PHYTOCHEMICAL, ANTIOXIDANT AND PROXIMATE ANALYSES OF *Citrullus lanatus* RIND EXTRACTS.

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ABSTRACT

This study was carried out to evaluate the phytochemical, antioxidant potentials, and proximate composition of extracts of Citrullus lanatus rind (CLR) in different solvents. Chloroform, n-hexane, ethyl acetate, ethanol, and aqueous extracts of CLR were prepared after which they were subjected to phytochemical, antioxidants, and proximate analysis using standard methods. The ethanol and aqueous extracts of Citrullus lanatus rind were rich in alkaloids, saponins, and terpenoids, while the chloroform and ethanol extract were rich in flavonoids. It was observed that the ethyl acetate, chloroform and ethanol extracts of Citrullus lanatus rind had the highest concentration of total phenolic substances when compared with other extracts considered. The DPPH antioxidant scavenging activity was significantly higher in the ethyl acetate, hexane, and chloroform extracts. The aqueous extract Citrullus lanatus rind was richer in moisture content, but chloroform and hexane extracts were richer in calorie or carbohydrate with a relatively high percentage of crude protein in the ethanol extract. With prescence of alkaloids, saponins, terpenoids, and phenolic compounds in ethanol and aqueous extracts of CLR. They may be beneficial in reducing cardiovascular diseases. Also, with improvement in quality of Citrullus lanatus rind, it may be of high medicinal value to man and livestock.

Keywords: Phytochemical, Antioxidant potentials, Proximate Analysis, *Citrullus Lanatus* Rind.

INTRODUCTION

Watermelon (*Citrullus lanatus*) is an edible flowering plant and a member of the *Cucurbitaceae* family, which is commonly grown throughout the year, worldwide[1]. The fruit has three parts which are the seed, juice part and the hard outer covering which is often referred to as the rind. These three parts are all edible and of benefit to man and livestock. The nutritive value is due to the presence of the dietary constituents (carbohydrate, crude protein, vitamins, minerals, and moisture contents and ash) found in all three parts of the *Citrullus lanatus* fruit[2].

It has also been reported that *Citrullus lanatus* fruits contain various nutrients and antioxidants that improve man and animal nutrition invariably providing valuable effects on health[3]. Hong *et al*[4] from their study, showed that CLR upregulate hepatic gene expression of endothelial nitric oxide synthase with downregulation of fatty acids synthase expression, cyclooxygenase -2, and nuclear factor-$_k$B p65. The changes in hepatic gene expression were due to the presence of L-arginine and some free scavenging antioxidant substances and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity found in all the parts of *Citrullus lanatus*. This assists the body by reducing cardiovascular diseases with lowered inflammatory activities and marked genetic expressions in the body[4]. As a result of the presence of phytochemical substances, antioxidant potential, and proximate values in different parts of CL, it exhibits some pharmacological attributes of biomedical importance. The *Citrullus lanatus* fruit was found to possess some antioxidant properties that makes it to exhibit anti-hyperlipidemic activities[5] as well as anti-giardial, anti-inflammatory, anti-secretory, laxative, gastroprotective, antiulcerative, antimicrobial, analgesic effects in man and animals[3]. Also, *Citrullus lanatus* fruit had been shown to have a hematinic effect[6] due to the presence of some phytochemical substances. The *Citrullus lanatus* rind (CLR) is edible and used as a vegetable in meals[7] this is emphasizing the nutritive values found in it. The *Citrullus lanatus* rind possesses cardio-protective effects[8] with the presence of some antioxidant's compounds. Moreover, fruits of CL have been shown to cause antipyretic and anti-inflammatory effects[3]. Also, the ethanolic extract of *Citrullus lanatus* seed was shown to have an anti-diabetic effect due to the presence of some phytochemical substances and antioxidant properties in the extract[5]. Undoubtedly, the presence of phytochemicals, antioxidant compounds and other compounds such as flavonoids, alkaloids, and phenolic substances in CLR would be responsible for the diverse and multifaceted biological effects of this plant. Indeed, some studies[7,9] have related level of various plant metabolites compounds (in specific solvent-extracts) to their enhanced biological effects such as higher presence of steroids, alkaloids, flavonoids, terpenoids and saponins in both the ethanol and petroleum ether extracts being positively associated with the anti-inflammatory effects of the plant. Studies

