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Research Article

Analysis of the Active Metabolites of Ethanol and Ethyl Acetate Extract of *Justicia carnea*

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ABSTRACT

Justicia carnea is a medicinal plant that has been used traditionally as a blood tonic and has other diverse therapeutic benefits for humans since time immemorial in Nigeria. This study is aimed at the analysis of the active metabolites in ethanol and ethyl acetate extracts of *Justicia carnea.* A powdered sample was extracted using absolute ethanol and ethyl acetate and concentrated with a rotary evaporator. Active metabolites were identified *via* column chromatography (CC) and gas chromatography-mass spectrometry (GC-MS). Ethanol was a more effective solvent for the extraction as most of the phytochemicals were identified in this extract. There was also extraction of other classes of active metabolites with a mixed solvent of petroleum ether, diethyl ether, and ethanol from crude ethyl acetate extract. Results from this study identified some active metabolites that possess a lot of therapeutic properties such as anti-microbial, anti-inflammatory, anti-diabetic, immune modulatory, and antimalarial activities that are of immense benefit to man.

Keywords: *Active metabolites, Justicia carnea, anti-microbial, anti-inflammatory, anti-diabetic, immune modulatory*

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INTRODUCTION

For thousands of years, plants have been used worldwide in numerous ways for their healing properties. Plant-based natural products have also been studied for use in pharmaceutical therapy because of their anti-inflammatory, antioxidant, and anticancer properties (Parsaeimehr *et al.*, 2017). In this field of study, the roots, stems, leaves, flowers, or seeds of plants have been used by researchers to improve healthy living, thus preventing disease and treating illness. Currently, medicinal research has estimated that 70–80% of the world population relies on herbal medicine for their primary healthcare needs (Akinyemi *et al.*, 2018).

The outbreak of modern illness, poverty, and the failure of orthodox medicine in western Africa has made many increase their faith in traditional medicine. It is also worthy of note that herbal medicine is cheap, affordable, and available. Thus, many families see it as a source of treatment (Kitadi *et al.*, 2019). Malnutrition as a result of poverty has left many people anaemic as well as vulnerable to various diseases. The non-affordability and non-accessibility of modern drugs has become a huge threat to most Nigerians, giving rise to plant therapy adaptation (Correa *et al.*, 2012; Anarado *et al.*, 2021).

Medicinal plants have been documented as having beneficial properties used for the management of various ailments because they have been discovered to contain bioactive compounds called phytochemicals (Fasuyi, 2007) and secondary metabolites that can protect humans against diseases (Kumar *et al.*, 2009). Examples of some documented plants and fruits used in the treatment of anaemic conditions are Cocos nuciferia (Benin Republic) (Tchogou *et al.*, 2016), leaf extracts of Tectona grandis (Togo) (Ani *et al.*, 2020) and Justicia secunda Vahl in Benin (Kpoviessi *et al.*, 2006). The availability of synthetic drugs used in the treatment of a specific disease is common, but because of the high cost and side effects associated with their use (Chattopadhyay & Bandyopadhyay, 2005; Oze *et al.*, 2008), attention is currently being focused on the use of medicinal plant products in the prevention or management of various diseases or ailments.

Justicia carnea is a medicinal plant that has been used conventionally as a blood tonic since time immemorial in Nigeria. The resultant liquid after boiling is usually crimson red (Anarado *et al.*, 2021). Local consumers of the leaves are mostly anaemic patients, women who want their blood replenished after the menstrual cycle, and pregnant women. It is generally considered an ornamental plant and is widely

distributed in various parts of Africa. In Nigeria, it is grown around homesteads and acts as a fence (Onochie *et al.*, 2020; Otuokere *et al.*, 2016).

Justicia Carnea has been proven by researchers who used animal models in a new study to possess blood-boosting properties more effective than blood tonics. This enables it to effectively restore blood levels to normalcy within a short period of treatment. Some tribes in Nigeria have given it names like "Hospital too far," "Blood of Jesus," "blood leaf," and "Jehovah Witness leaf" (Onyeabo *et al.*, 2017; Anarado *et al.*, 2021).

Traditionally, several species of Justicia Carnea are used in the management of inflammation, gastrointestinal disorders, respiratory tract infection, fever, pain, diabetes, diarrhoea, liver diseases, rheumatism, and arthritis (Badami et al., 2003; Corrêa & Alcântara, 2012). They also possess anti-inflammatory, anti-allergic, anti-tumoral, anti-viral, and analgesic activities (Radhika et al., 2013).

