



Comparative Study of Proximate and Phytochemical Analysis of the Roots of *Justicia carnea* Lindl. and *Justicia secunda* Vahl

Ajuru, M.G.* , Kpekot, A. K., Omubo, J. and Morrison, I.

Department of Plant Science and Biotechnology, Rivers State University, Nkpolu-Oroworuokwo, P.M.B. 5080, Port Harcourt, Rivers state, Nigeria.

*Corresponding Author: ajurumercygospel@yahoo.com,

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Abstract

Proximate and phytochemical analyses of the root extracts of *Justicia carnea* and *Justicia secunda* in the Acanthaceae family were investigated using standard procedures and compared. The result of the proximate analysis indicated that the roots of both species contained nutrients in varying levels. Protein (11.38%), lipid (4.81%) and moisture content (17.01%) were higher in the roots of *J. carnea* than in *J. secunda* (8.32%, 2.01%, 11.22%) respectively, while Carbohydrate (6.62%), Ash (16.75%) and Fibre (55.18%) were higher in *J. secunda* than in *J. carnea* (4.73%, 9.81%, and 52.29%) respectively. Qualitative phytochemical analysis showed the presence of tannin, flavonoid, alkaloids, saponin, phytate and cyanogenic glycosides whereas the result for the quantitative analysis showed that the quantity of phytochemicals in *Justicia carnea* and *Justicia secunda* were: tannin (46.75% and 41.01%), flavonoid (2.72% and 3.21%), alkaloid (10.16% and 11.21%), saponin (3.31% and 2.7%), phytate (30.54% and 33.86%) and cyanogenic glycoside (100.01% and 500%) respectively. *Justicia carnea* contained higher quantity of tannin and saponin while *Justicia secunda* contained higher amount of flavonoid, alkaloid, phytate and cyanogenic glycoside. These results conceivably indicated that the two species are good sources of essential nutrients which could be used in diets to supplement the daily nutrient needs in humans and animals, and phyto-nutrients which possess strong pharmacological activities, providing scientific credence for its therapeutic usage in folklore medicine.

Keywords: Acanthaceae, *Justicia carnea*, *Justicia secunda*, Phytochemical analysis, Proximate composition

Introduction

Many African studies have shown that numerous indigenous plants play important role in the diet of the populace, and are the cheapest and most available sources of important nutrients for the body (Amaechi, 2009; Muhammed *et al.*, 2011). Medicinal plants species contain substances needed for therapeutic purposes (Sofowora, 2008). Medicinal plants play important role in maintaining both the health and wealth of mankind. Most times, the world population use herbal medicines. Reports by the World Health Organization (WHO) show that approximately 21,000 plants have been used for medicinal purposes (Sofowora, 1993). Herbs are safe, efficient, and culturally acceptable with minimal side effects (Trease and Evans, 2002).

The wide diversity of foodstuffs found in the tropics plays a basic role in nutrition and healthy development of the bodies of a large number of the populations. An increased use of foods rich in carbohydrates, proteins, fats, etc can greatly reduce malnutrition especially in the rural communities. The dearth of information and insufficient development of nutritionally improved products from locally sourced materials have direct bearing on nutrition.

Currently, there has been increased interest in the use of herbal plants to improve health, and herbs are regarded as one of the first real functional food. Herbs enhance the flavour and taste of our foods as well as being useful as sources of dietary medicine, though people do not concern themselves with their nutritional composition (Oshodi, 1992; Ejoh *et al.*, 1996; Uhegbu *et al.*, 2011). Under-utilization of these herbs is as a result of ignorance of their nutritional properties and presence of some phytochemicals (Kaitadi *et al.*, 2019).

The presence of some secondary metabolites, collectively called phytochemicals, provides therapeutic power to the plants (Austin, 2004; Sofowora, 2008). These phytochemicals have potential to be developed as herbal medicines or could serve as precursors for modern medicine. Such plants have parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds used in the treatment of diseases and therefore contains chemical components that are medically active (Sofowora, 2008). Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic, lignins,

stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites which are rich source of free radical scavengers (Zheng and Wang, 2001; Gracelin *et al.*, 2012; Cathrine and Nagarajan, 2011). Our forefathers harnessed these phytochemicals to treat several diseases without the knowledge of the role played by phytochemicals in the treatment. In recent years, there has been a worldwide trend towards the use of the natural phytochemicals present in fruits and vegetables (John *et al.*, 2013; Wang and Jiao, 2000). Presently, phytochemicals such as glycosides, flavonoids, benzenoids, phenolic compounds, naphthoquinone and triterpenoids are active chemicals found in herbal plants (Awan and Aslam, 2014; Jayapriya and Gricilda, 2015).

