INVITRO ANTIOXIDANT ACTIVITY OF THE DIFFERENT SOLVENT FRACTIONS OF CRUDE METHANOLIC EXTRACT OF *JUSTICIA CARNEA*

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ABSTRACT
The necessity to examine medicinal plants for phytochemicals and bioactivities has been brought to light by the recent increase in interest in using native medicinal plants to treat diseases. This study aimed to assess the free radical scavenging activity of various solvent fractions of Justicia. carnea, and to evaluate and quantify the phenolic (TPC) and flavonoid (TFC) content in various solvent fractions of the methanolic extract of J. carnea. The TPC was discovered to be high in methanolic and n-hexane fractions at (31.28 ± 0.020 and 21.40 ± 0.020 mg GAE/g respectively) a similar trend was also observed in TFC with n-hexane and methanolic fractions having a highest concentration at (18.30 ± 0.015 and 19.29 ± 0.023 mg QE/g). The result of the DPPH assay showed a concentration-dependent increase in activity across all fractions with the n-hexane faction displaying the highest activity at 80.21±0.025%, the aqueous, ethylacetate and methanol fraction had DPPH radical scavenging activity of 60, 68 and 61.67%, respectively. The FRAP assays showed that all fractions had a concentration-dependent increase in invitro antioxidant activity with the n-Hexane, aqueous, methanol and ethylacetate fractions displaying the highest activity to be 63.92%, 74.26, 57.61 and 65.20%, respectively. The activity observed can be attributed to the flavonoid content of the fractions. The findings from this study show that J. carnea is a viable medicinal alternative in preventing and treating diseases associated with oxidative stress.

Keywords: antioxidant, flavonoid, fractions, invitro

1.0 INTRODUCTION

Plants have since earliest times been a source of medicine for mankind especially in developing countries. They are a rich store of molecules with pharmaceutical potential. The search for bioactive compounds from natural sources continues to be a viable research area and has always been of great interest to researchers looking for new sources of drugs useful in various diseases. *J. carnea* is a plant in the family Acanthaceae. (Otuokere, Amaku, Igwe, & Chinedum, 2016) identified several bioactive compounds in the ethanol extract of *J. carnea* leaves, suggesting its pharmacological importance. (Anigboro, Avwioroko, Ohwokevwo, Pessu, & Tonukari, 2021; Oloruntola et al., 2022) established the proximate composition of *J. carnea* as well as outlining its phytochemical profile. The anti-diabetic properties of J.carnea were observed by (Ani, Udedi, Akpata, Onyishi, & Nwakudu, 2020) suggesting its role in reducing blood sugar levels. The anti-inflammatory and antioxidant properties of *J. carnea* leaf powder was reported by (Anigboro et al., 2021) and suggested that the *J. carnea* leaf powder may play a significant role in acting as an antioxidant. Traditionally *J. carnea* has been used to replenish blood levels in anaemic patients. Studies have also revealed it to contain high concentrations of elements necessary for healthy blood, with potential uses in improving food quality and as a treatment for blood conditions like anemia (Anthonia, Ikechukwu, Uzoma, & Sunday, 2019), this is particularly important for developing countries considering this disproportionately affects its populations, pregnant women, and cancer patients. These findings encourage more research into identifying the bioactive compounds that are present in different fractions of this plant. Consequently, the goal of the research was to evaluate the phytochemical content of each solvent fraction of the *J. carnea* extract as well as each fraction's antioxidant capacity in vitro.

2.0 METHODOLOGY

Plant Collection and Identification

The *J. carnea* leaves was obtained from Ilaro, Ogun, Nigeria. It was identified and authenticated at the University of Lagos, Lagos, Nigeria, and a specimen was put in the University Herbarium with a voucher number LUH: 10064

Preparation of crude methanolic extract

The fresh *J. carnea* leaves were carefully washed with water, air-dried and milled with an electric blender. 500 g of the milled plant was soaked in 2.5L Methanol and left to macerate for (72 h) following this a wine-coloured filtrate was filtered by the use of a muslin cloth, the filtrate was concentrated using a rotary evaporator and a water bath at 60 °C was used to obtain the extract termed *J. carnea* methanol extract (JCmE).

