Antibacterial assay and reversion of carbon tetrachloride induced liver damage on wistar mice by *Vernonia amygdalina.* Delile

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Abstract: In this study *Vernonia amygdalina*. Delile stem and leaves were evaluated for anti-cancer and antibacterial actions against selected microorganisms. The crude plant extracts were extracted using methanol and fractionated into ethyl acetate, hexane, aqueous and crude extracts. Cancer inducing agent Carbon tetrachloride (CCl_4) was introduced into Wistar mice and screened with crude extracts of *Vernonia amygdalina*. Delile to evaluate the anti-cancer properties of the extracts. The effect of CCl_4 in mice was determined by isolating and quantifying damaged DNA fragments in liver and blood using DNA zol BD and diphenylamine (DPA). Results showed significant difference ($p < 0.05$) observed between the extracts and the controls. The observed changes obtained were concluded to be as a result of prophylactic effect of *Vernonia amygdalina*. Delile For the antibacterial studies, 20 mg/ml extracts fractions were tested against clinical isolates: *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi and Pseudomonas aeruginosa* using agar-well diffusion technique. The extracts exhibited selective inhibitions against the isolates. The diameter of the zones of inhibitions observed were between: 0.0 - 35.0 mm, with ethyl acetate fraction given the highest inhibition. It was evident from this investigation that crude extracts of *Vernonia amygdalina*. Delile effectively reduced cancer damage caused by CCl_4.

Keywords: *Vernonia amygdalina* Delile, DNA damage reversion, carbon tetrachloride, hepatoxicity, anticancer, antibacteria.

INTRODUCTION

Malignant neoplasm commonly referred to as cancer is a public health concern, having an effect on all age groups in human. Malignant neoplasm occurs when there is abnormal tissue mass, with uncoordinated growth which exceeds the normal tissue despite cessation of stimulus. This could occur as solid tumors in the liver, lung, intestine, breast, blood and lymphatic system-the bone marrow. Exposure to toxins partly is one of the conditions that could predispose one to cancer. Living cells have several protective mechanisms to counteract reactive oxygen species (ROS) generated when one is exposed to these toxicants. Conversely in a diseased state, the process may be truncated. ROS such as super oxide, hydrogen peroxide and hydroxyl are highly reactive causing DNA mutation, protein degradation, and lipid per oxidation and cell death (Cho *et al*., 2014).

Recently, interests in exploiting plants for medicinal purposes have been on the increase especially in Africa where endemic diseases caused by microorganisms are building up resistance to several synthetic drugs. These have created conditions where some of the general and cheap antimicrobial agents have lost their effectiveness (Nwinyi *et al*., 2008; 2009). In addition, access to modern health care facilities in developing countries are often inadequate or none- existence. Thus people consent to the use of herbal remedies due to low cost and trust in the safety and efficacy of traditional medicine. Ogbulie *et al*. (2007) reported that herbal medicine practitioners make use of the various parts of plants such as roots, leaves, flowers, stems, berries, plants to prevent, alleviate, and care for illness. This has been an age-long practice. In the twentieth century, it is noteworthy that many medications were developed from primeval healing traditions that treated ailments with specific plants. Medicinal plants exert bacteriostatic and bacteriocidal on bacteria and other microorganisms. In this day and age, science has separated the medicinal properties of abundance botanicals, and their healing components extracted and analyzed.

Vernonia amygdalina. Delile is the most widely cultivated species of the genus *Vernonia* with about 1000 species of shrubs. It is multipurpose plant found in natural forests, at home or commercial plantations with a number of potential uses. It is commonly identified as bitter leaf due the bitter taste of the leaves. *Vernonia amygdalina*. Delile belongs to the family Compositeae with the shrub growing to 2 to 10m tall and cultivated by stem- cutting at

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angle of 45^{o}C. *Vernonia amygdalina*. Delile grows under a variety of ecological zones in Africa with high biomass yield, adaptability and compatibility with other crops. The plant improves the soil nutrients and moisture and overall improves the soil fertility (Mekoya *et al*., 2008). *Vernonia amygdalina*. Delile has been named differently in various ethnic groups. For instance Bitter leaf is called Omjunso in East Africa especially Tanzania. In Nigeria, among the Igbo-tribe in South East it is called Onugbo, Orugbo among the Itsekiri and Urhobo tribes in Nigeria, Ewuro (Yoruba), Etidot (Ibibio), Ityuna (Tiv), Oriwo (Edo), Chusa-doki Shiwaka (Hausa), Muanya, 2013. Iwu, (2002); Sabiu and Wudii (2011) stated that with just a little amount of processing *Vernonia amygdalina* Delile, can be classified as healthy food because it contains active drug molecules and other substances that promote healthy development of the body and maintenance of physiological functions of the body. The use of *Vernonia amygdalina* Delile as medicinal herb started when zoopharmacologist discovered that during season sick chimpanzees with empty stomach sucked pith and juice from *Vernonia amygdalina* Delile plant stalk for self-deparatization, enhancement of body fitness, increase in strength or appetite and reduced constipation (Yeap *et al*., 2010). Since this discovery, subsequent researches had unveiled more bioactivities possessed by different extracts of this plant such as antidiabetic (Farombi, 2003; Erasto *et al*., 2006, Ebong *et al*, 2008), antibacterial (Madureira *et al*., 2002), antimalaria (Ajibola *et al*., 2011), antifungal (Ogbebor *et al*., 2007); antioxidant (Ayoola *et al*., 2008; Erato *et al*., 2007a,b; Iwalewa *et al*., 2005; Oboh,2005), Anti-cancer and cytotoxic effects(Fasakin *et al*., 2011, Izevbigie, 2003, 2004; Izevbigie *et al*., 2005; Howard *et al*., 2006) which are beneficial to health. Compounds including steroid glucosides, linoleic and linolenic, sesquiterpene lactones, alkaloids, saponins, vernonin, the sesuiterpenes, vernolepin and vernodalin, and the ubiquitous flavonoid, kaempferol and flavonoids have contributed to its bitter taste and bioactivities have also been isolated from this plant (Favi *et al*., 2008). Toxicology studies documented on this plant shows that *Vernonia amygdalina* Delile has little or no toxicity thus supporting the safe use of this plant for the benefits of health (Utoh-Nedosa, 2011).

