



## Characterization and Biological Activities of Ethanol Extract of *Justicia carnea*

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### ABSTRACT

For a long time, the blood tonic and other therapeutic qualities of the medicinal herb *Justicia carnea* have been utilized in Nigeria. The purpose of this research is to learn more about the biological activity and phytochemical profile of the *Justicia carnea* ethanol extract. The phytochemicals in *Justicia carnea* were identified using GC-MS and Fourier transform infrared spectroscopy. An 100% ethanol extraction was performed on the powdered sample, and the resulting extract was concentrated using a rotary evaporator. Among the many potential medical applications of the few active metabolites identified by the study are those for lowering inflammation, fighting diabetes, regulating the immune system, and even curing malaria. Extract from *Justicia carnea* was most effective against *Salmonella typhi*, an infectious disease, with a MIC of 0.001 mg/L. It is thought to have the most promising antioxidant properties at a concentration of 0.50 g/ml, inhibiting peroxide and superoxide radicals with an inhibition diameter of 70.41 and 74.57 mm, respectively. The therapeutic properties of *Justicia carnea* extract are being investigated by the medical community.

## 1. INTRODUCTION

Many people in Western Africa are increasingly relying on traditional medicine to help them deal with contemporary diseases, poverty, and the limitations of mainstream healthcare. The accessibility, cheap cost, and convenience of use are the three defining features of herbal medicine. That is why a lot of families use it as a kind of treatment [1]. Hunger brought on by poverty makes a lot of individuals weak and susceptible to several

ailments. Because contemporary pharmaceuticals are so expensive and difficult to obtain, many Nigerians resort to using treatments derived from plants [2, 3].

Therapeutic plants include bioactive molecules termed phytochemicals and secondary metabolites, which have shown promise in alleviating a wide range of ailments. One example of a fruit or herb that has been used to treat anemia

is *Justicia secunda* Vahl, which is native to the Benin Republic, while another is Togo. As an affordable and perhaps safer alternative to manufactured medications, which can be costly and come with side effects, medicinal plant products are becoming more popular for the treatment of many illnesses and conditions [7].

Blood tonics made from the medicinal herb *Justicia carnea* have a lengthy history of usage in Nigeria. The color of the resulting liquid is usually a bright crimson since water boils [3]. People who desire to restore blood after menstruation, women who suffer from anemia, and pregnant women are the main users of the leaves. Throughout most of Africa, you can find this plant, which is commonly used as an ornamental. A protective barrier, it is grown in close proximity to residences in Nigeria [8, 9]. Studies on animals show that *Justicia Carnea* has more potent blood-boosting properties than traditional blood tonics. A short term of medication is sufficient to restore normal blood levels. Numerous Nigerian tribes have bestowed onto it titles like "Hospital too far," "Blood of Jesus," "blood leaf," and "Jehovah Witness leaf" [10, 11]. Some of the many conditions that *Justicia carnea* has traditionally been used to treat include inflammatory diseases, rheumatism, arthritis, fever, pain, diabetes, diarrhea, liver disease, and respiratory infections [2]. Many beneficial effects, including pain relief, inflammation reduction, allergy alleviation, tumor targeting, and virus suppression, have been observed in these substances [12].

Research suggests that *Justicia carnea* may have therapeutic effects due to the presence of certain phytochemicals. According to Alamgir & Alamgir [13], the metabolites comprise a diverse array of compounds, such as vitamins, iridoids, triterpenoids, coumarin, lignans, and alkaloids. They may also contain triterpenoidal glycosides. It is believed that the majority of *Justicia* species contain lignans, which have a wide range of pharmacological actions including anti-

inflammatory, hallucinogenic, antibacterial, antisickling, immunomodulatory, antihypertensive, and antiviral properties [8, 11]. A wide variety of ailments, such as respiratory tract infections, inflammation, stomach issues, fever, diabetes, liver diseases, diarrhoea, arthritis, rheumatism, anaemia, cancer, HIV, and diabetes, have long been treated with members of the *Justicia* species in traditional medicine [2, 14, 15]. Finding out how human participants react to ethanol extracts of *Justicia carnea* leaves and what amounts of phytochemicals they contain is the driving force behind this study.

