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HPLC, GC QUANTITATIVE CHARACTERIZATION OF PHYTOCHEMICALS IN *JUSTICIA CARNEA* LEAF

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Abstract: **Phytochemicals are primary or secondary constituent depending on their role during metabolism in plant. The study investigated the quantitative characterization of phytochemicals present in leaves of *Justicia carnea.* Phytochemical screening of *Justicia carnea* was done using high performance liquid chromatography (HPLC) and gas chromatography (GC). The quantitative phytochemical screening of leaves of *Justicia carnea* indicated in descending order the presence of phytoconstituents; 744.24mg/100g (tanic acid), saponin 449.43mg/100g (Justisci-saponin-1 and sapogenin), sterols 30.58mg/100g (Sitosterol and Campesterol), , glycoside 39.27mg/100g (Kaemaferitrin and 0-sistosterol-3-B-glycoside) , terpenoids 8.55 x 10^{-1}mg/100g (Beta-amyrin and Alpha-amyrin) , oxalate 102.11ppm and phytate 12.36ppm. Leaves of *Justicia carnea* contain different phytochemicals with different therapeutic potentials which could serve as precursors in the synthesis of drugs as well as herbal agents in treatment of diseases.**

Keywords: Justicia carnea, **phytoconstituent, ethnomedicinal, chromatography.**

1. INTRODUCTION

The use of herbal plant in traditional medicine in the treatment of disease has been since time immemorial. Herbal plant has tremendously gained interest by researcher due to its pharmacotherapeutic agent as a result of the shortcomings of orthodox drugs available (Cordell, 1993). According to World Health Organization, medicinal plant is any plant in which one or more of its organ contains an active ingredient that can be used for therapeutic purpose or are precursors for the synthesis of useful drugs. An analysis on orthodox medicine revealed approximately one fourth of all medicine now in use are derived from over 2500 flowering plants (Dutta, 1999). The medicinal properties of these plants lie in the bioactive phytochemical constituent that produces definite physiological effect on human body. These natural substances formed the base of modern drugs that are in use (Edeoga *et al.*, 2005; Akinmo-laudn *et al.*, 2007; Rout *et al.*, 2009). Currently, over 25% of précised drugs are substance derived from plants (Zheng and Wang, 2001; Egwaikhide and Gimba 2007). The dependency of societies on herbal medicine is as a result of their effectiveness, affordability, availability, low toxicity and acceptability (Akharaiyi and Boboye, 2010). Natural substance contained in medicinal plant are complex chemical constituent derived from primary and secondary metabolite providing certain therapeutic effects (Firenzuoli and Gori, 2007; Doughari *et al.*, 2009). The different drugs obtained from different plant part show several therapeutic activities and different biologically and pharmacologically active compound which has been identified (Firn, 2010). These compounds are not used directly as therapeutic agents but as precursors for the synthesis of drugs or models for

pharmacologically active compounds (Li and Vederas, 2009). There are about 10,000 identified phytochemical and still a large number remains unknown. Phytochemical are biologically active, naturally occurring chemicals found in plants which provides health benefits for humans further than attributed to macronutrient and micronutrients (Hasler and Blumberg, 1999). Phytochemicals are classified as primary and secondary constituents. Primary constituent includes;common sugars, amino acids, proteins, chlorophyll, purines and pyrimidine of nuclei acids and secondary constituent includes; alkaloids, tannin, flavonoid, lignans, terpenoids, steroids, saponin and phenolic (Krishnaiah *et al.*, 2007; Barbosa *et al.*, 2013). They exhibit a wider range of biological activities, arising from their antioxidant properties, anti-inflammatory and analgesic strength and ability to boost the body's natural detoxification system (Fahey and Talalay, 1995).