Decoctions from the various parts of the plant are commonly used in traditional medicine. For instance, the aqueous extract of *Vernonia amygdalina* Delile showed an increasing effect against the growth of estrogen receptor negative ductal carcinoma (BT-549) cell line in a concentration dependent fashion with IC_{50} at 1000 µg/ml through inhibition of DNA synthesis (Opata and Izevbigie, 2006). Thus, the addition of *Vernonia amygdalina* Delile into the diet of cancer patients can improve their prognosis or quality of life (Gresham *et al*., 2008; Robinson *et al*., 2009). Khalafalla *et al*. (2009), reported of the induction of apoptosis against acute lymphoblastic leukemia and acute myeloid leukemia in patients with IC_{50} ranging between 5 to 10µg/ml when challenged with cold water, hot water and ethanol extracts of *Vernonia amygdalina*. Delile. Ajibola *et al*. (2011) documented that fractionation of the crude extracts of *Vernonia amygdalina* Delile could result in polyphenol fractions with higher potency. In addition, Froelich *et al*. (2006) demonstrated that combination of petroleum ether/ethyl acetate leaf extract of *Vernonia amygdalina* possessed cytotoxic effect towards human hepatoblastoma (HepG2) and urinary bladder carcinoma (ECV-304) cell lines.

Although the traditional claims of the use of *Vernonia amygdalina* Delile in the treatment of various ailment has been pharmacologically validated, Austin (2000) reported that geographical location can greatly influence the therapeutic activities of this all purpose plant. In furtherance, we demonstrated the antibacterial activities of the leaves and stems of *Vernonia amygdalina* Delile obtained from South- East and South-West geographical regions in Nigeria; against selected gram positive and gram negative bacteria and the reversion of DNA induced damage in the liver of Wistar mice using crude extract of *Vernonia amygdalina*. Delile. In this study, we used carbon tetrachloride (CCl_4) a toxic substance to induce the DNA damage. CCl_4 toxicity is known to cause changes in endoplasmic reticulum with loss of metabolic enzymes in the intracellular structures. The CCl_4 could be biotransformed by cytochrome P450 system to produce the toxic metabolite trichloromethyl free radicals that covalently binds to cell membranes and organelles to elicit lipid peroxidation. We report here-in, that despite the geographical location *Vernonia amygdalina* Delile exhibited antibacterial activities and also reversed the DNA damage caused by CCl_4 in wistar rats.

MATERIALS AND METHODS

Chemicals, reagents and media

Methanol, Hexane, Ethyl Acetate, Acetone, Ethanol of analytical grade were obtained from Sigma Aldrich. The agarose, electrophoresis grade was procured from Alfa, Aesar 26 Parkridge Rd Ward Hill, MA 01835 and DNAzol kit was obtained from Qiagen. The TAE (Tris Acetate-EDTA) buffer, Tris chloroacetic Acid solution (TCA), Olive oil, Grams staining reagents, 0.5% McFarland and other chemicals such as Diphenylamine, Diethyl ether, Formalin, Heparin, Isopropanol, carbon tetrachloride (CCl_4) were supplied by Microbiology laboratory, Covenant University. In addition, starch agar, methyl red and voges proskauer medium were also supplied by the Microbiology Laboratory, Covenant University. Mueller Hinton Agar, Nutrient agar (NA), Citrate and Christensen's urea agar, Peptone water were obtained from Lab M limited, Heywood Lancashire BL9 7JJ United Kingdom.