Fractionation

Separating funnel method: To commence the fermentation process, JCme is entirely dissolved or moistened with 250 mL of water. Following that, the material is transferred to a funnel for separation, shaken, and allowed to settle. In addition, 250 millilitres of n-hexane, the least polar solvent, were added and stirred. After the material had settled, the aqueous layer was removed by opening the bottom of the separating funnel. To extract the n-hexane fraction, the remaining material in the separating funnel was moved to a sterilized container. After adding the same amount of n-hexane again, shake well and separate. The addition was done until the top layer of n-hexane became transparent. A similar cycle was carried out with ethyl acetate. Since the crude extract was originally dissolved in water, the leftover portion after the fractionation was known as the aqueous fraction.

Qualitative Phytochemicals Analysis

Using standard laboratory procedures described by (Sofowora, 1996) the phytochemical analysis was carried out for qualitative assessment. Phenols, saponins, flavonoids, alkaloids, tannins, cyanogenic glycosides, and sterols are among the phytochemicals examined.

Phenol Analysis: methanol 20 millimetres was used to extract 2 milligrams of the material, which was filtered using filter paper. To One milliliter the filtrate was added one milliliter of 20% $NaCO_3$ and one milliliter of Folin-Clarkeon; the presence of phenol is shown by the dark blue color.

Alkaloids: 2ml of Wagner's reagent was combined with the extract, a reddish-brown precipitate formed, which suggested that alkaloids was present.

Cyanogenic Glycosides: Fehling's Test: 3 ml of the filtrate was combined with 5ml of Fehling's solution I and II in equal amounts, and the mixture was heated for 5 minutes. Glycoside presence was confirmed by a thicker brick red precipitate.

Saponins: To determine whether saponins were present, a foam test was conducted. After adding 6 ml of water to the 2 ml of extract in a test tube and giving it a good shake, the presence of saponins was confirmed by looking for the production of a persistent foam.

Flavonoids: Testing for the presence of flavonoids was done using an alkaline reagent assay. The extracts were combined with 2 millilitres of a 2% NaOH solution. The presence of flavonoids was revealed by the formation of a bright yellow hue that went colourless

and colourless upon the addition of a few drops of diluted acid H_2SO_4.

Tannins: A quantity of 5g was heated to a boil in 40 ml of water, filtered, and utilized for the ferric chloride examination. Ferric Chloride Test: A small amount of ferric chloride solution was added to around 3millilitress of the filtrate. The presence of tannin is indicated by a greenish-black precipitate.

Sterols: Salkowski test: A few drops of strong sulfuric acid were added to the test samples in chloroform, and sterols were detected by the appearance of red colour in the lower layer.

Determination of Flavonoid

Distilled water 1ml was used to dissolve about 0.25 g of the extract. Next, 5% $NaNO_2$ solution, 0.150 ml of freshly made ($AlCl_3$), and 1 M NaOH solutions were added. After letting the mixture remain for five minutes, a spectrophotometer measured the absorbance at 510 nm. The outcome was given as equivalents of quercetin (QE) (Seifu, Mehari, Atlabachew, & Chandravanshi, 2017).

Determination of Total Phenol

The folin-Ciocalteu technique (Singleton, Orthofer, & Lamuela-Raventós, 1999) was used to determine this. To 125 µl of the extract, Folin-Ciocalteu's reagent and distilled water was added. The 7% sodium carbonate solution was added after the mixture had stood for six minutes. The combination was let to stand for ninety minutes. At a wavelength of 760nm the absorbance of the mixture was measured and the result was expressed in gallic acid equivalents (GAE).