The genus *Justicia* belongs to Acanthaceae family and was named after the 18th century Scottish horticulturist James Justice (Correa and Alcantara, 2012). This perennial shrub has a characteristic pink to purple or orange flower colour which blooms all through the summer. It grows 3-4 feet wide and height of 1.8m (Wasshausen and Wood, 2004). The flowering plant is a native of South Africa, precisely Brazil and widely distributed in Africa, even Nigeria. It is propagated by pushing 1-2 inches of the stem into the soil and kept until new leaves appear (Mabberley, 1997). *Justicia carnea* function mostly as ornamental plant (Parker and Pearson, 2012).Medicinal properties of several species of *Justicia* include; respiratory, gastrointestinal disorder, inflammation including application in rheumatism and arthritis, headache and fever which may be associated with sedative and analgesic properties, cancer, diabetes and HIV (Correa and Alcantara, 2012). *Justicia neesii* has been reported to exhibit antitumoral activity (Rajasekhar and Subbaraju, 2010).). Characterization based on GC-MS analysis of ethanol extract of *justicia carnea* suggested the presence of isonicotonic acid N-oxide, phosphinodithioic acid, palmitic aci, 7H-purine, 7-benzyl-2,6-dichloro,9,12,15-octadecatrien-1-ol and2,2,3,3,4,4,5,5,5-Nonafluoro-pentanoic acid methyl ester (Otuokere *et al.*, 2016). The haematological and lipid modulatory effect of aqueous and ethanol extract of *justicia carnea* yielded a positive effect as described by Orjiakor, (2014); Onyeabo *et al.*, (2017). According to Alozie *et al.*, (2018), aqueous extract of *justicia carnea* demonstrated considerable antiplasmodial activity. Presently, the study is aimed at investigating the phytoconstituents in leaves of *Jusicia carnea*.

2. MATERIALS AND METHODS

2.1 Source and Identification of plant material

Fresh leaves of *Justicia carnea* were harvested from Obinze and Obiti communities in Owerri-west and Ohaji Local Government Area, Imo State, Nigeria. The plant sample was identified by Dr. Ekeke Chimezie at the herbarium unit of the Department of Plant Science and Biotechnology, University of Port Harcourt. The specimen was registered with voucher number UPH/PSB/2017/055 and specimen deposited at the herbarium .

2.2 Plant extraction

Fresh leaves of *Justicia carnea* were harvested, washed and air-dried under a shade for seven days. The dried leaves were pulverized into coarse powder. The pulverized powdered leaves weighed 2.5kg and stored in air-tight containers until when needed.

2.3 Phytochemical screening

2.3.1. Saponin extraction

The plant extraction was done using the modified method of analytical science (2009). The leaf was pulverized and the saponin was extracted thrice with redistilled methanol. The saponins were extracted with 20ml of the solvent for 20 minutes with the aid of sonication. The combined extract was concentrated to syrup under reduced pressure, and was suspended in water. The suspension was extracted with petroleum ether, chloroform and 1-butanol. The combined extracts were filtered and concentrated to 1ml. 1µl was introduced into the port of gas chromatography for analysis.

2.3.2.Tannin extraction

The extraction was done according to the modified method described by (Swain, 1979). Exactly 0.2g of the powdered sample was measured into 50ml borosilicate beaker and 20ml of 50% methanol was added. This was placed in a water bath after covering with paraffin and placed in a water bath at 80^0C for an hour. Lumps were avoided by stirring using a glass rod. The extract was then filtered into a 100ml volumetric flask and rinsed with 50% methanol. This was concentrated to 2ml in the borosilicate vial for the gas chromatography analysis.

2.3.3. Phytosterol extraction

The extraction of phytosterol was according to the modified AOAC 994.10 and AOAC 970.51 official methods. Powdered sample of 0.5g was weighed and transferred to Stoppard flask and treated with petroleum ether until the powder was fully soaked. The flask was agitated hourly for first six hours and after 24 hours. Extract was filtered after repeating the process for three days and the extract filtered and dried using nitrogen steam. Saponification was carried out on the extracts using 3ml of 10% potassium hydroxide (KOH) in the ethanol and 0.20ml of benzene was added to aid in the mixing. 3ml of de-ionised water and 2ml hexane was added to separate the non-saponifiable sterols. Complete extraction of sterols was achieved by extracting with 2ml hexane at 1 hour, 30 minutes and 39 minutes.

2.3.4 Glycoside extraction

The extraction was done as described by the modified method of food technique (2007). A weight of 1.0g of the pulverized sample was soaked for 24 hours with 10ml of 70% of alcohol and then filtered and concentrated. The redistilled hexane was employed to replace the initial solvent and the hexane was concentrated to 1ml in the vial for gas chromatography analysis and 1µl was introduced into the port of gas chromatography.

