INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Assessing *Ricinus communis* L. (castor) whole plant part for Phenolics and Saponins constituents for Medicinal and Pharmaceutical applications

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ABSTRACT

There has been an increasingly interest in medicinal plant researches owe to the organic richness and far less side effects. *Ricinus communis* L. commonly called castor plant; a strong perennial shrub of Euphorbiaceae plant family has been used in many parts of the world for treatment of various ailments. However in most case, the active phytochemical constituents that participated in alleviating these ailments cannot be justified. Using established protocols, the phytochemical screening of castor plant parts revealed that phenolics and saponins were quantitatively present in the seeds and roots; while saponins were additionally found in the leaves. Antioxidant activities of these phytochemicals when assayed at 100mg/ml were seen to be comparable with ascorbic acid; at the same concentration. At 50mg/ml saponins from leaves and seeds, and phenolics from the root showed appreciable anti-hemolytic activities. Antibacterial activities of these phytochemical, at 1mg/ml, 5mg/ml and 10mg/ml as tested against *Staphylococcus aureus* (14×10^4 CFU/ml) and *Klebsiella halize* (21×10^4 CFU/ml) showed differential trend but potential inhibition of these organisms, at increasing phytochemical concentrations. Amazingly, 5mg/ml and 10mg/ml of the leaves saponins completely inhibited these bacterial strains. It thus indicates that the phenolics and saponins of castor plant parts may be exploited as a natural antioxidant and as a potential agent against hemolysis of red blood cells. The effective antimicrobial activity of the leaves saponins is what should be further unravel as this has very great prospect for the treatment of pathogenic diseases such as those caused by the presence and proliferation of these organisms.

Keywords: Antioxidant, antihemolysis, antibacterial, pathogenic organism(s), phytochemicals, traditional medicine

1. INTRODUCTION

Alternative medicine in the use of medicinal plants in the treatment of many ailments is currently employed by large percent of world population [1, 2]. The continual use of this traditional system has increased the knowledge hub on vital phytochemicals that numerous plant and their parts possesses [2]. Medicinal plants generally referred to as plants in which one or more of its parts contains essential phytochemicals that may be exploited for therapeutic purposes, or such that may be used as precursors in chemo-pharmaceutical synthesis [3]. The presence of these phytochemicals in plants have been found to be very beneficial to human systems as most food consumed by human often contain less quantity of these biomolecules. Moreover their consumption results in far less side effects when compare to pharmaceutical synthetic drugs [4]. Thus their supplement with human food is that which cannot be over emphasized. Plant phytochemicals are known to posses essential biological activities among which are antioxidant, antimicrobial, anti-inflammatory, antiviral, antifungal, antihelmintic, anticoagulant, anti-carcinogenic, anti-asthmatic, anti-hypertensive, anti-allergic, diuretic, and even shown to improved fertility [5, 6, 7, 8]. Thus more and more of plants are being continually screened for their phytochemicals and evaluation of their possible biological activities.

Ricinus communis L. generally called castor plant is one of tropical flowering plant species of spurge family Euphorbiaceae and has been found to grow widely across the world [9]. It is a tall shrub of about 2 – 4 meters high, and its leaves are about 15- 45 cm long; long-stalked; palmate of 5 – 12 lobes with coarsely toothed segments (Figure 1). The different parts of the plant are been used by local communities and forest dwellers in the treatment of various ailments [10, 11]. Its prehistoric and modern therapeutic uses have been surveyed and reported and various parts of the plant such as root, stem, leaves, fruits, flowers, the complete aerial parts and the whole plant were shown to be used for medicinal purposes [12]. The extracts from plant parts were shown to posses various valuable effects such as analgesic, diuretic, antidiarrhea, antiasthmatic, anthelmintic and many other medicinal benefits [13, 14, 15, 16, 17, 18]. The leaves and roots extracts and the seed oil have been used in the traditional medicine as laxative, and in the treatments of headache, backache, rheumatism, abscesses, dropsy, warts, ringworms, hypoglycemia, inflammation and liver disorder [13, 19]. External application of the leaves extracts was shown to increase milk flow in nursing mothers while the oil supports pregnancy labour, resulting in quick delivery [19, 20].

Phenolics also referred to as polyphenolics are phytochemicals that contributes generally to the pigmentation of plants and their fruits, and were found to assist plants defence mechanisms against pathogens, herbivory attacks and combating abiotic stresses [21, 22, 23, 24]. Saponin on the other hand was coined from Latin word “sapo” which literally means soap. This was due to the fact that they form soap-like foams when shaken in water, thus saponins are defined as biological compounds with foaming characteristics [24]. They are high molecular weight, non- volatile surface-active compounds, and are widely distributed in numerous plants [25, 26, 27].

