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Whole plant screenings for flavonoids and tannins contents in Castor plant (*Ricinus communis* L.) and evaluation of their biological activities

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ABSTRACT
Castor plant (*Ricinus communis* L.) extracts have been used by many natives of the world in treatment of several ailments. However actual phytochemicals that confer these cures are mostly unidentified since crude extracts are employed. This study evaluated the distribution and biological activities of flavonoids and tannins in various parts of Castor plant. Flavonoids and tannins were found quantitatively in leaves, stems, seeds and roots, while capsules only contain flavonoids. High antioxidant and antihemolytic activities were shown by some of flavonoids and tannins extracts such as comparable to ascorbic acid and butylated hydroxyl anisole, which were used as standard antioxidant and antihemolytic agents respectively. Remarkably inhibitory effects on the population proliferation of *Streptococcus aureus* and *Krebsellia halize* were observed at increasing concentrations (1 mg/ml, 5 mg/ml and 10 mg/ml) of flavonoids and tannins extracts. It thus implies that Castor plant possesses essential phytochemicals, which could be exploited for medicinal/pharmaceutical applications.

Keywords: antioxidant, antihemolytic, antibacterial, medicinal plant, phytochemicals

1. Introduction
Wide variety of trees, shrubs, grasses, herbs and vegetables parts such as leaves, barks, roots, and seeds have been used by humans owe to their nutritional and/or medicinal values [1]. More of world populace is currently shifting towards the traditional medicine system and this is most likely to increase due to numerous side effects often encountered in the modern therapeutic system that involves use of synthetic drugs; which rarely occurs with use of natural substances obtained from plant sources [2].

Castor plant (*Ricinus communis* L.) belongs to perennial shrub family of Euphorbiaceae. It is popularly known as Castor oil plant in English; Arandi or Erandi in Hindi; Mexico weed, Palma Christi in English, German, Portuguese; Ricin commun in French; Ritsin in Russian; Rizinus in Danish, German; Rikinusu and Rishin in Japanese; etc [3, 4]. Castor is known locally in Nigeria as Era ogi in Bini; Kpamfini gulu in Nupe; Laraa in Yoruba; Jongo in Tiv; Ogilisi in Igbo; Zurman in Hausa [5]. For a comprehensive list of Castor plant names in other languages see [3].

The botanical name *Ricinus communis* was derived by Swedish naturalist Carlous Linnaeus in the eighteenth century. Ricinus is the Latin word for Mediterranean sheep tick (*Ixodes ricinus*) which the Castor plant seed has total resemblance to, and communis literally means common [6]. Castor plant has been cultivated as far back as 6,000 years ago. It is usually a small soft wooded tree that grows up to 6 meters with varying stem pigmentation; the leaves which is usually 30 – 60 cm in diameter may be green or reddish in colour made of about 5-12 coarsely toothed lobes; the fruits which is usually a three-celled thorny capsule covered with soft spins encloses the seeds (Figure 1) [3, 7].

Castor plant extracts have been used by numerous communities in different regions of the world for treatment and/or alleviation varieties of sicknesses. The extracts have been shown to possess essential and beneficial biological properties such as antioxidant, antimicrobial, antihelmintic, insecticidal, diuretic, anti-inflammatory, laxative; in the treatments of hypoglycemia, edema, rheumatism, headache, asthma, dermatitis, ringworm, warts, dandruff; external application on breast of nursing mothers shown to increase flow of milk and the oil shown to relieve labour pain and aid delivery [3, 7, 8, 9, 10, 11, 12]. The activity of the Castor plant seeds was studied on the male and female reproductive systems.

The seed extracts were shown to possess high anti-fertility activities by causing decrease in serum levels of testosterone, weight of the reproductive organs, sperm functions, disruption of seminiferous tubules and erosion of the germinal epithelium in male rats [9, 13]; and in reduction of progesterone levels, altering the oestrogen/progesterone balance, and abortifacient effect on the uterus and fallopian tube [9, 14].

