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Studies on *In vitro* Antioxidant and Anti-Inflammatory Activities of *Crinum jagus* Leaves and Bulb Extracts

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Authors' contributions

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

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ABSTRACT

Aims: The current study investigates the leaves and bulb extracts of *Crinum jagus* for antioxidant and anti-inflammatory activities.
Place and Duration of Study: Department of Chemistry, Federal University of Technology Akure, Nigeria between June 2016 and September 2017.
Methodology: The leaves and the bulb of the plant were subjected to successive extraction using hexane, ethylacetate and methanol. The phytoconstituents and total phenol contents were determined. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and ferric reducing antioxidant properties of the extracts (compared to that of the standard drug Ascorbic acid) were also determined. The membrane-stabilizing properties of the extracts, compared to that of indomethacin, were used to measure their anti-inflammatory properties.
Results: Phytochemical screening indicated the presence of flavonoids, alkaloids, tannins, saponins and terpenoids in the extracts. The total phenolic content of the extracts ranged between 33.230 and 98.340 gallic acid equivalence/g sample with the ethylacetate extract of the bulb having the

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highest phenol content. In the DPPH assay, the IC_{50} values of the extracts ranged between 0.503 and 1.050 with methanol extract of the leaves possessing the highest DPPH scavenging activity. The ferric reducing activities of the extracts ranged between 3.61 and 40.000 mg ascorbic acid equivalent / g of the sample with the ethylacetate extract of the bulb possessing the highest activity. Among the extracts screened for anti-inflammatory activity, the methanol extract of the leaves had the highest membrane stabilizing activity with value of 99.74 ± 0.68 at 0.5 mg/ml compared to indomethacin with a value of 52.65±1.18 at the same concentration.

Conclusion: The leaves and bulbs of *C. jagus* could be sources of antioxidant and anti-inflammatory compounds.

Keywords: Antioxidant; anti-inflammatory; Crinum jagus; DPPH.

1. INTRODUCTION

Reactive oxygen species, generated as by-products of biological processes, can cause various diseases including cancer, cardiovascular disease, inflammation and neurodegenerative disorders [1,2]. Furthermore, some synthetic antioxidants have been reported to be carcinogenic [3]. Hence, development of alternative antioxidants is a necessity. Phenolic compounds derive their antioxidant properties from their chemical structures enabling them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Flavonoids and phenolic acids (such as vanillic acid, ferrulic acid and caffeic acid) are typical examples of phenolics and have been widely reported in several plant species [4].

Inflammation, though part of the body's defence mechanism against injury, can induce, maintain or aggravate many diseases [5]. Chronic inflammation has been involved in the development of many diseases including diabetes, obesity, atherosclerosis, and several types of cancers [6]. Currently, steroidal and non-steroidal anti-inflammatory drugs are used in the treatment of inflammation. However, both classes of drugs have side effects associated with their use [7].

Crinum jagus, belongs to the family *Amaryllidaceae*. It is called *ogede-odo* in southwestern Nigeria. The bulbs and the leaves are used for the treating chronic cough, tuberculosis, rheumatism and withlow by herbalists in *Yorubaland*. In addition, it is used as anthelminthics, emetic, purgative and rubefacient. It is widely used by traditional practitioners in Africa for treatment of skin wounds and several other ailments, some of which have been scientifically validated [8,9]. *C. jagus* (Christopher lily) is commonly found in swampy locations and possesses white flowers that appear in the dry season [10]. Previous studies have examined the plant for anti-venom, antiashmatic, antihaemorrhagic and liver-protective activities [8,9,11,12]. In the current study, extracts of the leaves of *C. jagus* are screened for antioxidant and anti-inflammatory properties.

2. METHODOLOGY

2.1 Sample Collection and Extract Preparation

The leaves and bulbs of *Crinum jagus* were collected from the Botanical Gardens of the University of Ibadan, Nigeria. The samples were ground after drying under mild sunlight for several days. A 1 kg portion of ground sample was subjected to successive extraction using hexane, ethylacetate and methanol. The extracts were thereafter concentrated to dryness.

2.2 Phytochemical Screening of the Extracts

Phytochemical screening of the extracts was carried out using standard procedures.

Test for tannins: Two (2) drops of 5% $FeCl_3$ was added to 1 ml of the extract. A dirty green precipitate indicated positive test [13].

Test for glycosides: Ten (10) ml of 50% H_2SO_4 was added to 1 ml of extract in a test tube, this mixture was heated in boiling water for 5 minutes. 10 ml Fehling's solution A and B (5 ml each) were added and boiled. Brick red precipitate indicated positive test [14].