Table 1a: Experimental design of the ant-cancer assay

Groups	Purpose	Dosage
Group 1	Normal group	Tween 80 + olive oil
Group 2	Control group (negative)	Tween 80 + CCl_4 (7.5ml olive oil +2.5ml of CCl_4)
Group3	Study group	12.5mg/kg *Vernonia amygdalina* Del. crude extract + CCl_4 (7.5ml olive oil +2.5ml of CCl_4)
Group 4	Study group	25mg/kg *Vernonia amygdalina* Del. crude extract + CCl_4 (7.5ml olive oil +2.5ml of CCl_4)
Group 5	Study group	50mg/kg *Vernonia amygdalina* Del. crude extract + CCl_4 (7.5ml olive oil +2.5ml of CCl_4)
Group 6	Control group(positive)	10mg/kg of Quercetin+ CCl_4 (7.5ml olive oil +2.5ml of CCl_4)

Table 1b: Morphological and biochemical characteristics of the test organisms

Organisms	*Bacillus subtilis*	*Staphylococcus aureus*	*Salmonella typhi*	*Pseudomonas aeruginosa*
Grams reaction	+ve long rods in chains occurring singly	+ve grape like in shape occurring in clusters	-ve long rod shaped	-ve pink very short rods in clusters
Citrate test	+	-	+	+
Urease test	-	+	-	-
Oxidase test	-	-	+	+
Indole test	-	+	-	-
Starch test	+	-	+	-
Coagulase test	-	-	+	-
Catalase test	+	+	+	+

+: Positive Reaction; -: Negative Reaction

Table 2: Antibacterial activities of the Veronina amygdalina extract on the test organisms

Plant fractions	Test organisms	Mean zones of inhibition (mm)			
		LA	LB	SA	SB
Crude	*Bacillus subtilis*	21.5	-	-	-
	Staphylococcus aureus	18	-	-	-
	Salmonella tyhpi	8	-	-	-
	Pseudomonas aeruginosa	-	-	-	-
Aqueous	*Bacillus subtilis*	18.5	4.5	3	8
	Staphylococcus aureus	18.5	-	-	4.5
	Salmonella tyhpi	15.5	-	-	-
	Pseudomonas aeruginosa	8	-	7	-
Hexane	*Bacillus subtilis*	-	23.5	20.5	28
	Staphylococcus aureus	28	7.3	4	22.5
	Salmonella tyhpi	8	-	17.5	18.5
	Pseudomonas aeruginosa	8	8.5	22	15.5
Ethyl acetate	*Bacillus subtilis*	32.5	22.5	24	22
	Staphylococcus aureus	6	7	25	-
	Salmonella tyhpi	35	12.5	-	-
	Pseudomonas aeruginosa	10	13.5	7	8.5

LA – freshly obtained leaves and SA- freshly obtained stem of Vernonia amygdalina that were not stored , LB freshly obtained leaves and SB- freshly obtained stems of Vernonia amygdalina were stored for a period of time under controlled environmental conditions (-) no significant inhibition observed.

Collection of plant samples

In this study we used two types of *Vernonia amygdalina* Delile (bitter leaf) samples: the fresh leaves and stems obtained during the onset of the study, and leaves and stems that were dried were preserved for 6months. The sample collection and preparation was between collected in December, 2013 and July 2014. The fresh leaves and stem of *Vernonia amygdalina* Delile (bitter leaf) were collected from Mr. Isiaka compound at Iju, Ota, Ogun State Nigeria while the preserved leaves and stem samples were obtained from the compound of Chief Samuel C.O and Mrs. Rose O. Nwinyi of blessed memory of Urukpaleri village, Nawfia, Anambra State, Nigeria. All the *Vernonia amygdalina* Delile samples were identified

and authenticated by a qualified botanist Dr. Conrad A. Omonhinmin of the Department of Biological Sciences, Covenant University. The *Vernonia amygdalina* Delile samples were deposited in the Department. The fresh samples were air dried at room temperature, milled to uniformity and stored in a tight container until further use.

Preparation of leaves and stem extracts of Vernonia amygdalina. Delile

For ease of identification we named the freshly obtained leaves and stems as LA, SA while the preserved leaves and stems were named LB;SB respectively. Four hundred and fifty grams (450.0g) of the dried powdered leaves and stems *V. amygdalina* .Delile were respectively transferred into two separate tanks for cold extraction using methanol as solvent. For the preserved leaves and stems, 250g each of the dried powdered stem and leaves of *V. amygdalina* .Delile were also transferred into two separate tanks for cold extraction using methanol. After 8 days, the extracts were strained and filtered. The filtrates were dried in *vacuo* at 40^{o}C using rotary evaporator. The extracts were further dried over calcium chloride in a desiccator. The dried extracts were kept at 4^{o}C until further analysis.

Batch partitioning

The crude, methanolic extract of the plant samples were fractionated into hexane, ethyl acetate and aqueous layers successively by solvent-solvent extraction using the separating funnel. Each fraction including the aqueous was evaporated to dryness. The extracts obtained, were reconstituted with their corresponding solvents at a concentration of 20mg/ml for antimicrobial tests (Cos *et al*., 2006 Jorgensen *et al*., 1999; Joseph *et al*., 2006).