Medicinal plants have been of great significance to human health. The medicinal potentials of these plants results from several bioactive phytochemicals constituents such as alkaloids, anthrocyanins, flavonoids, phenolics, tannins, terpenoids, etc as well as vitamins that produce specific beneficial physiological and pharmacological functions in human body [15, 16, 17]. Phytochemical is coined from the Greek word *phyto* which means plant. Thus, phytochemicals encompass large group of bioactive, non-nutritive chemical compounds that confer disease protection/reduction abilities in human body [18, 19].

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

Flavonoids are low molecular weight secondary polyphenolic metabolites present in plants characterized by their flavan nucleus [20, 21]. There are over 700 characterized flavonoids and have been shown to be responsible for the flavor and colour pigment intensities in flowers, fruits and leaves [20, 21, 22]. Flavonoids have been found in many food products such as in colour intense fruits, red wine and in beverages [22, 23]. Their presences in plants assist in protection against UV radiation, pathogens and herbivores [22]. However they possess many beneficial medicinal advantages to human such as antioxidant and free radical scavenging activities, anti-inflammatory, anti-arrhythmic, antithrombotic, antimicrobial, anti-apoptotic, anti-ischemic, anti-hypertensive, anti-carcinogenic, anti-allergic, antitumor activities, and have been shown to possess therapeutic potential to prevent ulcers and alleviate several cardiovascular diseases [22, 24- 30].

Tannins are groups of plant polyphenolic high molecular weight secondary metabolites that have been used by human for decades. The name tannins came from the French word "*tan"* meaning the bark of the Holm oak tree used for tanning (ability to darken colour), and have been found to be present not only in the tree bark but also in leaves, stems, roots, buds and seeds [31]. They create dry, astringent and bitter taste in mouth when consumed in unripe fruit, strong tea or red wine [32]. Tannins like other phytochemicals possesses lots of medicinal benefits to human, such as stated previously in addition to been used in the treatment/control of diarrhea, rhinorrhea and leucorrhoea; treatment of wounds and burns; stoppage of bleeding; for sun screen or ultra-violet ray protection; combating obesity by its ability to increase digestion [32-35].

Although the crude plant extracts of Castor plant have been effectively used for the treatments of various ailments, very limited research work have been able to link the exact phytochemicals that confer such biological result. Furthermore, identification of these phytochemicals are mostly done [36-38], the exactly determination of the contents and distributions in the various plant parts are very limited. Therefore, studying the distribution of particular phytochemicals in the various plant parts and stating their biological activities cannot be overemphasized. This study thus pose in evaluating the distribution and contents of flavonoids and tannins in the various Castor plant parts (leaves, stems, roots, capsules and seeds) and to determine their biological properties as pertain to their antioxidant, antihemolytic and antibacterial activities. Since there are numerous classes of these phytochemicals, we believe that the different parts may contain varying classes which may be indicated in the degree of biological activities in one plant part extract to another. The information as may be generated will not simply add to the existing pool of biological activities of flavonoids and tannins, but could be further exploited for medicinal or pharmaceutical applications.

2. Materials and methods

2.1 Reagents: All reagents were of high analytical grade. Methanol, petroleum ether and diethyl ether were obtained from BDH Limited Poole England. Iron chloride, chloroform, ethanol, aluminum chloride, potassium hydroxide, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, butyl hydroxyl anisole (BHA), sodium chloride, hydrogen peroxide, phosphate buffer saline (PBS) tablets were obtained from Sigma-Aldrich Chemie GMBH. Nutrient agar powder was obtained from Limited Poole England.

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

2.3 Collection of plant material: Healthy Castor plants were located around Omu-Aran area in Kwara State, Nigeria in March 2014. Omu-Aran is located 88 km South of Ilorin, capital of Kwara State and 16 km North-East of Otun-Ekiti in Ekiti State, Nigeria on Latitude 8.13 ^{O}N and Longitude 5.1 ^{O}E. The whole plants were uprooted and transported to Biochemistry Laboratory in Biological Sciences Department, Landmark University, Omu-Aran, Kwara State, Nigeria. The whole plants were thoroughly washed with distilled water and the individual parts dissected. The parts (leaves, stems, roots, capsules and seeds) were dried in a Genlab oven at 50 ^{O}C until

constant dried weights were obtained. The parts were ground using Waring electric blender and stored in individual air tight containers until further use. Fresh healthy plant was sent for identification and authentication at the Herbarium of the Department of Plant Biology, University of Ilorin, Kwara State, Nigeria, where it was shown to be indeed as Castor plant (*Ricinus communis* L.) and was given a voucher number of UIH 001/965.

