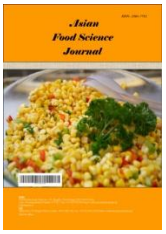

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Nutritional Composition and Antioxidant Property of Methanol Extract of *Corchorus olitorius* Leaf

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study investigates the phytochemicals, nutritional and antioxidant constituents of methanol extract of *C. olitorius* leaf using standard biochemical procedures.
Methodology: *Corchorus olitorius* (213.81 g) powdered leaves sample was soaked in 2.4 L of methanol respectively for 72 hr. Afterwards, the sample was filtered through a double-layered muslin cloth to obtain a filtrate which was placed in a rotary evaporator to dry off the solvent and stored. The proximate analysis, phytochemicals screening, mineral contents, antioxidant ability and phenolic compositions were determined for *C. olitorius.*
Results: The proximate analysis revealed that the *C. olitorius* extract contained 25.00% ash, 2.55% fat, 25.80% moisture, 5.50% crude fibre, 10.15% crude protein and 38.00% carbohydrate contents. Phytochemical screening indicated that flavonoids, tannins, cardiac glycosides, saponins, phenols and steroids were present in appreciable concentrations except for quinones and terpenoids. The mineral analysis of the extract showed considerable levels of potassium (1715.69 mg/100 g), calcium (33.43 mg/100 g), (sodium 49.62 mg/100 g), iron (16.78 mg/100 g) and manganese (9.44 mg/100 g) while magnesium (4.39 mg/100 g), copper (2.11 mg/100 g), zinc (2.94 mg/100 g) and lead (0.21 mg/100 g) were reduced. The extract showed high reducing power, diphenyl picrylhydrazine and H_20_2 radicals scavenging abilities. However, the total antioxidant capacity was low compared to the standard, ascorbic acid. High performance liquid chromatography result revealed that quercitrin, quercetin, chlorogenic acid, syringic acid, epicatechin and kaempferol were present in high amounts in the extract.

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Conclusion: Altogether, findings from this study indicated that *C. olitorius* leaf extract is a rich source of phytonutrients and mineral elements with ample antioxidative property (*in vitro*) that may be of relevance in the management of some degenerative conditions.

Keywords: Corchorus olitorius; phytochemicals; high performance liquid chromatography; In vitro antioxidant.

1. INTRODUCTION

Regular consumption of vegetables has been linked to the management of cancer [1], diabetics [2], high blood pressure [2] gonorrhoea [2] and stroke [1]. Currently, the emphasis is on foods that are high in dietary supplements or have positive health benefits [3]. The use of ingredients with increased doses of plant antioxidants, fibers, natural flavourings, mineral, phytonutrients, and less man-made materials, amongst many other things, has caught the awareness of shoppers [4]. In general, fruits and vegetables are widely consumed in human diets across the globe in which plants serve as a great caloric and restorative ingredient [5].

The traditional green vegetable, *Corchorus olitorius* belongs to the Malvaceae family [6]. Many agriculturally important species can be found in the genus, *Corchorus*, rich in medicinal properties. *C. olitorius* is made up of 40 species and about 30 species are native to Africa [5]. *C. olitorius* is known for having a large distribution and a lot of trans-diversity [7,8]. The leaflets are parallel, oval, lance-shade, and ridged, causing diverse morphotypes to be recognized [9,10].

***Corchorus olitorius* [11]**

The presence of various macronutrients, beta-carotene and folic acid in *C. olitorius*, makes this vegetable a major food component [12].

The leaves of *C. olitorius* have been applied in ethnomedicine in the management of gonorrhoea [2], chronic bladder infections [2], soreness [2], flu [2], and malignancies [2]. Furthermore, *C. olitorius* extracts could also be used to cure a variety of illnesses, including typhoid [9], anaemia [9] and ulcer [9]. As a result of its numerous ethnomedicinal uses, this study sets out to evaluate the proximate content, phytochemicals screening (qualitatively and quantitatively) and *in vitro* antioxidant properties of methanol extract of *C. olitorius* leaf.