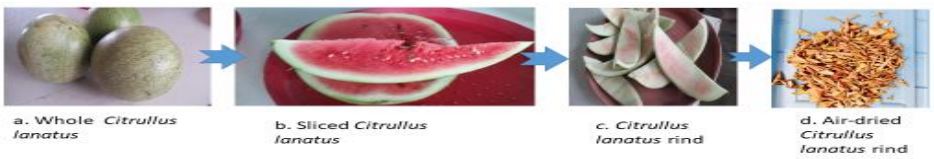
have shown different profile of compounds detected using different solvent in extraction procedures which could greatly influence pharmacologic properties[10, 11]. It has been established that *Citrullus lanatus* roots[12] and leaves[13] are rich in alkaloids, flavonoids, glycoside, saponins, tannins and phenols. The fruit of *Citrullus lanatus* has good anti-inflammatory and analgesic activities and can improve the nutritive values which are useful in human health[10]. It was also emphasized that the presence of terpenoids, anthraquinones, flavonoids in chloroformic, hexane and ethanolic extracts of *Citrullus lanatus* improved the pharmacological activities in animals [14].

This study is designed to evaluate the phytochemical contents, antioxidant potentials and proximate analysis of CLR, using different solvents for extraction. This is to determine the plethora of substances available within the plant in each medium, which will shed more light on the pharmacological values of the CLR and enable a comparison of the best solvent to use for harnessing these substances. Ultimately, the benefit of CLR will be revealed, providing a basis for its full exploitation or use in human and animal nutrition and reduction of waste associated with the normal practice of discarding the *Citrullus lanatus* rind.

MATERIALS AND METHODS

Collection of Citrullus lanatus rind (CLR) and Extraction process.

The CL fruits were purchased from a reputable fruit market at Abeokuta, Southwest Nigeria. The rind was removed from the fruit using a sharp knife, cut into small pieces and further processed to obtain air-dried *Citrullus lanatus* rind as shown in Picture 1.



Picture 1: Preparation of air-dried *Citrullus lanatus* rind for extraction.

The air-dried *Citrullus lanatus* rind was finely blended with a blender (Europremium blender)[R]. and later 130g of CLR was extracted with 100% methanol for 24 hours at room temperature (20-25^{0}C). The mixture was filtered with filter paper (Whatman No.42) to remove debris from the extract and then evaporated at 40^{o}C using rotary evaporator. The crude extracts were suspended in water and partitioned uninterruptedly with hexane, chloroform, ethyl acetate, ethanol, and water[15]. This led to preparation of different extracts of CLR (hexane, CLRH); (ethyl acetate, CLREA); (chloroform, CLRC); (ethanol, CLRET) and (aqueous, CLRA).

Determination of Phytochemicals of different extracts of CLR (CLRH, CLREA, CLRC, CLRET, and CLRA).

The qualitative and quantitative phytochemical analysis were evaluated using standard methods and analytical reagents to establish the presence and evaluate the quantity of alkaloids, steroid, and cardiac glycosides present in different extracts[16]. These were also done to establish tannin, saponin and flavonoids in different extracts using the method described by Park *et.al.*,[17] while terpenoids, and phenolic substances were evaluated[18].The quantitative phytochemical analysis was done in five replicates per extract with results presented as mean values.

Determination of Antioxidant properties of different extracts of CLR (CLRH, CLREA, CLRC, CLRET, and CLRA).

Determination of DPPH.

This was done in extracts of CLR (CLRH, CLREA, CLRC, CLRET, and CLRA). The analysis of DPPH is the free radical scavenging activity of the extracts was performed according to the method described by Abdul-azeez[7]. 0.5 ml of (extract) (100 mg/ml) and 0.3 ml of DPPH (0.5 mM) were added to 3 ml of methanol. The mixture was shaken vigorously for 30s in a vortex apparatus and allowed to stand in dark at room temperature for 30 minutes. The absorbance was measured at 517 nm. The blank was prepared by mixing 0.5 mL of the ascorbic acid with 3.3 ml of methanol, this was used as standard. Similarly, the control solution was prepared by mixing 3.5 mL of methanol and 0.3 mL of DPPH radical solution without the extract added.

This procedure was done in five replicates per extract with results presented as mean values.