2.4 Extraction of plant material for qualitative determination of flavonoids and tannins: 250 ml of 100 % methanol was added separately to 25 g of finely ground powder of individual plant part (leaves, stems, roots and capsules) in 1 litre sterile bottles. The bottles containing the plant material and methanol mixture were placed on Stuart orbital shaker SSL1 and shook vigorously at 300 rmp for 48 hours at room temperature. The contents were allowed to settle and the supernatants filtered separately through Whatman No. 1 filter paper. The resulting filtrates were centrifuged using a C5 bench top centrifuge for 10 minutes at 1000 rpm to remove any other insoluble particles and fatty layer. Obtained supernatants were concentrated in Stuart RE3008 water bath - rotary evaporator to 10 % initial volume and evaporated to dryness in Clifton water bath set at 80 ^{O}C. The dried plant extracts were stored individually in air tight correctly labeled containers.

For the Castor seed extract, the ground seeds were firstly defatted using chloroform and dried using diethyl ether before the commencement of the methanol extraction. 200 ml of chloroform was added to 25 g of the ground seeds powder in 1 Litre sterile bottle, placed on Stuart orbital shaker SSL1 and shook vigorously at 300 rmp for 2 hours at room temperature so as to remove oil and fats in the seeds. The mixture was allowed to settle and the supernatant which contains the oil and fats decanted. This was done twice using 100 ml chloroform and resulting precipitate was dried using 100 ml diethyl ether; this also carried out twice. The precipitate was spread on sterile paper towel and was ensure that it was completely dried before the methanol extraction as describe above was carried out.

2.5 Qualitative determination of flavonoids and tannins in the different plant parts: Qualitative determination for the presence or absence of flavonoids and tannins in different Castor plant parts were carried out according to the standard procedures as described by [24, 39, 40].

❖ **Test for flavonoids**

Each 0.5 g of the dried methanolic plant part extract was weigh separately into test tube; 5 ml petroleum ether was added and shaken vigorously for about 2 minutes in order to remove fatty materials. The defatted residue was dissolved in 20 ml of 80 % ethanol shaken vigorously for about 2 minutes and then filtered through Whatman No. 1 filter paper. The filtrate obtained from individual plant parts were used for the tests outlined below:

- 3 ml of the filtrate was mixed with 4 ml of 1 % aluminum chloride in methanol in a test tube and the colour was observed. Formation of yellow colour indicated the presence of flavonols, flavones and chalcones.
- 3 ml of the filtrate was mixed with 4 ml of 1 % potassium hydroxide in a test tube and the color was observed. A dark yellow color indicated the presence of flavonoids.

❖ **Test for tannins**

Each 0.25 g of the dried methanolic plant extract was dissolved in 10 ml distilled water and filtered through Whatman No. 1 filter paper. 3 ml of the filtrate was mixed with 3 ml of 1 % aqueous Iron chloride ($FeCl_3$) solution. The appearance of intense green, purple, blue or black color indicated the presence of tannins.

2.6 Quantitative determination of flavonoids and tannins in the different plant parts: Quantitative determination of flavonoids and tannins were carried out for the plant parts that gave positive test according to [41] for flavonoids and [42] with slight modification for tannins.

❖ **Flavonoid quantification**

100 ml of 80 % aqueous methanol was added to each 10 g of ground powdered plant part in sterile bottles and shaken vigorously for 6 hours at room temperature on a Stuart orbital shaker SSL1 at 300 rpm. The whole solutions were individually filtered through a Whatman No. 42 filter paper (125 mm), the filtrates transferred into separate crucibles and evaporated into dryness in a Clifton water bath set at 80 ^{O}C. 10 mg of each dried extract was dissolved in 4 ml 80 % aqueous methanol and assayed for flavonoids as described previously in order to establish its presence. The weights of the dried extracts were recorded. The percentage of flavonoids content was calculated as:

$$\% \text{ Flavonoid content} = \left(\frac{\text{Weight of dried plant extract}}{\text{Weight of plant material}} \right) \times 100$$

