum was expressed as U/ml (38).

Superoxide-dismutase (S.O.D.) activities were determined as described by Beyer and Fridovich. The method employs xanthine and xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitro phenol-s-phenyl tetrazolium chloride) to form a red formazan dye. The S.O.D activity was measured by the degree of inhibition of this reaction(39).

Glutathione reductase (G.S.H.) activity was determined by Fitri method. A total of 200µL of standards and samples were

added to the cuvettes. Then 200µL of chromogen was added to each cuvette, and 200µL of the enzymes was added to each of the cuvettes, mixed, and then incubated at room temperature for 5 minutes. A total of 200µL of NADPH was added to each cuvette. Changes in absorbance at 412nm for 3 minutes were recorded and observed(40).

Glutathione Peroxidase (GPx) activity was determined by the method of Rush and Sandiford (41). 0.2ml each of EDTA, sodium azide, G.S.H., H202, serum sample were mixed and incubated at 37°C for 10-minutes. The addition of 0.5ml of T.C.A. arrested the reaction, and tubes were centrifuged. To 0.5ml of supernatant, 3ml of phosphate solution, and 1 ml of D.T.N.B. were added and the colour developed was read at 420nm immediately using a spectrophotometer. GPx activity was expressed U/ml.

Total antioxidant capacity (T.A.C.) activity was estimated according to the method described by Rubio method (42).

High-Density Lipoprotein & Total Cholesterol

Total cholesterol and High-density lipoprotein-cholesterol (HDL-c) were determined using diagnostic reagent kits according to the method described by Farombi and Ige method (43). Low-density lipoprotein-cholesterol (LDL-c) and triglyceride were determined using the method described by Wu method (44).

Data analysis

Data obtained were subjected to S.P.S.S. version 25 (I.B.M., U.S.A., 2018). ANOVA was used to analyzed the lipid profile (total cholesterol, Triglyceride, High-density lipoprotein, and Low-density lipoprotein) and serum S.O.D., C.A.T., G.S.H., GPx, and T.A.C. followed by multiple comparisons using Turkey HSD. Values were presented as MEAN±STD, and data were considered significant at $p<0.05$.

RESULTS

Table 1 effect of Ethanolic extract of *Citrulus lanatus* on calcium carbide induced oxidative stress on serum catalase and total antioxidant capacity activity

Groups	Catalase (U/ml)	Total antioxidant capacity (U/ml)
	MEAN±STD	MEAN±STD
Group A (Control)	72.79±2.56***	837.76±2.57***
Group B (Calcium Carbide Only)	42.67±2.56	329.67±15.58
Group C (Calcium Carbide + 100mg/kg of E.C.L.S)	65.52±3.41 ***	816.58±1.53 ***
Group D (Calcium Carbide + 500mg/kg of E.C.L.S)	57.54±0.91**	771.59±15.15***

Data was analyzed using ANOVA followed by Post-Hoc Turkey HSD and values were considered significant at $p<0.05$. $p<0.001$***, $p<0.01$**, and $p<0.05$* Ethanolic extract of *Citrulus lanatus*; ECLS

Table 1 result revealed a significant ($p<0.05$) increase in CAT and TAC activity in group C and D, as compared to group B, while group B had a significant decrease ($p<0.05$) as compared to group A.

Table 2 effect of Ethanolic extract of *Citrullus lanatus* on calcium carbide induced oxidative stress on serum glutathione, superoxide-dismutase, and glutathione peroxidase activity

Groups	Glutathione reductase (U/ml)	Superoxide-dismutase (U/ml)	Glutathione Peroxidase (U/ml)
	MEAN±STD	MEAN±STD	
Group A (Control)	13.62±0.06***	17.46±0.36***	0.92±0.01***
Group B (Calcium Carbide Only)	6.08±0.07	9.03±0.01	0.57±0.01
Group C (Calcium Carbide + 100mg/kg of E.C.L.S)	11.16±0.05***	16.03±0.02**	0.86±0.02**
Group D (Calcium Carbide + 500mg/kg of E.C.L.S)	8.13±0.13 ***	9.80±0.50***	0.75±0.03***

Data was analyzed using ANOVA followed by Post-Hoc Turkey HSD and values were considered significant at $p<0.05$. $p<0.001$***, $p<0.01$**, and $p<0.05$* Ethanolic extract of *Citrullus lanatus*; ECLS

Table 2 result revealed a significant ($p<0.05$) increase in Glutathione reductase, Superoxide-dismutase, and Glutathione Peroxidase activity in group C and D, as compared to group B, while group B had a significant decrease ($p<0.05$) as compared to group A.