## 2. MATERIALS AND METHOD

### 2.1. Collection and Preparation of Samples

The juicy *Justicia carnea* leaves were retrieved from a botanical garden situated on the Abuja campus of the University of Port Harcourt in Rivers State, Nigeria. Transferring them to the university's Plant Science and Biotechnology lab allowed for their accurate identification. After letting it air dry for a week in the gloom of the chemistry lab, the fine powder was ground and placed in a sample vial.

### 2.2. Extraction with Ethanol

Using 1,250 milliliters of ethanol and 100 grams of powdered *Justicia Carnea* plant, an 18-hour soxhlet extraction was performed. A rotary evaporator was used to remove the solvent from the crude extract, which was then stored for later use.

### 2.3 Gas Chromatographic-Mass Spectrometric Analysis of Extracts

Applying gas chromatography-mass spectrometry (GC-MS) with the following parameters, the ethanol extracts were examined: ran at 70 eV in electron impact mode with a constant 1 mL carrier gas supply, 0.5 L injection volume with a 10:1 split ratio, and an injector temperature of 250°C. Withholding brought the oven temperature up from 280 °C to 110 °C in 2 minutes, and another 10 minutes brought it up to 200 °C. The database was utilized to provide more clarity to the GC mass spectra.

### 2.4 Antimicrobial evaluation

A variety of pathogenic bacteria were utilized to assess the antibacterial properties of plant extracts. The researcher from the University of Port Harcourt's Department of Microbiology was the one who isolated the pathogens. Diluting 100 milligrams of the extract in 10 milliliters of DMSO yielded a concentration of 10 mg/mL. With this concentration as a starting point, we could measure the antibacterial activity.

In order to determine the extracts' antibacterial potency, the agar diffusion method was employed. Mueller Hinton Agar (MHA) was utilized as the bacterial culture medium. The medium was made sterile, sterilized for 15 minutes at 121°C per the manufacturer's instructions, and then allowed to settle and harden. After that, the sterile medium was supplemented with the standard test microorganism inoculum, which amounted to 0.1 mL. The inocula was distributed uniformly over the surfaces of the media using sterile swabs. Use a standard cork borer to cut a 6 mm hole in the middle of each inoculation medium. After being diluted with 0.1 mL of distilled water to a concentration of 0.001 mg/mL, the extract was applied to each well. Each plate was checked for areas of growth inhibition after the polluted material was cultured for 24 hours at 37°C for bacteria. Using a transparent ruler, the zone of inhibition was measured and recorded in millimeters.

### 2.5 Hydroxyl and superoxide radical scavenging activity

The impact of plant samples on hydroxyl radicals was investigated in this study using the same methods as Vyas et al. [16]. The solutions in the test tubes consist of 75 µl of millet, sorghum, and wheat solution extract (5-100mg/ml in methanol), 150 µl of 2-deoxyribose (10 mM), 450 µl of sodium phosphate buffer (200 mM, pH, 7.0), 150 µl of H<sub>2</sub>O<sub>2</sub> (10 mM), 150 µl of FeSO<sub>4</sub>-EDTA (10 mM), and 525 µl of distilled water. Over the following four hours, the reaction was maintained at a temperature of 37 degrees Celsius. After mixing 750 µl of trichloroacetic acid (2.8% concentration) with TBA (1% in 50 mM NaOH), the reaction stalled. After 10 minutes of cooling under running water, the mixture was transferred back to the pot of boiling water. Spectrophotometer readings were then taken at 520 nm. Method: methanol as a blank, vitamin C as a standard.

$$\% \text{ Scavenging} = \frac{A_{540}(\text{Control}) - A_{540}(\text{Test A})}{A_{540}(\text{Control})} \times 100$$

## 3. RESULTS AND DISCUSSION

### 3.1 FTIR profile of leaf ethanol extract of *Justicia Carnea*

The result presented in Table 1 shows the functional group analysis of the leaf extract of *Justicia Carnea*.