2.3.5 Terpenes extraction

The extraction of terpenes was done according to the modified method of Romanian letters, (2009). The sample was pulverized and the terpenes constituent were extracted with redistilled chloroform. The terpenes were removed with 10ml of the solvent for 15minutes. The mixture was filtered and concentrated to 1ml and 1µl was introduced into the gas chromatography for analysts.

2.3.6 Flavonoids extraction

The pulverized sample was made to be devoid of water by drying to constant weight in an oven at 105^0C. About 1.0g of the sample was weighed into 250ml conical flask capacity with the addition of 100ml of de-ionised water. The mixture was boiled for 10 minutes. 100ml was added to methanol in ratio of 70:30 in a conical flask. The mixture was macerated for about 4 hours on the laboratory bench after which it was filtered using Whatman filter paper. The varying concentrations of the standard were arranged for injection into the high performance liquid chromatography (HPLC) system for calibration and correlation co-efficient establishment. Samples were injected into the HPLC system following the same procedure of standard mixtures.

2.3.7 Oxalate extraction

The modified method described by Day and Underwood (1986) was used to determine oxalate content of the sample . Exactly 1.0g of the sample was weighed into 100ml borosilicate glass flask. Then 75ml of 3M sulphuric acid was mixed with the solution and stirred carefully intermittently with magnetic stirrer for an hour and later filtered with Whatman filter paper. 25ml of the filtrate was collected and then titrated at the hot condition of 80^0C – 90^0C against 0.1M potassium tetraoxomanganate (viii) solution to the end point.

2.3.8 Phytate

Wheeler and Ferrel (1971) described the method used for phytate extraction. About 4.0g of the sample was macerated in 100ml of 2% HCl for 3 hours and later filtered with Whatman filter paper. 25ml of the filtrate was placed in a conical flask and 5ml of 0.3% ammonium thiocyanate was added, after which 53.5% of distilled water was added and titrated against a standard iron (III) chloride solution to end point. Phytate content was expressed as the percentage (%) phytate in the sample.

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3. RESULTS

Table 1: Quantitative phytochemical content of terpenoids in the leaves of *Justicia carnea*

TERPENOIDS	CONCENTRATION(mg/100g)
Euphorbioside-A	1.94×10^{-8}
Euphorbioside-B	5.55×10^{-8}
Taraxerol	6.42×10^{-8}
Taraxerone	3.29×10^{-8}
Alpha-amyrin	6.07×10^{-4}
Deglucosyleuphorbioside –A	3.94×10^{-8}
Clovandiol	8.35×10^{-8}
Beta-amyrin	8.55×10^{-1}
Lupeol	4.25×10^{-6}
Ephaginol	2.23×10^{-7}
Beuerenol acetate	4.71×10^{-5}
TOTAL	8.55×10^{-1}

Table 2: Quantitative phytochemical content of saponin in*Justicia carnea*

SAPONIN	CONCENTRATION (mg/100g)
Hispogenine	6.87×10^{-5}
Saponin B	1.37×10^{-4}
Saponin C	1.62×10^{-5}
Solagenin	1.87×10^{-5}
Dioscin	5.78×10^{-7}
Diosgenin	3.67×10^{-5}
Justisci-saponin-1	356.71
Tigogenin	2.50×10^{-6}
Neochlorogenin	3.71×10^{-2}
Hecogenin	8.84×10^{-5}
Sapogenin	69.43
Tribuloin	1.22×10^{-4}
Yanogenin	9.13×10^{-4}
Conyzorgin	6.20×10^{-4}
Gracillin	5.52×10^{-7}
Saponine	23.25
TOTAL	449.43

Table 3: Quantitative phytochemical content of sterols in*Justicia carnea*

STEROL	CONCENTRATION (mg/100g)
Cholesterol	2.75×10^{-9}
Cholestanol	2.27×10^{-3}
Tinyaloxin	3.47×10^{-9}
Daucosterol	1.52×10^{-8}
Ergosterol	1.83×10^{-3}
Campesterol	4.34
Stig-masterol	1.29
Savenasterol	3.62
Sitosterol	21.31
TOTAL	30.58

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Table 4: Quantitative phytochemicalcontent of glycoside in*Justicia carnea*

