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Antioxidant and sub-chronic toxicity studies on some biochemical parameters of the extract of *Rhipsalis neves armondii* K. Schuum. (Cactaeceae)

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ABSTRACT

Background: Rhisalis neves armondii K. Schuum. (Cactaceae) aerial parts preparation is used to treat inflammatory disorders and cancer in Nigerian ethnomedicine hence the need to study its antioxidant properties and to evaluate the toxicity profile. Methods: The dried, ground, aerial parts of Rhipsalis neves armondii were extracted with a mixture of methanol and dichloromethane (1:1). The obtained extract (RNE) was employed for antioxidant and sub chronic toxicity studies. Antioxidant activity was determined using two in vitro models: ferricyanide reducing power assay and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, while ascorbic acid was used as standard antioxidant agent. Sub chronic toxicity tests were performed on some hematological and biochemical parameters such as total leukocyte count, differential white blood count and levels of the liver marker enzymes, in rats. Results: The results showed that the RNE exhibited potent reducing power activity with mean absorbance value of 1.34 compared to 0.85 of ascorbic acid. The extract also produced a concentration-dependent percentage increase in DPPH radical scavenging activity at tested doses with IC40 values of 360 µg/ml and 130 µg/ml doses for RNE and ascorbic acid, respectively. There was no significant difference ($p<0.05$) in the liver marker enzymes (ALT, AST and ALT) and hematological parameters (neutrophils, lymphocyte, monocyte, eosinophil) of the animals at tested doses of the extract. The results also showed an LD50 of the extract greater than 5000 mg/kg body weight. Conclusion: The result indicated that the extract of Rhipsalis neves armondii possesses antioxidant activity with good toxicological and safety profile.

Keywords: Rhipsalis neves armondii; antioxidant; sub-chronic toxicity; biochemical parameters.

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1.0 Introduction

Oxidative stress is a major pathogenic event occurring in several diseases, ranging from metabolic to proliferation disorders such as coronary heart disease, inflammation, stroke, diabetes mellitus and cancer [1]. Reactive free radicals are highly responsible for oxidative stress and the pathological role of these free radicals in many ailments has been well established. Several biochemical reactions in our body system generate reactive oxygen species, which, if not effectively scavenged by cellular constituents, may lead to various morbid conditions [2]. This growing awareness of the role of reactive oxygen species (ROS) in disease conditions has led to a renewed interest in the search for novel antioxidant agents through extensive investigation of medicinal plants for novel antioxidant phytoconstituents. Antioxidants can be classified into enzymatic, non- enzymatic, and synthetic antioxidants. Enzymatic antioxidants are produced endogenously which include superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione S-transferase. The non-enzymatic antioxidants are variously obtained from medicinal plants and other natural sources and are mainly phenolic compounds such as tocopherols, carotenoids, ascorbic acid, flavonoids and tannins [3]. In addition, synthetic antioxidant compounds are butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which are commonly used in processed foods. However, reports have shown that they have several side effects such as risk of liver damage and carcinogenesis in laboratory rats [4, 5]. Many plant preparations and their bioactive constituents have already been reported to possess antioxidant activity by scavenging free radicals with therapeutic potential for free radical associated disorders [6, 7, 8]. *Rhipsalis neves armondii* K. Schuum. (Cactaceae) is among the numerous medicinal plants used in Nigerian ethnomedicine for the treatment of inflammatory conditions such as rheumatic pain and cancer disorders [9]. However, since these disorders could be attributable to oxidative stress, therefore, there the objective of this study was to evaluate the antioxidant potentials of *R. nerves armondii* as well as its sub chronic toxicological profile. Toxicological study of medicinal plants is of paramount importance as this will reveal their safety profile thus making them safe for both human and animal consumption.

2 Materials and methods

2.1 Animals

Adult Swiss albino mice (22-30 g) and rats (150-200 g) of both sexes were obtained from the Animal House Facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka and used for the study. The animals were housed under standard conditions (25 ± 2 °C) with free access to standard pellets (Guinea Feed Nigeria, Ltd.) and water. The animals were transferred to the research area 7 days before the experiment for acclimatization. All animal experiments were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals ((Pub No. 85-23, revised 1985) and in accordance with the University of Nigeria Ethics Committee on the use of laboratory animals, registered by the National Health Research Ethics Committee (NHREC) of Nigeria.

