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Phytochemical Screening, *in vitro* Antioxidant and Anti-inflammatory Activity of Aqueous Extract of *Jatropha curcas*

Adeyemo A. G. [a*], Awote O. K. [a], Bello O. P. [a], Sanusi R. B. [a], Akinyemi M. O. [a], Eshinlokun I. A. [a], Mabodu M. T. [a], Olarenwaju H. O. [a] and Amodu A. N. [a]

[a] Department of Biochemistry, Faculty of Science, Lagos State University, Ojo, Badagry-Expressway, Nigeria.

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

The wide range of secondary metabolites in medicinal plant extracts are used to treat and manage various diseases worldwide. Medicinal plants, such as *Jatropha curcas,* have played crucial roles in effectively treating a range of diseases, including bacterial and fungal infections. Therefore, the aim of this study was to determine the phytochemical constituent, *in vitro* antioxidant and anti-inflammatory activity of aqueous extract of *Jatropha curcas.* Phytochemical screening of *Jatropha curcas* was determined using standard procedures. The antioxidant activity of the aqueous extract of *Jatropha curcas* was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), and Hydrogen peroxide scavenging activity assays; and *in vitro* anti-inflammatory activity was monitored using protein inhibitory assay and albumin denaturation assays. The Phytochemical screening revealed

**Corresponding author: Email: adesegun.adeyemo@lasu.edu.ng;*

that aqueous extract of J. curcas had the presence of Tannins, Steroid, Saponin, Alkaloid, Cardiac glycoside, flavonoids, Terpenoids and Anthraquinones. The result also revealed that the DPPH scavenging activity and Hydrogen peroxide scavenging activity of the different concentrations (0.2, 04, 0.6, 0.8 and 1.0mg/ml) of the aqueous extract of *J. curcas* had a significant increase in the activity of scavenging DPHH compared to the standard (Ascorbic acid). Also, the protein inhibitory and albumin denaturation activity of the extract at different concentrations significantly increased compared to the standard (Aspirin). In conclusion, the aqueous extract of *Jatropha curcas* exhibited an antioxidative and anti-inflammatory effect possibly due to the inhibition of reactive oxygen species (ROS) generation.

Keywords: Jatropha curcas; antioxidant; anti-inflammation; phytochemical constituent.

1. INTRODUCTION

“The harmful effects of oxidative stress have become a serious human health problem, and the use of synthetic antioxidant molecules (butylated hydroxyanisole and butylated hydroxytoluene) is currently being questioned due to potential toxicological risks” (Lourenço *et al.,* 2019). “Also, anti-inflammatory drugs on the market have many side effects. Consequently, a priority for the pharmaceutical industry was to find new antioxidants and anti-inflammatory substances” (Mahdi, 2013). “Medicinal plants are an inexhaustible source of substances with many therapeutic effects. Medicinal plants, such as *Jatropha curcas*, have played crucial roles in effectively treating a range of diseases, including bacterial and fungal infections” [1]. The scientific designation for the physical nut plant is "*Jatropha curcas*." The genus name Jatropha originates from the Greek words jatr´os (doctor) and troph´e (food), indicating its association with medicinal applications [2]. “The foliage and other components of the plant are utilized in the management of diverse ailments. Extracted from the leaves of *Jatropha curcas* are several compounds, including the flavonoid apigenin and its glycosides vitexin and isovitexin, as well as the sterols stigmasterol, β-D-sitosterol, and its β-D-glucoside” [3]. “Additionally, the leaves of *Jatropha curcas* were found to possess steroid sapogenins, alkaloids, triterpenoid alcohol (1-triacontanol), and a dimer of a triterpenoid alcohol. A complex comprising 5-hydroxypyrrolidin-2-one and pyrimidine-2,4-dione was isolated from the *J. curcas* leaves through ethyl acetate extraction” [4]. “The leaves of *Jatropha curcas* have been employed in traditional medicine in Tonga, an island in Oceania, to address instances of vaginal bleeding” [5].