Test for resins: Two and a half (2.5) ml of Copper (II) Sulphate solution was added to 2.5 ml of the extract. The resulting solution was shaken vigorously and allowed to settle. A green colour indicated positive test [13].

Test for saponins (Frothing test): Two (2) ml of extract in water was vigorously shaken in test

tube for two minutes. Frothing indicated positive test [13].

Test for phlobatannins: Five (5) ml of distilled water was added to 5 ml of extract solution and boiled with 1%HCl for two minutes. A deep green colour indicated positive test [13].

Test flavonoids: Two (2) ml of the extract solution was heated with 10 ml of ethyl acetate on a water bath and cooled. The layers were allowed to separate and a colour of ammonia layer (red colouration formed) indicated positive test [15].

Test for sterols (Salkowski's test): Two (2) ml of conc. H_2SO_4 was added 2 ml of extract solution. A red precipitate indicated steroidal ring [16].

Test for Phenols: Equal volumes of extract solution and $FeCl_3$ were mixed. A deep bluish green solution confirmed the presence of phenols [15].

Test for carbohydrate. (Fehling test): Five (5) ml of the mixtures of equal volume Fehling solution A and B were added to 2 ml of the extract in a test tube. The resultant mixture was boiled for two minutes. A brick red precipitate of copper oxide indicated a positive test [15].

Test for alkaloids: One (1) ml of conc. H_2SO_4 was added to 3 ml of the extract, then treated with few drops of Wagner reagent. Reddish brown precipitate indicated positive test [17].

Terpenoid (Salkowski) test: 0.2 g of the extract sample was mixed with 2 ml of chloroform ($CHCl_3$) and conc. H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive result for the presence of terpenoids [16].

2.3 Determination of Antioxidant Activity

2.3.1 DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

The hydrogen or radical scavenging properties of the extract from the plant sample was determined by the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl radical) method as described by Ahmed, et al. [18]. To 1 ml of varying concentrations of the sample extract/standard (Ascorbic acid) was added 1 ml 0.3 mM DPPH in methanol and allowed to react. The mixture was vortexed and incubated in the dark for 30 min and the absorbance was measured at 517 nm against a DPPH negative control containing only 1 ml of methanol in place of the sample extract.

The percentage inhibition of the DPPH scavenging activity was calculated using the equation below:

$$\text{DPPH \%Inhibition} = [1-(A_{517nm\ sample}/A_{517nm\ control})] \times 100$$

Where;

$A_{517nm\ sample}$ is the absorbance of the sample (extract/standard) at 517 nm.
$A_{517nm\ control}$ is absorbance of the negative control at 517 nm.

Sample concentration providing 50% inhibition (IC_{50}) was obtained from the graph by plotting inhibition percentage against extract concentration.

2.3.2 Ferric reducing antioxidant power assay (FRAP)

This was carried out as described by Ahmed et al. [19]. A 300 mmol/L acetate buffer of pH 3.6, 10 mmol/L TPTZ [2,4,6 tri-(2-pyridyl)-1, 3, 5-triazine] and 20 mmol/L $FeCl_3.6H_2O$ were mixed together in the ratio of 10:1:1 respectively, to give the working FRAP reagent. A 50 µl aliquot of the sample extract at 1 mg/ml and 50 µl of the standard solutions of ascorbic acid (0.02, 0.04, 0.06, 0.08, 0.1 mg/ml) was added to 1 ml of FRAP working reagent. Absorbance measurement was taken at 593 nm exactly 15 min after mixing against reagent blank containing 1 ml of the FRAP working reagent and 50 µl of methanol.

All measurements were taken at room temperature and the reducing power was expressed as equivalent concentration which is defined as the concentration of antioxidant that gave a ferric reducing ability equivalent to that of the ascorbic acid standard (AAE).

$$AAE = c \times v/m$$

Where,

AAE= Ascorbic acid equivalent of sample extract (mg AAE/g of sample); c= concentration of ascorbic acid established from the standard calibration curve in mg/ml; v= volume of the sample extract in ml; m= weight of the sample extract in g.