Several studies have found many metabolites in Justicia Carnea that have been linked to certain illnesses. The metabolites include flavonoids, alkaloids, diterpenoids, vitamins, triterpenoids, iridoids, steroids, coumarin, lignans and triterpenoidal glycosides (Badami et al., 2003). Lignans have been reported to be major components of most extracts of species of Justicia and have shown some pharmacological actions such as anti-inflammatory, male contraceptive, hallucinogenic, superoxide anion radical scavenging, haematinic, antimicrobial, antisickling, immunomodulatory, antihypertensive, and antiviral activities (Corrêa & Alcântara, 2012; Ukpabi-Ugo et al., 2020). Many species of Justicia have been traditionally used in treating respiratory tract infections, inflammation, gastrointestinal disorders (Wood et al., 2020; Anarado et al., 2021), fever, diabetes (Khan et al., 2017; Manokari et al., 2019), liver diseases, diarrhoea (Aziz et al., 2017), arthritis, rheumatism (Arogbodo et al., 2020; Onochie et al., 2020), anaemia (Udedi et al., 2020), cancer, HIV, and diabetes (Corrêa & Alcântara, 2012).

Therefore, to substantiate the claim of the therapeutic properties and commercial values of Justicia Carnea, scientists have increased their investigation into the quantification and identification of active metabolites in the extracts of the plant. The aim of this study is the quantitative identification of the metabolites of ethanol and ethyl acetate extracts of the leaves of Justicia carnea and a review of their medicinal values for the benefit of man

MATERIALS AND METHODS

Collection and Preparation of Samples: Fresh leaves of *Justicia Carnea* plant were obtained from a botanical garden on the Abuja campus, University of Port Harcourt, Rivers State, Nigeria and taken to the Plant Science and Biotechnology laboratory of the University of Port Harcourt for proper identification with Ref No. UPH/PSB/2019/013. The fresh, healthy leaves of *Justicia Carnea* were air dried under shade for fourteen days and then grounded to form a fine powder.

Extraction with Ethanol: The powdered plant sample (100g) of *Justicia Carnea* plant was extracted with 1,250ml of ethanol using a soxhlet extractor for 18 hours. The solvent of the crude extract was evaporated *via a* rotary evaporator. The crude extract was subjected to GC-MS analysis for the identification of its components.

Extraction with Ethyl Acetate: Fresh leaf samples of the *Justicia Carnea* plant (2 kg) were boiled with 6 L of water for 50 min. The leaves were sieved out and a coloured solution of the plant was obtained. The solution was further boiled to evaporate the excess water. The solution was allowed to cool after evaporation, saturated with salt, and then decanted into a big conical flask ready for extraction. The extraction was done with ethyl acetate using a separating funnel. The extract obtained was dried with calcium chloride, filtered and simple distillation was used to concentrate the extract. The ethyl acetate extract (crude) of the leaves was subjected to thin layer chromatography (TLC) with the stationary phase of silica gel and the mobile phase of petroleum ether/diethyl ether/ethanol gradient, giving 8 fractions which were pooled based on similarities in Rf values, reducing the fractions finally to four. The fractions were further analysed using GC-MS equipment.

Figure 1: *Justicia carnea* leaves

Gas Chromatographic-Mass Spectrometric Analysis of Extracts: GC-MS analysis on the ethanol and ethyl acetate extracts was carried out using an Agilent 7890A5975C GC-MS system, employing the following conditions: operating at 70 ev in electron impact mode; carrier gas flow (constant) was 1 mL/min, injection volume was 0.5 L with a split ratio of 10:1, and injector temperature was 250°C.source temperature was 280 °C and the oven's initial temperature was 110 °C (hold for 2 mins). 110 to 200 °C at a rate of 10 °C/min

Identification of bioactive compounds: Elucidation of the GC mass spectrum was achieved using the database. The spectra of the unknown components were compared with the known components stored in the National Institute of Standards and Technology (NIST) library. The molecular weights and structures of bioactive compounds in the extracts were established in Table 1-5.

RESULTS

The bioactive compounds of ethanol from the leaf extract of Justicia Carnea are shown in Table 1, with twelve different compounds identified. The retention time, molecular formula, and weights are also listed. Most of the structures of compounds on the internet have been slightly modified..