2 MATERIALS AND METHODS

2.1 Reagents

Methanol, 2,2-diphenyl-1,1-picrylhydrazine (DPPH), ascorbic acid, potassium ferricyanide, trichloroacetic acid, ferric chloride and hydrogen peroxide were purchased from (Sigma Aldrich, Pan Reacc Appli, Darmstadt, Germany). Others were sodium (Asia-Pacific Specialty Chemicals, NSW, Australia), 2,2-diphenyl-1-picylhydrazyl (Alfa Aesar Ltd, Mumbai, India), sodium hydroxide (H.K Goel and Co. Ltd, Pitam Pura, New Delhi), phenolphthalein (Franklin Laboratories Ltd, Ludhiana, India)

2.2 Equipment/Apparatus

Water bath (Thermo Scientific Precision, General Purpose, Thermo Fisher, Loughborough, UK), Weighing balance, Conical flask, Beakers, Test tubes, Retort stand, Pipette, Spatula, Glass rod, Spectrophotometer (VGP 205, Buck Scientific, USA), Mortar and pestle, Oven (Memmert U.27,Phoenix furnace, model 534, SN: 524-85, Chapel town, Shefffield), Atomic Adsorption Spectrophotometer (AAS) (Systonic, Panchkula, Haryana) Centrifuge (VGP 205, Buck Scientific, USA), High pressure liquid chromatography (Thermo fisher, US), Muffle furnace(VGP 205, Buck Scientific, USA), Desiccator.

2.3 Collection of Plant Sample

Corchorus olitorius leaves were obtained from a vegetable farm in Ugbowo, Benin City, Edo State. The leaves were identified in the Department of Plant Biology and Biotechnology (Herbarium Unit), University of Benin, Nigeria.

The voucher specimen (voucher number: UBH-C558) was immediately deposited at the Herbarium of the department. The leaves were rinsed properly and air-dried at room temperature for 7 days, then hand crushed into coarse powder and weighed.

2.4 Preparation of *Corchorus olitorius* Extract

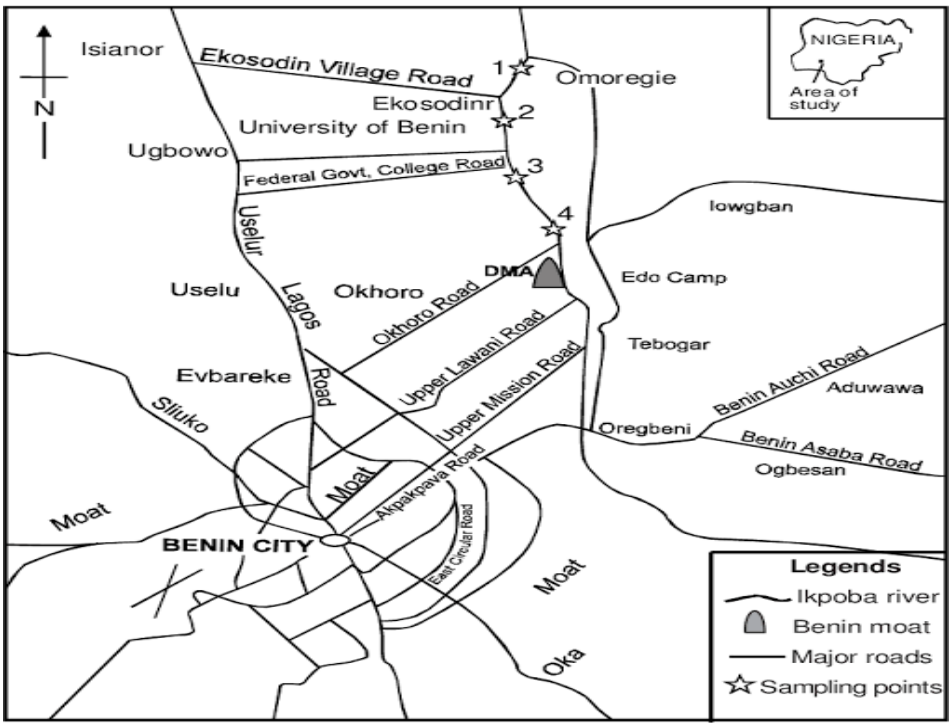
Corchorus olitorius (213.81 g) powdered leaves sample was soaked in 2.4 L of methanol respectively for 72 hr. Afterwards, the sample was filtered through a double-layered muslin cloth to obtain a filtrate which was placed in a rotary evaporator to dry off the solvent. The concentrate was stored in an airtight container at 4^{o}C to protect against sunlight and moisture [13].