“Inflammation is an integral component of the intricate biological reaction of vascular tissues to detrimental stimuli” [6]. “It serves as a defensive response of the body's cells to injury, infections, as well as allergic or chemical irritation” [7].” This response is identifiable by distinct inflammatory indicators such as redness, pain, swelling, heat, and reduced functionality. It occurs due to the dilation of blood vessels, which increases blood flow to the affected area. As a result, immune cells like neutrophils and macrophages, along with fluids causing edema, migrate towards the inflamed regions” [8]. “The process of inflammation is intricate and involves various factors, including molecules from bacteria to chemicals. These factors can lead to cellular trauma or death” [9]. “The trauma caused by tissue injury leads to the release of inflammatory mediators, which encompass reactive oxygen species (ROS) such as superoxide anion (O_2-), hydrogen peroxide (H_2O_2), contributing to oxidative stress. Additionally, nitric oxide and cytokines are also released in this process” [10]. “Elevated expression of pro-inflammatory mediators, such as cytokines, NADPH oxidase, NF kappa B, myeloperoxidase, and Inos, has been associated with immune system disorders” [11].

“Conversely, oxidative stress occurs when there is an imbalance between the production and buildup of reactive oxygen species (ROS) within cells and tissues and the biological system's capacity to eliminate these reactive substances” [12]. “Reactive oxygen species (ROS) have various physiological functions, such as cell signaling, and are typically generated as by-products of oxygen metabolism. However, environmental stressors such as UV radiation, ionizing radiation, pollutants, and heavy metals, as well as xenobiotics like anticancer drugs, significantly augment ROS production. Consequently, this imbalance leads to cellular and tissue damage, commonly referred to as oxidative stress” [13]. Several antioxidants have been exploited in recent years for their actual or

supposed beneficial effect against oxidative stress, such as vitamin E, flavonoids, and polyphenols. Understanding this has given a clue for target based therapeutic approach in search of new effective anti-inflammatory and antioxidant drugs. Owing to the fact that the pharmacological and physiological constituents of herbal medicines are known to regulate and modulate various functions of inflammatory response in the body either directly or indirectly and *Jatropha carcus* is rich in polyphenols therefore the aim of this study is to investigate the anti-inflammatory and antioxidant potentials of *Jatropha curcas*.

2. METHODOLOGY

Chemicals: All the chemicals acquired were of analytical quality and utilized without additional purification. All of the studies were conducted with distilled water and deionized water for preparing aqueous solutions.

Collection of plant sample: Fresh *Jatropha curcas* leaves were collected around Lagos State University in Ojo Lagos, Nigeria. The leaves were identified and authenticated by the Lagos State University Department of Botany.

Sample preparation and extraction: Fresh *Jatropha curcas* leaves were separated from the stems, sorted, and cleaned with tap water first, then with distilled water until no contaminants remained. The leaves were allowed to dry for three days before being chopped into smaller pieces. The aqueous extract of *Jatropha curcas* was made in a 2000ml conical flask with 400g diced leaves and 1200 ml deionized water. The mixture was allowed to sit for three days before macerating.The obtained extracts were passed through a Whatman No. 1 paper filter, and the filtrate was then heated at 50°C in a water bath to concentrate it. This filtrate was then kept for further study.

Qualitative phytochemical screening: Phytochemical screening was performed on all the extracted samples using standard procedures to identify the phytochemical constituents in the samples.

Test for saponins: 5ml of the extract solution was diluted with 5ml distilled water and shaken vigorously in a graduated cylinder for 3minutes for a stable persistent froth to form. They were all then observed for the formation of emulsion.

Test for phlobatannins: 2 ml of the extract solution of the samples were placed in a test tube with 2 ml of 1% aqueous hydrochloric acid (HCl) they were boiled for 3 minutes then the samples were observed for the formation of a red precipitate that shows the presence of phlobatannins in the extract.

Test for tannins: A few drops of 0.1% ferric chloride was added to 3 ml of the extracted sample solution and then they were all observed for brownish green or a blue-black colour formation.

Test for steroids: 2 ml acetic anhydride and 2 ml of sulphuric acid (H_2SO_4) were added to5 ml of each extracted sample solution. The samples were then observed for color changed from violet to blue or green indicating the presence of steroids.

Test for alkaloid: 5 ml of the extracted sample solution was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish-brown precipitate (with Draggendorff's reagent) confirmed the presence of alkaloids.

Test for flavonoids: Two methods were used to test for flavonoids. First, 5 ml of dilute ammonia was added to a to 2ml of each aqueous filtrate of the extract in the test tube then 1 ml Concentrated of sulphuric acid was added. The formation of yellow colouration that disappeared on standing indicated the presence of flavonoids. Second, a few drops of 1% aluminum chloride solution were added to a portion of the filtrate. And the formation of yellow colouration indicated the presence of flavonoids.