Table 1:
Compounds from Ethanol Extract

S/N	Name of compounds	Retention time (mins)	Formula	Molecular weight (g/mol)
1.	Cycloheptasiloxane, tetradecamethyl	7.463	$C_{14}H_{42}O_7Si_7$	518
2.	Limonene	8.486	$C_{10}H_{16}$	136
3.	D-Fructose,diethylmercaptal, pentaacetate	9.445	$C_{20}H_{32}O_{10}S_2$	496
4.	Copaene	10.139	$C_{15}H_{24}$	204
5.	∝ - Pinene	10.833	$C_{10}H_{16}$	136
6.	2,6-Octadien-1-ol, 3,7-Dimethyl,(Z)-	11.457	$C_{10}H_{18}O$	154
7.	Bicyclo [3.1.0] hex-2-ene, 2-methyl-5-(1-methylethyl) -	12.027	$C_{10}H_{18}$	136
8.	5-cyclopropylcarbonyl oxypentadecane	12.516	$C_{19}H_{36}O_2$	296
9.	Linalool	12.616	$C_{10}H_{18}O$	154
10.	Ascaridole	21.239	$C_{10}H_{16}O_2$	168
11.	Benzene,1.2.3-trimeth oxy-5-(2-propenyl)-	22.750	$C_{12}H_{16}O_3$	208
12.	Androstonane-11,17-dione,3-[(trimethylsily)oxy]-,17-[O -(phenylmethyl) oxime], (3∝, 5∝)-	22.915	$C_{29}H_{43}NO_3Si$	481

Table 2:
Compounds from Ethyl Acetate Extract

S/N	Name of compounds	Retention time (mins)	Formula	Molecular weight (g/mol)
1.	2-butyne-1,4-diol diformate	38.002	$C_6H_6O_4$	142
2.	Bicycle[2.2.1]heptan-2-ol,1,7,7-trimethyl-, (1 S-endo)-	38.267	$C_{10}H_{18}O$	154

Table 3:
Compounds from E_1 Fraction

S/N	Name of compounds	Retention time (mins)	Formula	Molecular weight (g/mol)
1.	Cyclobut-l-enyl methanol	4.140	C_5H_8O	84
2.	D:A-friedooleanan-7-one, 3-hydroxy	6.510	$C_{30}H_{50}O$	442
3.	Cyclohexane,l,l-oxybis-	8.433	$C_{12}H_{22}O$	182
4.	Benzene,(l-ethyl decyl)-	8.628	$C_{18}H_{30}$	246
5.	10,13-octadecadiynoic acid, methyl ester	9.757	$C_{19}H_{30}O_2$	290
6.	Phthalic acid, butyl undecyl ester	10.251	$C_{23}H_{36}0_4$	376
7.	Dibutyl phthalate	10.986	$C_{16}H_{22}O_4$	278
8.	Lormetazepam	12.769	$C_{16}H_{12}C_{l2}N_2O_2$	334
9.	Bis(2-ethylhexyl) phthalate	12.951	$C_{24}H_{36}O_4$	390

Table 4:
Compounds from E_2 and E_3 fraction

S/N	Name of compounds	Retention time (mins)	Formula	Molecular weight (g/mol)
1.	Pentanamide, N-(3,4- dichlorophenyl)-2-methyl-	8.322	$C_{12}H_{15}Cl_2NO$	259
2.	Thiopental	11.080	$C_{11}H_{18}N_2O_2S$	242
3	Butylated hydroxyl toluene	7.763	$C_{15}H_{24}O$	220
4	3-Cylopropylcarbonyl oxytridecane	11.998	$C_{17}H_{32}O_2$	268

Table 5:
Compounds from E_4 Fraction

S/N	Name of compounds	Retention time (mins)	Formula	Molecular weight (g/mol)
1.	1,2-Benzendicarboxylic acid, butyl octyl ester	10.416	$C_{20}H_{30}O_4$	334
2.	3-(Prop-2-enoyloxy) tetradecane	13.357	$C_{17}H_{32}O_2$	268
3.	Androstane-11,17-dione, 3-[(trimethylsily)oxy]-, 17-[O-(phenylmethyl)oxime], (3∝,5∝)-	14.139	$C_{29}H_{43}NO_3Si$	481
4.	1,8-Bis(spiro-4,5-dimethyl-1,3-dioxolan-2-yl)-8a-methyldecalin	27.150	$C_{19}H_{32}O_4$	324
5.	1,2-Benzisothiazol-3-amine	28.009	$C_{10}H_{14}N_2SSi$	222

The bioactive compounds of ethyl acetate of the leaf extract of *Justicia Carnea* are shown in Table 2, with two different compounds identified. The retention time, molecular formula, and weights are also listed.