2.5 Proximate Analysis of *Corchorus olitorius* Extract

The proximate composition (namely, crude protein, crude fibre, crude carbohydrate, moisture, crude fat and ash contents) of *Corchorus olitorius* leaf extract was carried out according to the Association of Official Analytical Chemists [14] methods.

2.6 Mineral Analysis of *Corchorus olitorius*

Five grams (5 g) of dried powdered leaf sample was weighed into a porcelain dish and further dried at 105ºC for 3 hr in an oven. The dish with content was transferred to a muffle furnace and ignited for 6 hr at 500ºC until free from carbon (residue appears greyish-white). This was removed from the oven and the ash was moistened with a few drops of water (to expose bits of unashed carbon). The ash was re-dried in the oven at 100ºC for 3 hr and re-ashed in the furnace at 500ºC for 1 hr. The content was removed from the muffle furnace, and placed in a desiccator until it cooled. The ash was dissolved in 10% nitric acid and filtered. The filtrate was further made up to 100 mL. The concentration of the mineral elements (including calcium, potassium, iron, lead, copper, magnesium, zinc, manganese and phosphorus) in *C. olitorius* leaf was analysed using an Atomic Adsorption Spectrophotometer (AAS) (Systonic, Panchkula, Haryana) [15].



Source: Map of Ugbowo, Benin city Edo State (13)

2.7 Preparation of Stock Solution

The stock solution was prepared by adding 0.1 g leaf extract of *C. olitorius* into a beaker containing 100 mL of ethanol.

2.8 Phytochemical Screening of *Corchorus olitorius*

The extract of *C. olitorius* were screened for the presence of phytochemicals namely; flavonoids, tannins, alkaloids, phenols, cardiac glycosides, saponins, steroids, terpenoids and quinones according to the method described by [16].

2.9 Reducing Power of *Corchorus olitorius*

The reducing power of *C. olitorius* extracts was determined according to the procedure described by [17,18] Exactly 800 µL of *C. olitorius* extract was mixed with 400 µL phosphate buffer (0.2 M, pH = 6.6) and 800 µL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]; the resulting mixture was incubated at 50°C for 20 min. Thereafter, 800 µL (10 %) of trichloroacetic acid (TCA) was added to the mixture and centrifuged for 10 mins (3000 r/t). The resulting supernatant (400 µL) was mixed with 400 µL of distilled water and 80 µL $FeCl_3$ (0.1%) and the absorbance was recorded at 700 nm against a blank reagent. The reducing power was reported in comparison with ascorbic acid. The results were expressed as µg ascorbic acid equivalent/mg extract.

2.10 Total Antioxidant Capacity of *Corchorus olitorius*

The total antioxidant capacity of *C. olitorius* was estimated by phosphomolybdenum assay of [19]. *C. olitorius* extract (1 mg/mL) was added to 3 mL of molybdate. The tube was incubated at 95°C for 90 min. After incubation, the tubes were normalized to room temperature for 30 min and the absorbance of the reaction mixture was measured at 695 nm. Ascorbic acid was used as the standard antioxidant compound.

2.11 Hydrogen Peroxide Scavenging Ability of *Corchorus olitorius*

Hydrogen peroxide was measured based on the method by [20]. Briefly, hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Ascorbic acid was used as standard antioxidant compound. The ability to scavenge the hydrogen peroxide was calculated using the following equation:

$$\text{Hydrogen peroxide scavenging activity } (\%) = \frac{A_0 - A_1}{A_o} \times 100$$

Where;
A_0 =Absorbance of control
A_1=Absorbance of sample

2.12 Diphenyl picrylhydrazyl Radical Scavenging Assay (DPPH) of *Corchorus olitorius*

The radical scavenging abilities of *C. olitorius* extracts against 1,1–diphenyl–2–picrylhydrazyl (DPPH) radicals were estimated by a slightly modified method of [21]. Exactly 0.5 mL of 0.1 mM DPPH solution in methanol was added to 2 mL of different concentrations (0.2 - 1.0 mg/mL) of *C. olitorius* extracts. The tubes were shaken and incubated for 15 min at room temperature in the dark. The absorbance was read at 517 nm. All tests were performed in triplicate. Ascorbic acid was used as standard antioxidant compound. A blank containing 0.5 mL of 0.3 mM DPPH and 2 mL methanol was prepared and treated as the test samples. The radical scavenging ability was calculated using the formula below:

$$\text{DPPH radical scavenging activity } (\%) = \frac{A_0 - A_1}{A_O} \times 100$$