Table 3 effect of Ethanolic extract of *Citrullus lanatus* on calcium carbide induced lipotoxicity on total cholesterol and triglyceride activity

Groups	Total cholesterol (mmol/L)	Triglyceride (mmol/L)
	MEAN±STD	MEAN±STD
Group A (Control)	281.94±7.23^{NS}	36.72±18.78^{NS}
Group B (Calcium Carbide Only)	248.51±0.90	25.23±2.81
Group C (Calcium Carbide + 100mg/kg of E.C.L.S)	281.04±35.25^{NS}	42.33±15.42^{NS}
Group D (Calcium Carbide + 500mg/kg of E.C.L.S)	260.67±2.23^{NS}	57.75±20.75*

Data was analyzed using ANOVA followed by Post-Hoc Turkey HSD and values were considered significant at $p<0.05$. $p<0.001$***, $p<0.01$**, and $p<0.05$* Ethanolic extract of *Citrullus lanatus*; ECLS

Table 3 result revealed a non-significant ($p>0.05$) increase in-group C and D in total cholesterol level when compared to group B, while group B had a non-significant decrease ($p>0.05$) as compared to group A. Triglyceride result revealed a non-significant ($p>0.05$) increase in-group C; group D had a significant ($p<0.05$) increase as compared to group B, while group B had a non-significant ($p>0.05$) decrease as compared to group A.

Table 4 effect of Ethanolic extract of *Citrullus lanatus* on calcium carbide induced lipotoxicity on High-density lipoprotein and Low-density lipoprotein activity

Groups	High-density lipoprotein (mmol/L)	Low-density lipoprotein (mmol/L)
	MEAN±STD	MEAN±STD
Group A (Control)	56.40±1.45 ^{NS}	243.93±10.69^{NS}
Group B (Calcium Carbide Only)	49.70±0.18	329.67±5.40
Group C (Calcium Carbide + 100mg/kg of E.C.L.S)	58.36±7.98*	413.88±100.22**
Group D (Calcium Carbide + 500mg/kg of E.C.L.S)	52.77±1.09 ^{NS}	422.11±130.39**

Data was analyzed using ANOVA followed by Post-Hoc Turkey HSD and values were considered significant at $p<0.05$. $p<0.001$***, $p<0.01$**, and $p<0.05$* Ethanolic extract of *Citrulus lanatus*; ECLS

Table 4 result showed an increase in the group in HDL-c in group C and D when compared to group B, but the increase is significant ($p<0.05$) in group C, while group D is not significant ($p>0.05$); group B had a non-significant decrease ($p>0.05$) as compared to group A. The LDL-c result showed a significant ($p<0.05$) increase in group C and D as compared to group B, while group B had a non-significant decrease ($p>0.05$) as compared to group A.

DISCUSSION

Oxidative stress is the body inability to prevent the excess production of reactive oxygen species and their reactive intermediates. The excess reactive oxygen species produced either can oxidize biomolecules or structurally modify proteins and genes to trigger signalling cascades that can lead to the onset and progression of inflammatory diseases (45,46). The results revealed in this study showed that calcium carbide administration had significant depletion of the antioxidant status (SOD, CAT, GSH, GPx, and TAC) compared with the control group. The depletion caused by calcium carbide is attributed to the presence of the impurities calcium arsenide (Ca3As2) and Calcium phosphide (Ca3P2) impurities found in calcium carbide (2) (Andre *et al.,* 2018), which causes the generation of reactive oxygen species. The finding of Danish (2015) has similarities with this report revealing a decrease in glutathione level. The findings of Palpandian *et al.,* (47) have similarities with this study revealing a decrease in GSH and CAT level. The presence of non-enzymatic antioxidants like glutathione, ascorbic acid and beta carotene could prevent damages caused by reactive oxygen species (ROS)on lipid membrane, nucleic acid and proteins (47).