2.2 Plant material

Fresh aerial parts of *Rhiphalis nerves armondii* were collected in the month of April before from Nsukka, Enugu State, Nigeria. The plant was identified and authenticated by Mr. Alfred Ozioko, a taxonomist, of the International Center for Ethno-medicine and Drug Development (InterCEDD), Nsukka, Nigeria. There the voucher specimen was preserved at the herbarium of the InterCEDD with the number INTERCEDD 08 11.

2.3 Preparation of plant material

The fresh aerial parts of *R. nerves armondii* were collected, cut, dried and pulverized. The powdered plant material was extracted by cold maceration with a mixture of methanol and dichloromethane (1:1 ratio) for 48 h. The mixture was then filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary vacuum evaporator under reduced pressure (40–50 °C) to obtain the *Rhipsalis neves-armondii* extract (RNE) which was stored in the refrigeration till usage.

2.4 Phytochemical analysis

Qualitative phytochemical analysis for identification of plant phytoconstituents was

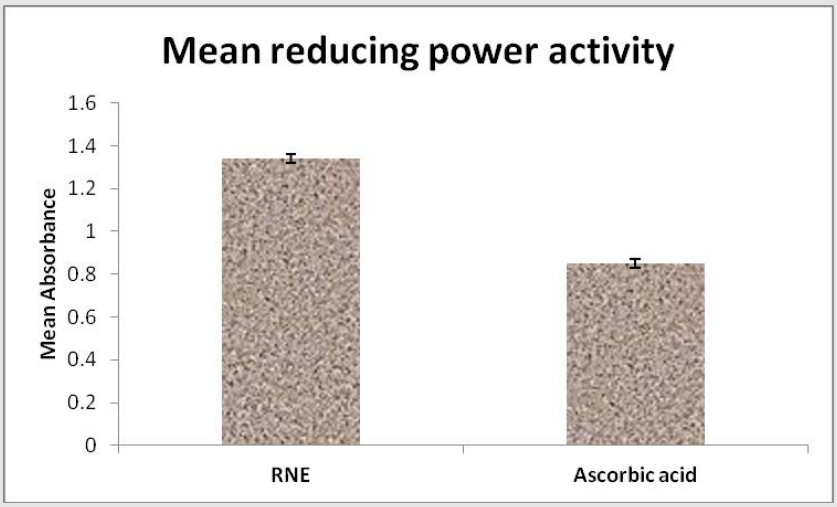

Figure 1: Mean reducing power activity

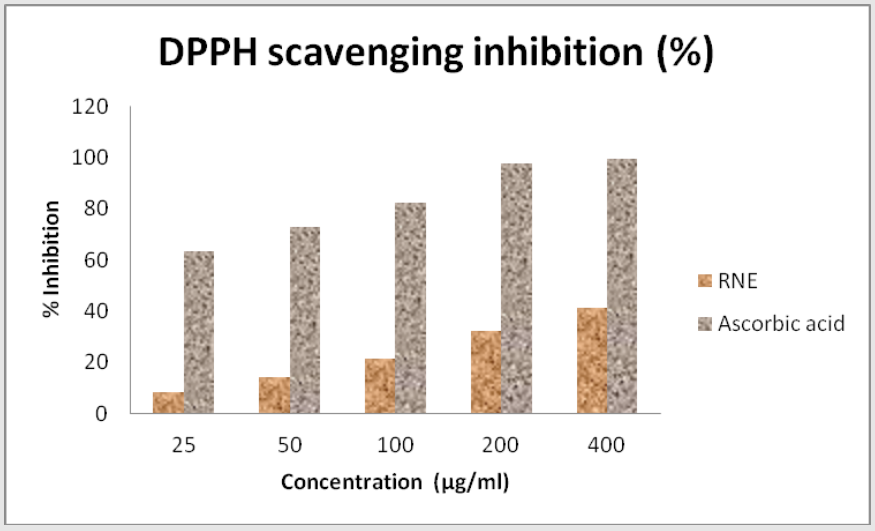

Figure 2: DPPH radical scavenging inhibition

Table 1: Phytochemical analysis

Phytoconstituent	Inference
Alkaloids	+
Flavonoids	+
Steroids	+
Saponins	-
Carbohydrates	+
Tannins	-
Terpenoids	+
Resins	+
Reducing sugars	+
Fats and oil	_
Glycosides	_

Key: + = presence; - = absence

performed on RNE using standard procedures outlined by Trease and Evans [10]. All reagents used for the phytochemical analysis were of analytical grade and freshly prepared.