DPPH (The percentage of scavenging activity (X %) was calculated according to the equation:

X% = (Absorbance of Sample – Absorbance of blank control) x100.

Determination of Total flavonoids.

This was done on extracts of CLR (CLRH, CLREA, CLRC, CLRET, and CLRA).

Total flavonoid content was determined following the method described by Park *et. al.*,[17]. In a 10 ml test tube, 0.3 ml of extracts, 3.4 ml of 30% ethanol, 0.15 ml of $NaNO_2$ (0.5 M) and 0.15 ml of $AlCl_3.6H_2O$ (0.3 M) were mixed. After 5 min, 1 ml of NaOH (1 M) was added. The solutions were mixed well and the absorbance was measured against the reagent blank at 506 nm. The sample, blanks, and standards were prepared in triplicate for each analysis, and the mean value of absorbance was obtained and expressed in terms of rutin equivalents (mg of RU/g of extract). The standard curve for total flavonoids was made using rutin standard solution (0 to 100 mg/l).

Determination of Total phenolic content (TPC) present in CLR.

This was done in extract of CLR (CLRH, CLREA, CLRC, CLRET, and CLRA).

The total phenolic content (TPC) was determined by the spectrophotometric method[15]. 1 ml of extract (1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 10 ml of a 7% Na_2CO_3 solution was added to the mixture followed by the addition of 13 ml of deionized distilled water and mixed thoroughly. The mixture was kept in dark for 90 min at 23°C, after which the absorbance was read at 750 nm. The TPC was determined from extrapolation of the calibration curve made by preparing the gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per g of each extract.

Determination of Free radical antioxidant potential (FRAP) present in CLR.

This was done in extract of CLR (CLRH, CLREA, CLRC, CLRET, and CLRA).

The measurement of FRAP was based on a reaction with reduction of colourless ferric complex (Fe^{3+} tripyridyltriazine) to blue-colored ferrous complex (Fe^{2+} tripyridyltriazine) by the action of electron donating antioxidants at low pH. This was measured according to the method of Oyaizu[18] with a slightest modification. The different extracts of CLR in different

concentrations ranging from 100 µl to 500µl were mixed with 2.5ml of 20Mm phosphate buffer and 2.5ml 1%w/v potassium ferricyanide were incubated at 50^0C for 30mins. Later 2.5ml of 10%,w/v trichloroacetic acid and 0.5ml 0.1%,w/v ferric chloride were added to the solution and allowed to react for 10 mins. Finally, the absorbance was measured at 700nm. Ascorbic acid was used as standard. This procedure was run in triplicate and mean value was determined.

Determination of Proximate Analysis of CLR.

The chemical composition (Calorie, Crude protein, Moisture content, Ash content, Crude lipids content, Total Organic Nitrogen) found in different media (n-Hexane, Chloroform, Ethyl acetate, Ethanol and Aqueous). These were evaluated in all the extracts of CLR (CLRH, CLREA, CLRC, CLRET, and CLRA). The proximate analysis of all the extracts were chemically analyzed with analytical reagents according to methods described by Association of Official Analytical Chemists[19]. All analyses were carried out in duplicate. The crude protein in the samples was determined by the routine semi-micro Kjeldahl, procedure/technique. This consists of three stages of analysis namely Digestion, Distillation, and Titration. This was completed with the method described by Association of Official Analytical Chemists[19]

Statistical Analysis.

The analysis and graphical illustration were done with Microsoft excel 2010.

RESULTS AND DISCUSSION

Qualitative Phytochemical analysis of different extracts of Citrullus lanatus rind.

The aqueous and ethanol extracts of CLR were moderately rich in saponin but it was absent in the hexane extract. Also, the aqueous and ethanol extracts were moderately rich in tannins, flavonoids, alkaloids, and phenolic substances, see Table1.

Table 1: Qualitative Phytochemical analysis of different extracts of *Citrullus lanatus* rind.

Constituents	Hexane	Chloroform	Ethyl acetate	Aqueous	Ethanol
Saponin	-	+	+	++	++
Tanin	-	++	++	++	++

Flavonoids	+	++	++	++	++
Cardiac glycosides	-	++	++	+	+
Anthraquinones	+	+	+	-	+
Terpenoids	+	+	+	+	++
Steroids	+	+	+	+	++
Phenol	+	+	+	++	++
Alkaloids	+	-	+	++	++

- =absent; + = mildly present; ++ = moderately present; ++ = severely present.