The study showed an attenuation of the antioxidants status (SOD, CAT, GSH, GPx, and TAC) at 100mg/ml and 500mg/ml *Citrullus lanatus*, which was significant (p<0.05). It is attributed to the secondary metabolites (flavonoids and polyphenol compounds) scavenging the free radicals generated by calcium carbide. The report of Palpandian *et al.,*(47) corroborates this study finding, revealing a significant increase in the GSH and CAT levels following administration of *C. lanatus* on CCL4 toxicity. The results of the study similarities to the study done by (48), which demonstrated that lycopene pretreatment, which is abundant in watermelon, remarkably improved the oxidant/antioxidant status and decreased the oxidative damage. Further, the hepatic-protective effect of watermelon on oxidative stress in mice has been demonstrated (27,49), revealing a decrease in the GSH activities. The report of Messaoudi *et al.,* (2019) revealed a significant ($p<0.05$) decrease in GSH and CAT activities following administration of *Citrullus lanatus*. The report of Hong *et al.,* (32) revealed a significant increase in TAC, SOD, and glutathione S-transferase following Watermelon consumption, which caused a reduction in oxidative stress.

Dyslipidemia is characterized by abnormally high lipids (total cholesterol level and low-density lipoproteins cholesterol, triglycerides) level or low level of high-density lipoprotein. High-density lipoprotein is good cholesterol due to its ability to remove excess cholesterol in the arterial wall, transport it to the liver for excretion or re-utilization, and prevent oxidation of LDL-c (50). Iheagwam *et al.,* (13) reported an inverse relationship between HDL-c and LDL-c and cardiovascular disease risk, which signifies an increased level of HDL-c in our study, highlights the effect of *Citrullus lanatus* on dose-related effect on lipid profile (13). Though Dyslipidemic and oxidative interruption of calcium carbide may not be due to it alone, it may also be related to calcium arsenide (Ca3As2) and Calcium phosphide (Ca3P2) impurities found in calcium carbide. These two toxic chemicals react with water to form arsenic (AsH3) and phosphine hydrides (PH3), respectively (2). These hydrides are fat-soluble as such can quickly diffuse to the body membrane layers causing severe health hazards. The study revealed a non-significant ($p>0.05$) decrease in the total cholesterol, triglyceride, HDL-c, and LDL-c levels in the calcium carbide group compared to the control group. However, at 100mg/kg and 500mg/kg of ECLS, the total cholesterol level shows a non-significant increase compared to the group that received calcium carbide only. Triglycerides show a non-significant increase at 100mg/kg, but at 500mg/kg, the *C. lanatus* showed a significant increase compared to the group that received calcium carbide only. The HDL-c shows a significant increase at 100mg/kg; in contrast, a non-significant difference at 500mg/kg *C. lanatus* group compared to the calcium carbide group only. The LDL-c shows a significant increase at 100mg/kg and 500mg/kg *C. lanatus* group compared to the calcium carbide group only. However, the decrease in the total cholesterol, triglyceride, and HDL-c, are not well understood but suggesting oxidative stress occurring through lipid-peroxidation. The attenuated effects were seen in the HDL-c at 100mg/kg of ECLS, and triglyceride at 500mg/kg is attributed to flavonoids present in the *C. lanatus*. This study disagrees with the report of Hong *et al.,* (32), revealing a decrease in triglyceride following administration of watermelon and l-arginine consumption. The study's findings disagree with the Francis *et al.,* (2018)(51) report revealing a significant decrease in HDL-c, Triglyceride, LDL-c, and Total cholesterol activity. Further, the study showed a significantly higher level of LDL-c at 100mg/kg and 500mg/kg of ECLS; the physiology behind these increases is not fully elucidated. The study contradicts Francis *et al.,* (51), who revealed a significant reduction in LDL-c level following the seed of *C. lanatus* administration in the diabetic model.