Column Chromatographic Analysis (CC): The column chromatographic analysis of the ethyl acetate extract showed four components. Its bioactive compounds are shown in Tables 3, 4 and 5. The tables contain their molecular masses, formulas, and retention times. The first fraction labelled E1 contains nine bioactive compounds, E2 and E4 each contain two bioactive components, and E3 consists of five bioactive compounds.

DISCUSSION

Ethanol and ethyl acetate extracts from the leaves of *Justicia Carnea* were used for the GC-MS analysis. This technique gave detailed information about the plant and its components. Results of the ethanol extract (Table 1) identified twelve bioactive compounds, while ethyl acetate extracts (Table 2) showed two compounds, respectively. Two of the bioactive metabolites that are found both in ethanol and ethyl acetate extracts have the same molecular mass and formula but different retention times. Most probably, they are isomers of the same compound.

Ascaridole is an analgesic, tranquilizing, and antifungal agent and worm-expeller (Dembitsky e*t al*., 2008). Its activities on *Leishmania amazonensis*, *Plasmodium falciparum*, and *Trypanosoma cruzi* show that it is a potential parasite inhibitor (Ukpabi-Ugo *et al*., 2019). Different kinds of tumour cell lines, such as breast cancer, human leukaemia, and promyelocytic leukaemia, have been suppressed by this ascaridole (Manokari *et al.,* 2019). The use of ascaridole for the management and treatment of eczema has been reported to be effective (Penner *et al.*, 2014).

Limonene is a naturally occurring terpene in most citrus plants. Several studies have shown that limonene is one of the natural plant products with several health benefits. Badami *et al*. (2003) reported that limonene can be used to promote weight loss, prevent cancer, and treat bronchitis (Emedicine health 2021). It has the ability to dissolve excess fats and cholesterol, reduce inflammation, and kill disease-causing germs. Limonene has been confirmed to be useful in the prevention or treatment of diabetes, metabolic syndrome, heart burns, gastroesophageal reflux disease (GERD) and gallstones (Sun, 2007). In manufacturing industries, limonene is also used as a raw material to produce hand sanitizers, perfumes, botanical pesticides, and chemical solvents (Onochie *et al*., 2020).

Linalool is very important in the release of vitamin E into the body for healthy functioning. It has been confirmed in medicine as an excellent sedative, anxiolytic, antiepileptic, anti-inflammatory, antipsychotic, and antidepressant. As a sedative, linalool is utilised as a treatment agent for anxiety. It is used as a fragrance agent by some manufacturing industries in the production of cleaning agents like soaps, detergents, shampoos, shower gels, and lotions. It is an effective mosquito repellent (Muller *et al*., 2009). Earlier studies have revealed that linalool possesses anti-epileptic activity that can prevent or remedy epilepsy by reducing the activity of brain chemicals involved in muscle contraction (Del-Re *et al*., 2000). Various rreports have confirmed that copaene possesses antimicrobial activity, anti-oxidant activity, anti-proliferative activity, anti-genotoxic activity, and cytotoxic activity (Martins *et al*., 2015). In medicine, 2,6-octadien-1-ol, 3,7-dimethyl-, (z)-(Nerol) is used to fight fungal infections and ant-food spoilage, as well as to treat spasms, calm down people who are stressed, and fight viruses in the aviary. It also has antineoplastic properties, which can fight avian leucosis and chick lymphoma in chicks (Marques et al., 2013). Silva *et al*. (2012) confirmed the fungicidal, antiviral, and antimicrobial properties of pinene. Further research established Pinene as an anti-breast cancer and leukaemia (Zhou & Yu, 2004), anti-inflammatory, anti-tumor, and chemotherapy supplement (Salehi et al., 2019).

Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl, (15-endo) in Table 2 has been discovered through research as an effective adjuvant that can improve drug delivery to the brain. It protects the structural integrity of the blood-brain barrier against pathological damage (Zhang *et al*., 2017) and is an efficient messenger drug in traditional Chinese medicine, enhancing the transport of multiple drugs to their particular sites and harmonising the effects of those drugs (Yin & Wang, 2017) and has anti-oxidative and anti-coagulative properties (Manokari *et al.,* 2019), protects cells from induced toxicity (Zhen *et al.* 2019), and anti-coagulative property with a reduced risk of heart disease. (Li *et al*., 2008).