Quantitative phytochemical analysis of different extracts of *Citrullus lanatus* rind

The ethanol and aqueous extracts of *Citrullus lanatus* rind were rich in alkaloids, saponin, terpenoids, and phenol. While the chloroform and ethanol extracts were rich in flavonoids. Generally, the ethanol extract of CLR had the highest concentration of all the phytochemical substances considered, see Figure 1.

Concentration of total flavononids of different extracts of *Citrullus lanatus* rind

Considering the concentration of total flavonoids of different extracts of CLR. The chloroform and ethanol extracts of *Citrullus lanatus* rind richer in total flavonoids at the highest concentration see Figure 2.

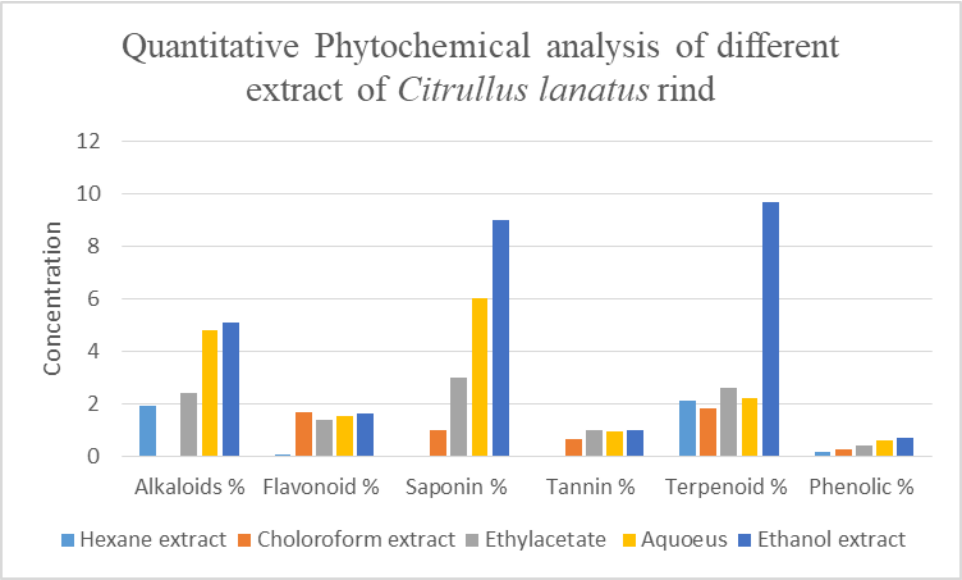


Figure 1: The quantitative phytochemical analysis of different extracts of *Citrullus lanatus* rind.

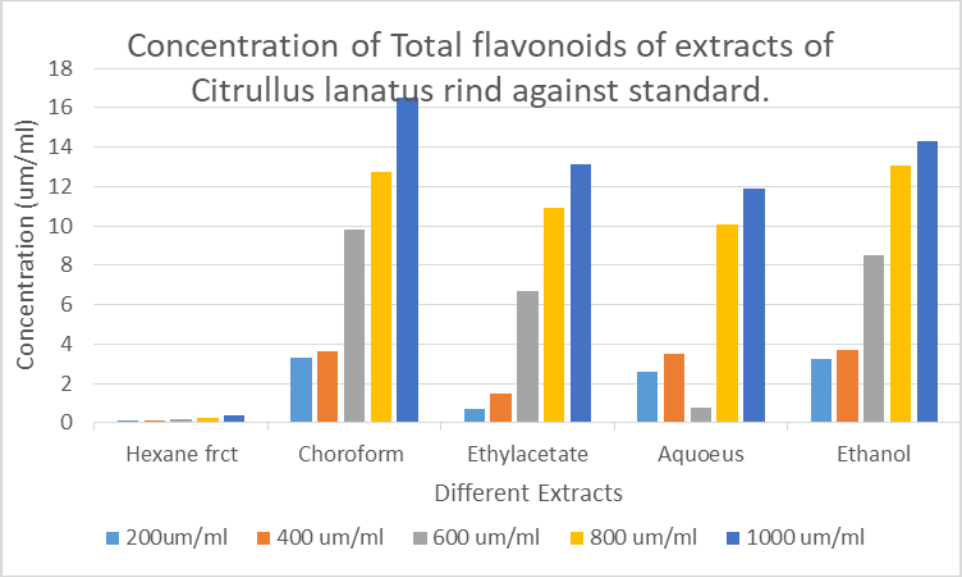


Figure 2: Concentration of otal flavonoids of extracts of Citrullus lanatus rind against standard.