Conclusion

In conclusion, the study showed that calcium carbide is toxic, and its utilization should be minimized with care despite its influence on fruit ripening and hot metal desulfurization. It shows that calcium carbide has a deleterious effect on the

antioxidant status and lipid profile. The ethanolic extract of *C. lanatus* pulp possesses high antioxidants activity against calcium carbide induced-oxidative stress. Also, it shows anti-lipidemic activity on calcium carbide dyslipidemia activity based on dosage-dependent. To the best of our knowledge, this is among the few research that focuses on administering calcium carbide directly on an experimental model to investigate its toxicity on antioxidants status and lipid profile and treatment with *C. lanatus*.

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Statement of competing interests: The authors have no competing interest.

List of Abbreviations

ECLS: Ethanolic extract of*Citrulus lanatus*
CaC2: Calcium carbide
GPx: Glutathione Peroxidase
GSH: Glutathione reductase
TAC: Total antioxidant capacity
SOD: Superoxide-dismutase
HDL-c: High-density lipoprotein-cholesterol
LDL-c: Low-density lipoprotein-cholesterol
ROS: Reactive oxygen species

REFERENCES

1. Olisah MC, Ilechukwu OU. Effect of Vitamin C against Calcium Carbide Induced Hepatotoxicity. Trop J Appl Nat Sci Trop J Appl Nat Sci [Internet]. 2017;2(1):74–7. Available from: https://doi.org/10.25240/TJANS.2017.2.1.12
2. Shar Andre G, Terver Sim U, Ungwanen J A, Oche Godwi O, Ikenna Ale N, M. Ikagu Y. Calcium Carbide Treatment on Some Physiochemical Characteristics of Broken and Mummy Mango Fruits. Am J Food Technol. 2017 Dec 15;13(1):23–31.
3. De I, S R, Kour A, Wani H, Sharma P, Panda JJ, et al. Exposure of calcium carbide induces apoptosis in mammalian fibroblast L929 cells. Toxicol Mech Methods [Internet]. 2021 [cited 2021 Apr 10];31(3):159–68. Available from: https://pubmed.ncbi.nlm.nih.gov/33190584/
4. Li T, Bai X, Gulzar U, Capiglia C, Bai YJ, Proietti Zaccaria R. Facile Synthesis of Highly Graphitized Carbon via Reaction of CaC 2 with Sulfur and Its Application for Lithium/Sodium-Ion Batteries. ACS Omega [Internet]. 2019 May 8 [cited 2021 Apr 10];4(5):8312–7. Available from: http://pubs.acs.org/journal/acsodf
5. Da Costa Nascimento R, de Oliveira Freire O, Ribeiro LS, Araújo MB, Finger FL, Soares MA, et al. Ripening of bananas using Bowdichia virgilioides Kunth leaves. Sci Rep. 2019 Dec 1;9(1).
6. Maduwanthi SDT, Marapana RAUJ. Induced ripening agents and their effect on fruit quality of banana. Vol. 2019, International Journal of Food Science. Hindawi Limited; 2019.
7. Jindal T, Agrawal N, Sangwan S. Issue 2• 1000159 J Clinic Toxicol ISSN: 2161-0495 JCT, an open access journal Research Article Open Access Jindal et al. J Clin Toxicol. 2013;3:159.
8. Sun H, Li Z, Bai J, Memon SA, Dong B, Fang Y, et al. Properties of Chemically Combusted Calcium Carbide Residue and Its Influence on Cement Properties. Materials (Basel). 2015;8:638–51.
9. Rodygin KS, Werner G, Kucherov FA, Ananikov VP. Calcium Carbide: A Unique Reagent for Organic Synthesis and Nanotechnology. Chem - An Asian J [Internet]. 2016 Apr 5 [cited 2021 Apr 10];11(7):965–76. Available from: http://doi.wiley.com/10.1002/asia.201501323
10. Teong SP, Zhang Y. Calcium carbide and its recent advances in biomass conversion. Vol. 5, Journal of Bioresources and Bioproducts. KeAi Communications Co.; 2020. p. 96–100.
11. Ranaweera SJ, Ampemohotti AALT, Arachchige USPR. BANANA RIPENING BY SMOKING CABINET. Available from: www.ijstr.org
12. Essien EB, Onyegeme-Okerenta M, Onyema JO. Calcium Carbide as an Artificial Fruit-Ripening Agent and its Physiological Effects on Wistar Rats. Clin Exp Med Sci [Internet]. 2018;6(1):47–61. Available from: https://doi.org/10.12988/cems.2018.865
13. Iheagwam PN, Onyeike EN, Amadi BA. Biochemical Parameters, Activity Levels of Marker Enzymes and Serum Electolytes of Wistar Albino Rats Fed Mango (Mangifera indica) Pulp Formulated Diets. Asian J Res Biochem [Internet]. 2019 May 21 [cited 2021 Apr 12];4(3):1–10. Available from: https://journalajrb.com/index.php/AJRB/article/view/30072
14. Igbinaduwa, Patrick O, Aikpitanyi-Iduitua, Rosemary O. Calcium Carbide-induced Alterations of Some Haematological and Serum Biochemical Parameters of Wistar Rats. Asian J Pharm Heal Sci. 2016;6(11):1396–400.
15. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. J Bot. 2012 Apr 24;2012:1–26.
16. Chen J yi, Ye Z xin, Wang X fen, Chang J, Yang M wen, Zhong H hua, et al. Nitric oxide bioavailability dysfunction involves in atherosclerosis. Vol. 97, Biomedicine and Pharmacotherapy. Elsevier Masson SAS; 2018. p. 423–8.
17. Tan BL, Norhaizan ME, Huynh K, Heshu SR, Yeap SK, Hazilawati H, et al. Water extract of brewers' rice induces apoptosis in human colorectal cancer cells via activation of caspase-3 and caspase-8 and downregulates the Wnt/β-catenin downstream signaling pathway in brewers' rice-treated rats with azoxymethane-induced colon carcinogenesis. BMC Complement Altern Med [Internet]. 2015 Jun 30 [cited 2021 Apr 11];15(1). Available from: https://pubmed.ncbi.nlm.nih.gov/26122204/
18. Francenia Santos-Sánchez N, Salas-Coronado R, Villanueva-Cañongo C, Hernández-Carlos B. Antioxidant Compounds and Their Antioxidant Mechanism. In: Antioxidants [Internet]. IntechOpen; 2019 [cited 2021 Apr 12]. Available from: www.intechopen.com
19. Shahidi F, Zhong Y. Measurement of antioxidant activity. Vol. 18, Journal of Functional Foods. Elsevier Ltd; 2015. p. 757–81.
20. Dworzański J, Strycharz-Dudziak M, Kliszczewska E, Kiełczykowska M, Dworzańska A, Drop B, et al. Glutathione peroxidase (GPx) and superoxide dismutase

