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Antioxidant activity and acute toxicity of two *Lagenaria siceraria* (Molina) Standl. varieties from Sudan

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Abstract: The present study was conducted to evaluate the antioxidant capacity and acute toxicity of the methanol extract of two *Lagenaria siceraria* (Molina) Standl. varieties of dried seeds, Sweet gourd Water Jug (WJ) and bitter gourd Basket Ball (BB). The seed extracts were tested for their total phenolic contents (TPC), total flavonoid contents (TFC), ferric reducing antioxidant power (FRAP) and scavenging activity to the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Both dried seed extracts were then tested for acute toxicity at doses 2 and 5 g kg^{-1} each. Sixty female and male rats were assigned into five groups per sex. Four groups were given low and high doses of each dried seed extract and the fifth group was given 10 % Tween 20 as a control. Kidneys and livers of all rats were assessed for biochemistry and histopathology. The seed extract of WJ revealed higher TPC, FRAP and DPPH activities compared to BB seeds, while TFC results were reversed. Toxicologically, no toxicity signs were recorded in animals. Biochemistry results were within the normal ranges with a slight increase in bilirubin and Alanine aminotransferase (ALT), and histology of kidneys and livers showed normal architecture. In conclusion, WJ and BB dried seed extracts exhibited high antioxidant activity suggesting promising therapeutic regimen against oxidative stress.

Key words: Lagenaria siceraria; antioxidant; acute toxicity

Antioksidacijska aktivnost in akutna toksičnost dveh sort vodnjače (*Lagenaria siceraria* (Molina) Standl.) iz Sudana

Izvleček: Raziskava je bila izvedena za ovrednotenje antioksidacijske sposobnosti in akutne toksičnosti metanolnih izvlečkov suhih semen dveh sort vodnjače (*Lagenaria siceraria* (Molina) Standl.)), sladke 'Water Jug' (WJ) in grenke vodnjače 'Basket Ball' (BB). V izvlečkih semen so bili analizirani celokupna vsebnost fenolov (TPC), celukupna vsebnost flavonoidov (TFC), velikost redukcije železa (FRAP) in lovilna aktivnost stabilnega prostega radikala 2,2-difenil-1-pikrilhidrazila (DPPH). Oba izvlečka sta bila potem preiskušena na akutno toksičnost z odmerkoma 2 in 5 g kg^{-1}. 60 podganjih samic in samcev je bilo razdeljeno glede na spol v pet skupin. Vsaka od štirih skupin je dobivala velike in majhne odmerke izvlečka semen, peta skupina pa je dobila 10 % Tween 20 kot kontrolo. Ledvice in jetra vseh podgan so bile ocenjene po biokemičnih in histopatoloških parametrih. Izvleček semen WJ je pokazal večjo aktivnost TPC, FRAP in DPPH v primerjavi s semeni BB, a rezultati TFC so bili obratni. Na živalih ni bilo opaziti nobenih znakov zastrupitve. Biokemični parametri so bili znotraj normalih meja z rahlim povečanjem vsebnosti bilirubina in aktivnosti alanin aminotransferaze (ALT). Tudi histološki pregled ledvic in jeter je pokazal normalno zgradbo. Zaključimo lahko, da imajo izvlečki suhih semen WJ in BB veliko antioksidacijsko aktivnost, kar obeta njihovo uporabo pri blaženju oksidacijskega stresa.

Ključne besede: *Lagenaria siceraria;* antioksidant; akutna toksičnost

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1 INTRODUCTION

Medicinal plants are still considered as cheap and safe, natural resource of drugs with less toxicity all over the world. Sudanese locals and people of other developing countries have relied on traditional herbs to treat their diseases (Elhadi et al., 2013). Therefore, it is useful to explore more about medicinal plants (Koko et al., 2008). Family Cucurbitaceae is a very large family composed of 118 genera and 825 species. One of the most important genera of this family is *Lagenaria* (Molina) which is also known as bottle gourd, calabash gourd or white flowered gourd plant (Fader et al., 2013). In addition to *Lagenaria siceraria,* genus *Lagenaria* consists of five other wild species, namely *L. brevifilora* (Benth) Roberty, *L. rufa* (Gilg) C Jeffrey, *L sphaerica* E Mey, *L. abyssinia* (Hook. F.) C Jeffrey and *L. guineensis* (G Den) C Jeffrey. *L. siceraria* is an indigenous species native to India (Shah et al., 2010) and became widely spread in Africa and the most cultivable species in Asia and America (Erickson et al., 2005). Shah et al. (2010) reported that *Lagenaria* has two distinguished varieties, one is bitter bottle gourd and sweet variety useful bottle gourd. The bitter variety is wild plant and used for the pharmacological application, while sweet variety is used as a vegetable and medicinal. In Sudan, varieties of *L. siceraria* ,the sorcerer is broadly diffused in different areas of the South and West, while some of them are edible such as snake gourd. Recently, researchers focused their study on *L. siceraria* varieties exploring more about their chemical composition. Studies reported that they can be used as nutritious source, in cosmetics and medicine (Mariod et al., 2015). Literature review reveals that *L. siceraria* showed a broad spectrum of pharmacological activities such as antioxidant (Mayakrishnan et al., 2013), antimutagenic (Thakkar, 2013), antiulcer (Srivastava et al., 2011), anti-inflammatory (Ghule et al., 2006), antidiabetic, hepatoprotective (Deshpande et al., 2008) and cardioprotective (Upaganlawar and Balaraman, 2011). Due to the presence of high percentage of alkaloids, phenolic glycosides, carbohydrates, proteins and minerals, *L. siceraria* can be suggested to replace allopathic medicines (Mariod et al., 2015; Sivannarayana et al., 2013). In folk medicine, different parts (leaves, stem, flower, root, and seeds, fresh and dry fruits) of *L. siceraria* have been used in the ointment for ailment of various diseases (Kumar et al., 2015).