GLYCOSIDE	CONCENTRATION(mg/100g)
Afzelin	6.24×10^{-4}
Arbutin	1.54×10^{-6}
Salicin	1.59×10^{-6}
Allantoin	6.82
Amygdalin	8.68×10^{-4}
Kaemferitrin	19.90
O-sitosterol-3-B-glycoside	12.54
Rutin	2.14×10^{-5}
B-sitosterol-glucoside	6.76×10^{-6}
Costusoside I	1.21×10^{-6}
Coustusoside J	1.37×10^{-6}
Kaemferol-3-o-glycopyran0side	2.32×10^{-6}
TOTAL	39.27

Table 5: Quantitative phytochemical content of flavonoids in*Justicia carnea*

FLAVONOIDS	CONCENTRATION(mg/100g)
(+)-Catechin	1.41×10^{-3}
(+)-Gallocatechin	3.28×10^{-5}
Coumarin	34.86
Dihydroxycoumarin	3.67×10^{-3}
Apigenin	6.03×10^{-4}
Butein	5.73×10^{-4}
Naringenin	4.31×10^{-3}
Luteolin	3.24×10^{-4}
Kaemferol	12.01
(-)-Epicatechin	7.85×10^{-2}
Quercitrin	1.01×10^{-1}
(-)-Epigallocatechin	5.87×10^{-5}
Myricitrin	1.65×10^{-4}
Quercetin	4.17×10^{-5}
(-)-Epicatechin-3-gallate	2.21×10^{-3}
Hyperin	265.33
(-)-Epigallocatechin-3-gallate	1.31×10^{-5}
Isorhamnetin	2.29×10^{-5}
Robinetin	3.91×10^{-3}
Abzelin	117.82
Myricetin	2.82×10^{-2}
Baicalein	3.06×10^{-3}
Isoquercitrin	1.78×10^{-4}
Baicalin	1.19×10^{-5}
Silymarin	3.68×10^{-7}
TOTAL	430.24

Table 6 Quantitative phytochemical content of tannin inJusticia carnea

Table 6: shows only tannic acid with total of 744.24mg/100g

TANNIN	CONCENTRATION(mg/100g)
Tanic acid	744.24
TOTAL	744.24

Table7 Quantitative phytochemical content of oxalate in *Justicia carnea*

Table 7: shows only oxalate phytochemical expressed as 102.11ppm

OXALATE	CONCENTRATION(ppm)
Oxalate	102.11
TOTAL	102.11

Table 8 Quantitative phytochemical content of phytate in *Justicia carnea*

Table 8: shows Phytate phytochemical whose concentration isexpressed in 12.36ppm

PHYTATE	CONCENTRATION (ppm)
Phytate	12.369
TOTAL	12.369

4. DISCUSSION AND CONCLUSION

Quantitative phytochemical screening of leaves of *Justicia carnea* revealed the presence of the following phytoconstituent ; alkaloids, terpenoids, saponin, sterols, phenolic acids, flavonoids, tannin, glycoside, oxalate and phytate in varying concentrations. Species of *Justicia* has been reported to contain mainly alkaloids, lignans, flavonoids and terpenoids (Correa and Alcantara, 2012).

Eleven terpenoid of which the highest concentration is beta-amyrin ($8.55x10^{-1}$mg/100g), followed by alpha-amyrin ($6.07x10^{-4}$mg/100g) Beuerenol acetate ($4.71x10^{-5}$mg/100g and Taraxerol ($6.42x10^{-8}$mg/100g) and the least is Euphorbioside-A ($1.93x10^{-8}$mg/100g) were discovered in Table 1 . Terpenoids improves the skin tone, increases antioxidants concentration in wounds, and restore inflammed tissues by increasing blood supply (Lyss *et al.*, 1998; Aggarwal *et al.*, 2006). It reduces diastolic blood pressure and lower the sugar level in blood in hypertensive and diabetic patients respectively (Grace *et al.*, 2013). It also exhibit anti-inflammatory, anticancer, antimalarial, inhibition of cholesterol synthesis, antiviral and antibacterial activities (Mahato and Sen 1997).

