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QUANTITATIVE DETERMINATION OF PHYTOCHEMICALS CONSTITUENTS OF *JUSTICIA SPP. (IRUGEJE) SEED*

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Abstract: **Phytochemicals are non- nutritive plant chemicals that have protective or disease preventive properties. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life. *Justicia spp.* (Irugege) is a spice used in the preparation of various dishes in the southern part of Nigeria. It grows in subtropical areas and is relatively drought resistant; it is believed to possess various nutritional properties. The aim of this work is to determine the quantitative phytochemical constituents of *Justicia spp.* Seed. The phytochemical constituent of the seed were carried out using gas chromatography flame ionization detector (GC-FID). Results revealed the presence of eleven phytoconstituents alkaloids (17.32/100g), terpenes (1.47mg/100g). tannins (4.59mg/100g), saponins (5.29mg/100g), anthocyanins (1.15mg/100g), phenols (119.40mg/100g), lignans (1.40mg/100g), glycosides (9.23mg/100g), phytosterols (45.52mg/100g), flavonoids (207.06mg/100g) and stilbenes (271.07mg/100g). *Justicia spp.*, high in stilbenes, flavonoids, and phenols concentration could be used as a potent herbal antioxidant, cholesterol lowering, antimicrobial and anti-cancer agents.**

Keywords: **Phytochemical, *Justicia Spp*, Quantitative, Antioxidant and Constituents.**

1. INTRODUCTION

Nature has been just to life, by providing abundant wealth which possesses medicinal values for all living creatures. Most plants have been used against various diseases since the ancient time. Therefore, there is a necessity to explore their uses and to conduct chemical screening of these plants to ascertain their chemical contents and therapeutic properties. Phytochemicals are non- nutritive plant chemicals that have protective or disease preventive properties. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect against diseases (Breslin *et al.*, 2017). *Justicia spp.* (Irugege) is a spice used in the preparation of various dishes in the southern part of Nigeria. It grows in subtropical areas and is relatively drought resistant; it is believed to possess various nutritional properties that have yet to be evaluated. Hence its mineral content with reference to the seed is properly evaluated to ascertain the presence of the various phytochemicals present therein. The seeds of *Justicia spp.* (irugege) are small, round and brown in shape comprising of a hard covering and consisting of a pungent smell which is a characteristic feature of its properties as a spice. *Justicia spp*. is a genus of flowering plants in the Acanthaceae family. It is the largest genus within the family, encompassing around 700 (Daniel, 2011) species with hundreds more as yet unresolved. The aimed is to determining the phytochemicals inherent in *Justicia Spp.* Seed. This plant has sweet and nice aroma hence commonly used as flavoring ingredient in preparation of ulam (salad), laksa and several other Malay food delicacies. The plant is found in Southeast Asian countries namely Malaysia, Indonesia, Thailand (Phak pai) and Vietnam (Nghể bé). The plant produces essential oil containing high levels of aliphatic aldehydes (72.54%) (Gor *et al.* 2011) and it has been recognized