Traditionally, *L. siceraria* seeds can be cooked, dried and served as snacks in some countries such as Egypt. Also can be ground and fermented for use as a flavor enhancer in gravies and soups in West Africa (Achigan-Dako et al., 2008). At the phytochemistry level, studies proved the presence of various phytochemicals like cardiac glycosides, terpenoides, carbohydrates, resins, saponins and phytosterols in *L. siceraria* seeds (Sood et al., 2012). Singh et al. (2012) suggested that the ethanol extract of *L. siceraria* seeds exhibits cardioprotective potential in experimental animals against cardiac toxicity induced by doxorubicin. Another study revealed that the ethanol extract of *L. siceraria* seeds showed excellent free radical scavenging activity (Gill et al., 2012). As an extension to our previous study on the eight Sudanese *Lagenaria* varieties (Mariod et al., 2015), the present study was conducted to determine the total phenol and total flavonoid contents, and acute oral toxicity of the methanol extract of sweet and bitter varieties of *L. siceraria* seeds from Sudan.

2 MATERIAL AND METHODS

2.1 IN VITRO ANTIOXIDANT CAPACITY STUDY OF WJ AND BB METHANOL EXTRACTS OF DRIED SEEDS

2.1.1 Plant material

Two varieties of *Lagenaria siceraria* dry fruits {Sweet gourd 'Water Jug' (WJ) and bitter gourd 'Basket Ball' (BB)} were obtained from the market of Omdurman, State of Khartoum, Sudan.

2.1.2 Preparation of seeds extracts

By the aid of 4.8 mm knife, the seeds were removed from the collected dried fruits. The removed seeds were properly washed and left dried at 50 °C in drying oven (FD 115; Fisher Scientific, Loughborough, Leicestershire, UK). The dried seeds were then ground to a powder using home blender and stored in polyethylene bags in the refrigerator at 4 °C till the onset of the experiment as previously done (Mariod et al., 2015). For seed extraction, the seed powder was firstly defatted using n-hexane at 50–60 °C in a Soxhlet apparatus for six hours following the AOCS method (Firestone, 2009). The dried seeds (seedcake) were extracted for 72 h in methanol 95 % at room temperature with occasional stirring. The solvent was filtered off using filter paper (Whatman No.1, Fitchburg, WI, USA) and the filtrate obtained was concentrated under reduced pressure on a rotary evaporator at 40 °C and finally freeze dried using a freeze-drying machine (LabConco, Kansas City, MO, USA). The dried seed extracts obtained from both water jug seeds (WJ) and basketball seeds (BB) were then stored at 4 °C for further use for various investigations.

2.1.3 Total phenol content (TPC) determination

Total phenolic content was determined using Folin-Ciocalteu reagent following the method of Singleton and Rossi, (1965) and using gallic acid as a standard. An amount of 10 µl of each WJ and BB dried seed extract solution (1 mg ml^{-1}) was added in a test tube followed by the addition of 0.5 ml of 1:10 Folin-Ciocalteu reagent. The mixture was incubated at room temperature for 5 minutes. Following incubation, 0.35 ml of 115 mg ml^{-1} natrium carbonate (Na_2CO_3) was added and mixed thoroughly. The mixture was then allowed to stand at room temperature for 2 hours. Absorbance reading taken spectrophotometrically at 765 nm and all determinations were done in triplicates. The total phenolic content of WJ and BB were expressed as milligrams of gallic acid equivalent to grams of dried seed extract.

2.1.4 Total flavonoid content (TFC) determination

Total flavonoid content of WJ and BB seed extracts were determined by aluminum chloride colorimetric method using quercetin as a standard (Chang et al., 2002). Briefly, 0.5 ml of each seed extract solution (1 mg ml^{-1}) was sampled in a test tube and mixed with 1.5 ml ethanol 95 %, 0.1 ml aluminum chloride and 2.8 ml distilled water. The mixtures were left to incubate for 30 minutes at room temperature followed by reading the absorbance at 415 nm. The total flavonoid content of WJ and BB were expressed as milligrams of quercetin equivalent to grams of dried plant material.

2.1.5 Ferric reducing antioxidant power (FRAP) determination

Ferric reducing activity of the WJ and BB seed extracts was estimated using the method developed by Benzie and Strain (1996), while vitamin C (Vit. C) was taken as reference. The principle of the method depends on the chelating power of substances to ferric tripyridyltriazine complex. Briefly, the working FRAP reagent was freshly prepared by mixing 300 mmol l^{-1} acetate buffer, 10 mmol l^{-1} TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mmol l^{-1} of HCL and 20 mmol l^{-1} of $FeCl_3.6H_2O$. The prepared mixture was then incubated at 35 °C in water bath for five minutes and then a blank reading was taken spectrophotometrically at 593 nm. A quantity of 10 µl of each extract/reference was sampled separately and mixed with 290 µl of the working FRAP reagent followed by vortexing and reading the absorbance immediately at 593 nm to get the record of zero minutes. Thereafter, the absorbance reading was taken every four minutes for a period of 2 hours. All the results were expressed as mmol ferric reducing activity of the extract per gram of dried weight based on three experiments.

2.1.6 DPPH free radical scavenging activity determination

In the DPPH radical scavenging test, the scavenging of DPPH is followed by monitoring the decrease in absorbance at 515 nm that occurs due to reduction by the antioxidant or reaction with a radical species. DPPH is widely used to test for the ability of compounds to act as hydrogen donors or free radical scavengers and to evaluate antioxidant activity of foods (Kadhim et al., 2019). Scavenging activity of the WJ and BB dried seed extracts to the stable 2, 2 diphenyl-1-picryl dyhydrazyl (DPPH) free radical was determined according to the method of Gorinstein et al. (2005) with slight modification. Briefly, the dried seed extracts and the reference standard (ascorbic acid) were prepared (1 mg ml^{-1}) as separate stock solutions and then serial dilution with 8 varying concentrations were prepared (0.37, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50 µl) from each stock solution. Five microliters of each sample was loaded in 96-well plate followed by the addition of 195 µl of DPPH reagent; the mixtures were then incubated in the dark at room temperature for 2 hours. At the end of the incubation period, the absorbance was measured spectrophotometrically at 515 nm with a Hitachi spectrophotometer (Hitachi, LTD.Tokyo, Japan), the DPPH percentage inhibition was calculated using the following formula:

DPPH (%) = [(Abs of blank-Abs of sample)/Abs of blank] ×100.