❖ **Tannin quantification**

100 ml of distilled water was added separately to 10 g of each ground powdered plant part in sterile bottles and shaken vigorously for 4 hours at room temperature on a Stuart orbital shaker SSL1 at 300 rpm. The whole solutions were also individually filtered through a Whatman No. 42 filter paper (125 mm), the filtrates transferred into separate crucibles and evaporated into dryness in a Clifton water bath set at 80 ^{O}C. 50 mg of each dried extract was dissolved in 2 ml distilled water and assayed for tannins as previously described in order to ascertain its presence. The weights of the dried extracts were recorded. The percentage of tannins content was calculated as:

$$\% \text{ Tannin content} = \left(\frac{\text{Weight of dried plant extract}}{\text{Weight of plant material}} \right) \times 100$$

2.7 Antioxidant assay

The antioxidant activities of the flavonoids and tannins extracts were determine following the procedure as described in [43]. To 1ml of each 50 mg/ml extract in methanol was added to 3 ml methanolic solution of 0.1 M DPPH and vigorously mixed together. The mixture was incubated in the dark for 30 minutes. Absorbance was measured at 517 nm using a Jenway UV/VIS spectrophotometer, using methanol as blank. 50 mg/ml and 100 mg/ml of ascorbic acid were used as the as the standard reference antioxidant compound to which the anti-oxidant and free radical scavenging activities of the extracts were compared. The anti-oxidant and free radical scavenging activities of the flavonoids and tannins extracts expressed in percent were calculated as follows:

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

Where, A_S is the absorbance value of sample + DPPH, and A_C is the absorbance value of only DPPH solution (i.e. the control).

2.8 Preparation of materials for hemolysis inhibition assay

- **Phosphate buffer saline (PBS) pH 7.4:**

Following the manufacturer's procedure, one tablet of phosphate buffer saline (PBS) was dissolved in 200 ml of deionized water to yield 0.01 M phosphate buffer saline pH 7.4.

- **20 ml 10 mM hydrogen peroxide (H_2O_2):**

22.67 µl of H_2O_2 equivalent to 0.0068 g was added to distilled water and made up to 20 ml to yield 10 mM H_2O_2.

- **Butylated hydroxyl anisole (BHA):**

200 mg of BHA was dissolved in 4ml of methanol to yield a final concentration of 50 mg/ml.

- **Plant extracts:**

200 mg each of dried plant flavonoids and tannins extracts were weighed and separately dissolved in 4 ml of methanol to yield a final concentration of 50 mg/ml each.

- **Blood collection and red blood cell (RBC) sample preparation**

Human blood sample (O^-) was obtained from the Blood Bank at Landmark University Medical Center, Omu Aran, Kwara State, Nigeria. 5ml of the blood was aliquot into EDTA (ethylene diamine tetra acetic acid) bottles. The blood samples were spun in C5 bench top centrifuge at 3000 rpm for 10 minutes. This was to remove the plasma, platelets and buffy coating from the sample. Resulting red blood cells (RBC) were washed twice with cold PBS; pH 7.4. 2 ml of the RBC (which is about 1 x 10^9 cells) was then subsequently used for the hemolysis inhibition assay. Table 1 illustrates the assay preparation mixture.

2.9 Hemolysis inhibition assay

This was carried according to the procedure as outlined in [44]. Table 1 illustrates the assay mixture preparation.

Table 1: Hemolysis inhibition assay mixture preparation

	RBC	PBS (pH 7.4)	10mM 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**

Assay mixtures were prepared for all the flavonoids and tannins extracts (50mg/ml), along with the BHA (50 mg/ml), positive and negative controls. The test tubes were incubated at room temperature, continuous shaking for 2 hours 30 minutes on Stuart orbital shaker SSL1 at 100 rmp, after which the contents centrifuged in C5 bench top centrifuge at 2000 rpm for 10 minutes. The absorbances of the supernatants obtained were read individually at 540 nm. Percent hemolysis inhibition was calculated as follows:

$$\frac{Abs\,(sample) - Abs\,(positive\ control)}{Abs\,(negative\ control) - Abs(positive\ control)} \times 100$$

$$\frac{Abs\,(BHA) - Abs\,(positive\ control)}{Abs\,(negative\ control) - Abs(positive\ control)} \times 100$$

In the negative control there was no hemolysis, whereas in the positive control there was hemolysis induced by H_2O_2. Results obtained for the flavonoids and tannins extracts were compared with that of BHA. BHA was used as a reference standard been a high antioxidant compound with great potential to prevent hemolysis.