Where;
A_0 =Absorbance of control
A_1=Absorbance of sample

2.13 Phenolic Composition Determination of *Corchorus olitorius* using High Pressure Liquid Chromatography

An aliquot (5 mL) of *C. olitorius* extract was injected through a conditioned solid-phase extraction tube at 5 mL/min using high pressure liquid chromatography (Thermo fisher, US). The tubes were placed under vacuum (60 kPa) until the resin was thoroughly dried after which the phenolic compounds were eluted with 1 mL of ethyl vials. The PPL tubes were conditioned by first passing 2 mL of ethyl acetate followed by 2 mL of water (pH < 2.0). Purified phenolic extracts (1 mL: 10:1 split) were analyzed for composition by comparison with phenolic standards and

chromatography with standards on a (Waters 600E, MA, USA) high performance liquid chromatography LCD system (Thermo fisher, US), equipped with waters 515 HPLC pump, waters 2487 UV/VIS detector (Systonic, Panchkula, Haryana),, C18 column with dimensions 5 mm, 4.6-250 mm with Hamilton microlitre syringe, and injection volume of 20 mL. The following conditions were employed per separation: wavelength, 280 nm; flow rate, 1.0 mL/min; gradient elution total run time of 31 min, having solvent A as acetonitrile, solvent B as 0.1% phosphoric acid in de ionized water, which was started with 85% A and held at this for 13 min. This was followed by 75% eluent B for 10 min and then the concentration of B was increased to 85% for another 8 min. The results were obtained from the chromatogram with various peaks.

2.14 Statistical Analysis

The results of this study were analysed using the Minitab version 17 package and Microsoft excel statistical package.

3. RESULTS AND DISCUSSION

3.1 Proximate Composition, Mineral Contents, Phytochemical Screening and Phenolic Composition of *Corchorus olitorius* Leaf Extract

The proximate composition of *Corchorus olitorius* revealed that ash, fat, moisture, crude fibre, crude protein and carbohydrate contents were 18.00%, 2.55%, 25.80%, 5.50%, 10.15% and 38.00% respectively (Table 1). The proximate composition result of *C. olitorius* in this study revealed appreciable amount of ash, crude fat, crude protein, crude moisture, crude fibre and crude carbohydrate contents. However, crude fat and fibre were relatively low. The ash, fat and moisture contents of *C. olitorius* as observed in this study were relatively higher when compared to previous work carried out on four tropical leafy vegetables by [22]. The moisture content of the leaf does not concur with the reports of [23]. The moisture content (25.80%) suggests that the *C. olitorius* leaf has a good shelf-life, improved processing characteristics and texture [24]. On the contrary, high moisture content may result to increased activity of water soluble enzymes that participate in the metabolic activities of leafy vegetables [25]. Ash is the inorganic residue left after the water and organic matter have been removed by burning the extract. In this study, *C. olitorius* recorded an appreciable amount (18.00%) of inorganic residue and mineral content. The protein content (10.15%) shows that *C. olitorius* leaf extract is a good source of protein needed for several functions in the body such as body tissue repair and biochemical functions. The ash content result asserts to the findings of [26]. The low fat content of *C. olitorius* in this study corroborated with the findings of previous studies which showed that leafy vegetables are poor sources of lipids [24]. Hence, it's important to note that food substance supplying 1 – 2 % of its caloric energy as fat is considered to be adequate to human beings, as excess intake of fatty food could conduce to cardiovascular disorders such as atherosclerosis [27], cancer [27] and aging [27]. Crude fibre measures the cellulose, hemicellulose and lignin content in a food sample [28]. In this study, the crude fibre content of *C. olitorius* was present in minute amount (5.50%) when compared to the previous study on four leafy vegetables by [22].The minute fibre content may be due to the presence of methanol added to the plant during extraction. Dietary fibres are known to enhance bowel motility [28], prevent constipation [28], and reduce the risk of colon cancer [28].