2.5 Ferricyanide reducing power assay

The reducing power of the extract was determined using the modified method of Athukorala [11]. Briefly, a volume of 1.0 ml of different concentrations of the extract (25, 50, 100, 200 and 400 µg/mL) in five (5) test rubes respectively were mixed each with 2.5 ml of phosphate buffer (200 mM, pH 5.5) and 2.5 ml of potassium ferricyanide (30 mM) and incubated at 50 °C for 20 min. Thereafter, 2.5 ml of trichloroacetic acid (600 mM) was added to each of the reaction mixture and centrifuged for 10 min. at 3000 rpm. The upper layer (supernatant) of each of the centrifuged solutions (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (6 mM) and their absorbance read at 700 nm. A standard (ascorbic acid) was used as the positive control. The absorbance of the standard was read and the values compared with that of the test extract. Increased absorbance of the reaction mixture indicated higher reducing power of the plant extract.

2.6 DPPH free radical scavenging assay

The DPPH free radical scavenging activity of the extract was measured using the modified method of McCune and Johns [12]. Briefly, 1.0 ml of different concentrations of the extract (25, 50, 100, 200 and 400 µg/ml) in five (5) test tubes respectively were mixed each with 1.0 ml of DPPH in methanol (0.3 nM), and 1.0 ml of methanol to make 3.0 ml of the reaction mixture. The reaction mixture was incubated for 10 min. in dark, and absorbance read at 517 nm. A standard (ascorbic acid) was used as the positive control. The percentage inhibition was calculated using the following formula: **Inhibition (%) = A_0 - A_1/ A_0 x 100.**

Where A_0 = the absorbance of control, and A_1 = the absorbance of the extract.

2.7 Acute toxicity study

The acute lethal dose (LD_{50}) of RNA was ascertained by the method described by Lorke [13]. Briefly, the study was performed in two phases. In the first phase, 9 mice were divided into 3 groups of 3 mice per group, and treated with the RNE at the doses of 10, 100 and 1000 mg/kg (p.o.) respectively. The animals were observed for 24 h for signs of toxicity. In the second phase, four mice were used. Three were treated separately with RNE doses of 1600, 2900 and 5000 mg/kg respectively, while the fourth (the control) received 10 ml/kg of distilled water. The animals were observed for 24 h period.

2.8 Sub-chronic toxicity test

Adult albino rats (150-200 g) were divided into four (4) groups (n=5) to receive 100, 400 and 1000 mg/kg of the methanol-dichloromethane extract and 2 ml/kg distilled water (vehicle), respectively. The doses were administered orally for 21 days with close monitoring and measurement of their body weight on weekly interval. The group that received vehicle served as the control. On day 22, blood samples were collected from overnight fasted rats by retro-orbital puncture. The collected blood samples were kept at room temperature for about 30 mins to clot after which they were centrifuged at 3000 revs/min to separate the serum from the blood cells. The clear serum supernatants were carefully aspirated with a syringe and stored in clean sample bottles for further biochemical analysis. The biochemical parameters assayed include alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) according to previously described methods [14, 15]. The hematological parameters; white blood cell (WBC) count and differential count were determined using a previously described method [16]. Later the animals were sacrificed and organs; liver, kidney and heart were collected for further evaluations.

2.9 Statistical Analysis

The data obtained were analysed using One Way Analysis of Variance (ANOVA) (SPSS Version 20) software and presented as mean ± SEM. Differences between means were considered significant at $p < 0.05$, while Post Hoc test was Dunnett's-test.