2.3.3 Determination of total phenol

This was carried out using procedures described by [20]. To a mixture of 0.1 mL of sample extract (1 mg/mL) or standard and 0.9 mL of distilled water was added 0.2 mL Folin's reagent. The mixture was vortexed. After 5 min, 1.0 mL of 7% (w/v) Na_2CO_3 solution was added and the solution was further made up to 2.5 mL by the addition of 0.3 mL distilled water, before it was finally incubated for 90 min at room temperature. The absorbance against a reagent blank containing 1 mL of methanol in place of the sample was measured spectrophotometrically at 750 nm. Gallic acid at different concentrations of 0.1, 0.08, 0.06, 0.04 and 0.02 mg/mL was used as the standard (Arvind, et al. 2012) [14]. The total phenolic content of the extracts was expressed as mg gallic acid equivalent per gram of extract (mg GAE/g) as shown below;

$$C = c \times v/m$$

Where:

C = total phenolic compound in gallic acid equivalent (mg GAE/g); c = concentration of gallic acid established from the calibration curve in mg/mL; v = volume of the extract in mL; m = weight of the extract in gram

2.4 Determination of *In vitro* Anti-Inflammatory Properties of Extracts

The membrane stabilizing properties of the extracts was used as a measure of their anti-inflammatory activity based on the procedure described by Oyedapo, et al. [21]. The assay mixture consisted of hyposaline (1 ml), 0.1 M phosphate buffer, pH 7.4 (0.5 ml), varying concentrations of the extract (0-0.5 mg/ml), varying concentration of normal saline and 0.5 ml of 2% (v/v) erythrocyte suspension in a total volume of 3 ml. The control was prepared as above without the drug while the drug control (3 ml) lacked erythrocyte suspension. The standard anti-inflammatory drug for the assay was Indomethacin. The reaction mixtures were incubated at 56°C for 30 min. The absorbance of the released haemoglobin was read at 560 nm against reagent blank. The percentage membrane stability was estimated using the expression:

$$\text{Percentage membrane stability} = 100 - \frac{\text{Abs}_{\text{test drug}} - \text{Abs}_{\text{drug control}}}{\text{Abs}_{\text{blood control}}} \times 100$$

The blood control represented 100% lysis.

3. RESULTS AND DISCUSSION

Table 1 shows the phytoconstituents detected in the extracts. The results showed that the extracts contain different phytoconstituents including alkaloids, tannins, flavonoids, sterols and phenols. The results showed that the extracts contain various phytoconstituents. The phytoconstituents are distributed between the solvents based on differences in their polarities. Phytoconstituents are responsible for the biological activities displayed by medicinal plants. Previous reports have shown that plants' phytoconstituents possess different bioactivities including anti-inflammatory [22], antioxidant [23], anticancer [24] and antimicrobial properties [25].

The total phenol contents of the extracts were obtained from a calibration curve obtained from a plot of absorbance against various concentrations of gallic acid (Fig. 1). Table 2 shows the phenolic contents of the extracts. The values ranged between 33.230 ± 3.060 mg GAE/g and 98.340 ± 7.890 mg GAE/g. The ethylactetate extract of the bulb and methanol extract of the leaves of *C. jagus* possess the highest phenolic contents with values of 98.340 mg GAE/g and 71.150 mg GAE/g respectively. The least values were obtained for hexane extracts of the bulb (33.230 mg GAE/g) and leaves (34.890 mg GAE/g) of the plant.

The ferric reducing antioxidant properties of the extracts were obtained from the calibration curve (Fig. 2). The results are presented in Table 2. The reducing capacity of extracts is an indication of their antioxidant properties. In the current study, the extract CJTEE has the highest ferric reducing property (40 mg ascorbic acid equivalent/g of sample) while CJAHE has the least (3.610 mg ascorbic acid equivalent/g). The reducing activities of CJAEE, CJAME and CJTHE ranged between 12.000 and 15.186 mg ascorbic acid equivalent/g (Table 2). Of the six extracts tested, the extract, CJTEE likely contains compounds with the highest ferric reducing properties.

Figs. 3a and 3b shows the trends observed in the DPPH scavenging properties of ascorbic acid and extracts respectively. The DPPH scavenging activities of the extracts/standard increased with the concentration of the extracts. Table 2 shows the IC_{50} values of the extracts.