Test for cardiac glycosides: 1ml of each extract were diluted with 5 ml in water and 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added which was then underlaid with 1 ml of concentrated sulphuric acid a brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. Then violet ring appeared below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer indicated the presence of cardiac glycosides.

Test for terpenoids: 2 ml of chloroform was added 1ml each of the extractand then 3 ml of Concentrated sulphuric acid (H_2SO_4) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for reducing sugars: 2ml of aqueous extract, 1ml of Fehling's A and 1ml of Fehling's B solutions were mixed in a test-tube and heated in boiling water bath for 10 min, appearance of yellow and then brick red precipitate indicates the presence of reducing sugars.

Test for Phenolic compounds: 5ml of aqueous extracts were treated with 2-3 drops of lead acetate. Formation of white-yellow colour indicates that the presence of phenolic compound.

Quantitative Phytochemical Screening: The quantitative photochemical analysis was performed at Nigerian institute of medical Research (HPLC) and spectrophotometry by little modification of the method(Harborne, 1998).

3. ANTIOXIDANT ASSAY

DPPH free radical scavenging activity: To create the radical solution (DPPH), 2.4mg of DPPH is dissolved in 100ml of methanol. Then, 50 µl of the test samples (different concentrations of J. curcas extract) is mixed with 2 ml of the methanolic DPPH solution. After thorough shaking, the mixture was left undisturbed at room temperature for a duration of 30 minutes, ensuring it was kept away from light. The absorbance of the resulting mixture was then measured using a spectrophotometer at a wavelength of 515 nm [14].

Ascorbic acid served as the standard, while water was utilized as the control in the experiment. The percentage of DPPH radical scavenging activity was determined using the following formula:

Formula 1:

Percentage Inhibition = [(Control – Test) / (Control) * 100]

Hydrogen Peroxide scavenging activity: A total of 0.1 ml of the test samples was combined with 0.3 ml of phosphate buffer (50 mM, pH 7.4) and 0.6 ml of hydrogen peroxide solution (2 mM in phosphate buffer, 50 mM, pH 7.4). The mixture was thoroughly mixed using a vortexer, and after a duration of 10 minutes, the absorbance was measured at a wavelength of 230 nm using a UV-Visible spectrophotometer [15]. Vitamin C was employed as the standard, and a phosphate buffer (50 mM, pH 7.4) was utilized as the blank in the experiment. The percentage of hydrogen peroxide scavenging activity was determined using Formula 1.

4. ANTI-INFLAMMATORY ASSAY

Protein Inhibitory Action: "A reaction mixture of 2 ml was prepared, consisting of 1 mg trypsin, 1 ml of 20 mM Tris HCl buffer (pH 7.4), and 50 µl of the test samples. The mixture was then incubated at 37°C for 5 minutes, following which, 1 ml of 0.8% (W/V) casein was introduced. The reaction was allowed to continue for an additional 20 minutes, after which 2 ml of 70% perchloric acid was added to stop the reaction. The cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank and water were used as control" [16]. Formula 1 was used to determine the percentage of inhibition of proteinase inhibitory activity.

Inhibition of albumin denaturation: A 1% stock solution of bovine serum albumin (BSA) was prepared using double distilled water. Additionally, a stock solution of *Jatropha curcas* was prepared at a concentration of 10 mg/ml. The reaction mixture consisted of 0.5 ml of BSA and 50 µl of the test samples. This mixture was then incubated at room temperature for a duration of 15 minutes. To induce denaturation, the reaction mixture was subjected to heating at 60°C for a period of 10 minutes. Subsequently, the absorbance of the mixture was measured at a wavelength of 660 nm using a UV-visible spectrophotometer [17]. Only BSA was used as a negative control. The formula shown below was used to determine the percentage inhibition of denaturation:

Percentage Inhibition = [(Negative control – Test) / (Negative control) * 100

5. RESULTS

Table 1 shows the qualitative phytochemical constituents of *J. curcas* aqueous extract. Tannins, flavonoids, saponins and reducing sugars were present while, cardiac glycosides, terpenoids, carbohydrates and proteins were absent.