The results were expressed as IC_{50} value which is the extract concentration required to reduce 50 % of the DPPH free radical.

2.2 IN VIVO ACUTE TOXICITY STUDY OF WJ AND BB METHANOL EXTRACTS OF DRIED SEEDS

2.2.1 Experimental animals

Sixty adult and healthy Sprague Dawley (SD) rats (6–8 weeks old) were obtained from the Animal House, Faculty of Medicine, University of Malaya, Kuala Lumpur (Ethic no.PM/30/05/2012/NSIAW (R). The body

mass of SD rats was between 195–210 g. The animals were fed standard rat pellets and tap water.

2.2.2 Acute toxicity test

The acute toxicity study was performed to determine the safe dosage of the dried seed extracts. Sixty SD rats (30 males and 30 females) were randomly and equally divided into 5 groups per sex. The groups were categorized as control (10 % Tween 20, 5 ml kg^{-1}), high dose WJ extract 5 g kg (HD-WJ), low dose WJ extract 2 g kg^{-1} (LD-WJ), high dose BB extract 5 g kg^{-1} (HD-BB), and low dose BB extract 2 g kg^{-1} (LD-BB) (Co-operation and Development, 2002). To prepare the animals for dosing, they were allowed to fast for 14 hours without accessing food but with free access to water. All the rats were weighed recording the body mass on day 0. After dosing, food was prevented for an additional four hours and the rats were observed for 30 min and 2, 4, 8, 24 and 48 hours, post dosing for the appearance of any clinical or toxicological signs such as respiration, salivation, diarrhoea, tremors, eyes and mucus membrane, skin and fur, sleep pattern and any signs associated with the nervous system. The presence of any morbidity, mortality or behavioural changes was recorded. At the end of the experimental period, the body mass of all the rats was recorded. On the 15th day, the animals were given over-dose of anaesthesia (xylazine with ketamine) then sacrificed to collect their blood for biochemistry study and kidneys and livers for histology study. Serum biochemical parameters were investigated at University of Malaya Medical Centre using a Hitachi Autoanalyzer, Japan, after optimizing the machine as previously performed (Al Batran et al., 2013). Liver function parameters were assayed for total protein, albumin, globulin and conjugated bilirubin. In addition aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl tansferase (GGT) were measured as markers of liver injury (Fujii 1997; Young et al., 2008).

2.3 STATISTICAL ANALYSIS

Statistical analysis of the data was carried out by using the statistical program for the social sciences (SPSS) version 16 while applying the ANOVA test for comparing means, followed by Post-Hoc Tukey test. All the data were expressed as mean ± standard deviation (SD) of triplicates of the *in vitro* study and n = 6 for the *in vivo* study. The statistical value $p \leq 0.05$ was considered significant.

3 RESULTS AND DISCUSSION

3.1 IN VITRO ANTIOXIDANT RESULTS

3.1.1 TPC and TFC of WJ and BB methanol extracts of dried seeds

Antioxidants are the compounds that when added to food products, especially to lipids and lipid-containing foods, can increase the shelf life retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage. Synthetic antioxidants, such as butylatedhydroxyanisole (BHA) and butylatedhydroxytolune (BHT), have restricted use in foods as they are suspected to be carcinogenic *via* their binding ability to DNA and cellu-

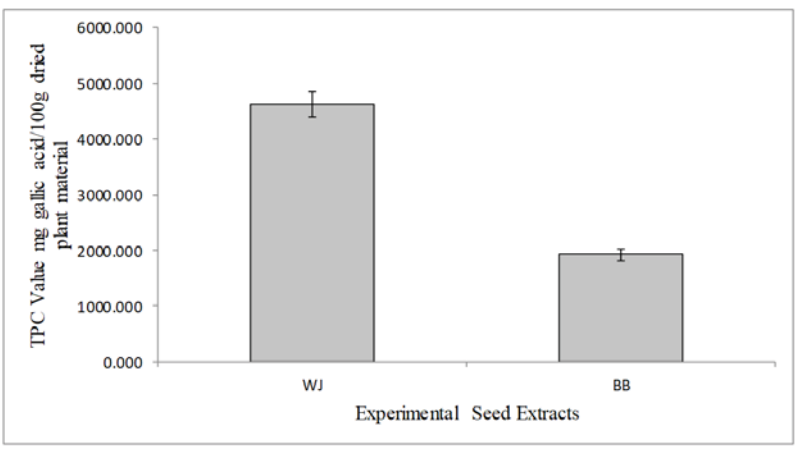

Figure 1: Total phenol content (TPC) of the methanol extracts of sweet gourd 'Water Jug' (WJ) and bitter gourd 'Basket Ball' (BB) dried seed extract

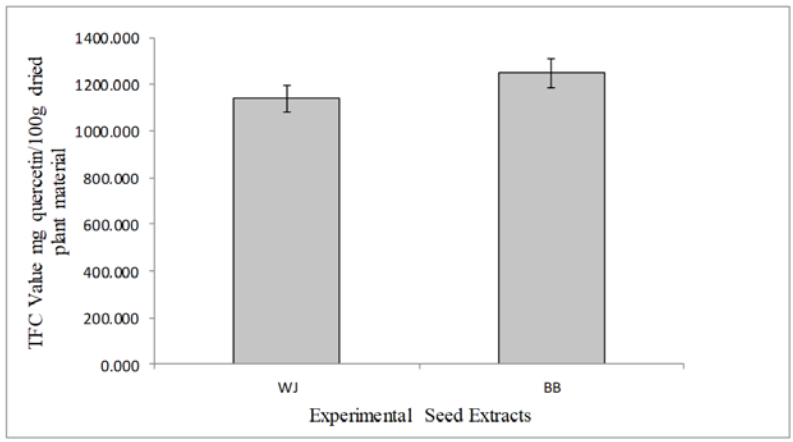

Figure 2: Total flavonoid content (TFC) of the methanol extracts of sweet gourd 'Water Jug' (WJ) and bitter gourd 'Basket Ball' (BB) dried seed extract

lar components (Dolatabadi and Kashanian, 2010; Mirza, Asema, and Kasim, 2017). Therefore, the exploitation of natural antioxidants, especially those of plant origin became necessary and attracted the attention of researchers in recent years (Moayedi et al., 2017). Aras et al. (2017) defined antioxidants as the substances that are capable of quenching or stabilizing free radicals.