Phenolics and saponins, just like their counterpart phytochemicals have been shown to have varieties of biological activities in human; such as outline previously. However limited information still exist as regards these, as most research works are mainly in qualitative terms of a particular plant part and not exactly on the distributions and quantities of the phytochemicals in the different plant parts. Furthermore, most of the works are solely on evaluating the biological activities of crude extract which is a mixture of so many phytochemicals. So knowing the phytochemical that is responsible for the reported biological activity is always dicey. Moreover, there are numerous classes of these phytochemicals, it is very likely that the class of a phytochemical present in a particular plant part may not necessary be present or be the same as in other parts of the plants. As an example, phenolics or saponins present in the stem may not necessarily be the same as the ones in the root or leaves, consequently they may not have the same biological activities. It is therefore imperative to screen various plant parts for a particular phytochemical and ascribe its biological activities accordingly.

Furthermore, information on whole plant profiling for phytochemicals is very limited. This study therefore seeks to study the distribution of phenolics and saponins in qualitative and quantitative terms, of the various plant parts (roots, stems, leaves, capsules and seeds) of *Ricinus communis* L. and also to unravel their biological activities as regards their antioxidant, antihemolysis and antimicrobial properties. We believe the amount of these phytochemicals present in the various plant parts will differ, so may their biological properties. Information obtained here will not only add to the existing knowledge of biological activities of phytochemicals, but may be exploited for medicinal or pharmaceutical preparations and applications.

Figure 1

A typical *Ricinus communis* L. (Castor) plant found around Omu-Aran environment, Kwara State, Nigeria.

2. MATERIALS AND METHODS

2.1. Chemicals and Other Reagents.

All reagents used were of analytical grade. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, butyl hydroxyl anisole (BHA), sodium chloride, hydrogen peroxide, chloroform, diethyl ether, n-butanol, petroleum ether, amyl alcohol, ethanol, methanol, ethylenediamine tetraacetic acid (EDTA), olive oil, nutrient agar were either from Sigma-Aldrich Chemie GMBH, Breckland Scientific or BDH Chemicals Limited.

2.2. Preparation of reagents

- **Preparation of 100 ml 5% aqueous NaCl**
  5 % aqueous NaCl was prepared by weighing 5 g of NaCl and dissolving in 100 ml of distilled water.
- **Preparation of 200 ml 20 % ethanol**
  20 % ethanol was prepared by measuring 40 ml of ethanol and adding to 160 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, according to the recommended manufacturer procedure.
- **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 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 nutrient agar**
  Nutrient Agar was prepared by weighing 28 g of the powder into conical flask and made to 1 Litre with deionized water. The mixture was allowed to dissolve totally and was sterilized by autoclaving for 15 minutes at 121 ^{O}C. It was then kept in water bath at 47 ^{O}C until further use.

2.3. Plant Material

Whole plants of *Ricinus communis* L. were collected in March 2014 from Omu Aran town; which is about 88 kilometers South of Ilorin, the capital of Kwara State, Nigeria. It is located on Latitude 8^{o}8' N and Longitude 5^{o}6' E. The plant was identified and authenticated at the Herbarium of Department of Plant Biology, University of Ilorin, Kwara State, Nigeria, and was given a voucher number of U1H00I/9652007. Healthy plants were uprooted from field and thoroughly washed in distilled water. The plants were dismembered into different parts (roots, stems, leaves, capsules and seeds), washed again with distilled water, blotted dry in-between clean sterile paper towel and oven dry in Genlab Oven (Genlab Ltd, Widnis, Cheshire, WA8 OSR) at 50^{O}C until a constant weight was achieved. The dried plant parts were ground in electric blender (Waring Products Division, Torrington, USA), fine powder were stored separately in air tight properly labeled container until further use.

2.4. Preparation of plant parts extracts for qualitative determination of phenolics and saponins

25 g of the finely ground powder of each plant parts (roots, stems, leaves and capsules) in separate sterile bottle were extracted each with 250 ml 100 % methanol. This was done by placing on a Stuart orbital shaker SSL1 (Bibby Scientific Ltd, UK) at 300 rmp and shaking vigorously for 48 hours at room temperature. The contents 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 (LW Scientific Inc. GA, USA) for 10 minutes at 1000 rpm to remove insoluble particles and any fatty layer. The supernatants were decanted and concentrated to about 10 % initial volume using a Stuart RE3008 water bath - rotary evaporator (Bibby Scientific Ltd, UK). The extracts in their individual labeled glass beakers were placed in Clifton water bath (Clifton Lab Equipment, UK) set at 80^{O}C and were evaporated to dryness. The dried plant extracts were then stored individually in air tight containers.