Test organisms

Four clinical isolates were used for the antimicrobial susceptibility tests. These organisms include *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus subtilis*. These are broad spectrum microorganisms, i.e., gram +ve and gram-ve, known to cause a wide range of ailments and discomfort ranging from: Stomach upsets, to dysentery, food poisoning, typhoid fever, skin infections etc. By using these organisms as test samples, the efficacy of *Vernonia amygdalina* Delile against either group of bacteria could be observed. These organisms were obtained from the Microbiology Laboratory, Covenant University, Nigeria. The selected organisms were tested for their viability by resuscitating them on buffered peptone broth. Thereafter, the organism were spread on nutrient agar slant and preserved on the slant at 4^{o}C. The isolates were sub-cultured in nutrient agar at 37^{o}C for 24 h prior to further studies.

Purification and characterization of the test organisms

Pure cultures from the stock culture were plated onto nutrient agar. This was incubated at 30±2^{o}C for 24hrs. Colonies were periodically transferred aseptically to fresh nutrient agar. These organisms were further validated by cultural, morphological and biochemical characterization.

For this, the standard cultural, biochemical and morphological techniques and comparison with standard reference organisms according to (Cowan, 1985, Olutiola *et. al*.1991) we used to re-validate the selected organisms. The following tests were carried out: Gram stain morphology, catalase, coagulase, colony motility, methyl red, voges proskauer, nitrate reduction, indole, spore test, gelatin hydrolysis, oxidase, starch hydrolysis, and citrate tests.

Antimicrobial susceptibility assay of the plant extracts (stem and leaves)

The clinical isolates *Pseudomonas aeruginosa*, *Salmonella typhi, Staphylococcus aureus and Bacillus subtilis* were selected and then placed in 2 ml sterile saline water (0.5%) respectively. The suspension was mixed using a vortex mixer and held side by side with 0.5 McFarland solutions. Agar well diffusion technique as described by (Adeniyi *et al*., 1996, Jorgensen *et al*., 1999; Joseph *et al*., 2006) was used to determine the antibacterial activity of the different crude, methanolic extract, hexane, ethyl acetate and aqueous extracts. Mueller Hinton (MH) test agar plates were seeded with 0.1ml of an overnight culture of each bacterial isolate (equivalent to 10^{5} CFU mL^{-1}).The seeded plates were allowed to set and a standard cork borer of 6 mm diameter was used to cut uniform wells on the surface of the agar (Nwinyi *et al*., 2009). The wells were then filled with 0.3 ml of each extracts at a concentration of 20mg $ml^{-1.}$ Methanol, ethyl acetate, hexane and distilled water served as controls were also put in separate wells. All the plates were incubated at 37^{o}C for 24 h. The assay was conducted at regular intervals of 24 h until marked decline in the potency of the extracts to inhibit the growth of the test organisms was noticed. Zones of clearance round each well means inhibition and the diameter of such zones were measured (See plate 1). The entire assays were carried out in triplicates. MH agar was selected as medium of choice because it illustrates satisfactory batch-to-batch reproducibility testing and its low content in sulfonamide, trimethoprim and tetracycline inhibitors. In addition, MH agar supports acceptable growth of most non-fastidious bacteria species and several data has been collected with reference to susceptibility tests carried out with this medium (Hudzicki, 2013).

Anti-Cancer Assay

Experimental animals

The experimental animals (albino mice of both sexes) weighing between 12-34kg were used for this study. The mice were obtained from the Biochemistry Department, University of Agriculture, Abeokuta, Ogun State, Nigeria. The mice were placed in the animal house of the

Department of Biological sciences, Covenant University. The experimental animals were handled and used in compliant with the international guide for the care and use of laboratory animals of the National Institute of Health. The experimental animals were kept in standard laboratory conditions. The mice had access to clean drinking water and the ambient environment maintained. Animals were fed with standard mice feeds and allowed to acclimatize for a period of 7 days before commencement of experiment.

Preparation of assay materials

Hundred millilitres (100ml) of 0.5% Tween-80 was measured and mixed with sterile distilled water. This was poured into sterile bottles. Quercetin was prepared by weighing 0.01g into 10ml of 0.5% Tween-80 this was poured into a 100ml beaker. 15ml of Tween-80 was added to the Quercetin in the beaker.

For *Vernonia amygdalina*, 1.0g of the crude extract was weighed and dissolved in sterile distilled water. Three different concentrations of the extracts were prepared: 12.5mg/kg, 25mg/kg and 50mg/kg. For Carbon tetrachloride (CCl_4) preparation, 7.5ml of Olive oil was added to 2.5ml of CCl_4.