In the present study, the seeds of dry fruits of two *Lagenaria siceraria* varieties, (sweet gourd Water Jug (WJ) and bitter gourd Basket Ball (BB) were ground, defatted and extracted in methanol and then tested for their total phenol and total flavonoid contents. The results of total phenol content (TPC) measured from the methanol extract of WJ and BB dried seeds are displayed in Fig.1. Results revealed that WJ seeds measured approximately two and half folds more than BB seeds indicating higher TPC of WJ seeds compared to BB seeds. The results of total flavonoid content (TFC) determined from the methanol extract of WJ and BB dried seeds are illustrated in Fig.2. Presented data recorded that the total flavonoid content of BB seeds was higher compared to that of WJ seeds.

3.1.2 FRAP and DPPH results of WJ and BB methanol extracts of dried seed

Free radicals, often called reactive oxygen species (ROS) and they are the core of any biochemical process representing a vital part in metabolism (Sangeetha and Venkatalakshmi, 2017). On the other hand, free radicals are highly generated in many pathological conditions and involved in the development and progress of common chronic degenerative diseases such as cardiovascular and neurodegenerative diseases, diabetes and cancer (Umeno et al., 2017). Disturbance in the balance between the formation of free radicals in the cells and their antioxidants load leads to oxidative stress that damages cellular components such as proteins, lipids and nucleic acids, and eventually leads to cell death (Shaker and Mnaa, 2017).

The FRAP values of WJ and BB seed extracts were displayed in Fig. 3 compared to vitamin C standard. The scavenging abilities of WJ and BB seed extracts to the stable DPPH free radical is diagrammed in Fig. 4 in comparison with ascorbic acid standard (vitamin C). Results recorded that IC_{50} of both dried seed extracts were significantly higher than that of ascorbic acid indicating less DPPH scavenging activity compared to ascorbic acid. However, the WJ seed extract showed higher DPPH scavenging activity as indicated by the lower IC_{50} value compared to BB seed extract.

Studying the chemical composition of natural products plays a crucial role in screening their biological activities. Natural products having a sufficient antioxidant capacity contain mainly phenolic and flavonoid compounds which exhibit a broad range of biological activities such as anticancer, anti-inflammation, anti-aging and anti-atherosclerosis (Rahman et al., 2013). Antia et al. (2015) studied the antioxidant activity of short-hybrid variety of *Lagenaria siceraria* seeds reporting that the methanol seed extract of that variety acquires significant antioxidant activity and the seeds can be used as excellent natural antioxidant. Later, another study conducted on the seed oil of short-hybrid variety and revealed significant DPPH scavenging activity (Antia et al. 2016).

In the present study, the seeds of dry fruits of two *Lagenaria siceraria* varieties, (sweet gourd 'Water Jug' (WJ) and bitter gourd 'Basket Ball' (BB) were ground, defatted and extracted in methanol and then tested for their total phenol and total flavonoid contents, their reducing power to Fe (III) and their ability to scavenge the stable DPPH free radical. The methanol extract of both seeds exposed high

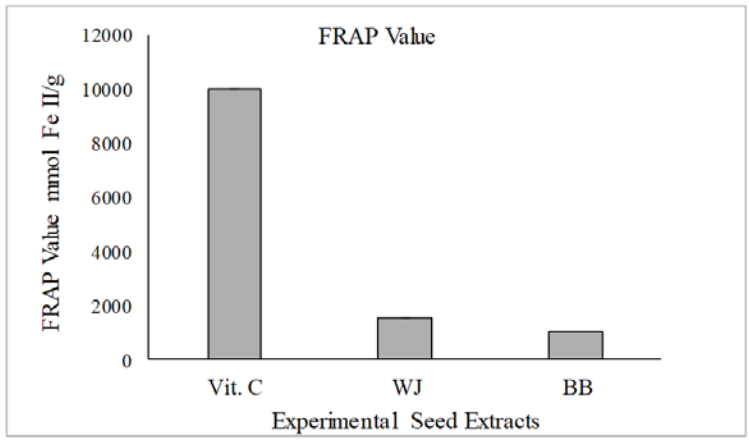

Figure 3: Ferric reducing antioxidant power (FRAP) of the methanol extracts of sweet gourd 'Water Jug' (WJ) and bitter gourd 'Basket Ball' (BB) dried seeds

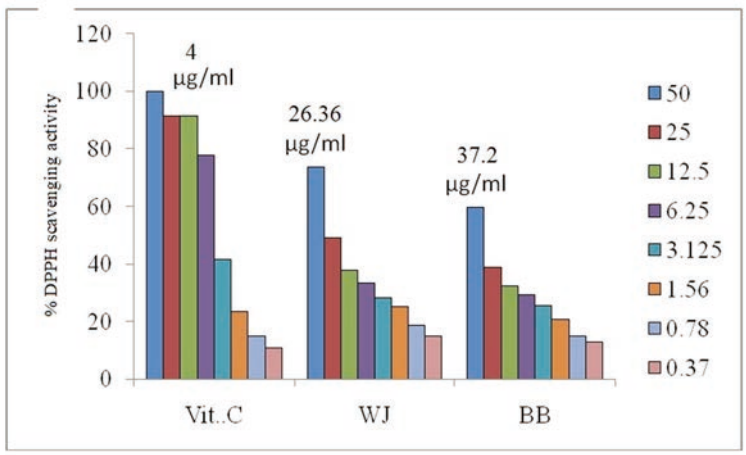

Figure 4: DPPH scavenging activities of the methanol extracts of sweet gourd 'Water Jug' (WJ) and bitter gourd 'Basket Ball' (BB) dried seeds compared to ascorbic acid standard (vitamin C). IC_{50} values are indicated on the bars.