**Table 1: FTIR bands of the leaf extract of *Justicia Carnea***

S/N	Wavelength (cm-1)	Functional group	Compound class
1	969.9349	C=C	Disubstituted(trans), alkene C=C bend
2	1657.185	C=C	Alkene(vinylidene), C=C stretch
3	2121.886	N=C=N	Carbodiimide, N=C=N stretch
4	2311.931	O=C=O	Carbondioxide, O=C=O stretch
5	2496.026	S-H	Thiol, S-H stretch(weak)
6	2898.378	C-H	Alkane, C-H stretch
7	3235.815	O-H	Carboxylic acid, OH stretch
8	3492.431	O-H	Alcohol,H-bonded, O-H stretch
9	3837.26	O-H	Alcohol(free), OH stretch

Disubstituted trans alkenes' C=C bending vibration was characterized by peak values at around 969.9349 cm<sup>-1</sup>. It was thought that the absorption at around 1657.185 cm<sup>-1</sup> was caused by the C=C stretching vibration of alkene (vinilydene) compounds. Based on previous research, it was shown that carbodimide compounds exhibited an absorbance of approximately 2311.931 cm<sup>-1</sup> for their O=C=O stretching vibration and a wavelength of around 2121.931 cm<sup>-1</sup> for their N=C=N stretching vibration [17]. The band surrounding 2496.026 cm<sup>-1</sup> was identified as the stretching vibration of the S-H bond in thiol compounds. Alkane C-H stretching vibration was determined to be the culprit at its peak at around 2898.378 cm<sup>-1</sup>. The O-H stretching vibrations of carboxylic acids were thought to be responsible for the absorptions at around 3235.815 cm<sup>-1</sup>, while those of alcohols were found to be at 3492.431 cm<sup>-1</sup>. The stretching vibration of alcohol-free molecular bonds was measured at a wavelength of around 3837.26 cm<sup>-1</sup> [18].

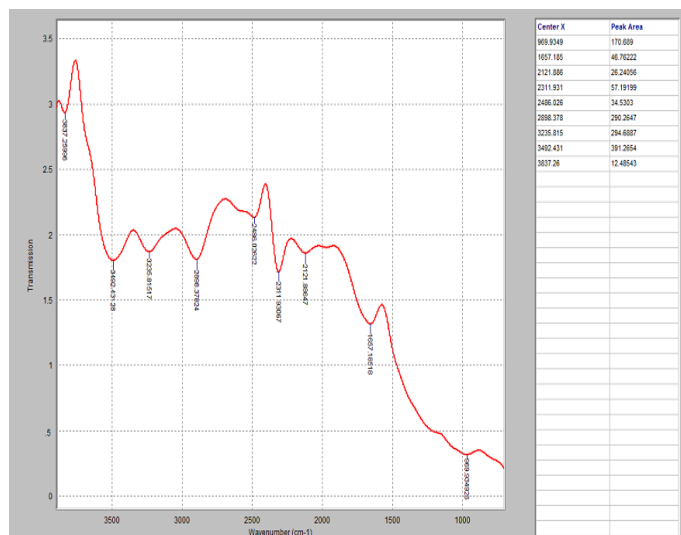


Figure 1: FTIR spectra of *Justicia carnea* leaf extract

### 3.2 GCMS analysis of *Justicia carnea* leaf extract

A total of twelve bioactive compounds derived from *Justicia carnea* extracts are listed in Table 2, along with their respective molecular formulas and retention times