DPPH radical scavenging assay

The method outlined was used to determine the hydrogen-donating or the investigated plant extracts' capacity to scavenge radicals, with ascorbic acid serving as a positive control (Baliyan et al., 2022). At 517 nm, the reaction mixture's color shift was seen. In summary, 2.5 ml of different sample concentrations and standard ascorbic acid were combined with a 1 ml aliquot of an ethanolic solution containing 0.3 mM DPPH. For 30 minutes the mixture was left in the dark after incubation, at which point the absorbance at 517 nm was measured. The blank used was ethanol. As a negative control, DPPH solution (1 ml, 0.3 mM) plus ethanol (2.5 ml) are used. The degree to which the tested extract decolourized DPPH from purple to yellow indicated how effective it was in scavenging. Three test runs (n = 3) were conducted, and average values were determined. Using the following formula, It was calculated what proportion of the radical scavenging activity was suppressed.

$$\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 = \text{DPPH radical scavenging activity (\%)}$$

Abs sample = Absorbance of DPPH radical + sample or standard,
Abs control = Absorbance of DPPH radicals + methanol.

Ferric Reducing Antioxidant Power (FRAP) Assay

In this method, the antioxidant ability in the extract is confirmed by measuring its reducing power. Depending on the reducing power of each molecule used in this assay, the test solution's yellow color changes to different colours of green and blue. The plant extract under test was found to have a Fe^{3+} reducing power using the approach described by (Hazra, Biswas, & Mandal, 2008). After mixing 0.5 ml of phosphate buffer (pH 6.6) and 0.5 ml of 0.1% potassium hexacyanoferrate [$K_3Fe(CN)_6$] with standard ascorbic acid in various concentrations (100, 200, 300, 400, and 500 µg/ml), the mixture was incubated for 20 minutes at 50°C in a water bath. 0.5 ml of 10% TCA was used to stop the reaction after incubation. A 0.1 ml solution containing 0.01% FeCl3 was included to the upper part of the solution (1 ml), which had already been combined with 1 ml of distilled water. After allowing the reaction mixture to sit at room temperature for 10 minutes, the absorbance at 700 nm was measured in comparison to the suitable blank solution. Three duplicates of each test were run (n = 3). A positive control was ascorbic acid. A blank solution made of phosphate buffer (PH 6.6) was utilized.

Statistical Analysis

GraphPad Prism 6 was used to analyze duplicate data, and results were given as mean ± SD. Invitro Antioxidant data was presented as percentage activity.

Result and Discussion

Table 1: Phytochemical distribution in the different fraction of *J. carnea.*

Phytochemicals	Aqueous JCE	Methanol JCE	Ethyl acetate JCE	n-Hexane JCE
Phenol	++	+++	+	++
Saponin	++	+	++	+
Tannin	+++	+++	+	++
Flavonoid	+	++	++	+++
Alkaloids	+	+	-	++
Cyanogenic Glycosides	-	+	-	+
Sterols	-	+	-	-

- = Absent
+ = Low ++ = Moderate +++ = High

Table 2: Total Phenolics and Total Flavonoids content of different fraction of *J. carnea.*

	Residual Aqueous JCE	Crude Methanol JCE	Ethyl acetate JCE	n-Hexane JCE
Total Phenolics (mg GAE/g)	20.86 ± 0.019	31.28 ± 0.020	10.19 ± 0.133	21.40 ± 0.020
Total Flavonoids (mg QE/g)	9.55 ± 0.032	18.30 ± 0.015	11.32 ± 0.032	19.29 ± 0.023