DPPH scavenging activity of different extracts of CLR.

The DPPH scavenging activity antioxidant potentials was highly found in the ethyl

acetate, hexane and chloroform extracts, when compared with Ascorbic acids which was used as standard, see Table 2.

Table 2: Antioxidant Potentials of extracts of *Citrullus lanatus* rind of DPPH Scavenging (%) activity against standard (Ascorbic Acid).

Conc.	Hexane	Chloroform	Ethyl acetate	Ethanol	Aqueous	Standard (Ascorbic acids)
50µg/ml	-	-	-	-	-	**95.09%**
100µg/ml	-	-	-	-	-	**95.53%**
200µg/ml	47.13%	49.12%	48.46%	47.87%	47.64%	**95.60%**
400µg/ml	47.21%	52.17%	57.48%	48.22%	48.11%	**95.68%**
600 µg/ml	48.14%	52.32%	63.06%	50.64%	49.63%	**95.68%**
800 µg/ml	51.42%	52.56%	64.35%	51.74%	50.28%	**95.82%**
1000 µg/ml	55.29%	55.49%	69.04%	54.24%	53.01%	**96.99%**

Free radical anti-oxidative potentials (FRAP) of different extracts of CLR.

The aqueous extract of *Citrullus lanatus* rind has the highest free radical antioxidant potential ability followed by ethanol extract of CLR when compared with other extracts considered,see figure 3.

Total antioxidant potentials of different extracts of CLR

The ethanol extract of CLR had the highest conc. of total antioxidant potentials against standard when compared with all other extracts considered, see Figure 4.

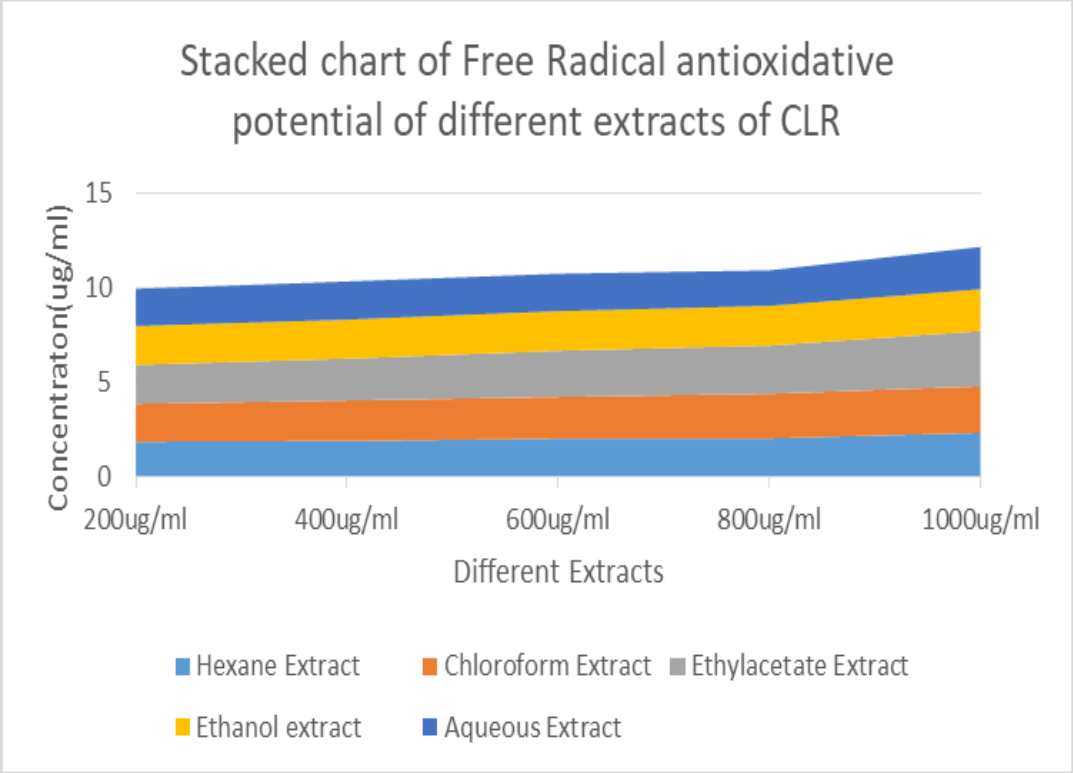


Figure 3: Stacked chart of Free radical antioxidative potentials (FRAP) of different extracts of *Citrullus lanatus* rind.

