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Research Article

Evaluation of Alkaloids and Cardiac Glycosides Contents of *Ricinus communis* Linn. (Castor) Whole Plant Parts and Determination of their Biological Properties

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ABSTRACT

Extracts from *Ricinus communis* L. (Castor) plant have been used from time memorial in traditional medicine for treatment of many ailments. However the bioactive phytochemicals that confer these biological effects are often not ascertained. Using established protocols we isolated, quantified and determined some biological properties of alkaloids and cardiac glycosides present in the various Castor plant parts (leaves, stems, roots, seeds and capsules). Alkaloids and cardiac glycosides were found to be concentrated highest in the leaves and stems extracts at 11.2 % and 63.60 % yields respectively. The alkaloids and cardiac glycosides extracts gave an appreciable antioxidant and antihemolytic activities when compared with ascorbic acid and butylated hydroxyl anisole which are two known antioxidant and antihemolytic compounds, respectively. The highest antioxidant and antihemolytic activities were expressed by leaves alkaloids and capsules cardiac glycosides at 57.61 % and 379.66 %, respectively. Furthermore extracts of these two phytochemicals also decreases the growth and proliferation of pathogenic *Klebsiella pneumonia* and *Staphylococcus aureus*. Thus *Ricinus communis* L. (Castor) plant phytochemicals are good source of alternative bioactive compounds which may be used in lieu of synthetic drugs that often comes with numerous side effects when administered.

Key words: antioxidant, antihemolytic, antimicrobial, medicinal plant, phytochemicals, pathogens, traditional medicine

INTRODUCTION

Ricinus communis L. (Castor) plant is a member of the Euphorbiaceae family. It was an indigenous plant of Eastern Africa, India and Southeastern Mediterranean Basin, but currently it is widely spread throughout in the tropical regions [1]. Parts of the plant extracts have been used in traditional medicine as laxative or purgative; used in treatments of abdominal/muscle pains, head ache, sinusitis, other inflammatory conditions; for treatments of ailments like eczema, rheumatism, swellings, lumbago, abscesses, head ache, treating skin disorders, abrasions, wound cuts, burns; to inducing labor and increasing flow of breast milk in lactating mothers [2, 3].

Currently Castor plant cultivation has been attracting industrial participation owe to is oil that has unique chemical structure that makes it valuable in many industrial applications, and this is supported by the fact that it could be easily planted in opened fields, uplands, river beds, stream banks, hillsides, marginal lands, even on salted lands [4]. These attributes is owe to its resistance/tolerance to many environmental stress conditions such as high salinity, drought, high soil moisture content, flooding, etc. [4]. Castor plant requires very low rainfall for its survival and can reach maturity and produces seeds after a short period of about 140 to 160 days [4].

Castor plant is commonly known with different names such as castor oil plant, castor bean plant, wonder boom, Datura, Eranda, Palma Christi [4]. In different Nigeria natives it is known as Jongo, Kpamfini gulu, Laraa, Ogilisi, or Zurman [5]. Name Castor was masterminded by English traders that confuse the Castor oil with that of Agno-castor (*Vitexagnus-castus*) [4]. However, *Ricinus communis* L. came from Swedish naturalist Carolus Linnaeus in eighteenth century when he was assigning scientific names to plants and animals. . Ricinus is part of Latin words specific for Mediterranean sheep tick (*Ixodes ricinus*). It was deduced that Linnaeus assumed the seeds looked like large ticks engorged with blood, while

Fig. 1: A picture of Castor plant (Ricinus communis L. indicated by black arrow) found within Omu-Aran area (Latitude 8.13 ^{O}N and Longitude 5.1 ^{O}E) in Kwara State, Nigeria in March 2014.

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Table 1: Anti-hemolysis assay preparation

	RBC	PBS (pH 7.4)	10 mM H_2O_2	Plant extract	Methanol	BHA
A (negative control)	2 ml	1.1 ml	-	-	1 ml	-
B (positive control)	2 ml	1.0 ml	0.1 ml	-	1 ml	-
C (plant extract; 50mg/ml)	2 ml	-	0.1 ml	1 ml	-	-
D (BHA; 50mg/ml)	2 ml	-	0.1 ml	-	-	1 ml

Table 2: Qualitative screening for alkaloids and cardiac glycosides in Castor plant parts extract.

Extracts	**Alkaloids**	**Cardiac glycosides**
Leaves	+	-
Stems	+	+
Roots	+	+
Capsules	+	+
Seeds	+	-

Key: (+); Present, (-); Absent

communis literally means common [6].

Castor plant is a perennial fast-growing shrub, which may be at times a small soft wooded tree of about 6 meter or more. Castor leaves are about a diameter of 30-60 cm and colour may be greenish, purplish or reddish (Figure 1) [7]. Usually the leaves contain about 5-12 deep lobes with coarsely toothed segments which are alternate and palmate, the stems pigmentation may however vary. In the past Castor plants were cultivated for the colour of its leaves and flowers and also for generating oil from the seeds [7]. However the traditional use of its other parts has increases is local cultivation.