Quantitative saponin characterization in Table 2 shows the presence of sixteen saponin of which the highest concentration is Justisci-saponin-1(356.71mg/100g), followed by Sapogenin (69.42mg/100g) Saponine (23.25mg/100g and Neochlorogenin ($3.70x10^{-2}$mg/100g). While the least is Saponin C ($1.62x10^{-5}$mg/100g). Saponins have hypocholesterolaemic, immunostimulant, hypoglycemic effects and anticarcinogenic properties (Ros, 2000). Saponins lower cholesterol level by forming large micelles that are then excreted in bile it also lowers serum levels of low density lipoproteins-cholesterol and decrease absorption of cholesterol in the intestines (Chung *et al.*, 2004). Saponins mediate immunostimulant (Guruvayoorappan *et al.*,2014) and superoxide scavenging effect (Zhu *et al.*, 2004).

Result in Table 3 revealed ten different sterols in the plant sample of which the highest in concentration is Sitosterol (21.311mg/100g), followed by Campesterol (4.34mg/100g), Savenasterol (3.62mg/100g) and Stigmasterol (1.29mg/100g) while the least is Ergosterol ($1.82 x10^{-3}$mg/100g) (Table 4.4). Clinical studies have shown that phytosterol intake leads to up to 15% reduction of LDL-cholesterol (Katan *et al.*, 2003; O'Neill *et al.*, 2005). β-sitosterol and β-sitosterol glycoside have been reported to reduce incidences of inflammatory diseases and carcinogen-induced cancer (Raicht *et al.*,1980; Ivorra *et al.*, 1987). These compounds also have insulin releasing effect, anti-complement and antipyretic activity(Ivorra *et al.*, 1987; Yamada *et al.*,1987). They increase the proliferation of TH1-type helper cells while inhibiting TH2-type helper cells, inhibit the secretion of IL-4 but increases the secretion of IFN-g and IL-2 (Harshal, and Prakash, 2014).

Result in Table 4 shows the presence of twelve glycoside of the highest concentration Kaemferitrin (19.91mg/100g), followed by O-sistosterol-3-B-glycoside (12.54mg/100g), Allantoin (6.82mg/100g) and Arbutin (1.5 x 10^{-6}mg/100g). While the least is Costusoside I (1.21 x10^{-6}mg/100g). Cardiac glycosides act by inhibition of Na+, K+-ATPase resulting to decreased intracellular K+ ions and increased intracellular Ca2+ and Na+ ions (Yeh *et al.*, 2003). Cardiac glycosides have been reported to inhibit the four genes that are overexpressed in prostate cancer cells including the inhibitors of apoptosis inhibitor survivin and transcription factors (Newman *et al.*,2007).

Twenty five flavonoids were characterized in Table 5 of which the highest concentration is Hyperin (265.331mg/100g), followed by Abzelin (117.82mg/100g), Coumarin (34.86mg/10 g) and Kaemferol (12.00mg/100g) and the least is Quercitrin (1.01 x10^{-1}mg/100g) Flavonoids possess antioxidant (Nema *et al.*, 2013), analgesic, anticancer, antiviral, antihepatotoxic activities (Uma-devi *et al.*, 1999). It has also been implicated in the inhibition of phospholipase A_2, hence provoking analgesic action (Amir *et al.*, 2015) Kaempferol promote β-cell survival, improve insulin secretory function and ameliorate hyperglycemia (Zhang *et al.*, 2011), cyanidin, keampferol and genistein may downregulate the expression of cancer-related genes to exert anticancer activity (Kumar *et al.*,2012; Xie *et al.*,2014).

Anti-nutrients such as oxalate, phytate and tannin interferes with absorption of nutrient (Beercher, 2003). Oxalate has been reported to have harmful effect on human health because it reduces the absorption of calcium and aids formation of kidney stone (Mutalik *et al.*, 2003).

Tannin in fruits is use for treating intestinal disorders such as diarrhoea, dysentery as well as treatment of swollen tissues and in prevention of cancer due to its astringent nature (Bajai, 2001; Kumar, 2001). Alkaloids and tannin have been reported to elicit analgesic action (Tylor *et al*., 2011).

5. CONCLUSION

Leaves of *Justicia carnea* contains in abundant different phytochemicals with different therapeutic potentials which could serve as precursors in the synthesis of orthodox drugs as well as herbal agents in treatment of diseases.

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Conflict of interest

The authors declare that they do not have any conflict of interest.

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