Table 1. Qualitative and Quantitative phytochemical analysis of *J. curcas* aqueous extract

Phytochemicals	Qualitative Analysis	Quantitative Analysis
Tannin	+	24.1± 0.1
Steroid	+	17.7±0.1
Saponin	+	14.1±0.1
Alkaloid	+	3.67±0.03
Reducing Sugar	-	-
Cardiac Glycoside	+	5.0 ± 0.1
Flavonoids	+	11.1±0.1
Terpenoids	+	10.5±0.3
Anthraquinones	+	1.2 ± 0.3

+ Present, - Absence The quantitative estimation of these secondary metabolites showed that tannins were most abundant (24.1 ± 0.1) followed by steroids (17.7 ± 0.1), saponins (14.1 ± 0.1), flavonoids (11.1 ± 0.1), and terpenoids (10.5 ± 0.3).where as the anthraquinones (1.2 ± 0.3), Alkaloid (3.67±0.03) and cardiac glycosides (5.0 ± 0.1) were least in quantity

Antioxidant Assay Result: Fig. 1 Shows H_2O_2 scavenging activity of *J. curcas* aqueous extract. It was observed that the H_2O_2 scavenging activity of *J. curcas* aqueous extract decreased as the dose increases, i.e., the higher the concentration of the extract, the higher the H_2O_2 scavenging activity and thus, the higher the antioxidant. Moreso, a statistically significant decrease in percentage H_2O_2 scavenging activity was observed at the concentration of 0.4 and 0.8 mg/ml as compared to the standard, ascorbic acid. However, 0.2 mg/ml and 0.4 mg/ml of the extract increased significantly compared to the standard. Antioxidant activities were significantly compared with Ascorbic acid, a positive control.

Fig. 2 Shows DPPH radical scavenging activity of *J. curcas* aqueous extract. It was observed that the DPPH radical scavenging activity of *J. curcas* aqueous extract decreased as the dose increases, i.e., the higher the concentration of the extract, the higher the DPPH radical scavenging activity and thus, the higher the antioxidant. Moreso, a statistically significant decrease in percentage DPPH inhibition was observed at the concentration of 0.1 and 0.2 mg/ml as compared to the standard, ascorbic acid. However, 0.2-1.0 mg/ml of the extract increased significantly compared to the standard. Antioxidant activities were significantly compared with Ascorbic acid, a positive control.

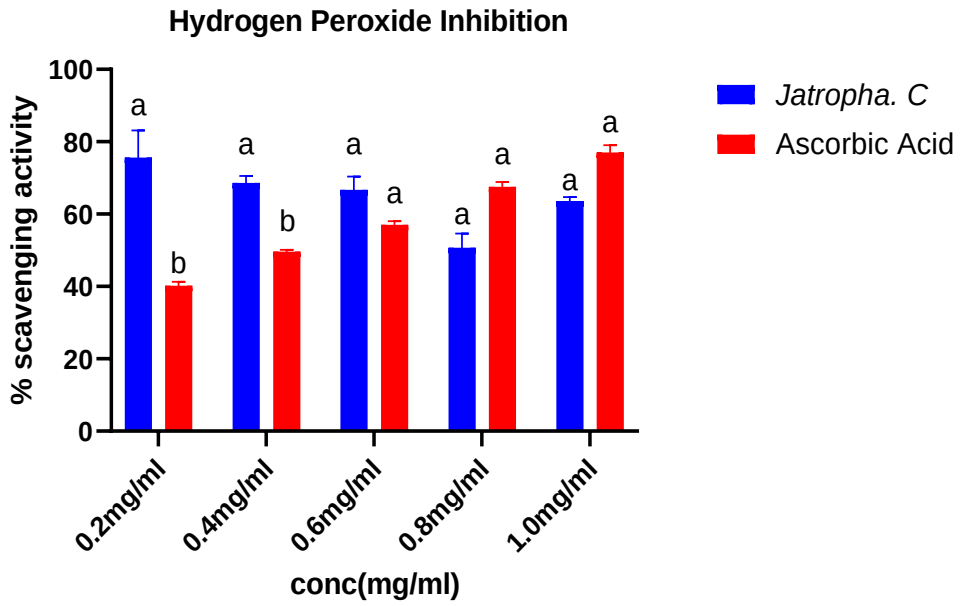

Fig. 1. H_2O_2 Scavenging Activity of *J. curcas* aqueous extract. All values are the mean ± SEM. Values with the same superscript are not significant at P<0.05