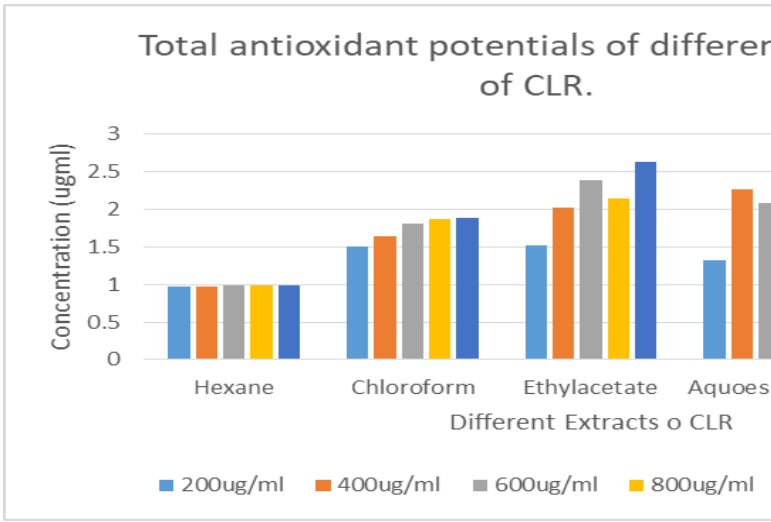


Figure 4: Total Antioxidant potentials of different extracts of *Citrullus lanatus* rind.

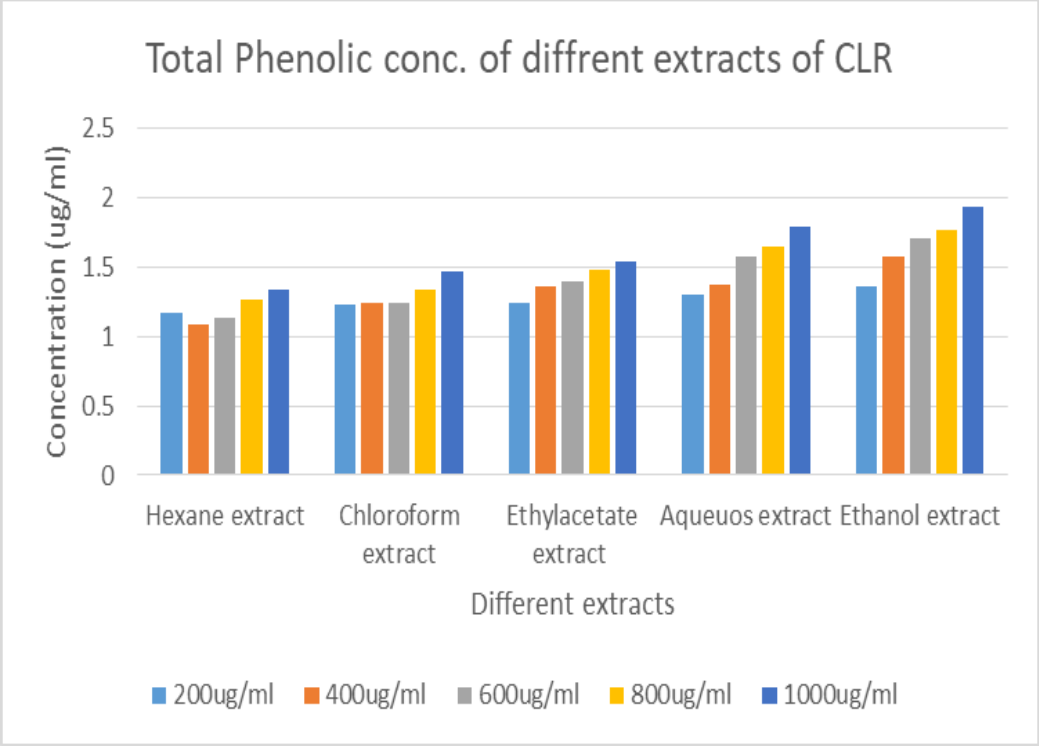


Figure 5: Total phenolic concentrations of different extracts of *Citrullus lanatus* rind.