2.10 Antibacterial activity assay

- **Test organisms**

Staphylococcus aureus and *Klebsiella halize* bacterial strains obtained from the Department of Microbiology, Landmark University, Kwara State, Nigeria. These two bacterial strains were used owe to their pathological effect on human, consequently been able to establish the possible inhibitory effects of the flavonoids and tannins extracts on their growth and propagation.

- **Preparation of nutrient agar incorporated with plant extract**

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 ^OC in Wisconsin Aluminum Steroclave 25X Bench-model Autoclave Sterilizer. The autoclaved agar was maintained at 47 ^OC by placing in Clifton water bath set at 47 ^OC. The flavonoid and tannin extracts from the different plant parts were prepared, and appropriate volume were added to 10 ml of nutrient agar to give a final concentrations of 1 mg/ml, 5 mg/ml and 10 mg/ml, and were poured into separate sterile petri dishes. This was done in triplicate.

- **Determination of antibacterial activity:**

Visualization of colony formation was used to access the inhibitory activities of the flavonoids and tannins extracts. *Staphylococcus aureus* and *Klebsiella halize* pre-cultures in nutrient broth were allowed to grow to their exponential phase before use. The amounts of microbial cells used were estimated in line with previous work that involves detecting bacteria culture turbidity at 540 nm and counting the number of cells against the optical density, as reported in "www2.mum.edu/ibrandon/Micro/enumeration.doc". The optical densities for *Staphylococcus aureus* and *Klebsiella halize* pre-cultures were 0.985 and 1.652 respectively. This gave an approximate 14 × 10^4 and 21 × 10^4 colony forming unit (CFU)/ml of *Staphylococcus aureus* and *Klebsiella halize* respectively. A volume of 200 µl of the microbial cells of *Staphylococcus aureus* and *Klebsiella halize* were spread evenly over the surface of separate agar plates (in triplicates) using a sterile glass tong. The plates were then incubated in Stuart orbital incubator S1500 for 24 hours at 37 ^OC; without

agitation. This volume was used so as to make certain that considerable amount of bacterial cells were used. The numbers of cells visualized and counted after this period.

2.11 Statistical Analysis
All analyzes were carried out in triplicates. Results were analyzed using the Graph Pad Prism software package. Data were represented as mean ± standard deviation. Results were further analyzed for correlation and test of significance by Student Paired t-test at $P \leq 0.05$.

3. Results
Table 2 below shows the results of qualitative determination for the presence of flavonoids and tannins in the Castor plant parts methanolic extracts. Analyzes revealed that flavonoids was present in all the 5 plant parts (leaves, stem, roots, seeds and capsules) examined, while tannins was present likewise in these plant parts with the exception of the capsules.

The quantitative analyzes showing the distribution and contents of flavonoids and tannins in the Castor plant parts expressed in percent yield is illustrated in Figure 2, where it shows flavonoids: leaves (7.78 %); stem (22.0 %); roots (22.8 %); seeds (6.66 %); capsule (6.75 %). For tannins: leaves (23.92 %); stem (34.0 %); roots (28.8 %); seeds (8.0 %).

Table 2: Qualitative analyzes for the presence of flavonoids and tannins in the Castor plant parts extract.

Plant Parts Extracts	Flavonoids	Tannins
Leaves	+	+
Stems	+	+
Roots	+	+
Seeds	+	+
Capsules	+	-