antioxidant activity. Although WJ seeds acquired higher phenolic contents, FRAP and DPPH activity more than BB seeds extract, but BB seeds showed higher TFC compared to WJ seeds. Our study is consistent with a previous study on the remarkable antioxidant capacity of *L. siceraria* seeds (Satvir et al., 2012). Based on the results of DPPH of ascorbic acid, plant extracts WJ and BB, the R-squared (R^2) values of the standard curve equations generated were 0.9516, 0.9478 and 0.9765, respectively. All values were close to 1.0 indicating acceptable standard curves for all the tested samples ascorbic acid, WJ and BB (Salama et al., 2013). Recent studies on the antioxidant power of natural products manifested that they are very well recognized antioxidants, playing vital role in protecting biological cells and tissues from the detrimental effects of free radicals (Zeb and Mehmood, 2004). Therefore, they can be promising pharmaceutical compounds in preventing cancer and degenerative diseases as well (Olszowy, 2019).

3.2 IN VIVO ACUTE TOXICITY RESULTS

Acute toxicity studies in animals are usually necessary for any pharmaceutical substance intended for human use. The information obtained from these studies is useful in selecting doses for repeat-dose studies, providing preliminary identification of target organs of toxicity, and, occasionally, revealing delayed toxicity. Acute toxicity studies may also aid in the selection of starting doses for Phase 1 human studies, and provide information relevant to acute overdosing in humans (CDER and FDA, 1996). Past studies on acute toxicity of petroleum ether, ethanol and chloroform extracts of *L. siceraria* fruits in

rats recommended safe dose up to 2 g kg^{-1} (Deshpande et al., 2008). Additionally, previous study on the acute toxicity and sub-chronic toxicity of the methanol extract of *L. siceraria* aerial parts showed that it is quite safe and can be used in the treatment of chronic diseases like diabetes (Saha et al., 2011). In the present study, the methanol extracts of WJ and BB dried seeds of *L. siceraria* revealed safe dose up to 5 g kg^{-1}.

The findings from the acute toxicity study did not indicate any morbidity or mortality from treating male and female SD rats with the methanol extracts of WJ and BB dried seeds at doses 2 g kg^{-1} and 5 g kg^{-1} throughout the 14 days study period. Physical observation of the treated rats throughout the study didn't show any changes on skin, fur, eyes, mucus membrane, behaviour pattern, tremors, salivation or sleep. In addition no diarrhea or coma observed on any of the treated rats from both sexes.

Table 1: Effects of WJ and BB dried seed extracts on the body mass of the rats

Groups	Body mass (g) day 0	Body mass (g) day 14
10 % Tween 20, male	202.50±4.4	208.16±3.7
10 % Tween 20, female	194.16±8.5	196.83±8.9
HD-WJ, male	205.16±4.5	207.66±5.3
HD-WJ, female	195.33±7.5	198.00±7.4
LD-WJ, male	205.16±4.8	207.66±5.1
LD-WJ, female	194.16±8.4	196.00±7.0
HD-BB, male	205.83±3.6	207.33±3.6
HD-BB, female	194.50±7.9	196.00±7.5
LD-BB, male	205.83±9.3	208.00±8.1
LD-BB, female	193.16±10.5	196.00±10.0

Values expressed as mean ± SD, the significant value was set at $p \leq 0.05$.

Table 2: Effects of WJ and BB dried seed extracts on renal function parameters

Groups	Sodium mmol l^{-1}	Potassium mmol l^{-1}	Chloride mmol l^{-1}	Carbon Dioxide mmol l^{-1}	Anion Gap mmol l^{-1}	Urea mmol l^{-1}	Creatinine umol l^{-1}
10 % Tween20 Male	146.5±2.3	5.66±1.20	105.00±1.2	26.06±1.10	23.70±0.89	5.00±1.90	36.30±2.14
10 % Tween20 Female	147.3±0.8	5.66±1.20	102.00±1.5	24.07±1.23	22.00±0.76	5.40±2.16	36.70±2.31
HD-WJ, Male	145.3±1.3	4.95±1.20	103.66±1.0	25.96±0.80	20.83±0.75	4.25±0.20	35.50±2`94
HD-WJ Female	146.3±1.2	4.66±.019	104.16±0.9	23.88±2.00	22.66±1.90	6.10±0.95	37.00±1.78
LD-WJ Male	144.3 ±1.3	5.85±0.72	103.58±1.6	24.25±1.70	23.00±1.30	5.81±0.33	33.33±2.64
LD-WJ Female	144.5±2.0	5.03±0.08	103.83±1.1	23.33±1.80	21.50±1.10	5.80±0.54	37.16±1.66
HD-BB Male	144.50±0.8	4.80±0.90	102.83±1.1	26.00±0.57	20.33±0.50	5.45±0.53	38.33±2.73
HD-BB Female	141.83±1.7	4.68±0.50	100.50±0.8	24.13±1.71	22.00±1.10	5.66±0.71	37.66±7.45
LD-BB Male	144.16±1.6	4.60±0.30	101.16±2.1	25.78±1.58	22.00±1.00	5.70±0.90	37.33±4.90
LD-BB Female	141.66±1.5	4.33±0.50	100.00±1.2	24.15±2.70	21.83±1.80	6.26±0.38	38.16±9.92

Values expressed as mean ± SD, the significant value was set at $p \leq 0.05$.