(SOD) activity in patients with diabetes mellitus type 2 infected with Epstein-Barr virus. Pagano JS, editor. PLoS One [Internet]. 2020 Mar 25 [cited 2021 Apr 12];15(3):e0230374. Available from: https://dx.plos.org/10.1371/journal.pone.0230374

21. Poduri A, Rateri DL, Saha SK, Saha S, Daugherty A. Citrullus lanatus "sentinel" (watermelon) extract reduces atherosclerosis in LDL receptor-deficient mice. J Nutr Biochem [Internet]. 2013 May [cited 2021 Apr 10];24(5):882–6. Available from: https://pubmed.ncbi.nlm.nih.gov/22902326/
22. Neglo D, Tettey CO, Essuman EK, Kortei NK, Boakye AA, Hunkpe G, et al. Comparative antioxidant and antimicrobial activities of the peels, rind, pulp and seeds of watermelon (Citrullus lanatus) fruit. Sci African. 2021 Mar 1;11:e00582.
23. Emmanuel AM, Phatlane WM, Phetole M. Comparative analysis of the chemical compositions of indigenous watermelon (Citrullus lanatus) seeds from two districts in Limpopo Province, South Africa. African J Biotechnol [Internet]. 2018 Aug 8 [cited 2021 Apr 12];17(32):1001–6. Available from: http://www.academicjournals.org/AJB
24. Messaoudi S, Tebibel S, Beladjila AK, Touhami FK, Kabouche Z. All Rights Reserved WJES © 2014 Available Online at : www.environmentaljournals.org Anti-hyperlipidemic , Anti-inflammatory and Antioxidant Activities of Citrullus lanatus. 2014;8(1):100–6.
25. Ouassor I, Aqil Y, Belmaghraoui W, El Hajjaji S. Characterization of two Moroccan watermelon seeds oil varieties by three different extraction methods. OCL - Oilseeds fats, Crop Lipids. 2020;27(6).
26. Folaranmi Olaniyan M, Oluwatosin R. Scholars Academic Journal of Biosciences (SAJB) Assessment of the Administration of Watermelon (Citrullus lanatus) on Lipid Profile in Rabbits Overdosed with Acetaminophen Extra. J Biosci [Internet]. 2016;4(10B):863–9. Available from: http://saspublisher.com/sajb/863www.saspublisher.com
27. Oyenihi OR, Afolabi BA, Oyenihi AB, Ogunmokun OJ, Oguntibeju OO. Hepato- and neuro-protective effects of watermelon juice on acute ethanol-induced oxidative stress in rats. Toxicol Reports [Internet]. 2016 [cited 2021 Apr 12];3:288–94. Available from: /pmc/articles/PMC5615783/
28. Ajiboye BO, Shonibare MT, Oyinloye BE. Antidiabetic activity of watermelon (Citrullus lanatus) juice in alloxan-induced diabetic rats. J Diabetes Metab Disord [Internet]. 2020 Jun 1 [cited 2021 Apr 10];19(1):343–52. Available from: https://pubmed.ncbi.nlm.nih.gov/32550185/
29. Abdel Aziz RL, Abdel-Wahab A, Abo El-Ela FI, Hassan NEHY, El-Nahass ES, Ibrahim MA, et al. Dose- dependent ameliorative effects of quercetin and L-Carnitine against atrazine- induced reproductive toxicity in adult male Albino rats. Biomed Pharmacother. 2018 Jun 1;102:855–64.
30. Joshi V, Joshi M, Silwal D, Noonan K, Rodriguez S, Penalosa A. Systematized biosynthesis and catabolism regulate citrulline accumulation in watermelon. Phytochemistry [Internet]. 2019 Jun 1 [cited 2021 Apr 10];162:129–40. Available from: https://pubmed.ncbi.nlm.nih.gov/30884257/