Table 1. Phytochemical screening of extracts

Tests	CJAHE	CJAEE	CJAME	CJTHE	CJTEE	CJTME
Tannins	+	+	+	-	+	+
Glycosides	-	-	+	-	-	-
Resin	+	+	-	-	+	+
Saponins	+	+	+	+	+	-
Phlobatannins	-	+	-	-	+	-
Flavonoids	-	+	+	+	+	-
Sterols	-	+	-	-	+	+
Phenols	-	+	-	+	+	+
Carbohydrates	-	-	+	-	-	-
Alkaloids	+	+	+	+	+	+
Terpenoids	+	-	+	-	-	+

+ = Present; - = Absent; CJAHE- Hexane extract of C. jagus leaves; CJAEE- Ethylacetate extract of C. jagus leaves; CJAME- Methanol extract of C. jagus leaves; CJTHE - Hexane extract of C. jagus bulbs; CJTEE - Ethylacetate extract of C. jagus bulbs; CJTME- Methanol extract of C. jagus bulbs

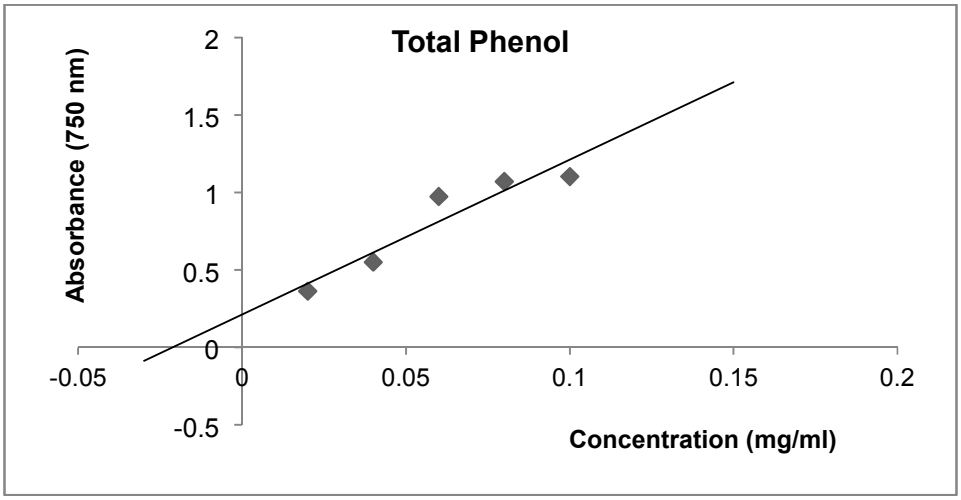

Fig. 1. A plot of absorbance against different gallic acid concentrations

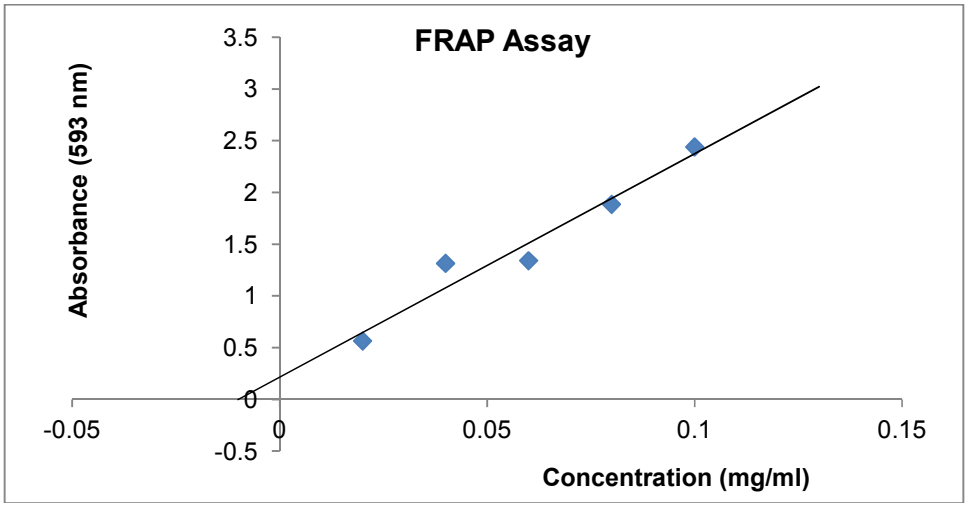

Fig. 2. Calibration curve: Plot of absorbance against different concentrations of ascorbic acid