The plant *J. secunda* belongs to the Acanthaceae family. It is locally called 'Asindiri' or 'Ohowaazara' (meaning medicine that gives blood) by the Ogbia people of Otuoke-Otuaba community in Ogbia Local Government Area of Bayelsa State, and other communities in the Niger Delta region of Nigeria (Osioda *et al.*, 2017; Perveen and Qaiser, 2010). It is used for the treatment of anaemia. Other common names are 'Hospital too far', 'Blood leaf' or Blood tonic. Its aqueous extract is normally served as a tea drink to the anaemic patients in these communities. *Justicia secunda* (Acanthaceae) is a plant used in traditional medicine in Cameroon in the treatment of anemia (Fongod *et al.*, 2013). Some studies have also shown that the plant possesses anti-anemic and antimicrobial properties (Chen *et al.*, 2012; N'Guessan *et al.*, 2010). Compared to many plants whose iron contents are known, *Justicia secunda* appears to be a very important source of iron. These high iron contents (240 mg/g 19) justify its use as anti-anemic agent in the Congolese popular medicine (Achi *et al.*, 2017; Moswa *et al.*, 2008).

Decoctions of the leaves of *J. secunda* are used traditionally for the treatment of anemia in humans (Fongod *et al.*, 2013). Usually these decoctions are prepared only when need arises and in quantities that can be consumed before deterioration. It is worth noting that empirical observations in the field have shown that it is difficult to store this beverage beyond 3 days. Since the leaves are equally very perishable, the availability of the leaves and/or its decoctions out of season is very limited. To prolong the shelf-life

of this drink, there is need to pasteurize and to stabilize it by the addition of an anti-bacteria agent which can delay the development of microorganisms during storage.

Justicia carnea is a flowering plant that belongs to Acanthaceae family, widely distributed in various parts of Africa. In Nigeria, the shrubs of *J. carnea* commonly called ‘Hospital Too Far or Blood of Jesus’ and are grown around homesteads and act as fences (Sharma, 2004), which are easy to grow and propagate from stem cuttings by pushing the stems 1 to 2 inches into the soil. Onyeabo *et al.* 2017 reported a survey among the Igbo local populace in Nigeria revealed that the plant under study is locally called “*ogwu obara*” meaning blood tonic. The deep purple coloured juice from the leaves of this plant is extracted either by soaking or boiling in water, which can be drunk as tea (Khan *et al.*, 2017; Onyeabo *et al.*, 2017). In other localities in Nigeria, the raw leaves are chewed and used together with “*nchu anwu*” as culinary vegetables to garnish yam porridge.

It is generally considered as an ornamental plant. This species of *Justicia*, like others, are widely used in folk medicine for the treatment of inflammation, respiratory, and gastrointestinal disorder (Parker and Pearson, 2012). Most of the medicinal properties exhibited by plant extracts such as antimicrobial, antioxidant, hypocholesterolemic and anti-cancerous are associated with their bioactive constituents mainly phenols and flavonoids (Chandon *et al.*, 2011; Janifer *et al.*, 2010; Uroko *et al.*, 2017). It has also been reported to be rich in both macronutrients and trace elements of which calcium and iron are in high quantity (Faiza *et al.*, 2013). *Justicia carnea* is used as blood tonic locally in many parts of Nigeria and there is no sufficient scientific evidence to support its use as blood tonic by traditional consumers. This research work is therefore focused on the determination of proximate and phytochemical contents of *Justicia carnea* and *J. secunda* in order to evaluate its pharmacological and nutritional values.

Materials and Methods

Sample collection and identification of the Plant Materials

The fresh and matured plants were collected from a farmland at Rumuigbo

residential area and by the roadside at Akwaka Junction, Rukpokwu area, all in Port Harcourt. Identification and authentication of the plants was done at the Herbarium in the Department of Plant Science and Biotechnology, Rivers State University. Voucher specimens were deposited in the Herbarium for reference purpose.

Preparation of Extracts

The fresh and matured roots of the collected and identified plants *Justicia secunda* and *Justicia carnea* were washed with distilled water, labeled accordingly and air-dried in the laboratory at ambient temperature (28-30°C) for 30 days. They were grounded into fine powder using sterile electric blender under laboratory condition. Fifty (50) grams of each of the powder was mixed with 500ml of Distilled water and ethanol in a sterile conical flask separately and stand for 3 days with intermittent shaking. The mixtures were filtered using filter paper and concentrated in water bath at 70 °C for 3 hours. The extracts were placed in two different well labeled sterile conical flask and refrigerated at 4 °C for further experiment.