Table 2 shows the results of the mineral analysis of *C. olitorius* leaf. Calcium (Ca), potassium (K), iron (Fe), sodium (Na), copper (Cu), magnesium (Mg), zinc (Zn), manganese (Mn) and lead (Pb) were present. Values were expressed in milligrams per 100 g (mg/100 g). The mineral analysis of *C. olitorius* extract revealed a high level of potassium (1715.69 mg/100 g). Furthermore, appreciable concentrations of sodium (49.62 mg/100 g), calcium 33.43 mg/100 g), and iron (16.78 mg/100 g) were also seen. However, copper, magnesium, zinc, manganese and lead were present in trace amounts in the leaf extract. The presence of high amount of certain minerals could be linked to the cultivar, different experimental analysis conditions and soil type [9]. Calcium is basic for blood coagulating [9], upkeep of pulse [9], and a cofactor in enzymatic procedures [9]. Potassium and sodium are important for the regular functioning of the sensory system [9] and also circulatory systems [9]. Potassium and sodium are important for the regular functioning of the sensory system and also circulatory systems [9]. Zinc, iron, magnesium and manganese constitute the basic components of the immune system and are essential for the build-up of haemoglobin [9].

The phytochemical screening of *C. olitorius* extract showed that flavonoids, tannins, cardiac glycosides, saponins, phenols and steroids were present in the leaf extract while quinones and terpenoids were undetected (Table 3) and the phenolic compositions of *C. olitorius* shown in (Table 4).

High-performance liquid chromatography of methanol extract of *C. olitorius* leaf is presented in Fig. 1 while the quantities of the phytochemicals present are shown in Table 4. The chromatograph (Fig. 1) showed that the leaf extract had high levels of some secondary metabolites especially quercetin (34.35 mg/100 g), kaempferol (15.93 mg/100 g), epicatechin (14.01 mg/100g), chlorogenic acid (13.93 mg/100 g), syringic acid (12.32 mg/100 g) and quercitrin (11.19 mg/100 g). The remaining metabolites, though of biological importance, occurred in small quantities (Table 4). The phytochemicals, including flavonoids, tannins, cardiac glycosides, saponins, phenols and steroids, present in the leaf extract of *C. olitorius* as observed in this study corroborates with the reports of [29;30]. Flavonoids help to regulate cellular activity and scavenge free radicals that cause oxidative stress [30]. Also, flavonoids have been reported to lower the risk of emerging chronic diseases [30]. Phenols regulate enzyme activity and cell receptors. Studies have shown the protective function of polyphenols such as quercitrin, quercetin, catechin, epicatechin, kaempferol and sinapinic acid in the management of cancer cardiovascular and neurodegenerative conditions [31-36]. These and other phytochemicals were quantified via HPLC in the leaf extract of *C. olitorius.*

Table 1. Proximate composition of *Corchorus olitorius* leaf extract

Proximate Contents	*Corchorus olitorius* (%)
Ash	18.00 ± 0.00
Fat	2.55± 0.33
Moisture	25.80 ± 0.59
Crude fibre	5.50 ± 0.05
Crude protein	10.15 ± 0.00
Carbohydrate	38.00 ± 0.69

All values were expressed as mean ± SEM n=3

Table 2. Mineral analysis of *Corchorus olitorius* leaf extract

Minerals	*Corchorus olitorius* (mg/100 g)
Ca	33.43
K	1715.69
Fe	16.78
Pb	0.21
Na	49.62
Cu	2.11
Mg	4.39
Zn	2.94
Mn	9.44

All values were expressed in mg/100 g

Table 3. Phytochemical screening of *Corchorus olitorius* leaf extract

Phytochemicals	*Corchorus olitorius*
Flavonoids	++
Tannins	+
Cardiac glycosides	++
Quinones	-
Saponins	+
Alkaloids	++
Phenols	++
Steroids	+
Terpenoids	-

Key: + Detected in low concentration, ++ Detected in moderate concentration, - Not detected