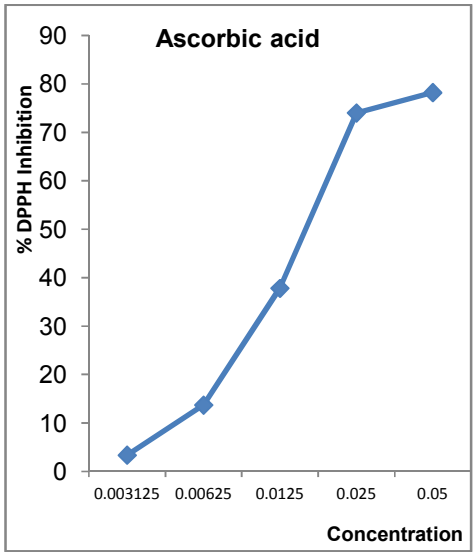

Fig. 3a

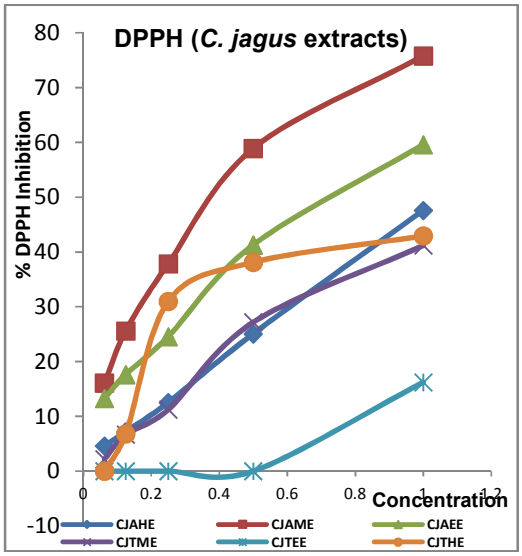

Fig. 3b

Figs. 3a & 3b. Graph of % DPPH Inhibition against different Concentrations of Ascorbic acid and *C. jagus* Extracts

Table 2. Total phenol content, DPPH scavenging and ferric reducing antioxidant potential of extracts

Extracts	Total phenol (Average mg GAE/g Sample) ± Standard deviation	DPPH (IC_{50})	FRAP (mg ascorbic acid equivalent / g of the sample)
CJAHE	34.890 ± 3.100	1.050	3.610
CJAEE	44.420 ± 1.360	0.765	15.186
CJAME	71.150 ± 9.700	0.503	13.936
CJTHE	33.230 ± 3.060	-*	12.000
CJTEE	98.340 ± 7.890	-*	40.000
CJTME	53.950 ± 8.320	1.160	3.703
Ascorbic acid	-	0.017	-

CJAHE- Hexane extract of C. jagus leaves; CJAEE- Ethylacetate extract of C. jagus leaves; CJAME- Methanol extract of C. jagus leaves; CJTHE - Hexane extract of C. jagus bulbs; CJTEE - Ethylacetate extract of C. jagus bulbs; CJTME- Methanol extract of C. jagus bulbs;
** - Could not be determined within the concentration range tested*

The IC_{50} values of the extracts ranged between 0.503 and 1.160. Compared to the standard (IC_{50} = 0.017), the extract, CJAME possess the highest activity. The IC_{50} values of CJTHE and CJTEE could not be determined within the concentration range tested. Phytochemical screening of the extracts showed that phenolics such as flavonoids, phlobatannins, phenols and alkaloids are present in the CJTEE and CJAME. Previous studies have shown that the plant contains high quantities of compounds containing phenolic groups. The result of the phytochemical screening of the extracts showed that they are rich in flavonoids (Table 1). Many flavonoids are known to be phenolic in nature [26]. These compounds could be partly responsible for the high phenolic contents observed in CJTEE and CJAME. The antioxidant properties observed could be partly due to the presence of these compounds in the extracts.

The DPPH scavenging properties of the leaves extracts increased with the phenolic contents of the extracts. Phenolics are, therefore, likely to have contributed substantially to the DPPH scavenging activities of extracts. Among the extracts, the polar solvent, methanol, appears to be more effective in extracting compounds with radical scavenging properties. The results showed the presence of phytochemicals such as saponins, tannins, flavonoids and terpenoids in the extracts. These classes of compounds have

been reported to exhibit antimicrobial and antioxidant effects [27].

Figs. 4a & 4b compares the membrane-stabilizing activities of the bulb and leaves extracts with that of the standard drug, indomethacin. Many of the extracts under study showed activities higher than that of indomethacin at one or more concentrations. The extracts, CJAHE (77.48 ± 14.84) and CJAEE (78.31 ± 13.46) showed activity higher than that of indomethacin (69.12 ± 6.65) at 0.1 mg/ml. The extract, CJTHE is found to possess higher stabilizing activity (85.19±6.74) than the standard drug (75.02±6.04) at 0.2 mg/ml. Similarly, CJAME showed higher activity (80.16 ±13.40) than indomethacin (73.51±1.88) at 0.4 mg/ml. Of all the extracts tested, the highest activity was observed for the extract CJAME (at 0.5 mg/ml) with a value of 97.74 ± 0.68. Table 3 shows the membrane-stabilizing activities of the extracts.