31. Figueroa A, Wong A, Jaime SJ, Gonzales JU. Influence of L-citrulline and watermelon supplementation on vascular function and exercise performance [Internet]. Vol. 20, Current Opinion in Clinical Nutrition and Metabolic Care. Lippincott Williams and Wilkins; 2017 [cited 2021 Apr 10]. p. 92–8. Available from: https://pubmed.ncbi.nlm.nih.gov/27749691/
32. Hong MY, Beidler J, Hooshmand S, Figueroa A, Kern M. Watermelon and L-arginine consumption improve serum lipid profile and reduce inflammation and oxidative stress by altering gene expression in rats fed an atherogenic diet. Nutr Res [Internet]. 2018 Oct 1 [cited 2021 Apr 10];58:46–54. Available from: https://pubmed.ncbi.nlm.nih.gov/30340814/
33. Bose KSC, Agrawal BK. Effect of lycopene from tomatoes (cooked) on plasma antioxidant enzymes, lipid peroxidation rate and lipid profile in grade-I hypertension. Ann Nutr Metab. 2007 Dec;51(5):477–81.
34. Maoto MM, Beswa D, Jideani AIO. Watermelon as a potential fruit snack. Int J Food Prop [Internet]. 2019 Jan 1 [cited 2021 Apr 12];22(1):355–70. Available from: https://www.tandfonline.com/doi/full/10.1080/10942912.2019.1584212
35. Al-Attar AM, Abu Zeid IM. Effect of tea (camellia sinensis) and olive (olea europaea L.) leaves extracts on male mice exposed to diazinon. Biomed Res Int [Internet]. 2013 [cited 2021 Apr 10];2013. Available from: https://pubmed.ncbi.nlm.nih.gov/23691503/
36. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol [Internet]. 1983 Dec [cited 2020 Aug 8];54(4):275–87. Available from: https://pubmed.ncbi.nlm.nih.gov/6667118/
37. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother [Internet]. 2010 Jul [cited 2021 Mar 8];1(2):87–93. Available from: /pmc/articles/PMC3043327
38. Hadwan MH. Simple spectrophotometric assay for measuring catalase activity in biological tissues. BMC Biochem [Internet]. 2018 Aug 3 [cited 2021 Apr 10];19(1):7. Available from: https://bmcbiochem.biomedcentral.com/articles/10.1186/s12858-018-0097-5
39. Beyer WF, Fridovich I. Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. Anal Biochem [Internet]. 1987 [cited 2021 Apr 10];161(2):559–66. Available from: https://pubmed.ncbi.nlm.nih.gov/3034103/
40. Fitri LE, Iskandar A, Sardjono TW, Erliana UD, Rahmawati W, Candradikusuma D, et al. Plasma glutathione and oxidized glutathione level, glutathione/oxidized glutathione ratio, and albumin concentration in complicated and uncomplicated falciparum malaria. Asian Pac J Trop Biomed. 2016 Aug 1;6(8):646–50.
41. Rush JWE, Sandiford SD. Plasma glutathione peroxidase in healthy young adults: Influence of gender and physical activity. Clin Biochem [Internet]. 2003 [cited 2021 Apr 10]; 36(5):345–51. Available from: https://pubmed.ncbi.nlm.nih.gov/12849865/
42. Rubio CP, Hernández-Ruiz J, Martinez-Subiela S, Tvarijonaviciute A, Ceron JJ. Spectrophotometric assays for total antioxidant capacity (TAC) in dog serum: An update. BMC Vet Res [Internet]. 2016 Aug 15 [cited 2021 Apr 10];12(1):166. Available from: http://bmcvetres.biomedcentral.com/articles/10.1186/s12917-016-0792-7
43. Farombi EO, Ige OO. Hypolipidemic and antioxidant effects of ethanolic extract from dried calyx of Hibiscus sabdariffa in alloxan-induced diabetic rats. Fundam Clin Pharmacol [Internet]. 2007 Dec [cited 2021 Apr 10];21(6):601–9. Available from: https://pubmed.ncbi.nlm.nih.gov/18034661/