3.2 High-Performance Liquid Chromatography Analysis of *C. olitorius* Extracts

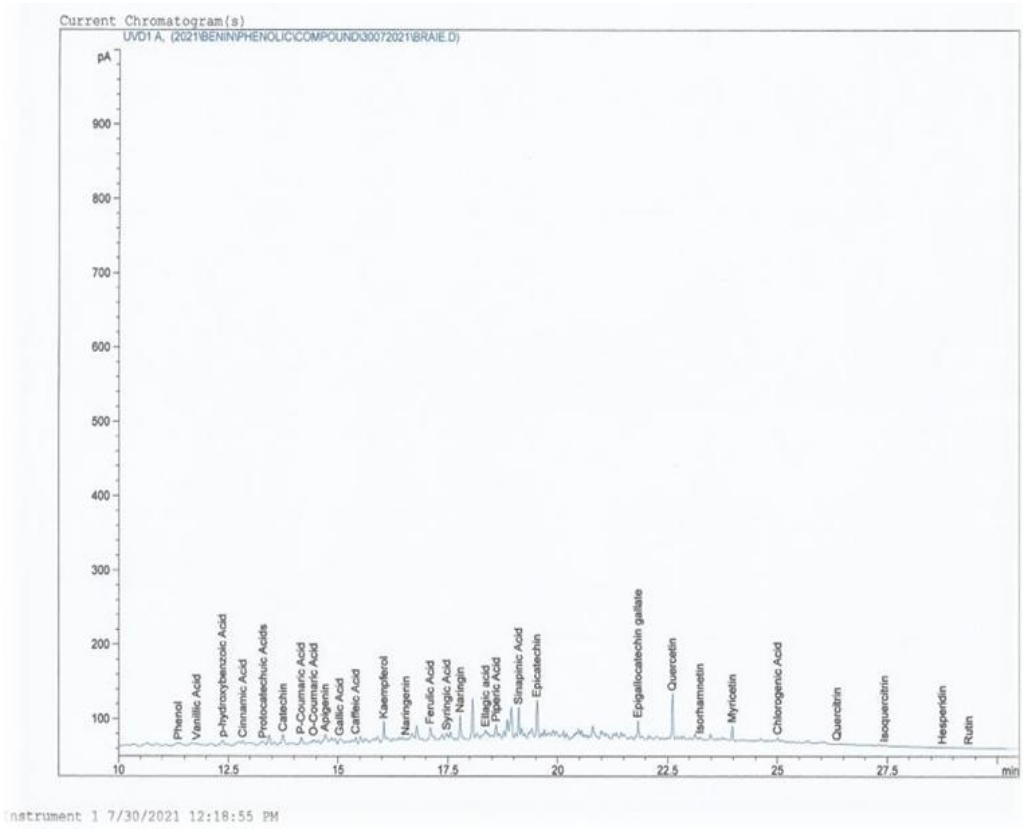


Fig. 1. HPLC chromatogram of methanol extract of *Corchorus olitorius*

Table 4. Phenolic composition of *Corchorus olitorius* leaf extract

Name	Amount (mg/100 g)
Quercetin	34.34994
Myricetin	5.94739
Chlorogenic acid	13.92977
Quercitrin	11.19065
Catechin	7.06486
Gallic acid	1.56594
Caffeic acid	4.95559
Kaempferol	15.93058
Naringenin	4.90425
Ferulic acid	8.71090
Syringic acid	12.31687
Sinapinic acid	$1.30345e^{-3}$
Epicatechin	14.00992
Epigallocatechin gallate	$7.14122e^{-1}$
Isoquercitrin	$3.45045e^{-1}$
Hesperidin	$1.30183e^{-2}$

3.3 *In vitro* Antioxidant Potential of *C. olitorius* Extract

Fig. 1 to 2.4 represent the *in vitro* antioxidant potential of *C. olitorius* extract in terms of total antioxidant capacity, reducing power, 1, 1–diphenyl–2–picrylhydrazyl (DPPH) radical scavenging and hydrogen peroxide radical scavenging capacities. The results showed that the total antioxidant capacity of the extract was

significantly (*P* = .05) low in contrast to the standard, ascorbic acid (Fig. 1). The reducing power of the extract increased in a concentration dependent manner (*P* = .05) but was not as high as the standard antioxidant, ascorbic acid (Fig. 2). In Fig. 3, *C. olitorius* extract was able to significantly (*P* = .05) scavenge DPPH radicals at comparable levels as the standard ascorbic acid with IC_{50} for the extract and ascorbic acid being 1.19 µg/mL and 1.10 µg/mL, respectively. The ability for *C. olitorius* extract to scavenge H_20_2 radicals significantly (*P* = .05) high but not commensurate with that of the standard antioxidant, vitamin C (Fig. 4). The antioxidant assays revealed that *C. olitorius* leaf extract has appreciably high antioxidant potential, especially in terms of its reducing power, total antioxidant capacity and hydrogen peroxide and DPPH radical scavenging abilities; though not as high as the standard antioxidant, ascorbic acid. The total antioxidant capacity assay is primarily based on the reduction of ferric ion to ferrous ion and molybdenum (VI) by the antioxidants in the samples, individually [30]. Thus, suggesting that *Corchorus olitorius* may possess the capacity of converting ferrous ion to their reduced form (ferric ion). In this study, the reducing power of *C. olitorius* extract may translate to its ability to donate electrons in oxidative stress situation [37]. The appreciable level of hydrogen peroxide scavenging effect of *C. olitorius* extracts signifies the reduction of H_20_2 radicals by making them less toxic. Therefore, the removal of hydrogen peroxide is critical for antioxidant defense in the cell [38].