Table 3 shows the membrane-stabilizing activities of the extracts at different concentrations. Compounds that can facilitate the stability of biological membranes when exposed to induced lysis can also prevent the triggering of inflammation. Some compounds isolated from plants have been previously reported to be capable of stabilizing red blood cells exposed to heat and/or hypotonic induced stress [21,28]. In the current study, the extracts of the leaves and bulb of *C. jagus* showed reasonably high membrane-stabilizing activities. The results of the phytochemical screening showed that terpenoids were detected in the hexane, ethylacetate and methanol extracts of the leaves of the plant. Many researchers have reported on the anti-inflammatory action of triterpenoids [29,30]. The presence of this class of compounds in the extracts could be partly responsible for the anti-inflammatory properties of the extracts.

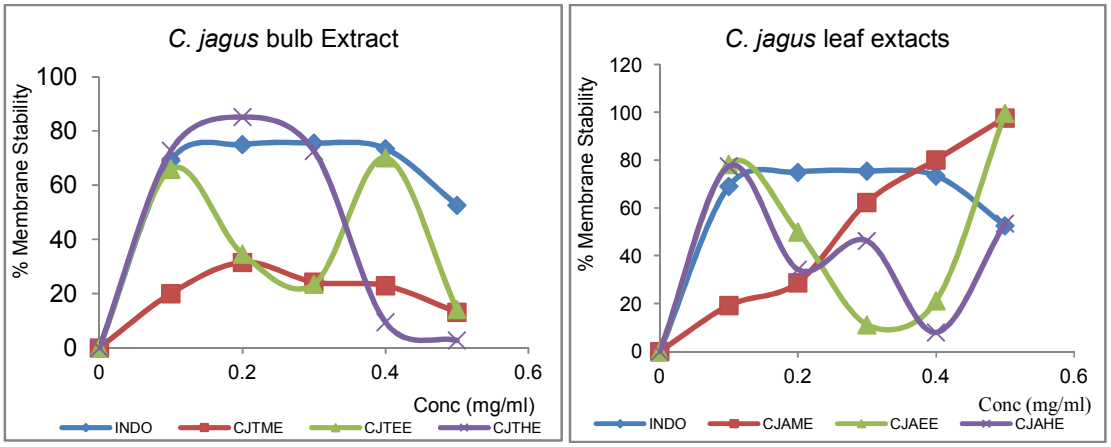

Fig. 4a&b. Graph of % membrane stability against concentration for *C. jagus* bulb and leaf extracts

Table 3. Membrane-stabilizing potential of extracts

Conc (mg/ml)	Mean percentage stability ± Stand. deviation						
	Indomethacin	CJTME	CJTEE	CJTHE	CJAME	CJAEE	CJAHE
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.1	69.12±6.65	20.03±4.79	65.96±5.14	72.78±14.25	19.23±0.82	78.31±13.46	77.48±14.84
0.2	75.02±6.04	31.53±1.34	34.64±2.36	85.19±6.74	28.82±8.14	50.03±4.63	34.25±16.76
0.3	75.55±2.16	24.28±0.68	23.50±42.77	72.64±22.86	62.33±3.55	11.15±9.77	46.25±8.10
0.4	73.51±1.88	22.92±0.71	70.20±6.66	9.46±14.38	80.16±13.40	21.22±17.42	7.98±4.35
0.5	52.65±1.18	13.16±1.21	14.16±14.18	2.73±1.54	97.74±0.68	99.70±25.65	53.59±12.75

CJAHE- Hexane extract of C. jagus leaves; CJAEE- Ethylacetate extract of C. jagus leaves; CJAME- Methanol extract of C. jagus leaves; CJTHE - Hexane extract of C. jagus bulbs; CJTEE - Ethylacetate extract of C. jagus bulbs; CJTME- Methanol extract of C. jagus bulbs

4. CONCLUSION AND RECOMMENDATION

The results of this study showed that the leaves and bulb of *Cinum jagus* possess significant anti-inflammatory and antioxidant activities. These plant parts could therefore be potential sources of anti-inflammatory and antioxidant agents. This study has further underscored the potency of medicinal plants in the treatment of many diseases by traditional medical practitioners. Further studies aimed at isolating and characterizing the compounds responsible for the observed activities should be carried out.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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