COMPARATIVE STUDY ON THE PHYTOCHEMICAL AND *IN VITRO* ANTIOXIDANT PROPERTIES OF METHANOLIC LEAF EXTRACT OF *JUSTICIA SECUNDA* VAHL

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Medicinal plants are a primary source of healing and widely used for the treatment of various ailments. This present study evaluates phytochemicals and *in vitro* antioxidant activities of the methanolic leaf extract of *Justicia secunda*, *Acanthaceae* family. Fresh leaves of the plant were collected, and shade dried. The dry leaves were pulverized, and the resultant powder extracted with 0.1% hydrogen chloride (HCl) in methanol, and 1% HCl in methanol respectively. Extracts obtained were subjected to qualitative phytochemical screening and *in vitro* antioxidant assays. Results obtained confirm the presence of saponinis, tannins, steroids, flavonoids, and alkaloids in equal degree in both 0.1% and 1% HCl methanolic leaf extract. Concentrations of anthocyanins, phenols and phlobatannins were quantitatively higher in 0.1% HCl extract as compared to 1% HCl extract. However, the presence of carbohydrates was not confirmed in either extracts. The results also indicated that phenolic and anthocyanins content of the leaf extracts were significantly higher ($p < 0.05$) in 0.1% HCl extract as compared to the 1% HCl leaf extract. Also, flavonoids contents were comparable ($p > 0.05$) in both extracts. The antioxidant scavenging efficacy showed that higher percentage inhibition was observed from the 0.1% HCl methanolic extract of *Justicia secunda* in its ability to scavenge hydrogen peroxide, 1,1 – diphenylpicrylhydrazyl, and superoxide anion. Overall, from this study, it could be concluded that *Justicia secunda* may be a good source of phytochemicals and natural antioxidants that will be useful in the food, medicine, and pharmaceutical industries.

Key words: *Justicia secunda*, anthocyanins, phenols, flavonoids, phlobataninins, phytochemicals.

INTRODUCTION

Justicia is a creeping perennial herb which grows on sand in shaded forest floor, and it is about 1 to 1.5 m height. Ethno pharmacological information according to Correa et al. (2012), suggests that lignans are the major components of the active extracts of the species of *Justicia* exhibiting important pharmacological properties such as antiviaral, antitumoral, anti-inflamatory and anti-platelet – aggregation.

Mpiana et al. (2010a) opine that *Justicia tenella*, *Justicia gendarussa*, and *Justicia insularis* possess high anti-sickling potency as a result of the high levels of anthocyanins they contain; while Mpiana et al. (2010b) reported the *in vitro* effects of anthocyanin extracted from *Justicia secunda* vahl on the solubility of sickle erythrocyte. Three compounds: lupeol, 16-hydroxyl lupeol and stigmasterol have also been isolated from *J. gendarussa* with promising antioxidant properties (Uddin et al., 2011).

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. It is used for the treatment of anaemia. Other common names are '*Hospital too far*', '*Blood leaf*' or *Blood tonic* (Roberts, 2016). Its aqueous extract is normally served as a tea drink to the anaemic patients in these communities.

Phytochemicals are naturally present in the medicinal plants, leaves, vegetables and roots that have protective mechanism and defend plants

from various infections; also called secondary metabolites. They are non – nutritive compounds that confer flavor and colour to the plants (Agbafor and Nwachukwu, 2011). These include alkaloids, terpenoids, and phenolic compounds.

Over the centuries, secondary metabolites in plants have played considerable role in medicine, being used in their natural state or their derivatives (Kinghorn et al., 2011; Newman and Cragg, 2012). One principal characteristic of phytochemicals that support its importance in medicine, food, pharmaceuticals, and nutraceuticals is their ability to function as antioxidants (Potterat, 1997; Khan et al., 2003; Prakash et al., 2012)

Antioxidants, according to Husseni et al. (2011) are substances that are capable of quenching the oxidation reaction of free radicals. Its mechanism may be through playing a direct role in radical scavenging or indirectly through destroying consumed substances that easily generate free radicals, thus preventing the occurrence of further reactions. However, there is a global shift from the use of synthetic antioxidants to naturally occurring antioxidants due to the former's harmful effect to human health due to their potential toxicity and carcinogenicity (Thaipong et al., 2006). Owing to the abundance of *J. secunda* in the lowland rainforests of the Niger Delta region of Nigeria, and its use by the indigenous people for curing certain ailments, this research reports its preliminary phytochemical and *in vitro* antioxidants properties.

MATERIALS AND METHODS

Plant materials

Fresh *J. secunda* leaves were collected from a garden in Otuoke village, Bayelsa State, Nigeria on May 2016. It was authenticated in the University of Port Harcourt herbarium by Dr. Ekeke Chimezie with voucher number UPH/V/1279.