Experimental Design

Twenty- seven albino mice of both sexes weighing between 12-34 kg were used for this experiment.. The mice were grouped into six (1-6) as shown in Table 1a, where each group had a minimum of 4 mice in all. The Animals of Group 1 served as normal group and were given Tween-80 solution. The Animals of Group 2, served as negative control. They were served with Tween-80 solution. Animals of Group 3, were given 12.5mg/kg of *V. amygdalina* Delile crude extract. The Group 4, was given 25mg/kg of extract and Group 5, was given 50mg/kg of *V. amygdalina* crude extract and Group 6 (positive control) was given 10mg/kg of Quercetin. The extracts were administered to the mice orally with the aid of a canular attached to a syringe. The animals were observed closely for the first 4 hours and thereafter daily for the next eight days. On the eight day, the mice were challenged with the cancer inducing agent (CCl_4). Each mouse was administered 0.02ml of the solution of CCl_4. Group 1 was given olive oil while groups 2-6 were given CCl_4. The dosages were according to the weights of the mice (0.1ml/10g) and after eight days they mice were subjected to 12 hours fasting and thereafter were sacrificed.

Blood collection and Preparation of sample for DNA isolation

Following the end of the experimental period, the experimental animals (mice) were sacrificed under light ether anesthesia. The bloods of the animals of the different groups were collected differently using the anticoagulant tubes. The animals were cut open and the liver removed. The liver was suspended in ice-cold solution and homogenized for the isolation and quantification of DNA. DNA was isolated using DNAzol according to manufacturer's instruction.

Quantitative assay of DNA fragmentation using diphenylamine (DPA) from the blood and liver

DNA fragmentation was accessed as described by Gercel-Taylor (2005). Briefly, 0.1g of the liver homogenates was added to (1000µl) lysis buffer solution. This was vortexed and centrifuged at 15000g for 15mins. For the blood, 1000µl of lysis buffer was added to the blood samples (100µl) and vortexed and stored for 30mins at 4^oC. It was then centrifuged at 15000xg for 15mins. In each of the liver and blood supernatants, 750µl of 20% TCA were added respectively and mixed by inversion until it pellets. Six hundred fifty microlitres (650µl) of 5% TCA were added to the different pellets after which each was incubated at 4°C overnight. The supernatant were centrifuged at 2500 x g for 10mins and the supernatant decanted and discarded. To the pellets, 650µl of 5% TCA was added, 2 tubes with 650µl of 5% TCA was prepared as blank. A hole was then made on top of each eppendorf tube and boiled for 15mins at 100°C in the water bath. It was allowed to cool to room temperature then centrifuged for 5mins at 2500 x g. Five hundred microlitre (500µl) of each supernatant was then transferred to another set of labeled Eppendorf tubes to which 1000µl of diphenylamine reagent (DPA) was added to the tubes. These were incubated at 37°C for 4 hrs and the absorbance read at 600nm.

Gel electrophoresis

In order to assess the genomic DNA fragmentation, extracted DNA samples were subjected to agarose gel electrophoresis on 7.5% agarose. At the end of electrophoresis, the gel was then placed on a UV-transilluminator for observation.

Authors declare that all experiments were scrutinized and endorsed by the appropriate Ethics Ccommittee and have therefore been executed in accordance with the ethical standards put forward in the 1964 Declaration of Helsinki."

RESULTS

The percentage yield of the crude extract is as shown in fig 1. In this we recovered between 10-14% crude extracts from the freshly obtained leaf and stem (SA, LA) than the preserved leaf and stem (SB,LB) that gave about 6 % yield of the crude extract of *V. amygdalina* Delile crude extract.

The four bacteria species obtained from the microbiology laboratory were re-validated by characterization using the morphological and biochemical characteristics as shown in table 1b. The clinical isolates *Bacillus subtilis* and *Staphylococcus aureus* were catalase positive, coagulase

negative and gram oxidase negative. The *Bacillus subtilis* and *Staphylococcus aureus* were all gram positive. The *Salmonella typhi* and *Pseudomonas species* were gram negative, oxidase and citrate positive. In the indole test the organisms were negative.

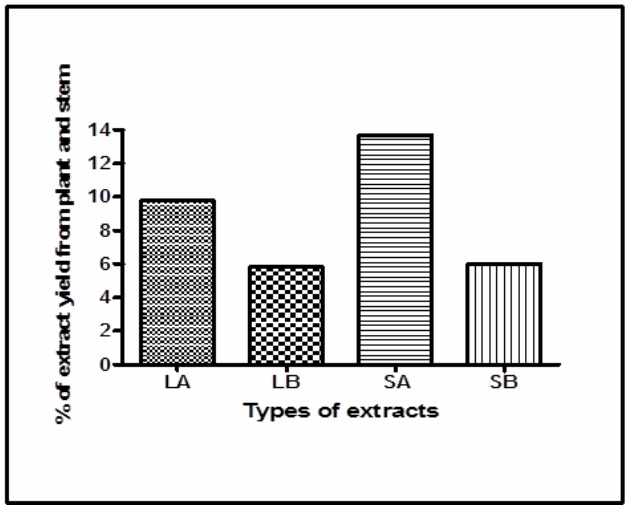

Fig. 1: Percentage %yield of leaf and stem samples of *V. amygdalina* Delile, calculated as: weight of plant samples extracted/ weight of plant samples after milling X 100.