3.2.1 Effect of WJ and BB dried seed extracts on body mass of rats

The body mass measurements of the treated and control rats on day 0 and day 14 are shown in Table 1. Data showed reasonable increase in the body mass of the treated and control rats. In addition, the body mass of the treated rats were insignificantly different as compared to control.

3.2.2 Effect of WJ and BB dried seed extracts on renal function test

Renal function parameters of both WJ-treated and BB-treated rats are presented in Table 2. From the results, the concentration of all measured parameters (sodium, potassium, chloride, carbon dioxide, anion gap, urea, and creatinine) in the animals treated with the low dose (2 g kg^{-1}) and high dose (5 g kg^{-1}) of both seed extracts were insignificant compared to control rats. The concentration of all measured parameters (sodium, potassium, chloride, carbon dioxide, anion gap, urea, and creatinine) is lying within the normal range, however there was slight increase in the concentration of anion gap and decrease in creatinine level, but they were alike in both control and treated groups indicating safe treatment. This showed that the seed extract at different levels tested did not produce considerable change in the levels of the different parameters studied.

3.2.3 Effect of WJ and BB seed extracts on liver function test

Table 3 reorded the effects of the low dose (2 g kg^{-1}) and high dose (5 g kg^{-1}) of both WJ and BB methanol extracts of dried seeds on liver function parameters. No significant changes were observed in the values of the parameters studied [(total protein, albumin, globulin, conjugated bilirubin, aspartate aminotransferase (AST) and gama glutamyl transferase (GGT),] in comparison with the control animals and the values obtained were within the normal biological and laboratory limit. On the other hand, the total bilirubin recorded slight decrease than normal ranges and showed significant difference in the low and high doses of BB-treated rats of both sexes compared to control. Additionally, alanine aminotransferase (ALT) revealed slight elevation than normal ranges.

Table 3: Effects of WJ and BB dried seed extracts on liver function parameters

	Total protein	Albumin	Globulin	Total bilirubin	ALT	AST	GGT
Groups	g l^{-1}	g l^{-1}	g l^{-1}	mmol l^{-1}	IU l^{-1}	IU l^{-1}	IU l
10% Tween 20 Male	69.33±2.9	40.33±0.80	28.67±1.20	3.50±1.00	46.83±2.00	47.12±9.1	<3
10% Tween 20 Female	72.66±4.3	37.83±0.70	34.83±1.90	3.50±0.54	45.16±2.30	46.66±8.3	<3
HD-WJ Male	68.16±3.3	36.83±0.40	31.33±0.51	3.00±0.00	49.16±300	44.92±5.6	<3
HD-WJ Female	70.60±40	35.00±0.80	35.60±2.00	2.83±0,40	41.50±2.10	48.33±60	<3
LD-WJ Male	68.83±0.88	39.00±0.63	29.83±2.10	2.83±0.46	48.66±2.70	49.45±7.2	<3
LD-WJ Female	66.66±1.4	43.16±1.30	23.50±1.80	3.33±1.80	46.00±1.90	46.66±11	<3
HD-BB Male	63.66±2.2	37.33±0.80	26.33±5.30	2.33±0.50*	41.83±8.70	49.66±10	<3
HD-BB Female	69.16±2.1	35.00±0.90	34.16±7.10	1.50±1.70*	38.33±6.20	45.00±13	<3
LD-BB Male	62.50±1.9	36.00±0.60	26.50±5.50	1.83±1.90*	43.83±5.10	42.83±16	<3
LD-BB Female	68.83±3.9	38.50±2.20	30.33±4.10	1.33±2.50*	40.16±3.40	47.50±9.5	4

Values expressed as mean ± SD, the significant value was set at $p \leq 0.05$. *p <0.05 compared to normal control

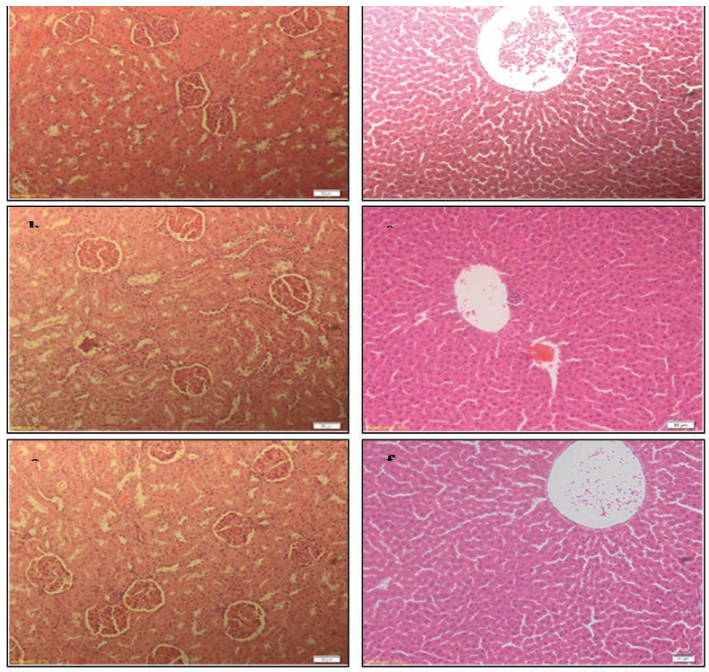

Figure 5: Histological sections (20 x magnifications) of kidneys and livers from the acute toxicity test on the methanol extract of WJ and BB dried seeds. (a & d) Vehicle kidney and liver respectively treated with 5 ml kg^{-1} (10 % Tween 20). (b & e) Kidney and liver respectively treated with WJ seed extracts (5 g kg^{-1}). (c & f) Kidney and liver respectively treated with BB seed extract.

3.2.4 Effect of WJ and BB dried seed extracts on the histopathology of liver and kidney of rats

The qualitative data from the histological sections of kidneys and livers collected from the control and rats treated with WJ and BB seed extracts at the highest dose tested (5 g kg^{-1}) are illustrated on Fig. 5. No significant difference was observed between the architecture of livers or kidneys collected from the WJ-treated and BB-treated animals compared to control group.