3.0 Results

3.1 Phytochemical analysis

Table 2: Effect of RNE on hematological parameters

Treatment	Dose (mg/kg)	Hematological Parameters (x10^3 /ml) or(IU/L)				
		WBC	Neutrophils	Lymphocytes	Monocytes	Eosinophils
RNE	100	14.92±1.44	3.69±0.65	10.77±1.16	0.29±0.09	0.17±0.02
RNE	400	16.02.±0.76	2.99± 0.68	12.41± 0.38	0.29±0.07	0.27±0.08
RNE	1000	15.15±2.03	2.43±0.33	12.10±1.57	0.39±0.22	0.22±0.04
Control	-	14.53±1.17	3.16±0.65	11.10±0.75	0.14±0.11	0.12±0.03

Values are expressed as mean ± SEM; n=5; * p<0.05 significant when compared with the control (One-way ANOVA; Dunett's post hoc)

Table 3: Effect of RNE on organ weight

Treatment	Dose (mg/kg)	Organ (g)		
		Liver	Kidney	Heart
RNE	100	5.80±0.41	1.21±0.03	0.62±0.01
RNE	400	5.75±0.28	1.12± 0.03	0.61±0.03
RNE	1000	5.59±0.55	1.04±0.10	0.76±0.1
Control	-	6.19±0.53	1.23±0.06	0.66±0.07

Values are expressed as the mean ± SEM; n=5; * p<0.05 significant when compared with the control (One-way ANOVA; Dunett's post hoc)

Preliminary phytochemical analysis of *Rhipsalis neves-armondii* extract revealed the presence of phytoconstituents such as flavonoids, alkaloids, terpenoids, reducing sugars and resins (Table 1).

3.2 Ferricyanide reducing power assay

The RNE at 400 µg/mL exhibited the highest reducing power activity with a mean absorbance value of 1.34 compared to 0.85 of ascorbic acid, indicating a higher reducing power activity compared with the control (Figure 1).

3.3 DPPH free radical scavenging assay

The RNE or extract showed non significant scavenging activity on the DPPH radical scavenging assay. Estimated IC_{40} scavenging activity of the RNE and ascorbic acid were 360 µg/ml and 130 µg/ml doses, respectively. Furthermore, ascorbic acid showed a DPPH scavenging activity of 99.71% at 400 µg/ml dose (Figure 2).

3.4 Acute toxicity study

The RNE exhibited an estimated LD_{50} greater than 5,000 mg/kg (p. o.) and did not cause any lethality or show any signs of acute intoxication after a 48-h observation period.

3.5 Sub-chronic toxicity tests

Daily oral administration of RNE at the tested doses for 21 days did not manifest any obvious symptoms of toxicity and/or mortality in the rats. Also the effect on hematological parameters and organ weight showed no significant ($p < 0.05$) changes compared to the control. There was either no significant change in the values of leukocytes differentials (Tables 2 and 3). On the liver marker enzymes, the extract did not cause any dose-related significant ($p<0.05$) elevation in the levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) when compared with the control. However, it caused a non-significant ($p< 0.05$) elevation of AST at 400 and 1000 mg/kg doses (Table 4). At the end of the treatment period, all the animals showed an increase in their body weights with the highest percentage increase of 28.1% and 21.4% at 400 mg/kg dose and the control respectively (Table 5). Also there were no obvious changes in the morphological appearance of the organs when compared with the control.

Discussion

This study investigated the *in vitro* antioxidant activities and the toxicity profile of the aerial parts of *Rhipsalis neves-armondii* extract (RNE). Physiologically, antioxidants protect the body from various diseases due to oxidative stress by scavenging the reactive oxygen species (ROS) or reactive nitrogen species (RNS) and maintenance of stable metabolic processes [17]. Oxidative stress may be defined as a series of events resulting in an imbalance between oxidant and antioxidant reactions which often leads to degenerative disorders such as cancer, inflammatory diseases and artherosclerosis [18]. In the reducing power assay the increase in the mean absorbance exhibited by the RNE implies increase in the reducing power activity which is an indication of potent antioxidant activity [19]. In addition, the DPPH scavenging activity of the extract did not indicate a very potent activity because the IC_{50} could not be ascertained instead the IC_{40} was determined at the doses tested. Therefore, ascorbic acid showed a better antioxidant activity in the DPPH radical scavenging activity and this could be due to experimentally related factors. Reports have shown that medicinal plants, fruits and vegetables elicit antioxidant activity due to the presence of polyphenol phytochemicals present therein [18, 20]. Polyphenolic phytoconstituents in plants are flavonoids, stilbenoids, tannins and phenolic acids. The result of the phytochemical analysis showed that RNE contains flavonoids as the only phenolic compound present. Flavonoids have been shown to possess antioxidant, anticancer, antidiabetic, antimicrobial, hepatoprotective, neuroprotective and cardioprotective properties [21]. By extrapolation, therefore, flavonoids components of RNE may be suspected to be responsible for its antixodant activity. Sub-chronic administration of the extract to rats showed no significant ($p< 0.05$) elevation or decrease in the serum levels of AST, ALT and ALP compared with the control and also no changes in the other biochemical parameters. This is an indication that the extract has no potential harmful effect on the liver and leukocytes. The slight increase in the AST at higher doses may not even debunk our safety profile claim as such doses for adult