by the Malaysian Government as an essential oil-producing crop in the Herbal Product Blueprint, (Wan Hassan, 2006). Traditionally P. minus has been used in herbal medicine as a cure for digestive disorders and dandruff in Malaysia despite of its regular uses as food flavoring agent and appetizer in Malays cuisine. The essential oil extracted from P. minus leaves is applied to hair to remove dandruff, used in aroma therapy (Almey *et al.* 2010) and in the perfume industry (Bunawan *et al.* 2011). Natural antioxidants play a key role in health maintenance and prevention of chronic and degenerative diseases, such as atherosclerosis, cardiac and cerebral ischema, carcinogenesis, neurodegenerative disorders, gestational diabetics, rheumatic disorder, DNA damage and ageing, (Uddin *et al*., 2008; Jayasri *et al*., 2009). Antioxidants exert their activity by scavenging the 'free oxygen radicals' there by giving rise to a fairly stable radical. The free radicals are metastable chemicals species, which tend to trap electrons from the molecules in the immediate surroundings. These radicals if not scavenged effectively in time, they may damage crucial bio molecules like lipids, proteins including those present in all membranes, mitochondria and the DNA resulting in abnormalities leading to disease conditions (Uddin *et al*., 2008). Many plants, citrus fruits and leafy vegetables are the source of ascorbic acid, vitamin E, carotenoids, flavonoids and phenolics which possess the ability to scavenge the free radicals in human body. Significant antioxidant properties have been recorded in phytochemicals that are necessary for the reduction in the occurrence of many diseases (Anderson and Teuber, 2001). The efficiency of some extracts in liquid medium and at low pH levels enhances their potency even in the human stomach. Their inhibitory effect on the intestinal and kidney Na/K ATPase activity and on alanine transport in jejunum has also been reported (Jakhetia *et al*., 2010). Essential oil of C. *osmophloeum* twig has excellent ant-inflammatory activities and cytotoxicity against HepG2 (Human Hepatocellular Liver Carcinoma Cell Line) cells. Previous reports also indicated that the constituents of C. *osmophloeum* twig exhibited excellent anti-inflammatory activities in suppressing nitric oxide production by LPS (Lipopolysaccharide) – stimulated macrophages (Jakhetia *et al*., 2010).

2. MATERIALS AND METHODS

The plant was collected from Odorubu Town in Patani Local Government Area of Delta State while the seed was brought from Choba market in Obio/Akpor Local Government Area of Rivers State and identified as *Justicia Spp.* by a botanist – Dr. C. Ekeke of the Department of Plant Science and Biotechnology from the University of Port Harcourt. The seeds of *Justicia Spp.* were washed thoroughly under running tap water and was grounded to a fine powder with a laboratory mortar and pestle, which was used for analysis.

QUANTITATIVE DETERMINATIONOF ALKANOIDS CONSTITUENT IN *Justicia Spp.*

The alkaloids extraction was carried out by the modified method of (Ngounou *et al.,2005).* An accurate weight of 5.0 g of the pulverized sample was macerated in hexane of 25ml for about 72 hours. The extract was filtered and the residue was air-dried, later treated with 10% aqueous ammonia ($NH_{3)}$ and macerated in trichloromethane ($CHCl_{3)}$ for 24 hours. After the filtration and evaporation at reduced pressure, the resultant crude extract was treated with 5% aqueous hydrochloric acid (HCl) of about 7.5ml. The aqueous phase was made alkaline with aqueous NH_3 and extracted thrice with $CHCl_3$. The $CHCl_3$ fraction was washed with water. The extract was poured into the round bottom flask of the rotatory evaporator arrangement. It was separated by driving the solvent off the extract. Then the concentrated extract was dried of water by using the anhydrous sodium sulphate. Gas chromatography (HP 6890 Powered with HP ChemStation Rev. A 09.01) was used for the quantification of the phytochemicals in the seed extract. The gas chromatography was equipped with a flame ionization detector, a column dimension (30m× 0.25mm×0.25µm) and a column type, DB-5MS capillary. The injection temperature was 250°C and in split injection of sample. The carrier gas used was nitrogen, at Hydrogen pressure (28psi). The initial oven temperature was 60°C for 5min and first ramping was at 10°C/min for 20min, then the second ramping was at 15°C/min for 4min, while the detector was operated at 320°C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of identified phytochemicals.