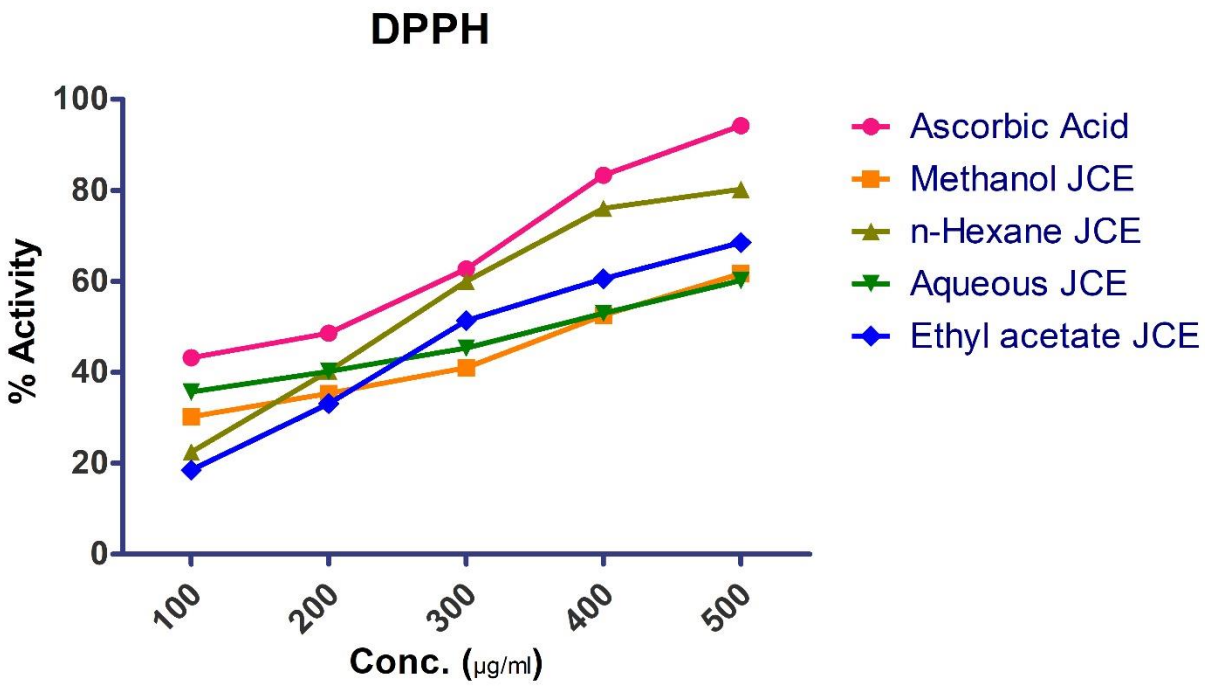

Fig 1: Showing the % DPPH Activity of the different fraction of *J. carnea*.

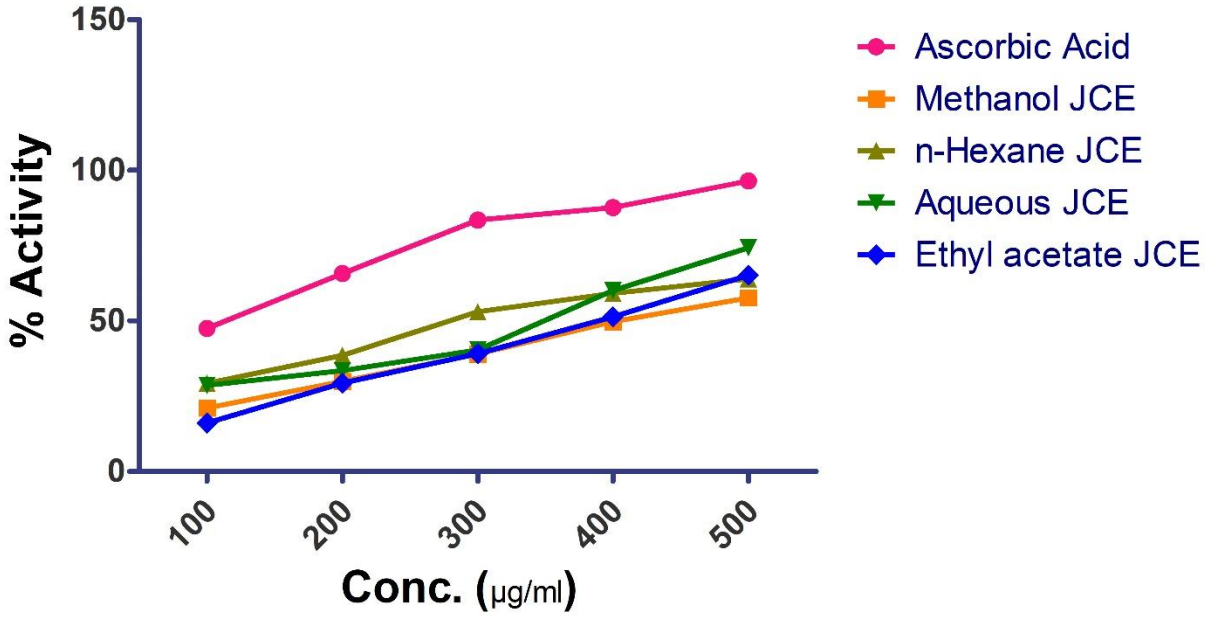

Fig 2: Showing the % FRAP Activity of the different fractions of *J. carnea*.