For seed extract, 25 g of the finely ground *Ricinus communis* L. seed powder was soaked in 200 ml chloroform in conical flask, placed on a Stuart orbital shaker SSL1 (Bibby Scientific Ltd, UK) at 300 rmp and shaking vigorously for 2 hours at room temperature in order to remove the fats and oil present in the seed powder. This was allowed to settle and the supernatant which contain fats and oil was removed. This process was repeated twice using 100 ml chloroform. The resulting precipitate was dried using 100 ml diethyl ether (done twice), later spread on a sterile paper towel to ensure complete dryness of solvent before the commencement of the methanol extraction as previously described.

2.5. Qualitative determination of phenolics and saponins in plant parts

Screening for presence or absence of phenolics and saponins in *Ricinus communis* L. plant parts were

carried out using the standard procedures as described in Sofowara[1], Mace [21], and Trease and Evans [28].

- **Test for Phenolics**

Ammonium hydroxide test: 50 mg of each plant part extract was diluted separately in a test tube with 5 ml distilled water. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were added and left for 30 minutes in dark. Development of bluish green colour was a positive presence of phenolics.

Ferric chloride test: 50 mg of each plant part extract was diluted separately in a test tube with 5 ml distilled water. 10 drops of neutral 5 % ferric chloride solution was added par test tube. The development of dark green colour was an indication of presence of phenolics.

- **Test for Saponins**

50 mg of each plant part extract was diluted separately in a test tube with 5 ml distilled water and was boiled in water bath for 2 minutes and filtered. The filtrate in test tube was shaken vigorously. The formation of a persistent froth indicates the presence of saponins. When frothing was mixed with 3 drops of olive oil and shaken vigorously, the formation of emulsion shows a characteristic of presence of saponins.

2.6. **Quantitative determination of phenolics and saponins in plant parts**

Ricinus communis L. plant parts that gave positive test were further characterized for their quantity and expressed as percentage of the dried material, following the procedures as described in Okwu and Josiah [29] for phenolics and Khan et al. [30] for saponins.

- **Phenolics quantification**

25 g of each ground plant parts powders were defatted separately in 500 ml of diethyl ether each using a soxhlet apparatus for 2 hours. The fat free samples were boiled separately in 500 ml of ether for 15 minutes for the extraction of phenolics components. 5 ml was taken from each extract and assayed as previously described in order to ascertain the presence of phenolics. The extracts were filtered and concentrated to 10 % of volume using a Stuart RE3008 water bath - rotary evaporator (Bibby Scientific Ltd, UK), the resulting extracts in their individual labeled glass beakers were placed in Clifton water bath (Clifton Lab Equipment, UK) set at 80^{O}C and were evaporated to dryness. About 10 mg of each dried extract was dissolved in 1 ml distilled water and assayed as previously described in order to ascertain and confirm the presence of phenolics. The dried extracts were obtained and weighed. The phenolic content of each extract was expressed as a percent of the starting dried plant material as follows:

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

- **Saponins quantification**

25 g of each ground plant parts powders were weighed into separate conical flasks and 125 ml of 20 % aqueous ethanol added. The flasks were heated in water bath at 55^{O}C for 4 hours with occasional stirring. The mixtures were separately filtered, the filtrates were kept and the residue re-extracted twice with 100 ml of 20 % aqueous ethanol. Each plant part filtrates were combined and reduced to 10 % of volume using a Stuart RE3008 water bath - rotary evaporator (Bibby Scientific Ltd, UK) and then transferred separately into 250 ml separatory flasks. 25 ml of diethyl ether was added into each flask and shaking vigorously. The diethyl ether layer was removed and this was repeated twice. The aqueous layer was recovered and 75 ml n-butanol was added and mixed thoroughly, and mixture washed twice with 12.5 ml of 5 % NaCl solution. The remaining solution was placed in hot water bath for solvent to evaporate, afterwards was placed in oven set at 60^{O}C to evaporate completely to dryness. About 10 mg of each dried extract was dissolved in 1 ml distilled water and assayed as previously described in order to ascertain and confirm the presence of saponins. The dried extracts obtained were weighed. The saponins content of each extract was expressed as a percent of the starting dried plant material as follows:

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

2.7. **Antioxidant Assay**

The antioxidant potentials of the phenolics and saponins extracts were adjudged following the method as described by Brand-Williams et al. [31]. 1 ml of extract (50 mg/ml in methanol) was added to 3 ml of 0.1 M DPPH in methanol solution and mixed vigorously. The mixture was incubated in the dark for 30 minutes. Absorbance was measured at 517 nm using a Jenway UV/VIS spectrophotometer (Bibby Scientific Ltd, UK) with methanol as blank. 50 mg/ml and 100 mg/ml of ascorbic acid were used as the standard reference antioxidant compound to which the activities of the extracts were compared. Antioxidants neutralize the DPPH free radical by donating hydrogen atoms to the free radical and causing the colour of the reaction mixture to change

from purple to yellow [32]. The degree of the discoloration indicates the scavenging potential of the antioxidants in the extracts. The anti-oxidant and free radical scavenging activities of the phenolics and saponins extracts as expressed in percent were calculated as:

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

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

2.8. **Hemolysis Inhibition Assay**

This was carried out according to the method as described in Tedesco et al. [33], with slight modification. Human blood sample (O^-) was collected from Blood Bank at the Medical Centre Landmark University, Omu Aran, Kwara State, Nigeria. The blood was aliquot in 5 ml into EDTA (ethylene diamine tetra acetic acid) bottles. The samples were centrifuged using C5 bench top centrifuge (LW Scientific Inc. GA, USA) at 3000 rpm for 10 minutes. This was to ensure the removal of plasma, platelets and buffy coat from the samples. The 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 used for hemolysis inhibition assay. Samples were prepared in test tubes as outlined in Table 1 below.

Assay mixtures were prepared for all the phenolics and saponins extracts (50 mg/ml), along with the butylated hydroxyl anisole (BHA; 50 mg/ml), negative and positive controls. The test tubes were incubated at room temperature with continuous shaking for 2 hours 30 minutes on Stuart orbital shaker SSL1 (Bibby Scientific Ltd, UK) at 100 rmp, afterwards the contents centrifuged at 2000 rpm for 10 minutes. The absorbance of the supernatant obtained was read at 540 nm. Percent hemolysis inhibition was calculated and compared with that of BHA. BHA is a known antioxidant with very high ability to prevent hemolysis; hence it was used as a standard to compare its activity on the RBC with that of the plant extracts.

The percent of hemolysis inhibition was calculated as follows:

$$\frac{\text{Abs}_{(\text{Plant extract})} - \text{Abs}_{(\text{Positive control})}}{\text{Abs}_{(\text{Negative control})} - \text{Abs}_{(\text{Positive control})}} \times 100$$

$$\frac{\text{Abs}_{(\text{BHA})} - \text{Abs}_{(\text{Positive control})}}{\text{Abs}_{(\text{Negative control})} - \text{Abs}_{(\text{Positive control})}} \times 100$$

Where in the negative control there was no hemolysis, whereas in the positive control there was hemolysis.

2.9. **Antibacterial Assay**

The antibacterial activities of the plant extracts were evaluated against *Staphylococcus aureus* and *klebsiella halize* growth. The organisms were obtained from the Department of Microbiology, Landmark University, Omu-Aran, Kwara State, Nigeria. These organisms have been implicated in many human dreadful conditions, hence were used to ascertain possible antagonist action of the extracts on their proliferation.

To the nutrient agar kept at 47^OC, appropriate quantities of phenolics and saponins extracts were separately mixed with 10ml agar to give final concentrations of 1 mg/ml, 5 mg/ml and 10 mg/ml, and were poured into separate sterile plates. This was done in triplicate.

The organisms were sub-cultured in nutrient broth maintained at 37^OC in a Stuart orbital incubator S1500 (Bibby Scientific Ltd, UK). The sub-cultured were allowed to grow into their exponential phase before use. The amounts of microbial cells used were evaluated according to previous work reported in [34] that involves detecting bacteria culture turbidity at 540 nm and counting the number of cells against the optical density. The optical density for *staphylococcus aureus* was 0.985 and *klebsiella halize* was 1.652, and this is approximately 14×10^4 and 21×10^4 colony forming unit (CFU)/ml respectively. Visualization of colony formation was employed, a volume of 200 µl of microbial cells of *Staphylococcus aureus* and *Klebsiella halize were* spread evenly over the surface of separate nutrient agar plates using a sterile glass tong. This volume was used in order to ensure that substantial amount of bacterial cells were used for the analysis. The plates were incubated in Stuart orbital incubator S1500 (Bibby Scientific Ltd, UK) for 24 hours at 37^OC; without agitation. This was carried out in triplicate.The numbers of cells were visualized and counted after this period.