Chemical Analysis

Proximate Analysis

The moisture content was determined by drying at 105°C in an oven until a constant weight was reached. For total ash determination, the leaves samples were weighed and converted to dry ash in a muffle furnace at 450 and at 550°C for incineration. The crude lipid content was determined by extraction with hexane, using a Soxhlet apparatus. All these determinations were carried out according to AOAC (1990). Kjeldahl method was used for crude protein determination. Carbohydrate content was determined by calculating the difference between the sums of all the proximate compositions from 100%. Crude fibre values were obtained by multiplying the carbohydrate, protein and fat by the Atwater conversion factors of 17, 17 and 37, respectively (Kilgour, 1987).

Phytochemical analysis

The phytochemical screening of the plants for various phytochemical constituents such as phytate, flavonoids, alkaloids, cyanogenic glycoside, saponin and tannin was conducted

using standard methods as described by Sofowora (1993) and Trease and Evans (2002).

Statistical analysis

All the data were subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences version 17.0 for windows, SPSS Inc. Means were separated using Duncan Multiple Range Test where significant.

Results

Proximate composition

The proximate composition of the roots of *J. carnea* and *J. secunda* presented in Table 1 showed that the roots of both species contain nutrients in varying levels. Protein (11.38%), lipid (4.81%) and moisture content (17.01%) were of a higher percentages in the roots of *J. carnea* than in *J. secunda* (8.32%, 2.01%, 11.22%) respectively, while the percentages of Carbohydrate (6.62%), Ash (16.75%) and Fibre (55.18%) were higher in *J. secunda* than in *J. carnea* (4.73%, 9.81%, and 52.29%) respectively.

Table 1: Proximate Composition of Dried Roots of *J. carnea* and *J. secunda*

Parameter	<i>J. carnea</i> root (% dry weight basis) Mean \pm SD	<i>J. secunda</i> root (% dry weight basis) Mean \pm SD
Carbohydrate	4.73 \pm 0.02	6.62 \pm 0.00
Protein	11.38 \pm 0.01	8.32 \pm 0.01
Ash	9.81 \pm 0.01	16.75 \pm 0.05
Lipid	4.81 \pm 0.01	2.01 \pm 0.01
Fiber	52.29 \pm 0.00	55.18 \pm 0.01
Moisture content	17.01 \pm 0,01	11.22 \pm 0.02

Phytochemical Composition

The qualitative phytochemical composition of ethanol extracts of *Justicia carnea* and *Justicia secunda* is presented in Table 2.

The result indicated the presence of alkaloid, flavonoids, phytate, saponin, tannin and cyanogenic glycoside.

Table 2: Qualitative phytochemical compositions of *J. carnea* and *J. secunda* ethanol extracts

Parameter	<i>J. carnea</i>	<i>J. secunda</i>
Tannin	+	+
Flavonoid	+	+
Alkaloid	+	+
Saponin	+	+
Phytate	+	+
Cyanogenic Glycoside	+	+

+ indicates presence

The phytochemical analysis as shown in Table 3 revealed that phytate, saponin, flavanoid, tannin, cyanogenic glycoside, and alkaloid were present in the roots. The percentages of tannin (46.75%) and saponin (3.31%) were higher in *Justicia carnea* compared to the quantity in *Justicia secunda*, 41.01% and 2.7% respectively,

while the percentages of flavonoid (3.21%), alkaloid (11.21%), phytate (33.86%) and cyanogenic glycoside (500%) were higher in *Justicia secunda*, compared to the quantity in *Justicia carnea*: 2.72%, 10.16%, 30.54% and 100.01% respectively.

Table 3: Quantitative phytochemical compositions of *J. carnea* and *J. secunda* ethanol extracts.