Total antioxidant capacity

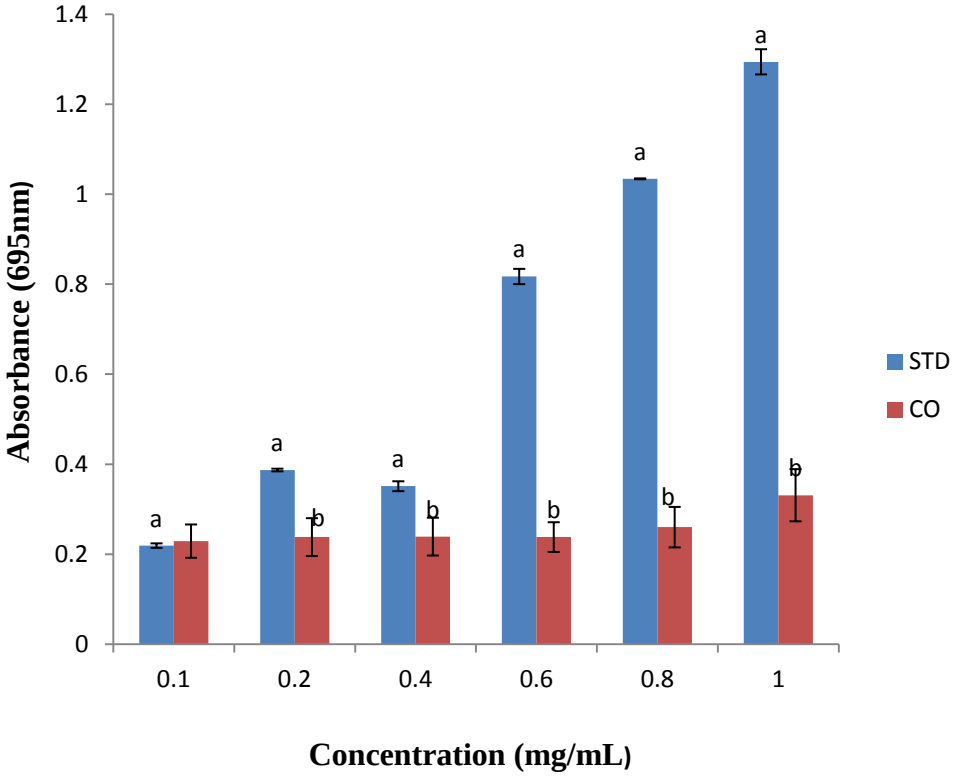


Fig. 2.1. Total Antioxidant Capacity (TAC) analysis of *Corchorus olitorius* extracts
Values were expressed as mean ± SEM where n = 3; (P = .05)
KEY: CO = Corchorus olitorius, STD = Standard (Ascorbic acid)

Reducing Power

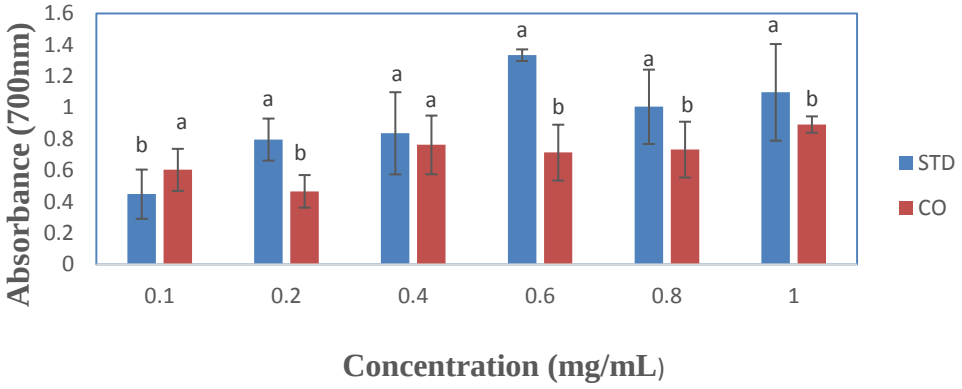


Fig. 2.2. Reducing power of *Corchorus olitorius* extracts

Values were expressed as mean ± SEM where n = 3; (P = .05). KEY: CO = Corchorus olitorius, STD = Standard (Ascorbic acid)