Phytochemicals are referred to as non-nutrient plant chemicals or biological active components in plants [8]. These bioactive components are classified into primary and secondary plant metabolites. The primary constitute compounds like sugars, proteins, amino acids, nucleic acids, chlorophylls, while the secondary among many others include alkaloids, anthrocyanins, flavonoids, phenolics, tannins, terpenoids, saponins, glycosides etc. [8]. The presences and quantities of these metabolites vary from one plant to another and from one plant part to another [9].

Alkaloids are the largest in these groups of secondary chemical plant metabolites and are made principally of ammonia compounds comprising of nitrogen bases that are synthesized from amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the polypeptide motifs [8]. Nitrogenous atoms in alkaloids (as 1^o, 2^o or 3^o amines) facilitate their alkalinic properties, making them turn red litmus paper to blue. The presence and exact location of these amines basically determines the alkalinity of alkaloid [10]. Alkaloids are classified mainly into 3: true alkaloids (have heterocyclic ring with amino acids nitrogen), proto alkaloids (does not have heterocyclic ring amino acids nitrogen) and pseudo alkaloids (have heterocyclic ring with nitrogen but not from amino acids) [11]. Alkaloids tend to have bitter taste and are suggested to be one of the basic compounds employed by plants in defense strategies against herbivores and pathogenic microorganisms. Hence they have been exploited in numerous pharmaceutical applications as anesthetics, analgesic, antibiotics, disinfectants, central nervous system (CNS) stimulants, sedatives, etc [8, 12]. Examples of alkaloids among many others include codeine and morphine (analgesic); berberine and sanguinarine (antibiotics); vinblastine (anti-cancer); scopolamine (sedative); atropine, ephedrine, ergotamine, caffeine, cocaine, codeine, morphine, nicotine, etc (CNS stimulant) [8].

Glycosides are plants secondary metabolites, often found in the cell sap and are colorless, water soluble crystalline compound made predominately of carbon, hydrogen and oxygen, but sometimes contain nitrogen and sulfur [8]. Glycosides are chemically formed from the condensation reaction that involves the participation of the hemiacetal entity of a carbohydrate (sugar) and a non-carbohydrate organic hydroxyl containing compound part (aglycone) made from alcohol, glycerol or phenol; thus yielding one or more reducing sugars upon hydrolysis by enzymes or chemical reagents [8]. Glycosides were originally named after their sources by the addition of a suffix 'in' as example the glycoside digitoxin was from Digitalis, salicin from Salix, prunasin from Prunus and cantharidin from Cantharides. However this has been substituted and are now principally categorized based on the type of sugar component present, chemical nature of the aglycone or their pharmacological action [8]. Basic examples of glycosides include chalcone glycoside (used as anticancer), cardiac glycosides (used in treatment of heart related illnesses), anthracene glycosides (used in treating skin diseases and as a purgative) [8]. Glycosides are often having an intense bitter taste that acts on gustatory nerves resulting in increased flow of saliva and gastric juices [8].

Extracts from *Ricinus communis* L. (Castor) plant has been reported to have high traditional medicinal values among others as a laxative, purgative, treating wounds and infections, for increasing flow of breast milk, etc [7]. However previous works on Castor plant were unable to categorically relate a particular phytochemical with the observed therapeutic effects. It is therefore expedient to investigate some of the Castor plant phytochemicals by exploring more on those that were shown to be involved in therapeutic effects in preliminary studies [13, 14, 15, 16]. Furthermore, whole plant profiling for phytochemicals in plant is very scarce, most times research is been done on a particular part of a plant, so the content and distribution of

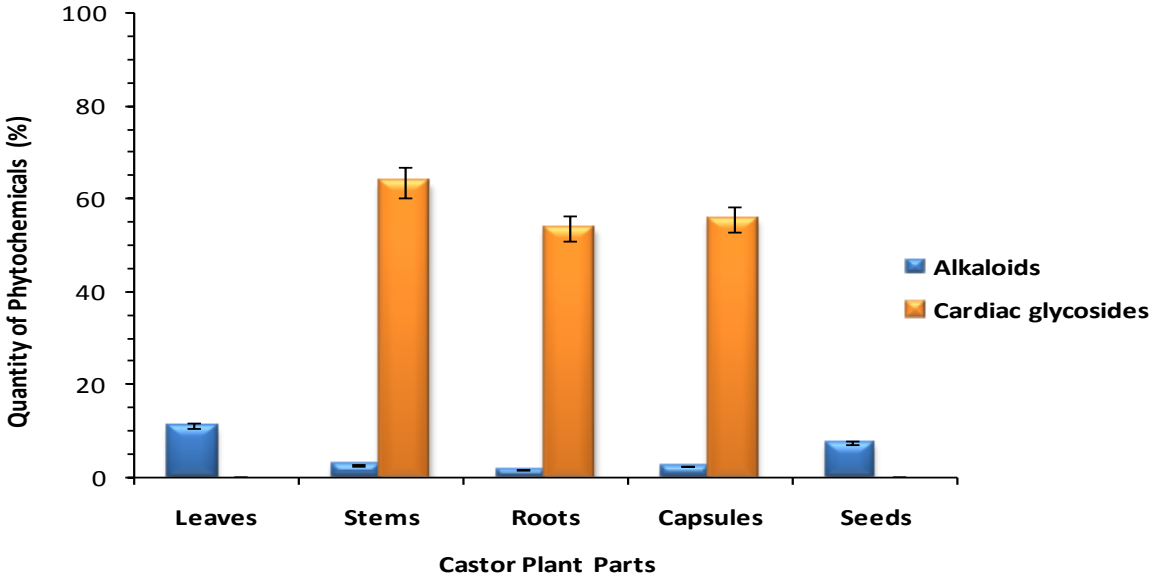

Figure 2: Quantitative determination of alkaloids and cardiac glycosides Castor plant parts. Results are the average of means (n = 3), and represented as mean ± standard deviation.