QUANTITATIVE DETERMINATION OF FLAVONOIDS CONSTITUENT IN *Justicia Spp.*

The flavonoids extraction was by the modified method of Oyedeji *et al*., (2015)

An accurate weight of 50.00g of the powdered sample was weighed and transferred to Stoppard flask and treated with ethanol until the powder was fully soaked. The flask was shaken every hour for the first six hours and then it was kept aside and shook after 24hours. This process was repeated for three days and then the extract was filtered. The extract was collected and evaporated to dryness by using nitrogen stream. An accurate weight of 0.5g of the concentrate was put into

conical flask of 250ml capacity with the addition of 100ml *of* de-ionised water and boiled for 10 minutes. The flavonoids extract was obtained by pouring 100ml of the boiling methanol: water (70:300, v/v) onto the materials. The homogenate was allowed to macerate for about 4 hours and then filtered through filter paper (Whatman No. 1). The filtrate was derivative for volatility in gas chromatography analysis. The mixture was concentrated to 2ml in Agilent vial. Gas chromatography (HP 6890 Powered with HP ChemStation Rev. A 09.01) was used for the quantification of the phytochemicals in the seed extract. The gas chromatography was equipped with a flame ionization detector, a column dimension (30m× 0.25mm×0.25µm) and column type, HP INNOW$_{ax}$. The injection temperature was 250°C and in split injection of sample. The carrier gas used was nitrogen, at hydrogen pressure (22psi) . The initial oven temperature was 50°C, and first ramping at 8°C/min for 20min, maintained for 4min and second ramping at 12°C/min for 4min, maintained for 4min. while the detector was operated at 320°C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of identified phytochemicals. The concentrations of individual phytochemicals were expressed in mg/g.

QUANTITATIVE DETERMINATION OF LIGNANS CONSTITUENT IN *Justicia Spp.*

The extraction was carried out by the modified method of James (2012). The sample was pulverized and the organic constituents extracted with methanol overnight with stirring. The lignan was removed by suction filtration and the filtrate shaken overnight with hexane/dichloromethane (60:40). The aqueous layer was removed in a separatory funnel; the organic solvent was washed with saturated sodium chloride, dried over sodium sulphate(Na_2SO_4) and the solvent was rotary evaporated to obtain viscous dark oil. Sample of oil was dissolved in acetone for the gas chromatography. The extract was concentrated to 1 ml in the vial, and 1 µl was injected into the injection port of gas chromatography. Gas chromatography (HP 6890 Powered with HP ChemStation Rev. A 09.01) was used for the quantification of the phytochemicals in the seed extract. The gas chromatography was equipped with a flame ionization detector, a column dimension (30 m × 0.25 mm × 0.25 µm) and column type, HP 5. The injection temperature was 250°C and in split injection of sample. The carrier gas used was nitrogen, at hydrogen pressure (28 psi). The initial oven temperature was 120°C, ramping at 10°C/min for 20min, while the detector was operated at 320°C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of identified phytochemicals.

QUANTITATIVE DETERMINATION OF GLYCOSIDE CONSTITUENT IN *Justicia Spp.*

The extraction was carried out by the modified method of James (2012)

An accurate weight of 1.0g of the pulverized sample was extracted by soaking for 2hours with 10ml of 70% alcohol and then filtered, and concentrated. The redistilled hexane was used to replace the initial solvent and the hexane was concentrated to 1 ml in the vial, and 1µl was injected into the injection port of gas chromatography. Gas chromatography (HP 6890 Powered with HP ChemStation Rev. A 09.01) was used for the quantification of the phytochemicals in the seed extract. The gas chromatography was equipped with a flame ionization detector, a column dimension (30 m× 0.25 mm × 0.25µm) and column type, AC-5 capillary. The injection temperature was 250°C and in split injection of sample. The carrier gas used was nitrogen with Hydrogen pressure (28 psi). The initial oven temperature was 70°C for 5min and ramping at 12°C/min for 20 min, while the detector was operated at 320°C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of identified phytochemicals. The concentrations of individual phytochemicals were expressed in mg/g.

QUANTITATIVE DETERMINATION OF TANINES CONSTITUENT IN *Justicia Spp.*

The extraction was carried out by following the modified method of Swain (1979).