Table 4: Effect of RNE on Liver marker enzymes

Treatment	Dose (mg/kg)	Liver marker enzymes (IU/L)		
		ALT	ALP	AST
RNE	100	17.67±1.83	60.34±1.95	124.81±6.88
RNE	400	20.39±0.92	57.09± 2.20	166.21±7.75
RNE	1000	16.78±2.17	53.62±0.36	157.97±9.90
Control	-	20.56±0.72	60.35±1.97	127.38±8.04

Values are expressed as the mean ± SEM. n=5; * p<0.05 significant when compared with the control (One-way ANOVA; Dunett's post hoc test)

Table 5: Effect of RNE on the body weight

Treatment	Dose (mg/kg)	Mean Body weight (g)				Change in weight (%)
		Day 0	Day 7	Day 14	Day 21	
RNE	100	140.00±0.00	145.00±3.08	164.00±5.80	156.94±4.65	12.1
RNE	400	120.00±0.00	134.05± 0.10	151.25± 8.00	153.71±0.58	28.1
RNE	1000	120.00±0.00	126.00±4.00*	142.50±0.80*	142.86±7.59	19.1
Control	-	133.00±18.57	152.00±8.10	175.00±9.1	161.42±0.20	21.4

Values are expressed as the mean ± SEM. n=5; * p<0.05 significant when compared with the control (One-way ANOVA; Dunett;s post hoc).

human weight may not actually be therapeutically feasible. The increased body weight of rats at all doses during the period of treatment by RNE is also an indication of good safety profile. This is more so because increase in body weights of animals is an indicator of safety, whereas decrease in body weight indicate relative toxicity [22]. The organ weights (liver, kidney and heart) remained comparably the same to the control, also indicating good safety profile as these organs are considered highly useful in toxicity studies because of their sensitivity to harmful compounds and their potential to predict toxicity [23]. Further work is ongoing towards ascertaining the specific mechanism of action of the extract.

Conclusion

In conclusion, the result of this study has revealed that the aerial parts of *Rhipsalis nerves armondii* possesse antioxidant activity with relatively good toxicological profile buttressing the folkoric usage in inflammatory diseases.

Declaration of interest

Authors hereby declare no conflict of interest.

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References

1. Tell G, Vascotto C, Tiribelli C. Alteration in the redox state and liver damage: Hints from the EASL Basic School of Hepatology. Journal of Hepatology, 2013; 58:365-374.

2. Ganie SA, Zargar BA, Masood A, Zargar MA. Hepatoprotective and Antioxidant Activity of Rhizome of *Podophyllum hexandrum* against Cabon tetrachloride induced Hepatotoxicity in Rats. Boimed Environ Sci, 2013; 26(3): 209-221.

3. Rice-Evans C. Flavonoids and Isoflavones: Absorption, Metabolism and Bioactivity. Free Rad. Biol. Med., 2004; 36:827-828.

4. Ito N, Fukushima S, Hagiwara A, Shibata M, Ogiso T. Carcinogenicity of Butylated Hydroxyanisole in F344 Rats. J. Nat. Cancer Inst., 1983; 70: 343-347.