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2.10. **Statistical Analysis**

All analyzes were carried out in triplicates. Results were analyzed using the Graph Pad Prism software. Data were presented as mean ± standard deviation (SD). Experimental results were further analyzed for

correlation and test of significance by Student Paired t-test at $P \leq 0.05$.

3. RESULT

3.1 Qualitative Analysis

Qualitative analysis of the *Ricinus communis* L. plant parts methanolic extracts revealed the presence of phenolics in the seeds and root extracts, while saponins was present in the leaves, root, and seeds extracts, as presented in Table 2.

3.2 Quantitative Analysis

The quantity of phenolics and saponins contents in the various *Ricinus communis* L. plant parts that showed positive results is as presented in Figure 2. For phenolics 6.6 % and 7.67 % were obtained for root and seeds extracts, while for saponins 7.2 %, 9.04 % and 6.7 % were obtained for root, leaves and seeds extracts, respectively.

3.3 Antioxidant Assay

The antioxidant and free radical scavenging activities of the phenolics and saponins extracts as adjudged using DPPH method is presented in Figure 3. The roots phenolics and leaves saponins gave very comparable antioxidant activities (89.95 % and 93.33 %, respectively) as recorded for ascorbic acid; 50 and 100 mg/ml (79.16 % and 95.61 %, respectively). The other plant parts such as seeds phenolics (26.30 %) and roots and seeds saponins (38.11 % and 40.19 %, respectively) gave good activity as well, but were not as high when compared to ascorbic acid.

3.4 Hemolysis Inhibition Assay

The percent hemolysis inhibition assay of red blood cells (RBC) shows that three of the five extracts, which include leaves and seeds saponins and roots phenolics gave positive antihemolysis activities, while the other two; roots saponins and seeds phenolics promoted hemolysis. The leaves (130 %) and seeds (175.8 %) saponins and roots (216.5 %) phenolics gave appreciable antihemolysis activities when compared with BHA (381.72 %) as shown in Figure 4. However, the roots saponins and seed phenolics promoted hemolysis by 25.52 % and 138.97 %, respectively.

3.5 Antibacterial Assay

Table 3 shows the results as obtained from the antibacterial assays against pathogenic organisms; gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Klebsiella halize.* For these two organisms, continuous lawn of bacterial colonies were observed in the control plates, which however reduces in some of the plates containing the plant extracts, in some there was no clear difference with control, while in some there was complete inhibition of bacterial growth. The observed bacterial continuous lawn colonies may be attributed perhaps to the morphological characteristics of the organisms or that the amount of bacterial cells inoculated onto the plates was high, hence the bacterial growth increase led to formation of continuous lawn. This phenomenon made it intricate to ascertain the number of cells present per plate, however judging by the cell proliferation, it was possible to see the inhibitory trend of the various concentrations of the *Ricinus communis* L. plant parts extracts on the bacteria survival and growth as illustrated in Table 3. The high amount of bacteria cells were used to ensure that considerable bacteria cells were used and to test the effectiveness of the extracts towards high bacteria population.

As shown above, there was a differential trend of bacterial growth inhibition. The seeds phenolics, and seeds and roots of saponins do not show any capable inhibitory effect on the bacteria survival and growth. The root phenolics showed inhibition at higher extracts concentrations, while the leaves saponins gave a tremendous inhibition of the two bacterial strains, which at 5 mg/ml concentration, there was complete inhibition of bacterial growth.

4. DISCUSSION

The medical and pharmacological actions of extracts from *Ricinus communis* L. parts been used from time memorial for treatment of various ailments could be said to be further strengthen and justified by this work. This work in part is related to previous work by Obumselu [35] where the presence of saponins in the leaves extracts of *Ricinus communis* L. was reported. However our reports further points to other parts of *Ricinus communis* L. that harbors this phytochemical, including phenolics.

The quantitative analyzes where the extraction method according to Okwu and Josiah [30] was employed and gave us higher yield of saponins when compared with the work reported in Obumselu [35] who used the method described by Harborne [36]. From our work 25 g of dried ground leaves material gave 9.04 % of saponins yield as compared to 0.46 % obtained in Okwu and Josiah [30] using 40 g of dried ground leaves material. Thus it may be advocated that method described in Okwu and Josiah [30] should be employed more for saponins extractions in order to obtain higher yield. Spectrophotometric quantification of phenolics using the Folin-Ciocalteau reagent method is also widely used, however interferences usually occurs as a result of the presence of other non-phenolic organic substances that may react with the Folin-Ciocalteau reagent to give an elevated phenolic concentrations Prior et al.