44. Wu LL, Warnick GR, Wu JT, Williams RR, Lalouel JM. A rapid micro-scale procedure for determination of the total lipid profile. Clin Chem [Internet]. 1989 Jul 1 [cited 2021 Apr 10];35(7):1486–91. Available from: https://academic.oup.com/clinchem/article/35/7/1486/5663301
45. Chatterjee S. Oxidative Stress, Inflammation, and Disease. In: Oxidative Stress and Biomaterials. Elsevier; 2016. p. 35–58.
46. Li WB, Qiao XP, Wang ZX, Wang S, Chen SW. Synthesis and antioxidant activity of conjugates of hydroxytyrosol and coumarin. Bioorg Chem. 2020 Dec 1;105.
47. Palpandian P, Shanmugam H, Alli Rani E, Prabu GTV. Determination of fruit quality of calcium carbide induced ripening in mango (Mangifera indica L. cv. alphonso) by physiological, biochemical, bio-enzymatic and elemental composition analysis (EDX). Indian J Biochem Biophys [Internet]. 2019;56(3):205–13. Available from: http://icargoares.in/wasp2.0/index.php
48. Saada HN, Rezk RG, Eltahawy NA. Lycopene protects the structure of the small intestine against gamma-radiation-induced oxidative stress. Phyther Res [Internet]. 2010 [cited 2021 Apr 12];24(SUPPL. 2). Available from: https://pubmed.ncbi.nlm.nih.gov/20041432/
49. Adebayo HA, Yakubu OF, Balogun TM. Protective Properties of Citrullus lanatus onCarbon Tetrachloride Induced Liver Damagein Rats. 2014 Jun 4 [cited 2020 Dec 30]; Available from: http://www.sciencedomain.org
50. Chiesa ST, Charakida M. High-Density Lipoprotein Function and Dysfunction in Health and Disease. Cardiovasc Drugs Ther [Internet]. 2019 Apr 15 [cited 2021 Apr 11];33(2):207–19. Available from: https://link.springer.com/article/10.1007/s10557-018-06846-w
51. Francis D, Ani C, Nworgu C, Okorie P, Igwe U, Uzoigwe J, et al. The Effect of Ethanolic Seed Extract of Citrullus lanatus (Watermelon) on Blood Glucose Level and Lipid Profile of Diabetic Wistar Rats. European J Med Plants. 2018;26(4):1–6.

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