The E_1 fraction of ethyl acetate extract obtained from column chromatography with the mobile phase of petroleum ether/diethyl ether/ethanol consists of the different nine

bioactive constituents shown in Table 3. These bioactive compounds are different from the crude extraction of ethyl acetate shown in Table 2 and ethanol extraction in Table 1. The prevailing compounds in the E_1 fraction are lormetazepan, known to be methyl-lorazepam, has been investigated by Domenick *et al.* (2020) and Nicholson and Stone (1982). They were with the affirmation that lormetazepan possesses anticonvulsant, sedative, hypnotic, skeletal muscle relaxant, and anxiolytic properties.

The E_2 fraction of ethyl acetate extract obtained from column chromatography with the mobile phase of petroleum ether/diethyl ether/ethanol consists of two bioactive constituents shown in Table 4. Maddison *et al*. (2008) associate thiopental with the ability to reduce intraocular pressure, so it can be administered to patients with problems like glaucoma, deep corneal ulcers, and penetrating eye injury. Pentanamide, N-(3,4-dichlorophenyl)-2-methyl, is another component of E_2 that has been used to inhibit the growth of Mycobacterium tuberculosis (Badami *et al.,* 2003). The bioactive components of E_3 are butylated hydroxyl toluene and 3-Cylopropylcarbonyl oxytridecane, with their retention times, molecular masses, and formula shown in Table 4. 3-Cylopropylcarbonyl oxytridecane induced leucopenia, neutrophilia, and marked lymphocytopenia in both non-vaccinated and vaccinated mice (Tryphonas *et al.,* 1999). Previous findings revealed that 3-Cylopropylcarbonyl oxytridecane has a suppressive effect on lymphocyte functions with a significant increase in granulocytes and a marked decrease in leucocytes (Tryphonas *et al.*, 1999; De Stefani *et al.,* 2004). There is a lot of good evidence that BHT can help treat genital herpes and AIDS (De Stefani *et al.,* 2004).

The E_4 fraction of ethyl acetate extract obtained from column chromatography with the mobile phase of petroleum ether/diethyl ether/ethanol consists of five bioactive constituents shown in Table 5. 1,2-Benzendicarboxylic acid, butyl octyl ester, and 1,2-Benzisothiazol-3-amine have the highest percentage abundance. Viani *et al*. (2017) confirmed the efficacy of 1,2-Benzisothiazol-3-amine derivatives on Escherichia coli (Gram-negative), Pseudomonas aeruginosa (Gram-negative), Bacillus subtilis (Gram-positive), Streptococcus faecium (Gram-positive), and Staphylococcus aureus (Gram-positive). Cotter *et al.* (2005) affirmed that 1,2-Benzendicarboxylic acid and butyl octyl ester are basically fatty acids with antimicrobial, antioxidant, cytotoxic, antifungal, and antitumor properties. The other bioactive compounds in this fraction are in table 5 and will aid in the biological importance of *Justicia Carnea.*

In conclusion, *Justicia Carnea* is a beneficial therapeutic plant used for the management and cure of many ailments. The GC-MS analysis of ethanol and ethyl acetate extractions identified some phytochemical metabolites of high medicinal value. The column chromatographic analysis of ethyl acetate extracts afforded four fractions whose GC-MS analysis gave rise to eighteen different compounds, which were confirmed by the Rf values and the molecular masses. A total of thirty different bioactive compounds were identified from the ethanol and ethyl acetate of the leaf extract of the plant. The chemical molecular formulae, molecular weight, retention times, and signal intensities were all elucidated in the mass spectrum of the identified compounds. Ethanol was more efficient than ethyl acetate in the extraction. The presence of Thiopental and 1,8-bis(spiro-4,5-dimethyl-1,3-dioxolan-2-yl)-8a-methyldecalin should attract more investigation to this method of ethyl acetate extraction. Extracts from the leaves of the plant possess a lot of therapeutic properties which are of immense benefit to man. However, we suggest that more research be carried out on the absolute characterization, toxicity, and mode of action of the bioactive compounds isolated to guarantee their use in modern medicines

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