4 CONCLUSION

In conclusion, the current study showed that the methanol extracts of both WJ and BB dried seeds of *L. siceraria* possesses high antioxidant power. The WJ seeds variety reported higher TPC, FRAP and DPPH values than BB variety, while the TFC results of BB seeds variety recorded higher value than that of WJ. The acute toxicity test of the methanol extract of both seed varieties reported safe dose up to 5 g kg^{-1} in both sexes of rats.

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6 REFERENCES

Achigan-Dako, E. G., Fagbemissi, R., Avohou, H. T., Vodouhe, R. S., Coulibaly, O., Ahanchede, A. (2008). Importance and practices of Egusi crops (*Citrullus lanatus* (Thunb.) Matsum. Nakai, *Cucumeropsis mannii* Naudin and *Lagenaria siceraria* (Molina) Standl. 'Aklamkpa') in sociolinguistic areas in Benin. *Biotechnologie, Agronomie, Société et Environnement, 12*(4), 393-403.

Al Batran, R., Al-Bayaty, F., Al-Obaidi, M. M. J., Abdulla, M. A. (2013). Acute toxicity and the effect of andrographolide on *porphyromonas gingivalis*-induced hyperlipidemia in rats. *BioMed research international*, Volume 2013, Article ID 594012, 7 pages http://dx.doi.org/10.1155/2013/594012

Antia, B. S., Essien, E. E., Udoh, B. I. (2015). Antioxidant capacity of phenolic from seed extracts of *Lagenaria siceraria* (short-hybrid bottle gourd). *European Journal of Medicinal Plants, 9*(1), 1-9. https://doi.org/10.9734/EJMP/2015/18242

Antia, B. S., Essien, E. E., Peter N. S. (2016). Antioxidant capacity and total polyphenolics from seed oils of various cultivars of *Lagenaria siceraria*. *Indo American Journal of Pharmaceitic Science*, *3*(5).

Aras, A., Silinsin, M., Bingol, M. N., Bursal, E. (2017). Identification of Bioactive Polyphenolic Compounds and As-

sessment of Antioxidant Activity of *Origanum acutidens*. *International Letters of Natural Sciences, 66*, 1-8. https://doi.org/10.18052/www.scipress.com/ILNS.66.1

Benzie, I. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry, 239*(1), 70-76. https://doi.org/10.1006/abio.1996.0292

CDER, and FDA. (1996). Guidance for industry: single dose acute toxicity testing for pharmaceuticals (Final).

Chang, C.-C., Yang, M.-H., Wen, H.-M., Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis, 10*(3), 178-182. https://doi.org/10.38212/2224-6614.2748

Co-operation, O. f. E., & Development. (2002). *Test No. 423: Acute Oral Toxicity-Acute Toxic Class Method*: OECD Publishing.

Deshpande, J., Choudhari, A., Mishra, M., Meghre, V., Wadodkar, S., Dorle, A. (2008). Beneficial effects of *Lagenaria siceraria* (Mol.) Standley fruit epicarp in animal models. *Indian Journal of Experimental Biology, 46*, 234-242.

Dolatabadi, J. E. N., & Kashanian, S. (2010). A review on DNA interaction with synthetic phenolic food additives. *Food Research International, 43*(5), 1223-1230. https://doi.org/10.1016/j.foodres.2010.03.026

Elhadi, I. M., Koko, W. S., Dahab, M. M., El Imam, Y. M., & El Mageed, M. A. E. A. (2013). Antigiardial activity of some Cucurbita species and *Lagenaria siceraria*. *Journal of Forest Products and Industries, 2*(4), 43-47.

Erickson, D. L., Smith, B. D., Clarke, A. C., Sandweiss, D. H., & Tuross, N. (2005). An Asian origin for a 10,000-year-old domesticated plant in the Americas. *Proceedings of the National Academy of Sciences of the United States of America, 102*(51), 18315-18320. https://doi.org/10.1073/pnas.0509279102

Fader, M., Gerten, D., Krause, M., Lucht, W., Cramer, W. (2013). Spatial decoupling of agricultural production and consumption: quantifying dependences of countries on food imports due to domestic land and water constraints. *Environmental Research Letters, 8*(1), https://doi.org/10.1088/1748-9326/8/1/014046

Firestone, D. (2009). *Official methods and recommended practices of the AOCS*: AOCS.

Fujii, T. (1997). Toxicological correlation between changes in blood biochemical parameters and liver histopathological findings. *Journal of Toxicological Sciences, 22*, 161–83. https://doi.org/10.2131/jts.22.3_161

Ghule, B., Ghante, M., Upaganlawar, A., Yeole, P. (2006). Analgesic and anti-inflammatory activities of *Lagenaria siceraria* Stand. fruit juice extract in rats and mice. *Pharmacognosy magazine, 2*(8), 232-238.

Gill, N., Singh, S., Arora, R., & Bali, M. (2012). Evaluation of ethanolic seed extract of *Lagenaria siceraria* for their therapeutic potential. *Journal of Medical Sciences, 12*(3), 78-84. https://doi.org/10.3923/jms.2012.78.84

Gorinstein, S., Drzewiecki, J., Leontowicz, H., Leontowicz, M., Najman, K., Jastrzebski, Z., Katrich, E. (2005). Comparison of the bioactive compounds and antioxidant potentials of fresh and cooked Polish, Ukrainian, and Israeli garlic. *Journal of agricultural and food chemistry, 53*(7), 2726-2732. https://doi.org/10.1021/jf0404593

Kadhim, N. J., Al-Rekaby, L. S., Abdul Redha, A., Chappell, J. (2019). Chemical composition and antioxidant capacity of eggplant parts during vegetative and flowering stage. To cite: *Journal of Physics*: Conf. Series 1294 (2019) 092013 IOP Publishing, https://doi.org/10.1088/1742-6596/1294/9/092013.