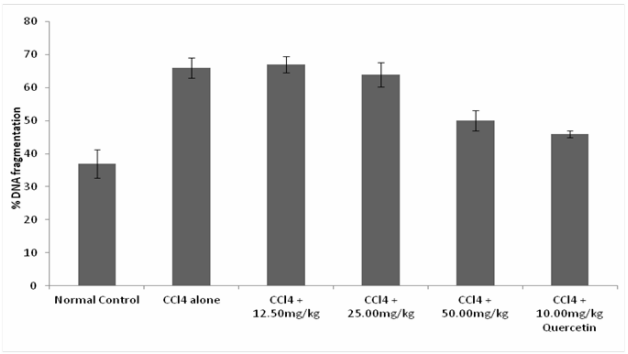

Fig. 2: Shows the Bar Chart level of DNA damage induced with CCl_4 in the liver of the mice.

The table 2, showed the data obtained from the antibacterial susceptibility tests of the crude, aqueous, hexane and ethyl acetate extract of *Vernonia amygdalina* Delile. The determination of the antibacterial activity of the crude extract from the freshly obtained leaves of *Vernonia amygdalina* Delile showed that the extracts possessed antibacterial activity against all the test organisms except *P. aeruginosa.* The crude extracts of the stem of the freshly prepared *Vernonia amygdalina* Delile and the preserved stem and leaves (SB, LB) showed no activity on the test organisms (*S. aureus, P. aeruginosa, Salmonella typhi and Bacillus subtilis*). From the result obtained for the crude extract, *Bacillus subtilis* was the most inhibited with the mean zone of inhibition of 21.5 mm and the least inhibition 8mm was obtained for *Salmonella typhi*. *Bacillus subtilis* and *S. aureus* was the most sensitive organisms to the aqueous extract of *Vernonia amygdalina* Delile with the zone of inhibition of 18.5 mm for the freshly obtained leaves and the least sensitive organisms to the aqueous extract from freshly obtained stem (SA) was *Bacillus subtilis* with the zone of inhibition of 3.00 mm at the same concentration (20 mg/ml). The SB and LB showed antibacterial activities on *Bacillus subtilis* and *S. aureus* with mean zones of inhibition from 4.5- 8.00 mm. The hexane extract of the different types of *Vernonia amygdalina* Delile samples (SA, LA, SB, LB) showed antibacterial activity against the test organisms with the mean zones of inhibition ranging from 28- 4mm. The highest zone of inhibition 28 mm on *Bacillus subtilis* and *S. aureus* were obtained from LA and SB extracts. The ethyl acetate fractions of the SA, LA, SB and LB all showed inhibitions on the test organism however the SB and SA fractions had no activity on the *S. aureus* and *Salmonella typhi.* In terms of highest zones of inhibition recorded in this study, the ethyl acetate fraction of the freshly obtained leaves showed the mean zone of inhibition of 35 -32.5mm on *Salmonella typhi* and *Bacillus subtilis.* From this investigation, it was observed that ethyl acetate and hexane fractions had more inhibitory activity against the test organisms when compared to other fractions (aqueous, crude and ethyl acetate) at same concentration (20 mg/ml).

Plate 1: Inhibition of the test organisms by different fractions of the LA extracts of *Vernonia amygdalina* Delile

In all, *V. amydalina* extracts (SA, LA, SB and LB) had bacteriostatic effect on all test organisms.

In fig. 2, the bar chart depicts the 1st group of mice (normal control), that were given only Tween-80 and Olive oil. The second group (negative control), were mice that were given Tween-80 challenged with carbon tetrachloride. . The 3rd, 4th and 5th groups, served as the study group and were served with (CCl_4) and 12.50, 25.00, 50.00mg/ml respectively for 3rd 4th and 5th groups. The 6th group were served with CCl_4 + 10.00mg/kg Quercetin.

STATISTICAL ANALYSIS

The liver assay results obtained were reported as mean and standard deviation. One-way ANOVA (Analysis of Variance) with Tukey's multiple comparison tests were done to determine the overall significant level of the obtained data. The significant difference was observed when *P*-value was $p < 0.05$.