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

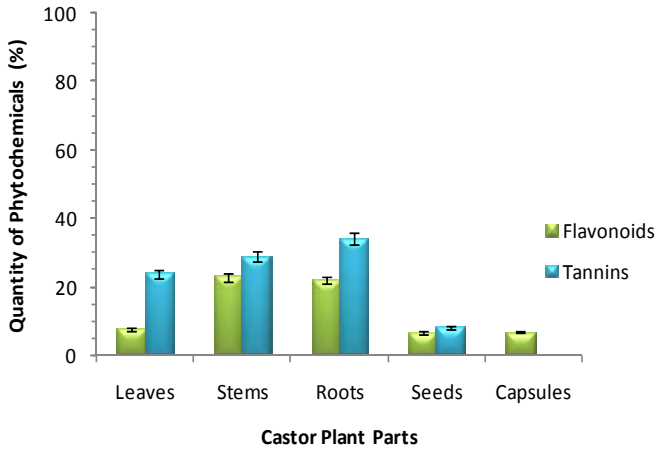

Fig 2: Quantitative analyzes of flavonoids and tannins distribution and contents in the different Castor plant parts. Results are the average of means (n = 5), and represented as mean ± standard deviation.

The free radical scavenging and antioxidant properties of the flavonoids and tannins extracts as accessed using the DPPH assay is illustrated in Figure 3 below. Very high antioxidant activities were observed in leaves flavonoids (99.23 %), stems flavonoids (99.7 %), seeds flavonoids (99.6 %) and capsules flavonoids (99.75 %) while in leaves tannins (73.78 %), which are very similar to ascorbic acid; 50 mg/ml and 100 mg/ml (79.16 % and 95.61 %, respectively). The other plant parts such as roots flavonoids (49.45 %) and stems tannins (31.51 %); seeds tannins (58.2 %) and roots tannins (34.69 %) gave appreciable activity but not as high as ascorbic acid.

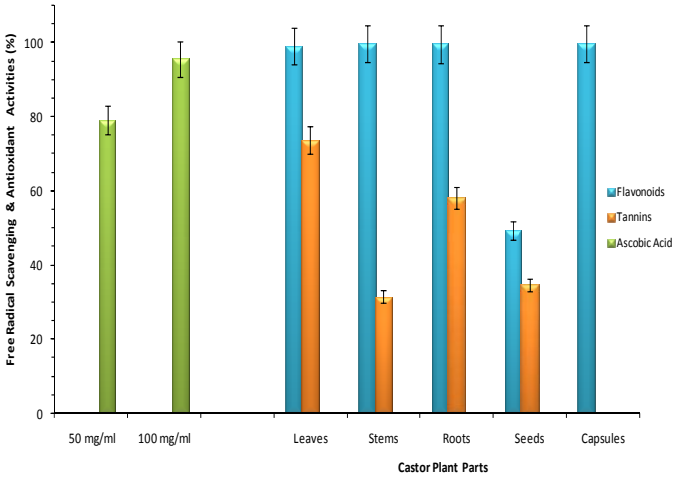

Fig 3: The antioxidant and free radical scavenging activities of flavonoids and tannins Castor plant parts extracts. The experiment was performed in triplicates. Values were the averages of means, and represented as Mean ± standard deviation. Statistical analyses were performed using student paired T-test. Results were considered statistically significant at $p \leq 0.05$.

The effect of the flavonoids and tannins Castor plant parts extracts in protecting the red blood cells (RBC) against the hemolytic activity H_2O_2 is presented in Figure 4. All the flavonoids and tannins extracts gave comparable anti-hemolytic activities with butylated hydroxyl anisole (BHA); which was used as the reference anti-hemolytic compound, except for the stems flavonoids that promoted hemolysis. Figure 4 shows the following anti-hemolytic activities: leaves flavonoids (327.38 %), roots flavonoids (146 %), seeds flavonoids (209.89 %) and capsules flavonoids (242.96 %), leaves tannins (182.5 %), stem tannins (400.38 %), seeds tannins (138.78 %), roots tannins (353.23 %), while 50 mg/ml BHA gave 536.5 % anti-hemolytic activity. The stems flavonoids however promoted the hemolysis by 166.54 %.