About 0.2g of the pulverized sample was measured into the 50ml borosilicate beaker 20ml of the 50% methanol was added and covered with paraffin and placed in a water bath at 80°C for 1 hour. The content was stirred with a glass rod to prevent lumping. The extract was quantitatively filtered using a double layered Whatman No 1 filter paper into a 100ml volumetric flask using 50% methanol to rinse. This was concentrated to 2ml in the borosilicate vial, l.0µl was injected into the injection port of the gas chromatography. Gas chromatography (HP 6890 Powered with HP ChemStation Rev. A 09.01) was used for the quantification of the phytochemicals in the seed extract. The gas chromatography was equipped with a flame ionization detector, a column dimension (30m× 0.25mm×0.25µm) and column type. The injection temperature was 250°C and in split injection of sample. The carrier gas used was Hydrogen pressure (22psi). The initial

oven temperature was 120°C and ramping at 10°C/min for 20min, while the detector was operated at 320°C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of identified phytochemicals.

QUANTITATIVE DETERMINATION OF PHENOLS CONSTITUENT IN *Justicia Spp.*

Two stage extraction procedures followed for the effective removal of the polyphenols' phenolic compounds described by the following stages;

Stage1: About 50.0mg of the sample was extracted with 5 ml of 1 M sodiumhydroxide (NaOH) for 16hours on a shaker at ambient temperatures as described by (Simait *et al,2017).* After extraction, the sample was centrifuged (5000 x g). rinsed with water, centrifuged again and the supernatants were combined and placed in a disposable glass test tube and heated at 90°C for 2h to release the conjugated phenolic compounds as supported by Whitehead et al. (1983). The heated extract was cooled, titrated with 4M hydrochloric acid (HCl) to pH <2.0, diluted to 10 ml. with de-ionised water, and centrifuged to remove the precipitate. The supernatant was preserved for subsequent purification and the residue was extracted further in stage 2.

Stage 2: The residue from stage 1 above was extracted with 5 ml of 4M NaOH, heated to 160°C in Teflon as described by (Simait *et al, 2017).* After cooling, the mixture was filtered.Supernatant was collected and the residue washed with water (de-ionised). The \supernatants were combined and adjusted to pH <2.0 with 4M HCl. The filtrates were combined for further purification.

Purification of Extracted Phenolic Acids

An aliquot (5-15mL) of the various supernatants was passed through a conditioned Varian (Varian Assoc..Harbor City.CA) Bond Elut PPL (3-mL size with 200-mg packing) solid-phase extraction tube at -5ml. min^{-1}attached to a Visiprep (Supelco. Bellefonte, PA). The tubes were then placed under a vacuum (-60 kPa) until the resin was thoroughly dried after which the PAs were eluted with I ml. of ethyl acetate into gas chromatography autosampler vials. The PPL tubes were conditioned by first passing 2mL of ethyl acetate followed by 2mL water (pH <2.0). The phenolic acid standards used were purchased from Aldrich (Aldrich Chemical Co.. Milwaukee. WI).

QUANTITATIVE DETERMINATION OF SAPONIN CONSTITUENT IN *Justicia Spp.*

The extraction was carried out by the modified method of James. (2012). The sample was pulverized and the saponin was extracted three limes with redistilled methanol. The saponins were removed with 20ml of the solvent for 20 minute with the aid of the sonication. The combined extracts were concentrated to syrup under reduced pressure, and then suspended in water. The suspension was extracted with petroleum ether, chloroform and 1-butanol saturated with water, successively, to give the respective extract after removal of the solvent. The combined extract was filtered and concentrated to 1 ml in the vial for gas chromatography analysis and 1 µ1 was injected into the injection port of gas chromatography. Gas chromatography (HP 6890 Powered with HP ChemStation Rev. A 09.01) was used for the quantification of the phytochemicals in the seed extract. The gas chromatography was equipped with a flame ionization detector, a column dimension (30m× 0.25mm×0.25µm) and column type capillary, DB-225MS, . The injection temperature was 250°C in split injection of sample. The carrier gas used was nitrogen with Hydrogen pressure (28psi). The initial oven temperature was 60°C for 5min and first ramping at 12°C/min for 18min and second ramping at 15°C/min for 5min. while the detector was operated at 320°C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of identified phytochemicals.