3.0 DISCUSSION

Numerous plants have demonstrated a variety of health advantages, notably green vegetables, which have been shown to promote excellent health and wellness due to the presence of phytochemicals. Phytochemicals have a wide range of activities which help the immune system fight against diseases and their abundance in a plant could be a pointer to its pharmacological activity (Yadav, Khare, & Singhal, 2017). This may also contribute significantly to the antioxidant capacity. The phytochemical analysis of the methanolic extract of *J. carnea* and its fractions of n-hexane, ethyl acetate and the residual aqueous fraction showed that phenol, flavonoid, saponin, tannins, alkaloids were present in the crude methanol fraction which agrees with research carried out by (Ajuru, Kpekot, Robinson, & Amutadi, 2022). These compounds have been observed to have various pharmacological activities. Saponins have been established to have antifungal, cytotoxic, bacterial, and antiviral activities (Kregiel et al., 2017), tannins can also act as an antioxidant with free radical scavenging activity, antibacterial, anti-cancer, and

cardio-protective qualities (Smeriglio, Barreca, Bellocco, & Trombetta, 2017). The total phenolic and total flavonoid content showed that the crude methanolic JCE and n-Hexane fraction of JCE had the highest phenolic content with 31.28 ± 0.020 and 21.40 ± 0.020 respectively, this agrees with the work by (Mehmood, Javid, Khan, Ahmad, & Mustafa, 2022) their report showed that methanolic fraction of some selected medicinal plants all had total phenolic and total flavonoid rich methanolic fraction. Flavonoids are polyphenolic chemicals with pharmacological effects such as antioxidative, hepatoprotective, antibacterial, anti-inflammatory, anticancer, and possible antiviral activities, in addition to their well-known antioxidant activity (Kumar & Pandey, 2013). n-Hexane fraction had the most flavonoid content at 19.29 ± 0.023, the residual Aqueous JCE had the lowest flavonoid concentration at 9.55 ± 0.032.

In vitro antioxidant activity may be measured using a variety of techniques, including FRAP and DPPH tests. The DPPH radical scavenging test, which is commonly used to assess a compound's capacity to operate as a hydrogen source and free-radical scavenger, is quick, simple, and affordable for determining antioxidant capabilities. The DPPH test is based on the elimination of DPPH, a stabilized free radical (Baliyan et al., 2022). Fig 1 shows the % scavenging activity of the different fractions of *J. carnea* equated with the standard. The result shows that all fractions showed a concentration-dependent increase in activity with the n-Hexane fraction displaying the highest activity at 80.21% ± 0.015. The n-Hexane activity can be attributed to the rich flavonoid content of the fraction (Mehmood et al., 2022). Free radicals have been linked to several illnesses, including cancer, diabetes, and neurological disorders. Antioxidant-rich plant sources continue to be a source of free radical prevention, and the antioxidant activity reported in different fractions of *J. carnea* suggests that it may have medicinal value as a chemopreventive.

CONCLUSION

In the present study, several fractions of *J. carnea*'s methanol crude extract were analysed to assess phenolic and flavonoid content, as well as antioxidant potential. Our data indicate that the methanol and n-hexane fractions of *J. carnea* are high in TPC, and hence have strong antioxidant activity, implying that the bioactive component contained in *J. carnea* may be in the n-hexane and methanol fractions. Further fractionation might be performed on the fraction to isolate the bioactive component. In vivo, investigations on the anti-cancer and cardioprotective properties of *J. cornea's* n-hexane fraction can be conducted to broaden its biological uses.

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