Kumar, D., Sharma, C., Singh, B., Singh, D. (2015). Pharmacognostical, phytochemical and pharmacological profile of natural remedy *Lagenaria siceraria* (Mol.) Standly: A Review. *British Journal of Pharmaceutical Research, 7*(5), 340-352. https://doi.org/10.9734/BJPR/2015/17641

Mayakrishnan, V., Veluswamy, S., Sundaram, K. S., Kannappan, P., & Abdullah, N. (2013). Free radical scavenging potential of *Lagenaria siceraria* (Molina) Standl fruits extract. *Asian Pacific journal of tropical medicine, 6*(1), 20-26. https://doi.org/10.1016/S1995-7645(12)60195-3

Mirza, S. K., Asema, U., Kasim, S. S. (2017). To study the harmful effects of food preservatives on human health. *Journal of Medicinal Chemistry and Drug Discovery, 2*(2), 610-616.

Moayedi, A., Mora, L., Aristoy, M.-C., Hashemi, M., Safari, M., Toldrá, F. (2017). ACE inhibitory and antioxidant activities of peptide fragments obtained from tomato processing by-products fermented using *Bacillus subtilis*: Effect of amino acid composition and peptides molecular mass distribution. *Applied biochemistry and biotechnology, 181*(1), 48-64. https://doi.org/10.1007/s12010-016-2198-1

Olszowy, M. (2019). What is responsible for antioxidant properties of polyphenolic compounds from plants?. *Plant Physiology and Biochemistry*, *144*, 135-143. https://doi.org/10.1016/j.plaphy.2019.09.039

Rahman, H., Manjula, K., Anoosha, T., Nagaveni, K., Eswaraiah, C., Bardalai, D. (2013). *In-vitro* antioxidant activity of *Citrullus lanatus* seed extracts. *Asian Journal of Pharmaceutical and Clinical Research, 6*(3), 152-157.

Saha, P., Mazumder, U., Haldar, P., Islam, A., & Kumar, R. S. (2011). Evaluation of acute and subchronic toxicity of *Lagenaria siceraria* aerial parts. *International Journal of Pharmaceutical Sciences and Research, 2*(6), 1507-1512.

Salama, S. M., AlRashdi, A. S., Abdulla, M. A., Hassandarvish, P., & Bilgen, M. (2013). Protective activity of *Panduratin A* against thioacetamide-induced oxidative damage: demonstration with in vitro experiments using WRL-68 liver cell line. *BMC complementary and alternative medicine*, *13*(1), 1-9. https://doi.org/10.1186/1472-6882-13-279

Sangeetha, M. and Venkatalakshmi, P. (2017). *In Vitro* antioxidant activity of the aqueous extract of *Andrographis paniculata* and *Carica papaya* leaves. *World Journal of Pharmacy and Pharmaceutical Sciences, 6*(5), 1631-1643. https://doi.org/10.20959/wjpps201705-9229

Satvir, S., Gill, N., Arora, R. (2012). Study the antioxidant activity of *Lagenaria siceraria* seeds. *International Journal of Natural Product Sciences, 1*, 224.

Shah, B., Seth, A., & Desai, R. (2010). Phytopharmacological profile of *Lagenaria siceraria*: a review. *Asian Journal of Plant Sciences, 9*(3), 152-157. https://doi.org/10.3923/ajps.2010.152.157

Shaker, E. and Mnaa, S. (2017). Protective effect of some lo-

cal plants against oxidative stress caused by hydrogen peroxide. *Journal of Environmental and Toxicological Studies, 1*(1), https://doi.org/10.16966/2576-6430.104

Singh, M., Mohd, F., Ayaz, A., Ankur, S., & Jyoti, Y. (2012). Protective effect of *Lagenaria siceraria* against doxorubicin induced cardiotoxicity in wistar rats. *International Journal of Drug Development and Research, 4*(2), 298-295.

Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture, 16*(3), 144-158.

Sivannarayana, T., Hussain, S. S., & Jithendra, K. P. (2013). Pharmacological and pharmaceutical applications of *Lagenaria siceraria*. *International Journal of Pharmacy and Pharmaceutical Sciences, 1*(4), 288-292.

Sood, A., Kaur, P., & Gupta, R. (2012). Phytochemical screening and antimicrobial assay of various seeds extract of cucurbitaceae family. *International Journal of Applied Biology and Pharmaceutical Technology, 3*(3), 401-409.

Srivastava, V., Rao, C., & Panday, A. (2011). ntiulcer activity of methanolic fruit extract of *Lagenaria siceraria* (Mol.) Standly. *International Journal of Pharmaceutical Research and Development, 3*(7), 187-192.

Thakkar, J. H. (2013). Evaluation of invitro antimutagenic potential of *Lagenaria siceraria* using Ame's test. *American Journal of Cancer Biology, 1*(2), 57-61.

Umeno, A., Biju, V., Yoshida, Y. (2017). In vivo ROS production and use of oxidative stress-derived biomarkers to detect the onset of diseases such as Alzheimer's disease, Parkinson's disease, and diabetes. *Free Radical Research, 51*(4), 413-427. https://doi.org/10.1080/10715762.2017.1315114

Upaganlawar, A. and Balaraman, R. (2011). Cardioprotective effects of *Lagenaria siceraria* fruit juice on isoproterenol-induced myocardial infarction in wistar rats: a biochemical and histoarchitecture study. *Journal of Young Pharmacists, 3*(4), 297-303. https://doi.org/10.4103/0975-1483.90241

Young, T. H ., Tang, H. S., Chao, Y. C., Lee, H. S., Hsiong, C. H., Pao, L. H., Hu, O. Y. P. (2008). Quantitative rat liver function test by galactose single point method. *Laboratory Animals*, *42*(4), 495-504. https://doi.org/10.1258/la.2007.06040e

Zeb, A., & Mehmood, S. (2004). Carotenoids contents from various sources and their potential health applications. *Pakistan Journal of Nutrition, 3*(3), 199-204. https://doi.org/10.3923/pjn.2004.199.204