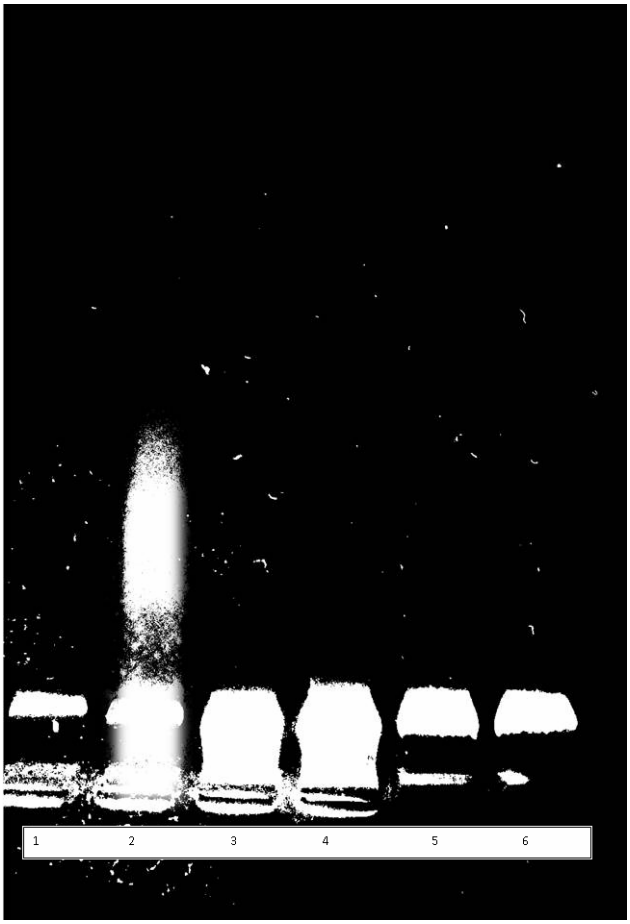

Plate 2: Ethidium bromide stained gel electrophoresis of DNA damage induced in the liver of the mice.

Representative image of the gel electrophoresis showing the normal control and those treated with *Vernonia amygdalina*. Delile and carbon tetrachloride as presented in fig. 2, exhibited a reversal effect on the genotoxic damage on the DNA of the mice. As shown in this fig. there was significant difference ($P<0.05$) between the normal group and the second group (negative control) amended with carbon tetrachloride (CCl_4), the 3rd and 4th groups were amended with 12.5 and 25.00mg/kg respectively of *Vernonia amygdalina* extracts and the carbon tetrachloride (CCl_4). In fig. 2, the study groups (2nd, 3rd and 4th groups) showed no significant difference in the level of DNA damage between the negative control and mice fed with carbon tetrachloride (CCl_4) and 12.50 and 25.00mg/kg of *Vernonia amygdalina* extracts. It was obvious that the hepatoprotective effect of *Vernonia amygdalina* had little or no pronounced effect on the percentage of the DNA fragmentation. Assessing the level of significance in terms of DNA damage between the mice in groups 5 and 6 (positive control), fed with (50.00 mg/kg) of *Vernonia amygdalina* and 10.00 mg/kg Quercetin respectively, and those in group 2 (negative control) amended with carbon tetrachloride CCl_4 $P< 0.05$. Associating the group 3 (mice fed with carbon tetrachloride CCl_4 and 12.5mg/kg of *Vernonia amygdalina*) to those of groups 5 amended with 50.00 mg/kg of *Vernonia amygdalina*, there was significant difference in the hepaprotective potential of *Vernonia amygdalina*. Comparing the extent of DNA damage between mice in groups 5 and 6, those fed with 50mg/kg and 10mg/kg of *Vernonia amygdalina* and quercetine, there was no significant difference in the values obtained. This technique has shown that *Vernonia amygdalina* leaf extracts, reverses DNA damage in a dose-dependent manner.

DISCUSSION

In this study, we have assessed the anticancer effects of *Vernonia amygdalina* Delile (VA) crude extracts on DNA induced damage caused by carbon tetrachloride and antibacterial activities of the leaf and stem extracts. Results obtained from anticancer assay in group 5 of our study (mice fed with 50mg/kg of VA) strongly suggest that VA crude extracts could significantly inhibit genotoxic DNA damage caused by exposure to toxic contaminant that can result to malignant neoplasm. The obtained results was in agreement to the findings of Arhoghro *et al*., 2009 that reported of 15% extract VA reverting completely liver damage of treated animals to normal. Tekobo *et al*. (2002) and Hsiang *et al*. (1989) corroborated to our findings, and stated in their reports that agents that have the potentials to reverse DNA damage are generally excellent options for cancer therapy. In furtherance, Khalafalla *et al*. (2009) demonstrated that the cold water, hot water and ethanol extracts of VA could induce apoptosis against acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Likewise, Froelich *et al*. (2006), stated that the combination of petroleum ether/ethyl acetate leaf extracts of VA showed cytotoxic effect in human hepatoblastoma (HepG2) and urinary bladder carcinoma (ECV-304) cell lines. Taken together, our result on the anticancer effect of crude extract of VA and those from previous reports it may be considered that VA could prevent or delay process of cancer initiation in human.

In the antibacterial susceptibility study, the extracts exhibited bacteriostatic effect on test organisms, although there were variations in the extent of antibacterial sensitivities of the extracts on the isolates (table 2). The observed variation perhaps could to be due to each