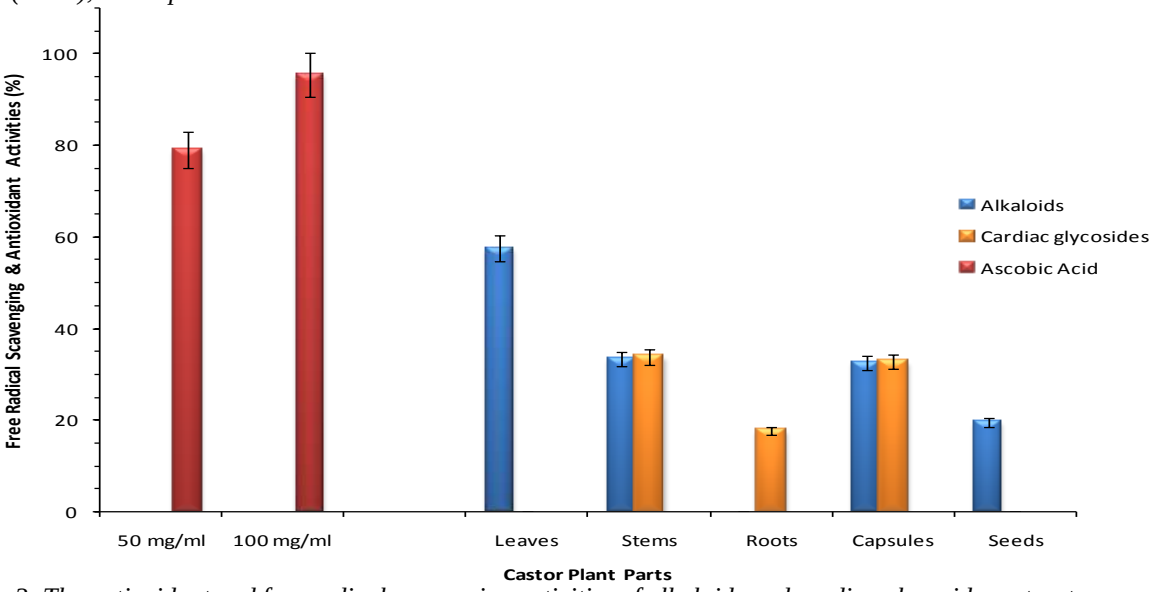

Figure 3: The antioxidant and free radical scavenging activities of alkaloids and cardiac glycosides extracts as accessed by the DPPH assay. The experiments were carried out in triplicates. Values were the averages of means, and represented as Mean ± standard deviation. Statistical analyses were performed using student paired T-test. Results were considered statistically significant at $p \leq 0.05$.

a particular phytochemical is often unknown. Of particular interest in Castor plant is the neglected capsule part (harbors the seeds) which could be of great medicinal value if characterized. We therefore evaluate the distribution and characterize some biochemical properties of alkaloids and cardiac glycosides in the whole Castor plant parts. Information obtained from this research may be further exploited for medicinal/pharmaceutical applications.

MATERIALS AND METHODS

Reagents: All reagents were of high analytical grade. Ascorbic acid, glacial acetic acid, hydrochloric acid, ethanol, methanol, mercuric chloride, potassium iodide, ferric chloride, hydrogen peroxide, sulphuric acid, lead acetate, di-sodium hydrogen phosphate, concentrated ammonium hydroxide, 2,2-diphenyl-1picrylhydrazyl (DPPH), EDTA bottles, butylated hydroxyl anisole (BHA), phosphate buffer saline (PBS) tablets, nutrient agar powder; were either manufactured by Sigma-Aldrich Chemie, GMBH or by BDH Limited, Poole England.

Equipments: Stuart vortex Mixers - SA7, Stuart orbital shaker SSL1, Stuart RE3008 water bath - rotary evaporator, Stuart orbital incubator S1500 and Jenway UV/VIS spectrophotometer were from Bibby Scientific Ltd, UK. C5 bench top centrifuge was from LW Scientific Inc. GA, USA. Oven was from Genlab Ltd, Widnis, Cheshire, WA8 OSR. Electric blender from Waring Products Division, Torrington, USA. Clifton water bath was from Clifton Lab Equipment, UK. Wisconsin Aluminum Steroclave 25X Bench-model Autoclave Sterilizer was from Labequip Ltd, USA.