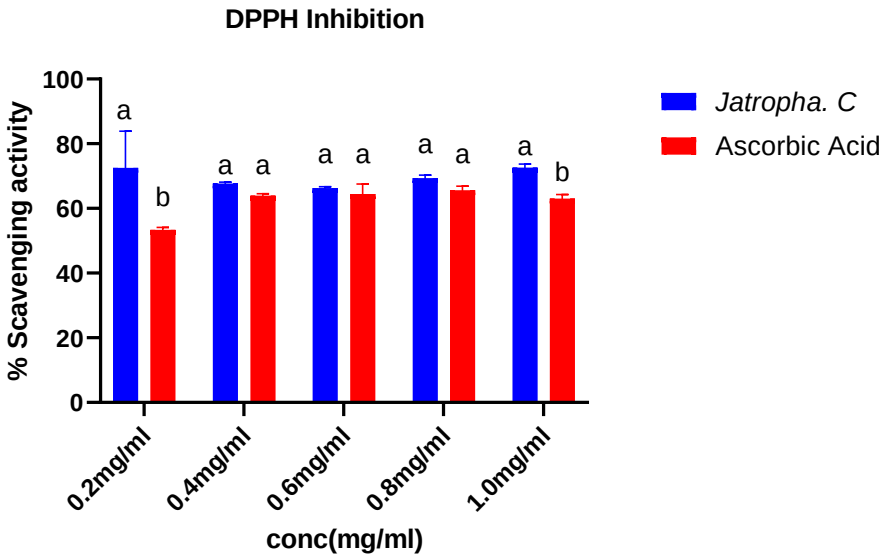

Fig. 2. DPPH Scavenging activity of *J. curcas* aqueous extract. All values are the mean ± SEM. Values with the same superscript are not significant at P<0.05

Fig. 3 showed that there was a significant difference between the albumin denaturation inhibition activity of the extract and the standard. The albumin denaturation inhibition activity of the extract was dose dependent and the highest activity was seen at 0.5mg/ml.

Fig. 4 showed there was a significant difference between the extract and the standard (Aspirin) although the graph also showed that the extract possesses protein inhibitory activity with the highest inhibition at 02mg/ml, this may suggest that the protein inhibitory activity of the extract was not dose dependent.

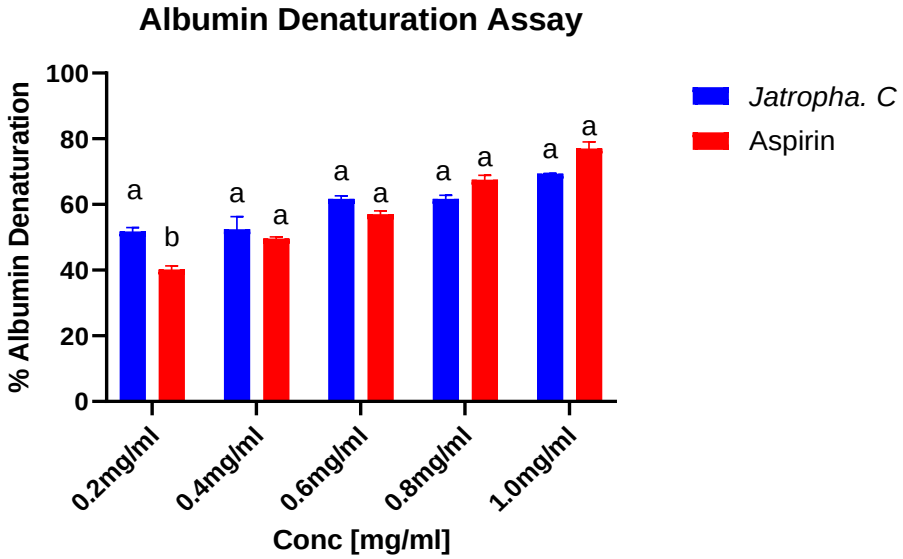

Fig. 3. Graph comparing the Albumin denaturation activity of hexane extract of *Sargassum fluitans*to Aspirin. All values are the mean ± SEM. Values with the same superscript are not significant at P<0.05