DPPH Radical Scavenging Ability

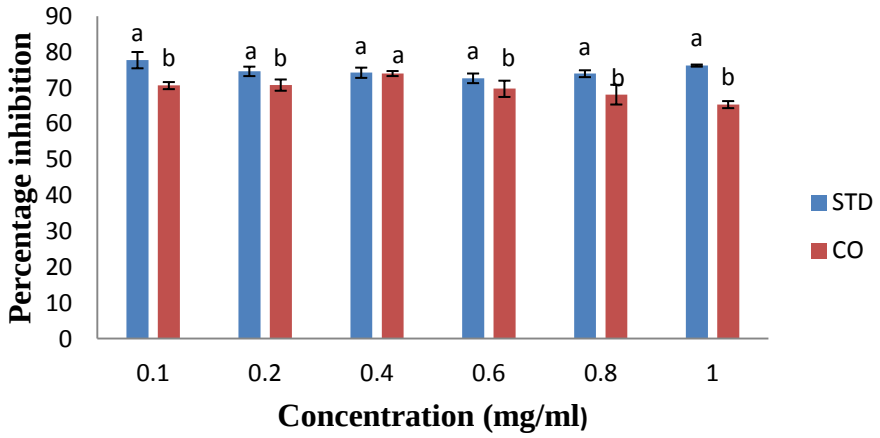


Fig. 2.3. DPPH radicals scavenging ability of *Corchorus olitorius* extracts

Values were expressed as mean ± SEM where n = 3; (P = .05). KEY: CO = Corchorus olitorius STD = Standard (Ascorbic acid)

Hydrogen Peroxide Radical Scavenging Ability

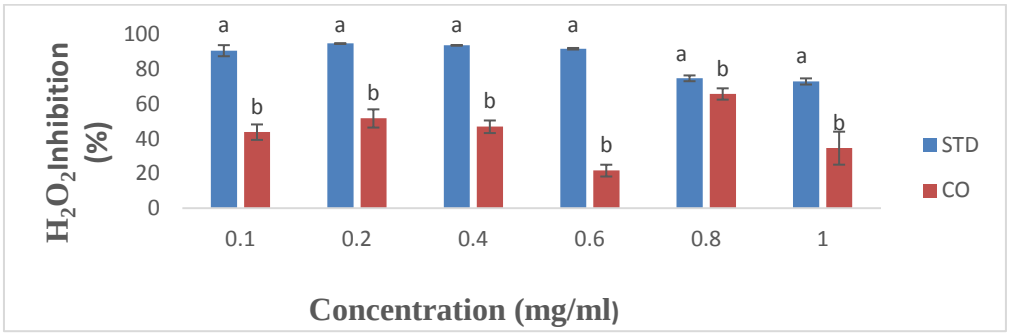


Fig. 2.4. Hydrogen peroxide radical scavenging ability of *Corchorus olitorius* extracts

Values were expressed as mean ± SEM where n = 3; (P = .05). KEY: CO = Corchorus olitorius, STD = Standard (Ascorbic acid)

Table 5. DPPH IC_{50} value of *Corchorus olitorius* Leaf Extract

Extract	Concentration (ug/mL)
Ascorbic acid	1.10
Corchorus olitorius	1.19

In this study, the DPPH radical scavenging capacity of the extract was noticed to be almost at par with that of ascorbic acid standard suggesting that the extract may act more as a scavenger of free radicals generated and thereby inhibiting autoxidation of lipids in the cells [39,40].

4. CONCLUSIONS

In conclusion, findings from this study reveal that methanol extract of *C. olitorius* leaf extract has appreciable nutritional values and could be considered as a rich source of antioxidants. The extract contained important secondary metabolites which may be of relevance in the management of some degenerative conditions; gonorrhoea, chronic bladder infections, malignancies, flu, soreness. Furthermore, this study has shown that *C. olitorius* leaf extract may be considered as a good candidate for drug development. Hence *in vivo* and molecular studies are recommended to be carried out on the leaf extract of *C. olitorius.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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