QUANTITATIVE DETERMINATION OF PHYTOSTEROLS CONSTITUENT IN *Justicia Spp.*

The phytosterol extraction and analysis were carried out by the modified of (Anderson *et al.* 1985). About 5.00g of the powdered sample was weighed and transferred to Stoppard flask and treated with petroleum ether until the powder was fully soaked. The flask shook every hour for the first six hours and then it was kept aside and shook after 24hours. This process was repeated for three days and then the extract was filtered. The extract was collected and evaporated to dryness by using nitrogen stream about 0.5g of the extract from the sample was added to the screw-capped test tube. The sample was saponified at 95°C for 30 minutes by using 3ml of 10% potassium chloride (KOH) in ethanol to which 0.20ml of benzene had been added to ensure miscibility. 3ml of de-ionised water was added and 2m 1 of hexane was used in

extracting the non- saponifiable materials, e.g. sterols. Three extractions, each with 2ml of hexane were carried out for 1 hour, 30min and 39mins respectively to achieve complete extraction of the sterols.

The hexane was concentrated to 2ml in Agilent vial. Gas chromatography (HP 6890 Powered with HP ChemStation Rev. A 09.01) was used for the quantification of the phytochemicals in the seed extract. The gas chromatography was equipped with a flame ionization detector, a column dimension (30m× 0.25mm×0.25µm) and column type HP INNOW$_{AX}$, . The injection temperature was 250°C in split injection of sample. The carrier gas used was nitrogen with Hydrogen pressure (22psi). The initial oven temperature was 60°C and first ramping at 10°C/min for 20min, maintained for 4min and second ranging at 15°C/min for 4min, maintained for 10min. while the detector was operated at 320°C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of identified phytochemicals.

QUANTITATIVE DETERMINATION OF ANTHOCYANINS CONSTITUENT IN *Justicia Spp.*

Accurately weighed 5 g of the finely powdered sample was placed in a round bottom flask and then 100ml methanol was added accurately. Then, the sample was heated under reflux for 2.0h, cooled, and replenished the loss of the solvent with methanol, filtered through a 0.45µm nylon membrane analysis. High performance liquid chromatography(Agilent 1100 series) was used for the quantification of the phytochemicals in the seed extract. The High performance liquid chromatography with detector agilent 1260 and wavelength;535$_{nm}$.A column of YMC-Pack Pro C18 RS, C18 with column temperature 30°C, and dimension (5µm, 4.6mm×250mm), with flow rate of 1.0ml/min. The concentrations of individual phytochemicals were expressed in mg/g.

QUANTITATIVE DETERMINATION OF TERPENES CONSTITUENT IN *Justicia Spp.*

The extraction was carried out by the modified method of Serap (2009). The sample was pulverized and the terpenes constituents extracted with redistilled chloroform. The terpenes were removed with 10ml of the solvent for 15 minute. The mixture was filtered and concentrated to 1 ml in the vial and 1 ml was injected into the injection port of gas chromatography. Gas chromatography (HP 6890 Powered with HP ChemStation Rev. A 09.01) was used for the quantification of the phytochemicals in the seed extract. The gas chromatography was equipped with a flame ionization detector, a column dimension (30m× 0.25mm×0.25µm) and column type HP 5MS . The injection temperature was 250°C in split injection of sample. The carrier gas used was nitrogen with Hydrogen pressure (22psi). The initial oven temperature was 40°C and first ramping at 5°C/min to 200°C for 2mins. While the detector was operated at 300°C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of identified phytochemicals. The concentrations of individual phytochemicals were expressed in mg/g.

QUANTITATIVE DETERMINATION OF STILBENES CONSTITUENT IN *Justicia Spp.*

The sample was made to be free of water by ensuring constant weight after series of oven dried at 105^{0}C for a period of time in the laboratory.