[37]. Thus we believe weighing the dried sample and calculating the percentage yield as employed in this work will give a better and accurate content of the total phenolics.

Plants phytochemicals confers many beneficial biological activities to humans, such as those properties that have been previous described, which may include among many others antioxidants, antibacterial, antiviral, antifungal, anti-inflammatory, anticoagulant, etc. Saponins have been considered to help fight infections caused by parasites by boosting the immune system. This was attributed to the antioxidant activity of the non-sugar moiety of saponins [38]. Saponins was shown to bind and react with the cholesterol and cholesterol rich membranes of cancer cells; thereby preventing cholesterol to be reabsorbed into the system and excreted out of the body, and restricting cancer cells proliferation respectively [38]. This consequently reduces cancer and other cholesterol associated diseases such as stroke, heart and central nervous system diseases [38]. Phenolics on the other hand were shown to prevent diseases associated with oxidative stresses by mopping up the free radicals from biological system using their hydroxyl moieties on their phenolic ring [39]. Such properties among others include anti-inflammation, anti-apoptosis and cardiovascular protection [40]. Phenolics are also used in combination with other natural compounds in cosmetics applications as exfoliants, blemish removal, antipruritic, skin lightening, topical anesthetic and in cosmetic surgery [41]. These have been attributed to the antioxidant potentials of phenolics [41]. Using the DPPH free radical method [31] to asses the antioxidant and free radical scavenging properties of phenolics and saponins of *Ricinus communis* L. parts, showed that the roots phenolics and leaves saponins have very good potential to scavenge free radicals as their activity are very similar to ascorbic acid, and as such they may be exploited in the treatment of degenerative diseases resulting from or associated with oxidative stresses. The other extracts; roots and seeds saponins and seeds phenolics though gave lower antioxidant activities, they may still be used in treatment of oxidative stress diseases, it may just mean that the amount that will be used may be slightly higher compared to roots phenolics and leaves saponins.

Red blood cells (RBCs) are often subjected to oxidative damages by reactive oxygen species [such as O_2^-, OH^- NO^-, $ONOO^-$, $RO(O)^-$, $LO(O)^-$ and H_2O_2], owe to the presence of high amounts of poly-unsaturated fatty acids at their outer membranes and the hemoglobin molecules redox potentials that is associated with O_2 transport that consequent results in the hemolysis of the RBCs leading to numerous cardiovascular illness [42, 43]. Phytochemicals with very good antioxidant properties have proven to protect or increase resistance of RBCs to oxidative damages [43, 44]. This work showed that leaves and seeds saponins and roots phenolics are potential anti oxidative stress compounds as they protected the RBCs from hemolysis in a very close range to butylated hydroxyl anisole (BHA). Hemolysis was however promoted by the seeds phenolics and roots saponins. This hemolytic action was previously reported in Oda et al.[45], but this work has shown that not all saponins will promote hemolysis. This has revealed that the saponins in the roots, leaves and seeds, and the phenolics in the root or seeds may not necessarily have the same total chemical composition with one another, although they may belong to the same class of phytochemicals.

The fact that bacteria genera are assuming higher level of resistance to conventional synthetic drugs has made the screening of new antimicrobial compound from natural products a great priority in therapeutic medicine. This study shows the great potentials that the saponins and phenolics extracts have on the growth proliferation of the two studied pathogenic bacteria; *Staphylococcus aureus* and *Klebsiella halize*. Differential inhibition of these two bacteria were observed when treated with the *Ricinus communis* L. parts extracts, however of prominence were the root phenolics and seed saponins that showed increased inhibition of the organisms at increasing extract concentration (Table 3). Remarkably inhibitory results were seen at 5 mg/ml and 10 mg/ml of leaves saponins that completely inhibited the growth of the organisms. The potential antibacterial activity of *Ricinus communis* L. is well documented [7, 13, 15, 16, 17]; however these assays were carried out using crude extracts which doesn't involved one phytochemical, but a collection that may have worked together to confer the reported antimicrobial activity. Thus, this work revealed that phenolics and saponins extracts of *Ricinus communis* L. parts could be exploited for their antibacterial activity. More importantly the leaves saponins that completely inhibited the bacteria growth at just 5 mg/ml is worthy investigating further for its other biological properties such as antifungal, antiviral, anticoagulant, anti-hypercholesterolaemic activities. The fact that it could completely inhibit both gram positive bacteria; *Staphylococcus aureus* and gram negative bacteria; *Klebsiella halize* is an indication that it could have a wide range of inhibition on many other bacteria, and more importantly its inhibitory potential on enteric pathogenic bacteria may assist in curbing the current menace of antibiotics resistance bacteria strains. It will be of great medical significance if *in-vitro* / *in-vivo* cytotoxicological test