Preparation of reagents

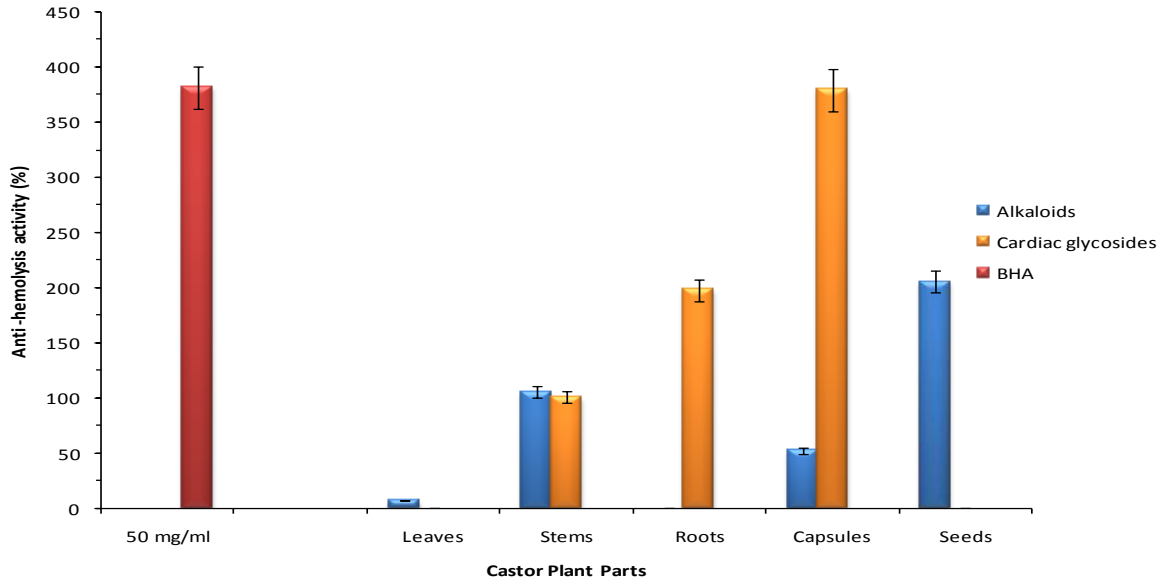

Figure 4: Anti-hemolysis activities of Castor plant alkaloids and cardiac glycosides extracts in protecting the red blood cells (RBC) against hemolytic effect of H_2O_2. The experiments were carried out in triplicates. Values were the averages of means, and represented as Mean ± standard deviation. Statistical analyses were performed using student paired T-test. Results were considered statistically significant at $p \leq 0.05$.

Preparation of Maeyer's reagent: 0.355 g of mercuric chloride was dissolved in 60 ml of distilled water. 5.0 g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water. The reagent was then kept in a brown bottle and stored in the dark.

Preparation of 100 ml 1% HCL: 1 % HCL was prepared by diluting 1 ml of concentrated HCL in 99 ml of distilled water in a conical flask.

Preparation of 100 ml 70 % ethanol: 30 ml of distilled water was added to 70 ml of absolute ethanol.

Preparation of 100 ml 12.5 % lead acetate: 12.5 g of lead acetate was weighed and dissolved in 100 ml of water.

Preparation of 100 ml 10 % acetic acid in ethanol: 10 % acetic acid in ethanol was prepared by adding 10 ml of glacial acetic acid into 90 ml of ethanol.

Preparation of 100 ml 4.77 % di -sodium hydrogen phosphate: 4.77 g of di sodium hydrogen phosphate was dissolved in 100 ml of distilled water.

Preparation of concentrated ammonium hydroxide 70 % ethanol: 80 ml of ammonia was dissolved in 515 ml of distilled water.

Preparation of phosphate buffer saline (PBS; pH 7.4): One tablet of phosphate buffer saline from SIGMA was dissolved in 200 ml deionized water to yield 0.01 M phosphate buffer saline pH 7.4. This is following SIGMA recommended procedure.

Preparation of 20 ml 10 mM hydrogen peroxide (H_2O_2): 0.0068 g (22.67 µl) of H_2O_2 was pipette and made up to 20 ml with distilled water to yield 10 mM of H_20_2.

Preparation of butylated hydroxyl anisole (BHA): 200 mg of BHA powder was dissolved in 4ml ethanol to yield a final concentration of 50 mg/ml.

Preparation of nutrient agar for antibacterial assay: 28 g of the nutrient agar powder was weighed into conical flask made up to 1 Liter with deionized water. It was allowed to completely dissolve and was sterilized by autoclaving for 15 minutes at 121 ^{O}C in Wisconsin Aluminum Steroclave 25X Bench-model Autoclave Sterilizer. The sterilized agar was maintained at 47 ^{O}C by placing in Clifton water bath set at 47 ^{O}C.

Collection and preparation of the plant material: Uninfected healthy Castor plants were collected from Omu Aran environ (located on Latitude 8º8' N and Longitude 5º6' E) in Kwara State, Nigeria, in March 2014. The plant was authenticated in the Herbarium Unit of Plant Biology Department, University of Ilorin, Nigeria where a voucher number of UIH001/965 was assigned to it. The plants were thoroughly washed with distilled water and dissected into the various parts (leaves, stems, roots, capsules and seeds). They were washed again with distilled water, blotted dry in-between clean sterile paper towel and oven dried until a constant weight in Genlab Oven set at 50°C. The dried plant parts were ground in Waring electric blender into fine powder, and were stored separately in air tight properly labeled container until further use.