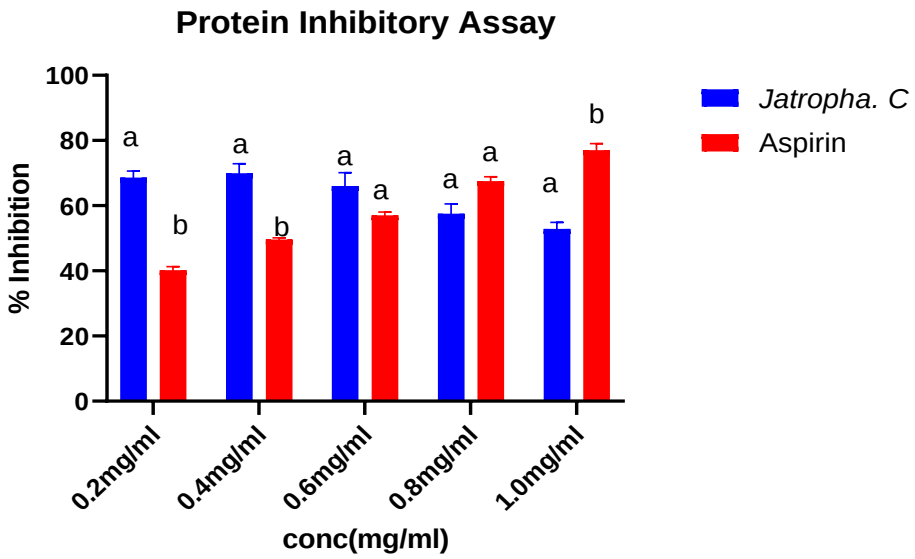

Fig. 4. Graph comparing the protein inhibitory activity of hexane extract of *Sargassum fluitans*to Aspirin. All values are the mean ± SEM. Values with the same superscript are not significant at P<0.05

6. DISCUSSION

The extracts' antioxidant activity was determined by measuring their Hydrogen peroxide and DPPH free radical scavenging activity [18]. The hydrogen peroxide assay measures the ability of an antioxidant to reduce hydrogen peroxide, which is a reactive oxygen species [19]. The extract showed a dose-dependent reduction of hydrogen peroxide, with an IC_{50} value of 0.3µg/mL. The extract's hydrogen peroxide reducing activity was comparable to or better than that of other natural antioxidants, such as ascorbic acid and α-tocopherol [20].

"The DPPH radical has been extensively used as a free radical to test extracts for their reductive ability of extracts as free radical scavengers or hydrogen donors, as well as to evaluate plant extract antioxidant capacity [21]. Antioxidant compounds react with DPPH, reducing it to 1,1-diphenyl-2-hydrazine (DPPH-H) by providing electron or hydrogen atoms. The extract showed a dose-dependent scavenging activity of the DPPH radical, with an IC_{50} value of 0.2µg/mL. The extract's DPPH scavenging activity was comparable to or better than that of other natural antioxidants, such as catechin and gallic acid" [22,23].

Proteinases have been associated with arthritic reactions. Neutrophils, in their lysosomal granules, carry much serine proteinases. Proteinases of leukocytes play a major role in the development of tissue damage during inflammation [24,25]. A significant level of protection is provided by proteinase inhibitors [26]. Various recent studies have shown that many flavonoids contribute significantly to the antioxidant and anti-inflammatory activities of many plants [27]. Therefore, the presence of bioactives present in *J. curcas* may contribute to its anti-inflammatory activity. From the result, there was a significant difference between the protein inhibitory activity of the hexane extract of *J. curcas*and the control standard (Aspirin) although the result also showed that the extract has protein inhibitory activity which was not dose dependent.

"Protein denaturation is a perfectly documented reason for the inflammation in conditions as rheumatoid arthritis" [28]. "The prevention of protein denaturation is the main mechanism of action of non-steroidal anti-inflammatory drugs (NSAIDs) [29]. Therefore, the ability of the studied extract to prevent the denaturation of proteins could be responsible for their anti-inflammatory properties. The hexane extract of *J. curcas* could inhibit protein denaturation (albumin) and its percentage inhibition was low when compared to the standard aspirin. This is in concordance with findings from" [30] who reported that *J. curcas* can manage protein denaturation.

7. CONCLUSION

In conclusion, the presence of bioactive secondary metabolites in *J. curcas* extracts, revealed by the preliminary phytochemical study, makes it an effective gift of nature with important anti-inflammatory and antioxidant activity. However, more research is needed to understand the mechanisms of action and to evaluate the safety and efficacy of these extracts before they can be used in clinical settings. Overall, this study contributes to the growing body of research on natural products as potential sources of therapeutic agents for various diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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