The antibacterial effects of the flavonoids and tannins Castor plant parts extracts on the growth and proliferation of *Staphylococcus aureus* and *Klebsiella halize* strains are as outlined in Table 3. The bacterial culture plates were visualized for their colony formation and differential trend of inhibition on the growth at increasing phytochemical concentrations were observed. Continuous lawns (biofilms) were mostly observed in the control plates of the two bacterial strains which however reduce at increasing phytochemical concentrations in the experimental plates. This perhaps may be due in part to the morphology of the two bacteria strains used or may be as a result of high number of colony forming units (CFU) of bacteria that were used, which facilitates the bacteria to proliferate rapidly [45, 46, 47, 48]. Thus the amount of CFU on the control and experimental could not be properly ascertain, however it was possible to see the antibacterial effects judging by the decrease in the continuous lawns in the experimental compared to the control. The high CFU were used in order to assess the full efficiencies of these extracts towards high

bacteria population.

Table 3: Antibacterial activities of flavonoids and tannins Castor plant parts extracts against *Staphylococcus aureus* and *Klebsiella halize*

Phytochemicals	*Staphylococcus aureus*			*Klebsiella halize*		
	1 mg/ml	5 mg/ml	10 mg/ml	1 mg/ml	5 mg/ml	10 mg/ml
Leaves flavonoids	--	--	+	--	+	+
Stems flavonoids	--	--	+	--	--	+
Roots flavonoids	+	+	++	+	+	++
Seeds flavonoids	--	--	-	--	--	--
Capsule flavonoids	+	++	++	+	++	++
Leaves tannins	--	+	++	--	+	+
Stems tannins	--	+	++	--	+	++
Roots tannins	+	++	++	+	+	++
Seeds tannins	--	--	--	--	--	--

Key: (--) no inhibition; (+) inhibition; (++) high Inhibition

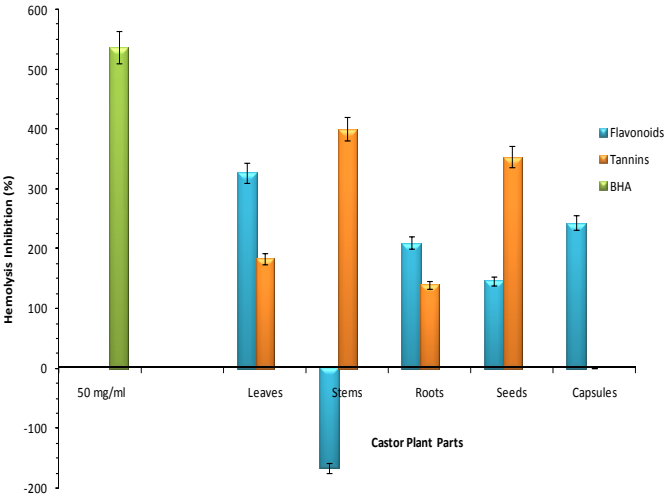

Fig. 4: Hemolysis inhibition assay of flavonoids and tannins Castor plant parts extracts in protecting the red blood cells (RBC) against hemolytic effect of H_2O_2. The experiment was performed in triplicates. Values were the averages of means, and represented as Mean ± standard deviation. Statistical analyses were performed using student paired T-test. Results were considered statistically significant at $p \leq 0.05$.

As illustrated in Table 3, the roots and capsules flavonoids and roots tannins gave an increasing trend of antibacterial activity with increasing phytochemical concentrations. The stems flavonoids and stems tannins only gave inhibitory effects at high phytochemical concentrations (5 mg/ml and 10 mg/ml). The flavonoids and tannins extracted from the Castor plant seeds however gave no bacteria inhibitory effect at all as there was no clear difference between the experimental and control plates, even at higher phytochemical concentrations.

4. Discussion

Medicinal plants are said to have therapeutic effects against free radicals, pathogenic organisms and various forms of ailments. The effects of such medicinal plants arise as a result of various secondary metabolites which are present in these plants [15]. Such metabolites are responsible for the various biological actions that occur. This has led to the need to identify the specific phytochemical that is responsible for such interactions.

Research works have documented various biological activities of solvents extracts from Castor plant (*Ricinus communis* L.) parts however there exist very limited information as regards the quantity and biological activities of flavonoids and tannins present in this plant [36, 37, 38, 49, 50, 51]. Thus, our study was able to ascertain the presence, quantity and also evaluate some biological activities of flavonoids and tannins in the various Castor plant parts. Results show differential presence of flavonoids and tannins (Table 2) where the roots gave the highest flavonoids content and the stems gave the highest tannins content (Figure 2).