Extraction of plant materials for alkaloids and cardiac glycosides qualitative analyzes: 25 g of finely ground powder of each Castor plant part was added separately into sterile bottles containing 250 ml 100 % methanol. They were placed on Stuart orbital shaker SSL1 set at 300 rmp and were shaking vigorously for 48 hours at room temperature. The contents in the bottles were allowed to settle and the supernatants were filtered through Whatman No. 1 filter paper. Resulting filtrates were centrifuged using a C5 bench top centrifuge set at 1000 rpm for 10 minutes in order to remove fatty layer and insoluble particles. The process of extraction was repeated using 100 ml 100 % methanol and filtrates obtained were mixed with the previous ones and concentrated to about 10 % of initial volume using Stuart RE3008 water bath - rotary evaporator. The extracts poured into clean sterile individually labeled glass beakers, placed in Clifton water

Table 3: Antibacterial activity of Castor plant alkaloids and cardiac glycosides extracts against Klebsiella pneumonia and Staphylococcus aureus growth and proliferation.

Phytochemicals	Klebsiella pneumonia			Staphylococcus aureus		
	1 mg/ml	5 mg/ml	10 mg/ml	1 mg/ml	5 mg/ml	10 mg/ml
Leaves alkaloids	+	+	++	+	+	++
Stems alkaloids	--	+	+	--	+	++
Roots alkaloids	ND	ND	ND	ND	ND	ND
Capsules alkaloids	--	+	+	+	++	++
Seeds alkaloids	++	++	**	+	++	++
Stems cardiac glycosides	--	++	**	--	+	++
Roots cardiac glycosides	--	--	+	+	+	++
Capsules cardiac glycosides	+	+	++	+	+	++

(--) No inhibition; (+) Inhibition; (++) Strong inhibition; (**) total inhibition; (ND) not determined

bath set at 80 ^{O}C and evaporated to dryness. The dried plant extracts were then stored individually in correctly labeled air tight containers.

Before the seeds were extracted for their alkaloids and cardiac glycosides contents, they were firstly defatted using chloroform and dried using diethyl ether. 200 ml of chloroform was added to 25 g of finely ground Castor seeds powder and was placed on Stuart orbital shaker SSL1 set at 300 rmp and shaking vigorously for 2 hours so as to remove fats and oils in the seeds. This was allowed to settle and supernatant containing the fats and oil was decanted. This was repeated using 100 ml chloroform. The resulting precipitate was dried by adding 100 ml diethyl ether to it, shaken vigorously and the supernatant decanted. This was repeated and the resulting precipitate spread on clean sterile paper towel to absorb any solvent leftovers and ensure complete dryness before commencing on the methanolic extraction as previously describe.

Qualitative determination of alkaloids and cardiac glycosides in the Castor plant parts: Qualitative test for the presence or absence of alkaloids and cardiac glycosides in different Castor plant parts were carried out according to the standard procedures as described in [17, 18].

Test for alkaloids (using Maeyer's reagent): 50 mg of each Castor plant part extract was dissolved in 8 ml of 1 % HCL, warmed and filtered through Whatman No. 1 filter paper. 2 ml of the filtrate was treated with few drops of Maeyer's reagents. Turbidity formation or white creamy precipitate indicated the presence of alkaloid.

Test for cardiac glycoside (using Keller-Killani Method): 50 mg of each Castor plant part extract was dissolved in 2 ml of glacial acetic acid containing 2 drops of 2% solution of $FeCl_3$. The resulting mixture was filtered through Whatman No. 1 filter paper and added to into another test tube containing 2 ml concentrated H_2SO_4. Formation of brown ring at the interface indicated the presence of cardiac glycosides.

Quantitative determination of alkaloids and cardiac glycosides in the Castor plant parts: Quantitative analyzes of alkaloids and cardiac glycosides were carried out for Castor plant parts that gave positive result in the qualitative test. Alkaloids and cardiac glycosides were determined as described in [17, 19].

Alkaloids quantitative determination: 25 g of ground Castor plant parts were weighed separately into 250 ml sterile bottles and 200 ml of 10 % acetic acid in ethanol was added into each. Bottles were covered and placed on Stuart orbital shaker SSL1 set at 300 rmp and were shaking vigorously for 4 hours at room temperature. They were separately filtered through Whatman No. 1 filter paper and were concentrated on a Clifton water bath set at 80 ^{O}C to one quarter of their initial volume (about 50 ml). Concentrated ammonium hydroxide was added drop wise to the concentrated filtrate until precipitate formation was complete. The resulting solutions were allowed to stand until all flocculating particles settle. Solutions were then individually filtered through Whatman No. 1 filter paper and precipitates were collected. The precipitates were washed with 1 % ammonium hydroxide and solution allowed to drain completely. The resulting precipitate on the filter paper is the alkaloids present in the ground Castor plant part. These were put into Genlab Oven set at 80 °C until complete dryness and constant weight was obtained. 50 mg of each precipitate was dissolved in 8 ml of 1 % HCL and assayed for alkaloids as described previously in order to establish its presence. The percent alkaloids content was calculated as:

$$\% \, alkaloid = \frac{weight \ of \ dried \ extract}{weight \ of \ dried \ ground \ plant \ sample} \ X \ 100$$