Phytochemicals	<i>J. carnea</i> (Quantitative Analysis) (%)	<i>J. secunda</i> (Quantitative Analysis) (%)
Tannin	46.75 \pm 0.00	41.01 \pm 0.01
Flavonoid	2.72 \pm 0.01	3.21 \pm 0.00
Alkaloid	10.16 \pm 0.00	11.21 \pm 0.01
Saponin	3.31 \pm 0.01	2.7 \pm 0.00
Phytate	30.54 \pm 0.01	33.86 \pm 0.00
Cyanogenic Glycoside	100.01 \pm 0.00	500 \pm 0.00

Discussion

Proximate composition

The crude fibre contents in *J. carnea* (52.29 ± 0.00), was lower than that of *J. secunda* (55.18 ± 0.01). Epidemiological evidence suggest that increased fibre consumption may cause a reduction in the incidence of certain diseases including colon cancer, coronary heart diseases, diabetes, high blood pressure, obesity, and various digestive disorders (Food and Agriculture Organization, 1990; Scientific Advisory Committee on Nutrition, 2008).

The carbohydrate contents in *J. carnea* root were 4.73 ± 0.02 and in *J. secunda* root was 6.62 ± 0.00 . The nutrient was higher in *J. secunda* than in *J. carnea*. This means it could serve as source of energy. Carbohydrate is the principal constituent of all normal diets, providing most of the energy required. Carbohydrates are plant products which are synthesized as the by-products of photosynthetic processes. They are consumed by man and animals as the major source of energy. Carbohydrates are hydrolyzed in the body to yield glucose which can be utilized immediately, or stored as glycogen in the muscles and liver for future use (Raven *et al.*, 1999; Okeke *et al.*, 2008). The crude protein contents were 11.38 ± 0.01 in *J. carnea* root and 8.32 ± 0.00 in *J. secunda* root (8.32 ± 0.00). According to Pearson (1976), plant foods that contain more than 12% of its caloric value from protein are considered good source of protein. Therefore, both species are not a good source of protein, but they could still be used as supplements in protein deficient food. This is comparable to the daily protein requirement of 23-56g (FAO/WHO/UNU, 1991; Chaney, 2006). Proteins are important in the body for the production of hormones, enzymes and blood plasma. They are immune boosters and can help in cell division as well as growth (Okeke and Elekwa, 2006). Protein is an essential component of human diet needed for the replacement of dead tissues and for the supply of energy and adequate amount of required amino acids (Igite *et al.*, 2013).

The moisture contents in *J. carnea* root were 17.01 ± 0.01 and in *J. secunda* root was 11.22 ± 0.02 . Moisture or water is a universal solvent; it dissolves other substances, carries nutrients and other materials throughout the body, making it possible for every organ to perform its function effectively (McDonald *et al.*, 1998). The

moisture content of any food is an index of its water activity (Olutiola *et al.*, 1991; Pearson, 1994), and is used as a measure of stability and susceptibility to microbial contamination (Uraih and Izuagbe, 1990). The moisture content is within the range of required value as safe storage limit for plant food materials (Umar *et al.*, 2007). This indicates that the roots can be stored for a long time without the development of mould.

Ash contents were 9.81 ± 0.01 in *J. carnea* and that of *J. secunda* was 16.75 ± 0.05 . The ash content is a measure of the total mineral content of a food (Vunchi *et al.*, 2011). Mineral is required by the body for proper physiological functioning. Ash content of a plant based food is the function of the mineral elements present. Dietary ash has proved helpful in establishing and maintaining acid-alkaline balance of the blood system, as well as in controlling hyperglycaemia condition (Gokani *et al.*, 1992). The values compared favourably with the values reported in some Nigerian leafy vegetables.

Lipid contents in *J. carnea* was 4.81 ± 0.01 and in *J. secunda* 2.01 ± 0.01 . This is low and it suggests that it can be easily incorporated in weight reducing diet. The result indicated that the roots of both species are poor sources of plant lipid, which agrees with the general observation that leafy vegetables are low lipid containing food. Fats/oils are very important in human health as it serves as sources of energy and components of biological membranes (Michelle *et al.*, 1993). Fats are secondary plant products that yield more energy per gram than carbohydrates. Dietary fats are important not only because of their high energy value but the fat-soluble vitamins and essential fatty acids contained in the fat of natural foods. Fats and oils help to regulate blood pressure and play useful role in the synthesis and repair of vital cell parts (Dutta, 2003). The high content of these nutrients in the roots of these species makes them a good source of energy and crude fat.

Fibres are parts of fruits, grains and vegetables which can neither be digested nor absorbed by the human system (Agarwal and Rastogi, 1974). As an important part of any diet, fibre aids indigestion. An average adult is recommended to consume 18 to 32 grams of fibre daily. Generally, dietary fibres function in the body to slow down the rate of glucose absorption into the bloodstream, thereby reducing the risk of

hyperglycaemia (Boutwell, 1998). They also reduce the levels of plasma cholesterol and prevent colon cancer and cardiovascular diseases (Davidson et al., 1995). Fibre soften stool and therefore, prevents constipation (Ayoola and Adeyeye, 2009).