The abilities of the flavonoids and tannins extracts from the Castor plant parts to act as an antioxidant and free radical scavenger as adjudged by DPPH assay, show that flavonoids from stem, seed, capsule and leaves and tannin from leaves have very high potential to act as a very good natural antioxidant as the antioxidant values obtained from these sources (Figure 3) were very similar to the activity of 100 mg and 50 mg ascorbic acid. Similar antioxidant activities have been documented in other plant flavonoids [27, 28, 52]. However the value obtained for Castor flavonoids show very high antioxidant potentials as compared to what has been previously obtained in other plants extracts. Thus it will be imperative that the study of Castor flavonoids be further elucidated in order to establish the specific class of flavonoid that is involved in the antioxidant and free radical scavenging activities.

Hemolysis is the destruction or breakdown of red blood cells (RBC's) which causes the release of its internal components such as hemoglobin into its surrounding environment [53]. This subsequently results in the removal of red cells circulation within the body faster than their normal life span of 120 days [54].

Studies have shown that sudden hemolysis of red blood cells results from the attack of free radicals and many other reactive oxygen species produced during disease states such as cardiovascular diseases, diabetes, cancer and in ageing processes [55]. Our results show that flavonoids and tannins from various Castor plant parts have the ability to counter the effect of reactive oxygen species (such as H_2O_2) on red blood cells, as very high antihemolytic results were obtained when red blood cells were treated with H_2O_2 with inclusion of 50 mg Castor flavonoids and tannins extracts, which gave comparable antihemolytic activity with 50 mg of butylated hydroxy anisole (BHA) (Figure 4). BHA is a well-known antioxidant and anti-

hemolytic compound [56, 57]. However the flavonoids extract from Castor stem aided hemolysis; this may be connected to the same fact that different classes of flavonoids are present in plants. Thus the flavonoids obtained from the Castor stem may not have the same structural/biological activity as what may be present in other plant parts. Similar results of extracts increasing red blood cell hemolysis have been reported in 400 µg of ethanolic extract of *Ixora coccinea* Linn plant extracts and 5 mg/ml *Raphiostylis beninensis* extracts [58, 59]. Thus, further structural characterization will provide the appropriate distinctions between these phytochemicals, and those with good human biological effects will be known and separated from the not so good ones.

Differential antibacterial activities were observed on the treatment of flavonoids and tannins extracts of Castor plant parts as illustrated in Table 3. The roots and capsules flavonoids and roots tannins gave the highest antibacterial activities at increasing phytochemical concentrations, while the seeds flavonoids and tannins gave no inhibitory effects on the two pathogenic bacteria. Thus Castor plants may have evolved in distributing substantial antimicrobial agents to parts that are more susceptible to microbial attacks such as the roots and capsules (which harbors the seed that is rich in carbon content a good platform for microbial proliferation). It will be interesting to unravel the chemical components/structural form of the flavonoids present in the root and capsule, so also will be tannins present in the roots. In future this may be a target that could be exploited in the formulation of pharmaceutical drugs that may have high antimicrobial potency.

Judging by the overall evaluation of biological activities of flavonoids and tannins in Castor plant parts carried out in this research, it could be deduced that Castor capsule flavonoids has better biological activities compared to the others.

5. Conclusions

This study has revealed the distribution of flavonoids and tannins in Castor plant parts and also elucidated their antioxidant, antihemolytic and antibacterial effects. It would be of great medicinal importance if further works could be done which may include cytotoxicological test, anticoagulant activity and structural characterization of the various classes of flavonoids and tannins present in the different Castor plant parts. These will offer in-depth knowledge of flavonoids and tannins and how they may be of medicinal or pharmaceutical applications. The structural information could provide a platform for the design of synthetic drugs which will have structural resemblance to these phytochemical and thus provide the same/similar biological activities. More importantly it will be interesting to establish further biological properties of capsule flavonoids since it gave a better biological activity as regards antioxidant, antihemolysis and antimicrobial properties. To the best of our knowledge, this is a pioneer work into important biological activities that Castor capsules may possess aside from just housing the seeds.

Thus far, we will advise that the use of the various Castor plant parts should be administered with caution as some parts may have negative effect which may promote the progress of certain health conditions as revealed in this study.

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