Cardiac glycosides quantitative determination**:** 25 g of ground Castor plant parts were weighed separately into 250 ml sterile bottles and 200 ml of 70 % ethanol was added into each bottle. Bottles were covered and placed on Stuart orbital shaker SSL1 set at 300 rmp and were shaking vigorously for 6 hours at room temperature. They were separately filtered through Whatman No. 1 filter papers were each transferred into 1 Litre volumetric flasks. 500 ml of distilled water was added into each flask followed by 100 ml of 12.5 % lead acetate (to precipitate tannins, resins and pigments). Volumes were made to 800 ml with distilled water and vigorously shaken on Stuart orbital shaker SSL1 set at 300 rmp for 10 minutes. To the 800 ml, 200 ml of 4.77 % disodium hydrogen phosphate (Na_2HPO_4) solution was added (to precipitate the excess Pb^{++} ions). Resultant solutions were filtered separately through Whatman No. 1 filter papers to give a clear filtrate. The filtrates were then individually evaporated to dryness. 50 mg of each dried extract was dissolved in 2 ml of glacial acetic acid containing 2 drops of 2% solution of $FeCl_3$ and assayed cardiac glycosides as described previously in order to establish its presence. The percent cardiac glycosides content was calculated as:

$$\% \, Cardiac\ glycosides = \frac{weight\ of\ dried\ extract}{weight\ of\ dried\ ground\ plant\ sample} \ X \ 100$$

Determination of antioxidant/free radical scavenging activities: Antioxidant/free radical scavenging activities of Castor plant parts alkaloids and cardiac glycosides were carried as described in [20]. Plant extracts samples were prepared by dissolving 200 mg of each alkaloids and cardiac glycosides extract separately in 4 ml of methanol to give a final concentration of 50 mg/ml. To 1 ml of each 50 mg/ml extract in test tube was added 3 ml methanolic solution of 0.1 M DPPH and was mixed vigorously and incubated in the dark for 30 minutes. After the incubation, the antioxidant/free radical scavenging activities of the extracts were determined by the decrease in the absorbance as measured at 517 nm using Jenway UV/VIS spectrophotometer and methanol as blank. 50 mg/ml and 100 mg/ml ascorbic acid were used as antioxidant standards to which the antioxidant/free radical scavenging activities of the extracts were compared. The antioxidant/free radical scavenging activity of each extract was expressed in percent as follows:

$$\% \text{ Antioxidant activity} = \left(1 - \frac{A_s}{A_c}\right) \times 100$$

Determination of anti-hemolysis activity**:** Estimation of the inhibitory effect of the Castor plant parts extracts on red blood cell (RBC) hemolysis was carried out according to the method described by [21]. Human blood sample (O^-) was obtained from Blood Bank Landmark University Medical Centre, Omu Aran, Kwara State, Nigeria. The blood was aliquot in 5 ml EDTA bottles and aliquots were centrifuged for 10 minutes using C5 bench top centrifuge (LW Scientific Inc. GA, USA) set at 3000 rpm. This was to remove plasma, platelets and buffy coat from the samples. Resulting RBC were washed twice with cold Phosphate buffer saline; pH 7.4. 2 ml of the RBC (which is about 1 x 10^9 cells) was used for anti-hemolysis assay. Samples were prepared in test tubes as shown in Table 1 below.

Anti-hemolysis assay reaction mixtures in test tubes were prepared for all alkaloids and cardiac glycosides extracts (50 mg/ml), including butylated hydroxyl anisole (BHA; 50 mg/ml), negative and positive controls. Reactions were allowed at room temperature with continuous shaking for 2 hours 30 minutes on Stuart orbital shaker SSL1 set at 100 rmp. Thereafter test tube contents were centrifuged at 2000 rpm for 10 minutes. The absorbances of the supernatants obtained were read at 540 nm. Percent anti-hemolysis activities were calculated and compared with that of BHA. BHA is an antioxidant with high anti-hemolysis potentials; hence it was used as a reference standard and its activity on the RBC was compared with that of the alkaloids and cardiac glycosides plant extracts. Anti-hemolysis activity was calculated as:

$$\% \, Anti - \text{hemolysis activity} = \frac{Abs(extracts; BHA) - Abs(+ve\ control)}{Abs(-ve\ control) - Abs(+ve\ control)} \times 100$$

There was no hemolysis in the negative (-ve) control, while there was hemolysis induced by H_2O_2 in the positive control there was.

Determination of antibacterial activity: In order to establish possible inhibitory effects of alkaloid and glycoside on the cell growth and proliferation on bacteria, two human pathogenic organisms; *Klebsiella pneumonia* (Nosocomial isolate) and *Staphylocuccus aureus* (NCIM 2079) were used. These organisms were obtained from Department of Microbiology, Landmark University, Kwara State, Nigeria. Appropriate quantities of alkaloids and cardiac glycosides extracts were separately added to 10 ml sterile nutrient agar kept at 47 ^{O}C, to give a final concentration of 1 mg/ml, 5 mg/ml and 10 mg/ml, and were poured into separate sterile plates. This was done in triplicate.

The two organisms were sub-cultured for about 18 hours in nutrient broth maintained at 37 ^{O}C in a Stuart orbital incubator S1500. The cultures were allowed to grow to their exponential phase before use. The amount of microbial cells were adjudged in line with previous work reported in "www2.mum.edu/ibrandon/Micro/enumeration.doc" that related bacteria culture turbidity and its optical density measurement at 540 nm with the number of cells present in the culture. The optical density as obtained for *Klebsiella pneumonia* was 1.652, and *staphylococcus aureus* was 0.985, which is approximately 21×10^4 and 14×10^4 colony forming unit (CFU)/ml respectively.