Sample preparation: Accurately weight 0.3g of the finely powdered sample was placed in a round-buttom flask and then 50ml methanol was added accurately. Then,the sample was heated under reflux for 2.0 h, cooled and replenish the loss of the solvent with methanol, filtered through a 0.45 µm nylon membrane prior to the analysis. High performance liquid chromatography(Agilent 1200 series) was used for the quantification of the phytochemicals in the seed extract. The High performance liquid chromatography with detector agilent 1260 and wavelength;295$_{nm}$.A column of Kromasil, C18 with column temperature 40°C, and dimension (5µm, 4.6mm×250mm), with flow rate of 1.0ml/min and pressure (180 × 10^{s} Pa) The concentrations of individual phytochemicals were expressed in mg/g.

3. RESULTS AND DISCUSSION

Phytochemicals Analysis of *Justicia spp. Seed*

Results in the table 1 revealed ten different phytochemical constituents which are arranged in increasing order of concentration. Terpenes (1.13mg/100g), Tanins (3.66mg/100g), Saponins (6.57mg/100g), Anthocyanins (9.83mg/100g), Lignans (9.83mg/100g), Alkaloids (13.89mg/100g), Glycosides (19.85mg/100g), Phytosterols (36.89mg/100g), Phenols (110.28mg/100g), Flavonoids (201.71mg/100g) and Stilbenes (280mg/100g) with the highest concentration.

Table 1: QUANTITATIVE PHYTOCHEMICALS CONTENT OF *JUSTICIA SPP. SEED (IRUGEJE)*

S/N0	PHYTOCHEMICALS	CONCENTRATION mg/100g
1	Stilbene	271.07
2	Flavonoids	207.06
3	Phenolic	119.40
4	Phytosterol	45.52
5	Alkaloids	17.32
6	Glycoside	9.23
7	Saponins	5.29
8	Terpenes	1.47
9	Lignans	1.40
10	Antocyanides	1.15
11	Tanines	4.59

The presence of eleven phytochemical constituents namely: terpenes, tannins, saponins, anthocyanins, lignans, alkaloids, glycosides, phytosterols, phenols, stilbenes and flavonoids. However, the concentrations of three phytochemicals were above 100mg/100g, namely; Stilbenes, flavonoids and phenol. Stilbenes being the highest with concentration of (280.09mg/g) have various biological activities alongside its derivatives such as anti-cancer, anti-bacterial and fungi static activity. Some of its derivatives have positive influences on cardiovascular system and also have high hopes in pharmacological use and curing different diseases (Ireneusz, 2009).

Flavonoids being the second highest with concentration (201.71mg/100g), have gained recent attention because of their broad biological and pharmacological activities. In these order flavonoids have been reported to exert multiple biological property including antimicrobial, anti-toxicity, ant-inflammatory as well as antitumor activities but the best described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species.

The capacity of flavonoids to act as antioxidants depends on their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities (Tim *et al*., 2005).

On the other hand, flavonoids such as luteolin and cathechins, are better antioxidants than the nutrient antioxidants such as vitamin C, vitamin E and beta carotene. Flavonoids have been stated to possess many useful properties, containing anti-inflammatory activity, enzyme inhibition, antimicrobial activity, oestrogenic activity, anti-allergic activity, antioxidant activity, vascular activity, enzyme inhibition, antioxidant activity, vascular activity and cytotoxic antitumor activity (shashank and Abhay, 2013).

Flavonoids constitute a wide range of substances that play important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA (Atamani *et al.*, 2009).

Phenols being the third highest in concentration (110.28mg/g) can infer that *Justcia spp.* is not a bad source and hence increases bile secretion, reduces blood cholersterol and lipid levels and antimicrobial activity against some strains of bacterial activity against some strains of bacteria such as staphylococcus aureus are some of the biological activities of phenolic acids

4. CONCLUSION

The phytochemical screening of *Justcia spp.* indicates the presence of flavonoids, stilbenes and phenols in very high concentrations inferring that they could be used as a potent herbal antioxidant, blood cholesterol lowering agent, antimicrobial agent, could influence the cardiovascular system and possibly act as an anti-cancer agent.

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