solvent ability to recover the different bioactive compounds present in VA. The hexane fraction showed more antibacterial activity against all the test organisms (4-28mm) when compared with the zones of inhibition from other fractions. The ethyl acetate fraction exhibited relatively higher activity than the aqueous and finally the crude fractions. The obtained result was in agreement with Foo *et al*. (2014) that reported that the choice of solvents and extraction method plays important role in the preparation of plant extracts. In furtherance of their study, they showed that the use of solvents of different polarity affects not only the extraction yield but also the content of the total phenolic and total phenol content, the free radical scavenging activities, the bioactive compounds that can be obtained and the antimicrobial activity of the extracts. Thus they inferred that more polar solvents gave higher extraction yields than the less polar solvents. Taken this together, it is obvious that different bioactive compounds were extracted by the different solvents used. The hexane fraction, exhibited more bacteriostatic action on the gram positive (*Bacillus subtilis; Staphylococcus aureus*) organisms than the gram negative organism (*Salmonella typhi* and *Pseudomonas aeruginosa* . This could be due to the differences in the cell wall architecture of the gram positive and gram negative organisms. Cos *et al.* (2002) demonstrated that *Vernonia amygdalina* Delile was more sensitive towards the gram positive bacteria than gram negative bacteria. For the gram negative bacteria species that exhibited minimal antibacterial sensitivities, it could be due to their possession of lipopolysaccharide structure that are amphipathic, negatively charged and highly conserved. The negative charge of the lipopolysaccharide could repel hydrophobic molecule, serve as barrier for chemical defense especially many antibiotics.

The antibacterial activities of the ethyl acetate extract showed almost similar antibacterial potential as the hexane extract. However this, it was obvious that extracts from fresh leaves (6-32.5mm) and stems (4-25mm) showed more activity than those obtained from the preserved sample of stem and leaves. This shows that there is likelihood of loss in effective antimicrobial activities when leaves and stems of medicinal plants are stored for longer duration. This is usually a common practice among trado-medical practitioners that preserve their medicinal plants over long duration. Several studies on *V. amygdalina*, have reported that this plant possesses antibacterial activity. Newbold *et al*. (1997) showed that *V. amygdalina*, has mild antimicrobial effect on rumen bacteria and protozoa while Kambizi and Afolayan (2001) proved that acetone extract of *V. amygdalina* possesses antibacterial activity towards *Bacillus cereus*, *Bacillus pumilus*, *Bacillus subtilis*, *Micrococcus kristinae*, *Staphylococcus aureus*, *Enterobacter cloacae* and *Escherichia coli* growth with minimum inhibition concentration (MIC) of 5 mg/ml.

In addition, Akinpelu, (1999); Kambizi and Afolayan, (2001) stated that methanol extract of *Vernonia amygdalina* Delile did not only inhibit growth of the gram positive bacteria such as *B. cereus*, *B. pumilus*, *B. subtilis*, *E. cloacae*, *S. aureus* and *M. kristinae* but was also effective against gram-negative bacteria including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Shigella dysenteriae* and *E. coli*. In the report of Ogbulie *et al*. (2007); Kola (2007), ethanolic extracts of VA showed antibacterial effect against both gram-negative (*E. coli* and *Salmonella typhi*) and gram-positive (*Clostridium sporogenes*, *Staphylococcus pyogenes* and *S. aureus*) bacteria. However these findings, there are contradictions concerning the activity of ethanol extract of *Vernonia amygdalina*. Delile. Ogbulie *et al*. (2007) suggested that ethanol and soxhlet extractions could be the best solvent and the best method to give optimum antibacterial effect of VA. However this report, Pesewu *et al*. (2008) stated that ethanol extract could not inhibit the methicillin-resistant (MRSA UELSHB 102, UELSHB) and methicillin-sensitive (MRSA NCTC 6571) strains of the bacteria. However, the water and blender extracts showed antibacterial effect on *Streptococcus pyogenes*, *E. coli* and *P. vulgaris* with minimum bactericidal concentration (MBC) higher than 50 mg/ml. Taiwo *et al*., (1999) reported of *V. amygdalina* root water extract showing antibacterial activity on *Streptococcus gordonii*, *Porphyromonas gingivalis*, *Porphyromonas nigrescens*, *Prevotella intermedia*, *Fusobacterium nucleatum* and *P. aeruginosa* with MIC at 100 mg/ml. Although VA water extract does not exhibit strong antimicrobial activities, Kambizi and Afolayan, (2001) reported that VA water extract still remain as the most common practice in traditional medicine and have demonstrated success in treating patients with sexually transmitted diseases. This success may be due to the absence of upper limit in the concentration of *V. amygdalina* one can consume, thus this could contribute to the increased efficacy of this extract in traditional practices.

CONCLUSION

In conclusion, this study has confirmed the antibacterial and anti-cancer activities of *Vernonia amygdalina* Delile despite being obtained from different geographical regions. The results obtained from this study supports previous claims of the medicinal properties of this plant. Thus *Vernonia amygdalina* Delile can serve as starting materials for drug development in the developing countries were ample populations depend on this plant to satisfy their health care requirements. Given the relevance of this study to herbal medicine, the result of this study could provide direction on the appropriate time to use the plant (fresh) rather than being preserved over long period such that efficacy of *Vernonia amygdalina* Delile could reduce. For future studies, in-depth studies will be carried

out on *Vernonia amygdalina* Delile minimum inhibitory concentration (MIC), minimum bacterial concentration (MBC) and structural elucidation of the bioactive compounds using high performance liquid chromatography (HPLC) and Nuclear magnetic resonance (NMR).

ACKNOWLEDGEMENTS

The authors appreciate the inputs of the reviewers whose contributions improved the quality of this manuscript. Authors are grateful to Covenant University for defraying the cost of publication.

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