Table 2: Compounds in leaf ethanol extract of *Justicia Carnea*

S/N	Name of compounds	Retention time (mins)	Formula Molecular
1	Cycloheptasiloxane,	7.463	C <sub>14</sub> H <sub>42</sub> O <sub>7</sub> Si <sub>7</sub>
2	Limonene	8.486	C <sub>10</sub> H <sub>16</sub>
3	Diethylmercaptopal	9.445	C <sub>20</sub> H <sub>32</sub> O <sub>10</sub> S <sub>2</sub>
4	Copaene	10.139	C <sub>15</sub> H <sub>24</sub>
5	α - Pinene	10.833	C <sub>10</sub> H <sub>16</sub>
6	2,6-Octadien-1-ol,3,7-Dimethyl,(Z)-	11.457	C <sub>10</sub> H <sub>18</sub> O
7	Bicyclo [3.1.0] hex-2-ene	12.027	C <sub>10</sub> H <sub>18</sub>
8	5-cyclopropylcarbonyl oxypentadecane	12.516	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>
9	Linalool	12.616	C <sub>10</sub> H <sub>18</sub> O
10	Ascaridole	21.239	C <sub>10</sub> H <sub>16</sub> O <sub>2</sub>
11	Benzene,1.2.3-trimeth oxy-5-(2-propenyl)-	22.750	C <sub>12</sub> H <sub>16</sub> O <sub>3</sub>
12	Androstonane-11,17-dione,3-[(trimethylsilyloxy]-,17-[O -(phenylmethyl) oxime], (3α, 5α)-	22.915	C <sub>29</sub> H <sub>43</sub> NO <sub>3</sub> Si

Along with its many other uses, ascaridonole can alleviate pain, relax the nervous system, and even eliminate worms and fungi [18, 19]. Being able to

limit the growth of *Plasmodium falciparum*, *Leishmania amazonensis*, and *Trypanosoma cruzi* gives it potential anti-parasitic properties [20].

Manokari et al. [21] reported that this ascaridole suppresses multiple tumor cell lines, including human leukaemia, breast cancer, and promyelocytic leukaemia. This information comes from their research. Based on their findings, Sharma et al. [21] concluded that ascaridone was useful in the treatment and management of eczema. Research has demonstrated that limonene and other compounds derived from plants can have positive effects on human health. According to studies done by Okocha et al. [11], limonene could have multiple medical uses, including aiding in weight loss, preventing cancer, and alleviating bronchitis. Destroying harmful microbes, lowering inflammation, and breaking down extra lipids and cholesterol are all within its capabilities. Several medical issues, including as diabetes, metabolic syndrome, gallstones, heartburn, and gastroesophageal reflux disease (GERD), have been found to benefit from limonene, according to research [20]. One of the key components of vitamin E absorption is linalool. Some of the many medical conditions it has been shown to alleviate include depression, anxiety, seizures, inflammation, psychosis, and lethargy. According to Martins et al. [23] and others, copaene has a number of useful qualities, including antibacterial, anti-oxidant, anti-proliferative, anti-genotoxic, and cytotoxic effects. One of these is that it increases the antioxidant capacity in cultures of human lymphocytes.

Among 2,6-octadien-1-ol, 3,7-dimethyl-, (z)- (Nerol)'s many medicinal applications are the

following: alleviation of spasms and fungal infections; soothing of anxious individuals; and prevention of viral infections in birds. Also, it helps battle chick lymphoma and avian leucosis thanks to its antineoplastic characteristics [24]. According to Silva et al., pinene has fungicidal, antiviral, and antifungal effects [25]. Because of its anti-inflammatory, anti-tumor, and chemotherapy-supportive properties, pinene has also demonstrated efficacy against leukemia and breast cancer [26]. Adjuvant research has shown that bicyclo [3.1.0] hex-2-ene significantly enhances drug delivery to the brain. Useful in preventing pathological damage to the blood-brain barrier and enhancing the delivery of many drugs to their intended sites while balancing their effects, this potent messenger drug is a staple of traditional Chinese medicine [27]. Moreover, it protects cells from induced toxicity and has anti-coagulative and anti-oxidative effects, which reduce the risk of cardiovascular disease [28].