Visualization of colony formation was used to adjudge the antimicrobial effect of the alkaloids and cardiac glycosides extracts. 200 µl of microbial cells of *Klebsiella pneumonia* and *Staphylococcus aureus were* evenly spread onto the surface of separate nutrient agar plates using a sterile glass tong. The plates were carried out in triplicates and were incubated in Stuart orbital incubator S1500 for 24 hours at 37 ^{O}C; without agitation. This volume of bacterial cells was used in order to ensure that extracts contend with significant amount of bacterial cells; as a result be able to see the indication on the organisms' growth and proliferation. The bacteria cells were visualized and counted/estimated after this period.

Statistical analysis: All analyses were carried out in triplicates. The software Graph Pad Prism was used to analyze results. Data were presented as mean $\pm$ standard deviation (SD). Experimental results were further analyzed for correlation and tested for significance by student's T-test at $P \leq 0.05$.

RESULTS

The results of analyzes carried out on the Castor plant parts (leaves, stems, roots, capsule and seeds) are as presented below.

Qualitative screening for alkaloids and cardiac glycosides: The result of the qualitative analysis is presented in Table 2. From the analysis alkaloids were found to be present in all the Castor plant parts, while cardiac glycosides were only present in stems, roots and capsules.

Quantitative analyzes of alkaloid and glycoside: Quantitative analyzes of the Castor plant parts that showed

the presence of alkaloids and cardiac glycosides is presented in Figure 2. The result shows alkaloids: leaves (11.12 %), stems (2.8 %), roots (1.8 %), Capsules (2.45 %) and seeds (7.4 %). Cardiac glycosides: stems (63.6 %), roots (53.6 %) and capsules (55.55 %). This result revealed that cardiac glycoside is more abundant in the Castor parts where they are found as compared to alkaloids.

Antioxidant/free radical scavenging activities: The antioxidant and free radical scavenging potentials of alkaloids and cardiac glycosides Castor plant parts as accessed by the DPPH assay are as presented in Figure 3. Low antioxidant and free radical scavenging activities were observed when compared with that of 50 mg/ml and 100 mg/ml ascorbic acid (79.16 % and 95.61 %, respectively) that were used as reference standards, although an appreciable activity was produced by leaves alkaloids. Activity as recorded for alkaloids: leaves (57.61 %), stems (33.38 %), capsule (32.54 %) and seeds (19.51 %), while for cardiac glycosides: stems (33.83 %), roots (17.65 %) and capsule (32.81 %). The low extract yield of roots alkaloids made the determination of its antioxidant and free radical scavenging activity unattainable.

Anti-hemolysis activities: The ability of the Castor plant alkaloids and cardiac glycosides extracts to inhibit hemolysis of Red blood cells (RBC) induced by H_2O_2 is presented in Figure 4. All extracts showed anti-hemolysis activity at differential degree; some gave low activities while others gave activities such as comparable with butylated hydroxyl anisole (BHA); which was employed as the standard reference anti-hemolysis compound. Anti-hemolysis activity as recorded for alkaloids: leaves (7.59 %), stems (105.30 %), capsule (52.41 %) and seeds (205.86 %). For cardiac glycosides: stems (101.03 %), roots (197.93 %) and capsule (379.66 %), while BHA gave 381.72 % anti-hemolysis activity. Likewise the low extract yield of roots alkaloids made its anti-hemolysis assay unachievable.

Antibacterial activities: The antibacterial activity of Castor plant alkaloids and cardiac glycosides extracts against *Klebsiella pneumonia* and *Staphylococcus aureus* growth and proliferation is presented in Table 3.

Although biofilms/continuous lawns growths were observed on the culture plates after 24 hours incubation, these greatly reduce as the extracts concentration increases. In some instances, there were no clear difference between the control and experimental culture plates, some showed appreciable decrease in growth while there was total inhibition of bacterial growth in some. The observed biofilms/continuous lawns may in part be due to the high amount of bacteria colony forming units (CFU) applied onto the plates or be related to the morphologies of these two organisms that are known to propagate by forming biofilms [22, 23, 24, 25]. Thus it was not possible to ascertain the exact bacterial CFU; however it was possible to observe the antibacterial effects on the culture plates judging by the decrease in the biofilms formation. We employed high amount of bacterial CFU at the inception of the experiment in order to evaluate the extracts efficacy towards high bacteria population.

Table 3 shows differential antibacterial activities of the extracts at increasing concentration with seeds alkaloids and stems cardiac glycosides producing the most potent inhibitory effects on the two bacteria tested as 10 mg/ml was able to totally inhibit the growth of *Klebsiella pneumonia*. The antibacterial activity of root alkaloids was not tested owe to its low yield during extraction.

DISCUSSION

Use of plants extracts in traditional field of medicine has provided alternatives to synthetic drugs. Currently, searches for new medicines that will pose less side effects have also increased the number of plants that are been analyzed day-in day-out for their potential phytochemical constituents. Different parts of Castor (*Ricinus communis* L.) plant have been used by indigenous people even before the advent of modern medicine, however every few information exist as regards the phytochemicals that bequeath these biological effects leading to healing. The biological properties of Castor plant alkaloids and cardiac glycosides extracts as presented in this research report lend strong credence to its bio-efficacy in alleviating or complete healing of sicknesses and diseases as used in ancient times.