5. Oyedemi SO, Bradley G, Afolayan AJ. *In-vitro* and *in-vivo* antioxidant activities of aqueous extract of Strychnos henningsii Gilg. African Journal of Pharmacy and Pharmacology, 2010; Vol. 4(2):070-078.

6. Jamuna S, Paulsamy S, Karthika K. Screening of in vitro antioxidant activity of methanolic leaf and root extracts of Hypochaeris radicata L. (Asteraceae). Journal of Applied Pharmaceutical Science, 2012; 02 (07):149-154.

7. Prior RL and Cao G. Antioxidant Phytochemicals in Fruits and Vegetables: Diet and Health Implications. Hortic. Sci., 2000; 35:588-592.

8. Chanda S, Dave R. *In vitro* model for antioxidant activity Evaluation and some medicinal plants possessing antioxidant properties: An Overview. African Journal of Microbiology Research, 2009; 3(13):981-996.

9. Akunne TC, Akah PA, Nwabunike IA, Nworu CS, Okereke EK, Okereke NC and Okeke FC. Anti-inflammatory and anticancer activities of extract and fractions of Rhipsalis neves-armondii (Cactaceae) aerial parts. Cogent Biology, 2016; 2:1237259. http://dx.doi.org/10.1080/23312025.2016.1237259.

10. Trease GE, Evans WC. Test Book of Pharmacognosy. 11th ed. Brailliare Tindall and Macmillian Publishers, London, 1989; 176- 180.

11. Athukorala Y, Kin KN, Jeon YJ. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga *Ecklonia cara*. Food Chem. Toxicol., 44:1065-1074.

12. McCune LM and Johns T. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American Boreal forest. J. Ethnopharmacol., 2002; 82:197-205.

13. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicology, 1983; 54:272-289.

14. Reitman S and Frankel S. A colorimetric method for determination of serum glutamic oxaloacetic and glutamic transaminases. American Journal of Clinical Pathology, 1957; 28: 56-62.

15. Okoye TC, Akah PA, Ezike AC, Okoye MO, Onyeto CA, Ndukwu F, Ohaegbulam E, Ikele L. Evaluation of the acute and sub acute toxicity of *Annona senegalensis* root bark extracts. Asian Pacific J. of Tropical Medicine, 2012; 5(4): 277-282.

16. Schalm OW, Jain NC, Carroll EJ. Veterinary Haematology. 3rd ed. Lea & Febiger eds., Philadelphia, 1975; pp:129-25.

17. Karagöz A, Artun FT, Özcan G, Melikoğlu G, Anıl S, Kültür S and Sütlüpınar N. In vitro evaluation of antioxidant activity of some plant methanol extracts, Biotechnology & Biotechnological Equipment, 2015; 29(6):1184-1189.

18. Lima GPP, Vianello F, Corrêa CR, da Silva Campos RA, Borguini MG. Polyphenols in fruits and vegetables and its effect on human health, Food and Nutrition Sciences, 2014; 5:1065-1082.

19. Alam MN, Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods of evaluation of antioxidant activity. Saudi Pharmaceutical Journal, 2013; 21(2):143-152.

20. Crozier A, Jaganath IB, Clifford MN. Phenols, Polyphenols and tannins: an overview, in plant secondary metabolites: occurrence, structure and role in the human diet (eds. Crozier A, Clifford MN and Ashihara H), Blackwell Publishing Ltd, Oxford, UK, 2016.

21. Gul MZ, Ahmad F, Kondapi AK, Qureshi IA, Ghazi IA. Antioxidant and antiproliferative activities of *Abrus precatorius* leaf extract-*in vitro* study. BMC Complementary and Alternative Medicine, 2013;13:53.

22. Okoye TC, Akah PA, Ezike AC, Okoye MO, Onyeto CA, Ndukwu F, Ohaegbulam E, Ikele L. Evaluation of the acute and subacute toxicity of Annona Senegalensis root bark extracts. Asian Pacific Journal of Tropical Medicine, 2012; 5(4):277-282.

23. Bello I, Bakkouri, AS, Tabana YM, Al-Hindi B, Al-Mansoub MA, Mahmud R and Asmawi MZ. Acute and sub-acute toxicity evaluations of the methanolic extract of *Alstonia scholaris* stem bark. Med. Sci., 2016; 4(4):1-14