Phytochemicals

The results of the present study suggested that several phytochemicals are present in the ethanol extracts of the two species. Phytochemicals bestow colour, flavour, smell on plants, protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites and are part of plants natural defense system. The phytochemicals saponin, flavonoid, tannin, alkaloid, cyanogenic glycoside and phytate were present in *J. carnea* and *J. secunda* ethanolic extracts according to this study.

Tannin was found to be present in the root of both species of *Justicia* in the following quantity: 46.75 ± 0.00 and 41.01 ± 0.01 in *J. carnea* and *J. secunda* respectively. This is in line with the findings of Bedoya et al. (2008). The presence of tannin in the medicinal plant suggests the ability of these plants to play key roles as antifungal, antidiarrhoeal, antitumoral, antioxidant, antidiabetic and antihemorrhoidal agent. Tannins are regarded as anti-nutrient (Doss et al., 2011). The presences of tannins are responsible for the astringent flavour in plants.

The quantity of flavonoid was 2.72 ± 0.01 in *J. carnea* and 3.21 ± 0.00 in *J. secunda*. Similar result was reported by Adams et al. (2007). Flavonoids are the compounds which possess antiprotozoal, antibacterial and antiviral actions. These compounds having the capability of inhibiting important viral enzymes like reverse transcriptase and protease (Havsteen, 2002). Several mechanisms for anti-inflammatory action of flavonoids have been reported (Rathee et al., 2009). Progression of tumors (Kim et al., 1994); reduction of coronary heart disease has been reported to be associated with intake of flavonoid (Hertog et al., 1993). The presence of flavonoid in the roots supported the report of Edeoga et al., (2005); flavonoids are well known antioxidants (Tiwari and Rao, 2002).

The quantity of alkaloid in the roots of *J. secunda* was 11.21 ± 0.01 , greater than in *J. carnea* which was 10.16 ± 0.00 . The Same result

was also reported by Adams et al. (2007). Alkaloids are very important in medicine and constitute most of the valuable drugs. They have marked physiological effect on animals (Edeoga et al., 2005). Alkaloids in these species can be exploited for their importance in traditional pharmaceutical usage as they have a wide range of pharmacological activities including antimalarial (e.g., quinine), anticancer (e.g., homoharringtonine) (Kittakooop et al., 2014), antibacterial (e.g., chelerythrine) (Cushnie et al., 2014), and antihyperglycemic activities (e.g., piperine) (Qiu et al., 2014).

Saponin in the root of *J. carnea* and *J. secunda* were 3.31 ± 0.01 and 2.7 ± 0.00 respectively. This is in line with the investigation by Sridhar et al. 2014. Saponins are very important metabolites of plant and are known to possess both beneficial (cholesterol-lowering) and deleterious (cytotoxic permeabilization of the intestine) properties and also exhibit structure dependent biological activities. Elekofhinti (2015) reported that saponins protects against microbial attack in plants; Saponin is used in medicine and pharmaceutical industries because of its foaming ability with frothy effect.

Phytate and cyanogenic glycosides were both found in the root of the two *Justicia* species. The quantity of Phytate was 30.54 ± 0.01 in *J. carnea* and 33.86 ± 0.00 in *J. secunda*, while the quantity of cyanogenic glycosides was 100.01 ± 0.00 in *J. carnea* and 500 ± 0.00 in *J. secunda*. The glycosides result is in line with the findings of Bedoya et al. (2008). Glycosides are the well-known and well established class of phytochemicals which are having cardiogenic, antibacterial, antiviral, anticancer, antioxidant, anti-inflammatory, neuroprotective, hepatoprotective and immunomodulatory actions. They can also inhibit the oxidase enzymes like tyrosinase and useful in dermatological treatments (Fu et al., 2008).

Conclusion

Based on the findings of the present study, proximate and phytochemical constituents of *J. carnea* and *J. secunda* were determined. The phytochemical components of the two species contain alkaloid, saponins, flavonoids, cyanogenic glycoside, tannin and phytate. The results of the proximate analyses of the roots

indicated the presence of considerable amount of nutrients. The presence of the phytochemicals has authenticated its usefulness by traditional herbalists in ethno medicine and potentials in drug formulation and development. In addition to that, the presence of nutrients indicates that these plants can be used as food supplement.

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