### 3.3 *Biological activity of of Justicia carnea leaf extract*

#### 3.3.1 *Antimicrobial activity of of Justicia carnea leaf extract*

The antimicrobial activity of *Justicia carnea* extract is shown in Table 3, with four clinical pathogens.

**Table 3: Bioactivity of of *Justicia carnea* leaf extract**

S/No.	Pathogens	Concentrations (g/L)/Inhibition diameter (mm)					
		100 mg·L	10 mg/L	1 mg·L	0.1 mg/L	0.01 mg/L	0.001 mg/L
1	<i>E. coli</i>	13.5	11.2	10.4	9.0	*8.5	0
2	<i>S. aureus</i>	7.6	7.0	*6.5	0	0	0
3	<i>S. typhi</i>	10.8	9.8	9.1	7.0	5.0	*4.5
4	<i>B. subtilis</i>	6.3	*4.7	0	0	0	0

Findings demonstrated that, when tested against *Salmonella typhi*, the extract from *Justicia Carnea*

had the highest minimum inhibitory concentration (MIC) at 0.001 mg/L. An MIC of 0.01 mg/L indicated that the extract had a secondary impact against *Escherichia coli*. Because its MIC was 10.0 mg/L, not even *Bacillus subtilis* could survive. *Aspergillus flavus* has an inhibitory zone of 20 mm, which is consistent with what Xia et al. [29] discovered. *Jatropha curcas* possesses a wide, and

higher inhibitory zone against many clinical isolates, as demonstrated by Nna et al. [14].

**Antioxidant activity of *Justicia carnea* leaf extract**

The hydroxyl and superoxide radical scavenging activity of *Justicia carnea* extract is shown in Table 4, with ascorbic acid with used as a standard.

**Table 4: Hydroxyl and superoxide radical scavenging activity of *Justicia carnea* leaf extract**

Concentration	Peroxide radical	Superoxide radical	Ascorbic acid
0.05 mg/L	50.19	55.66	40.16
0.10 mg/L	55.75	57.60	55.20
0.5 mg/L	70.41	74.57	70.84
1.0 mg/L	65.43	67.56	68.78

The results demonstrate that the *Justicia Carnea* extract has the maximum capacity to inhibit radicals at a concentration of 0.50g/ml. A quantity of 0.05g/ml neutralizes the peroxide and superoxide radicals to the lowest possible level. As with the control condition, the compound's ability to neutralize peroxide and superoxide radicals starts to diminish at concentrations greater than 0.50 g/ml. Trials with the *Justicia Carnea* ethanol leaf extract had less than desirable outcomes compared to ascorbic acid [30, 31]. According to Sharma et al. [22], the ethanolic extract of the green shell of *Juglans regia* shown enhanced activity.

Many plant phytochemicals have the ability to bind to free radicals and protect cells from their harmful effects [11, 14]. Vitamins, alkaloids, and flavonoids are found in the ethanol leaf extract of *Justicia carnea* plants, which gives them antioxidant qualities. See how the abundance of inhibitory compounds in plants relates to their antioxidant capacity in Fig. 1. The figure shows that the radical scanning potential with superoxide radical is highest for *Justicia carnea* ethanol leaf extract.

**4.0 Conclusion**

A wide range of ailments can be cured or alleviated with the medicinal herb *Justicia Carnea*. The ethanol extracts were analyzed using GC-MS, and several phytochemical components were found. These components show promise as therapeutic agents. The *Justicia Carnea* extract shows a wide range of antibacterial properties, making it a possible candidate for antimicrobial treatment. The extensive range of medical applications made possible by its wide activity spectrum justifies its recommendation. The pharmacological and bioactive components of the extract make it a potential candidate for use in the development of novel pharmaceuticals.

**Conflict of Interest Declaration**

The authors declare that there is no conflict of Interest.

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