Total phenolic content (TPC) of different extracts of CLR.

The ethanol and aqueous extracts of *Citrullus lanatus* rind had the highest concentration of total phenolic substances when compared with other extracts considered, see figure 5.

Proximate analysis of different extracts of CLR.

It was observed that the chloroform and hexane extracts were rich in calorie level or carbohydrate (CHO) with a relatively slight high % of crude protein in the ethanol extract of the *Citrullus lanatus* rind. But the aqueous extract of *Citrullus lanatus* rind was noticed to be very rich in moisture content, see figure 6.

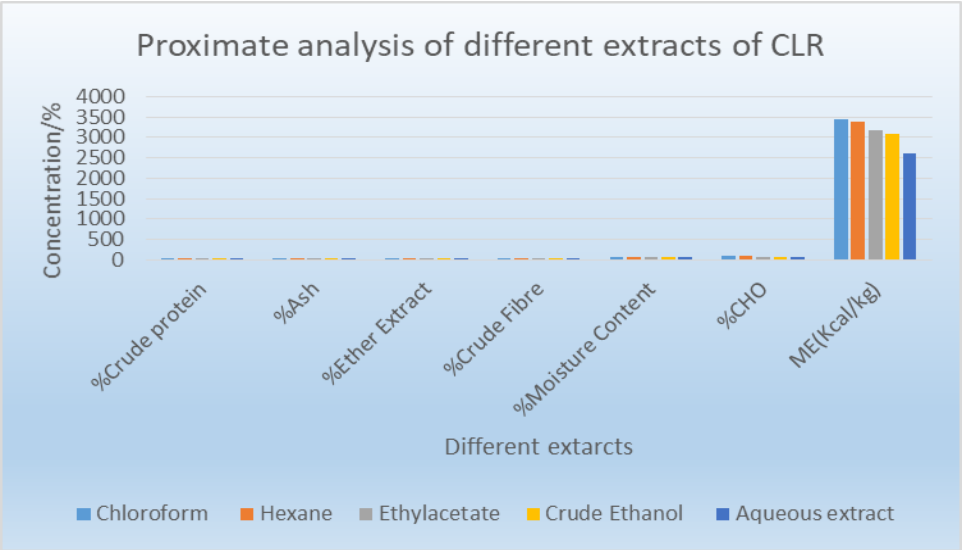


Figure 6: Proximate analysis of different extracts of *Citrullus lanatus* rind.

Discussion

The ethanol and aqueous extracts of *Citrullus lanatus* rind were found to be rich in alkaloids, saponin, terpenoids, and phenol in this study. This can be inferred from the study of Kolawole *et al.*,[3] that with the presence of nutrients and antioxidants it can improve man and animal nutrition invariably providing valuable effects on health. Also Hong *et al.*,[4] emphasized in there study that the presence of flavonoids, alkaloids and phenolic substances in *Citrullus lanatus* can cause diverse and multifaceted biological effects[10] as well as improving the hematinic[6] and cardioprotective[7] effects in animals. With this, it can be inferred that the ethanol and aqueous extracts of *Citrullus lanatus* rind can assist to reduce inflammatory activities and cardiovascular diseases in man and livestock. Also Aruna[9] *et al.*, stated in there study that the presence of crude protein with high moisture level in diet can perform effective pharmacological activities and establish good biological importance in the body. Thus it can be concluded that with this high presence of crude protein in aqueous and ethanol extracts of *Citrullus lanatus* rind they can perform effective pharmacological activities and ensure homeostasis of biological importance[9] in the body.

CONCLUSION

Generally, it may be inferred from this study that improving the quality of *Citrullus lanatus* rind in different media to produce different extracts especially production of ethanol and aqueous extracts of *Citrulllus lanatus* rind can improve the metabolic activities in the body and enhance good health when they are consumed by man and animals.

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