could be carried out in order to establish the safety and tolerance of its human oral use and to further purify and characterize the saponins in order to know it's molecular and structural properties and establishes its mechanism of action. These properties may be exploited in the design of synthetic drugs that may confer similar biological activities.

5. CONCLUSION

This work has shown that *Ricinus communis* L. parts is a great source of essential phytochemicals like phenolics and saponins. It lends a great support to its tradition use in treatment of ailments since it contains essential phytochemicals with great biological activity that may aid in the relief of these sicknesses. Owe to its ease of cultivation and survival in virtually all vegetations, *Ricinus communis* L. could be produce enormously and these phytochemicals extracted. The extracts may be used for therapeutic purposes or use in combination therapy along with other pharmaceutical formulations. This may help in reducing cost of treatment as compared to synthetic drugs that often comes with lots of side effects. This work has evaluated the distribution of phenolics and saponins in the various parts of *Ricinus communis* L. and the obtained high antioxidant, antihemolytic and antibacterial activities are worth been noted, and should be put to judicious medical and pharmaceutical applications.

6. ACKNOWLEDGMENTS

The authors are grateful for the laboratory spaces provided by Landmark University, Nigeria and technical assistances by the laboratory Technologist, Department of Biological Science Landmark University, Nigeria.

Table 1
Hemolysis Assay sample 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**

Table 2
Result of phytochemical screening of methanolic extracts of *Ricinus communis* L. Plant parts.

Plant Parts	Phenolics	Saponins
Roots	**+**	**+**
Stems	**-**	**-**
Leaves	**-**	**+**
Capsules	**-**	**-**
Seeds	**+**	**+**

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

Table 3
Antibacterial analyzes of various concentrations of *Ricinus communis* L. plant parts extracts

Phytochemicals	*Staphylococcus aureus*			*Klebsiella halize*		
	1 mg/ml	5 mg/ml	10 mg/ml	1 mg/ml	5 mg/ml	10 mg/ml
Roots phenolics	--	+	++	--	+	+
Seeds phenolics	--	--	+	--	--	--
Leaves saponins	+	**	**	+	**	**
Roots saponins	--	--	+	--	--	--
Seeds saponins	--	+	++	--	+	+

Key: (--); No inhibition, (+); Inhibition, (++); Strong inhibition, (**); complete inhibition

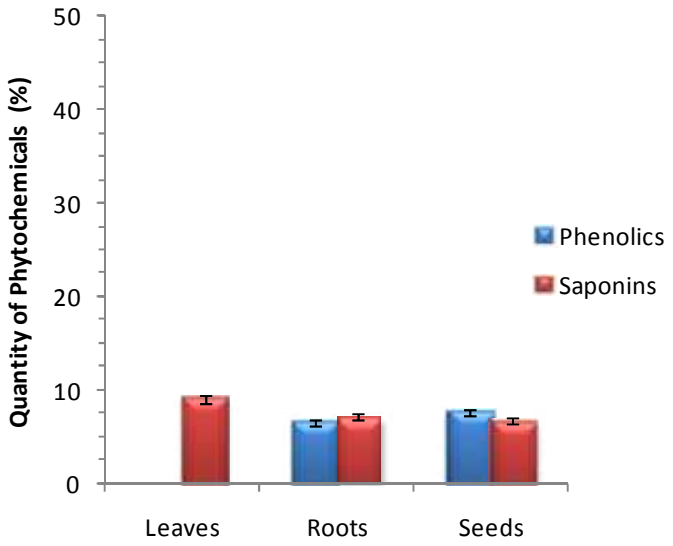

Figure 2
Quantitative analyzes of phenolics and saponins contents in the different *Ricinus communis* L. plant parts extracts. Results are the average of means (n = 5), and represented as mean ± standard deviation.