Previous research works have reported the presence of alkaloids and cardiac glycosides in plant extracts; however they often failed to distinctively isolate them, quantify and determine their biological activities [13, 26, 27, 28]. Besides, the reported biological activity is a combinatorial effect of mixtures of these phytochemicals. It is therefore imperative to isolate, quantify and determine the biological effects of particular phytochemicals. Presented in the result section above are differential presence of alkaloids and cardiac glycosides in the various Castor plant parts; their quantities in the parts and some of their biological effects. Our work lends more credence to previous works [3, 5, 13, 26, 27, 28, 29] that have differentially detected alkaloid and cardiac glycosides in Castor plant parts. Most of these research works reported alkaloids mainly in the leaves, however we also found alkaloids in the other Castor plant parts analyzed. Alkaloids in the leaves were presumed to act as a cytotoxin to waive off plants microbial pathogenic or herbivory invaders, and may be employed to neutralize poisons [3]. Few reports found cardiac glycoside in the leaves extracts of Castor plant [26, 28] however in our work it was absent. Thus the presence or absent of a phytochemical in a particular plant organ may be connected to lots of variations such as the geographical location of the plant, season, soil topography and nutrients content, plant age; as plants do posses different levels of secondary metabolites subject to their metabolic state that is well connected to their cellular, developmental and environmental statuses [30]. Very high yield of cardiac glycosides were found in the stems, roots and capsules of the Castor plant parts. This may be well connected to the facts that these organs could well serve as a storage organs as glycosides are predominately made of carbohydrates (sugars). Since alkaloids are more of natural defence metabolites, it is thus imperative then that their

concentrations in the various plant parts are kept at the optimum tolerable amount to the plant so has not to have adverse effects on the plant they are protecting against foreign invaders. Presumably, the observed low contents of the alkaloids in the Castor plant parts.

Antioxidants principal function is to mop up free radicals and oxygen reactive species which are released from the mitochondria as byproduct of normal cellular respiration process [31, 32]. Theses free radicals are responsible for numerous diseases in human, thus the search for antioxidants from natural sources that will have therapeutic effects against these free radicals cannot be over emphasized. Although when compared to ascorbic acid, the alkaloids and cardiac glycosides gave about half of the antioxidant effect, we believe doubling the concentrations of these phytochemicals for therapeutics will definitely produce an effect that will be comparable to the synthetic ascorbic acid. The leaves alkaloids on the other hand gave higher antioxidant activity, thus it will be of importance if this is further studied in other to know the specific class(es) of the alkaloid(s) present. Furthermore, antioxidants are know to protect red blood cells (RBC) from free radical attacks, thus allowing the RBC to live longer, consequently help in combating RBC sickling effects [33]. The capsules and roots glycosides and seeds alkaloids extracts showed very high potential towards protecting RBC from oxidative damage such as may be induced by H_2O_2 as they gave comparable activity with butylated hydroxyl anisole (BHA); a potent antihemolysis drug. Thus, these phytochemicals may play beneficiary medical roles in humans that suffer from frequent RBC lyses.

At increasing phytochemical concentrations, all alkaloids and cardiac glycosides gave relative increasing antibacterial activity against the two pathogenic bacteria (*Klebsiella pneumonia* and *Streptococcus aureus*) tested. Of particular interest are the seeds alkaloids and stem glycosides extracts that gave complete inhibition of the two organisms. Antibacterial activity by measuring the diameter of zone inhibition method has been employed in many experimental works; however this approach may seems limited as only the organisms within the vicinity where the extracts can diffuse to will experience the inhibitory effect of the extracts. Thus the observable result is subject to the rate of diffusion of the extracts within the agar. However visualization of colony forming unit method which is obtained by the incorporation of the extracts into the agar, ensures that the extracts are evenly distributed in the entire agar, hence the result obtained will give the true inhibitory reflection of the extract on the growth and proliferation of the organisms since the extracts is not concentrated within a particular vicinity. Reports of antibacterial activity of Castor plants extracts predominately are on the whole crude extract [13, 15, 26, 27, 34], so knowing the exact phytochemical(s) that is (are) conferring the effects is always very dicey. Thus the antibacterial activities of alkaloids and cardiac glycosides of Castor plant parts that potentially inhibit the two pathogenic bacteria give definite abilities of these phytochemicals to potentially inhibit these bacteria, and they may also confer such similar activity on other human pathogenic bacteria. Furthermore, molecular and structural characterization of the (seeds alkaloids and stem glycosides) that gave the best antibacterial activity should be further exploited for pharmaceutical applications in the production of drugs that may have similar molecular and structural properties as these two phytochemicals and as a consequent similar biological activity.

CONCLUSION

Thus far this study has revealed the distribution and potential biological properties of alkaloids and cardiac glycosides extracts of Castor plant parts. It will be imperative that further work is done to establish the cytotoxicological activities of these phytochemicals in order to ascertain its biosafety for human use. With the ever increasing resistance of pathogenic organisms to synthetic antibiotics, the best way out is what the nature has to offer, which are phytochemicals with high inhibitory potencies that produces very low side effects.

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