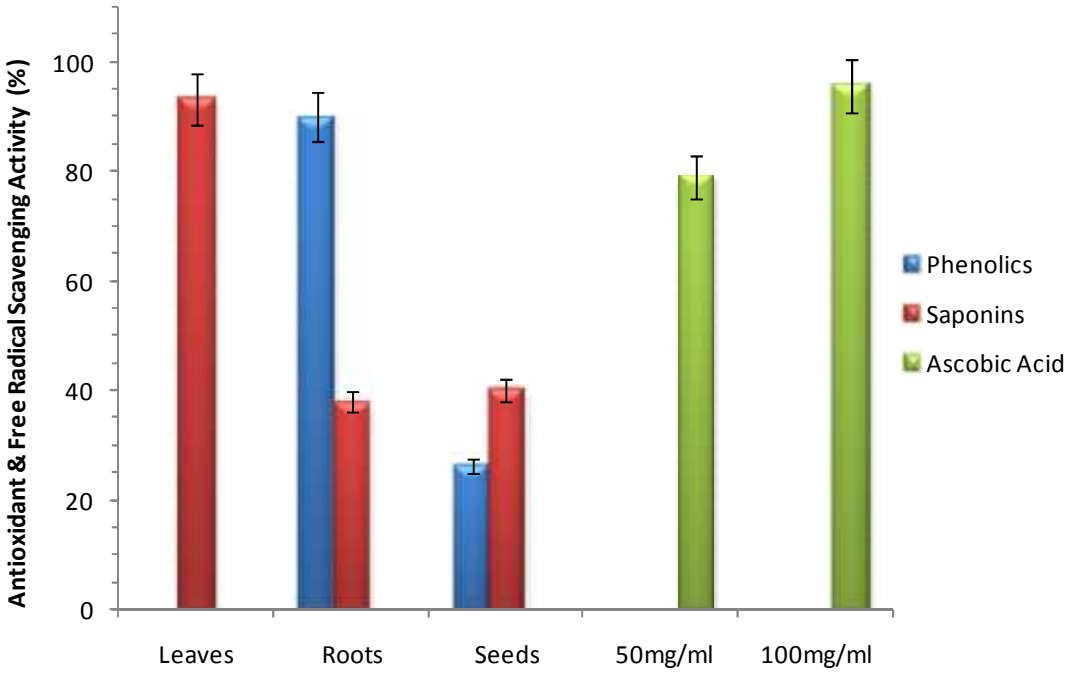

Figure 3
Antioxidant and free radical scavenging activity of *Ricinus communis* L. plant parts extracts. The experiment was carried out in triplicates. Values were the averages of means, and represented as mean ± standard deviation. Statistical analyses was carried out using student paired T-test. Results were considered statistically significant at p≤0.05.

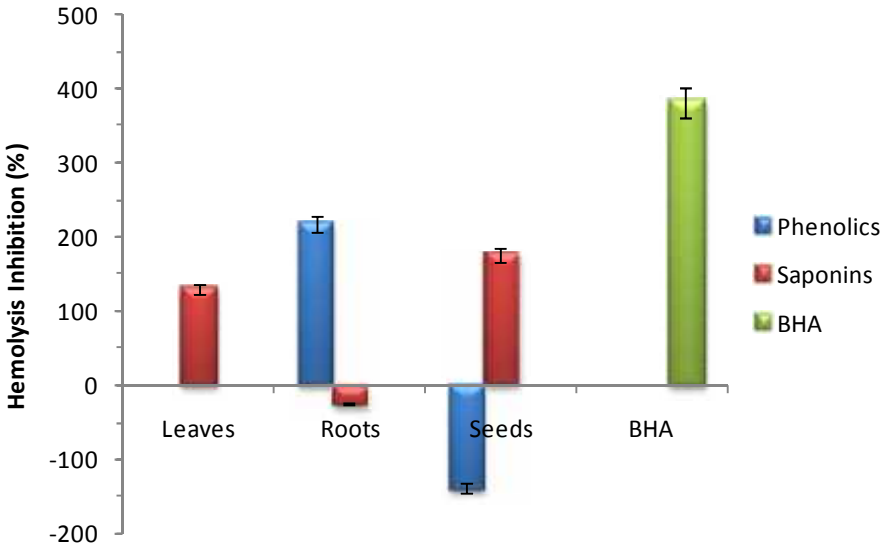

Figure 4

Hemolysis inhibition assay of *Ricinus communis* L. plant parts extracts on human RBC. The experiment was performed in triplicates. Values were the averages of means, and represented as mean ± standard deviation. Statistical analyses was carried out using student paired T-test. Results were considered statistically significant at p≤0.05.

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