Preparation of extract

The leaves of the plant were carefully cleaned and washed with tap water to remove impurities followed by shade drying. The air-dried leaves were pulverized and the resultant powder was stored in air-tight bottles at 20°C. One part (600mg) of the powdered sample was soaked in 1500ml of 0.1% HCl methanol; while another part was soaked in 1% HCl methanol for 72 h at room temperature (25°C), and then filtered through a cotton plug followed by Whatman filter paper No.1. The parent extract was concentrated with a Rotary Evaporator and stored in the refrigerator until required for use.

Qualitative phytochemical screening

Phytochemical screening was performed on parent plant extracts using standard procedures to qualitatively identify its constituents. The re-constituted aqueous crude extract (10mg / 20ml of distilled H_2O) obtained was used for the experiment. The methods of Evans (1997) and Sofowara (2006) were used for the detection of alkaloids and flavonoids respectively. The presence of tannins, phenolics, and saponins were detected by employing standard methods by Segelman et al. (1969), Mukherjee (2002) and Kokate (1999) respectively. The method of Edeogal et al. (2005) was employed to confirm the presence of phlobatannins and steroids in the plant extract. Screening for anthocyanins and carbohydrates were done using standard methods by Paris and Moyse (1969), and Rajagopal and Ramakrishanan (1993) respectively.

Estimation of *In vitro* Antioxidant Properties in Methanolic Leaf Extract of *J. secunda*

Employing previously described standard procedures, flavonoids and total phenolic content in the methanolic leaf extract of *J. secunda were* estimated according to Chang et al. (2002) and Singleton and Rossi (1965). The concentration of anthocyanins was determined according to the method by Lapornik et al. (2005). The hydroxyl radical scavenging ability was assayed by the method of Kaur et al. (2006), while the methods of Abdel – Hameed (2009) and Fontana et al. (2001) were respectively employed for the assay of DPPH radical and superoxide anion scavenging activities.

Statistical analysis

The values of all the parameters determined are expressed as Mean ± SD for triplicate determinations, and all data were analyzed using one-way ANOVA. The level of significance was placed at $p < 0.05$. All statistical

analyses were performed using SPSS version 16.0.

RESULTS

Qualitative phytochemical screening

The results of the qualitative phytochemical screening of the methanolic leaf extracts of *J. secunda are* presented in Table 1. The presence of flavonoids, tannins, phenols, saponinis, and steroids were qualitatively confirmed to the same degree for both 0.1% and 1% HCl methanolic leaf extracts of *Justicia secunda*. Alkaloids, phlobatannins, and anthocyanins were observed more in 0.1% HCl methanolic leaf extract as compared with 1% HCl methanolic leaf extract of *Justicia tenuipes*. The table also revealed that carbohydrate was not observed in either 0.1% or 1.0% HCl extract of the plant by the screening procedure used.

Table 1. Phytochemical constituent of the methanolic leaf extracts of *J. secunda.*

Phytochemicals	0.1% HCl in methanol extract	1.0% HCl in methanol extract
Saponins	+ + +	+ + +
Tannins	+ + +	+ + +
Carbohydrate	– – –	– – –
Steroids	+ + +	+ + +
Flavonoids	+ + +	+ + +
Anthocyanins	+ + +	+ +
Phenols	+ + +	+ +
Alkaloids	+ + +	+ + +
Phlobatannins	+ + +	+ +

+= Presence of constituent in the leaf extract; ¯ = Absence of constituent in the leaf extract.

Concentrations of flavonoids, phenols and anthocyanins

The concentrations of flavonoids, total phenolic and anthocyanin contents in both 0.1 and 1.0% HCl methanolic leaf extracts of *J. secunda are* depicted in Table 2. The results indicated that 0.1% HCl methanolic leaf extract of the plant showed a significantly ($p < 0.05$) higher total phenolic and anthocyanin content as compared with 1.0% HCl plant extract. However, the concentrations of flavonoids were comparable between 0.1 and 1.0% HCl methanolic leaf extracts.

Table 2. Flavonoids, total phenolic and anthocyanin contents in 0.1 and 1% HCl methanolic leaf extracts of *J. secunda.*

Phytochemicals	0.1% extract	1.0% extract
Flavonoid content (mg/g of rutin)	2.67 ± 0.31^{a}	2.56 ± 0.1^{a}
Phenolic Content (mg/100ml of extract)	6.88 ± 0.33^{a}	4.47 ± 0.41^{b}
Anthocyanin content (µg/g cyanidin)	9.72 ± 0.41^{a}	8.59±0.50b

Values are expressed as Mean ±SD for triplicate determinations; Means sharing different superscript alphabet on same row differ significantly at $p < 0.05$.

In vitro free radical scavenging efficacy

In vitro free radical scavenging efficay of 0.1 and 1.0% HCl methanolic leaf extract of *J. secunda were* assessed by its ability to scavenge hydrogen peroxide, diphenylpicrylhydrazyl (DPPH) and superoxide anion radical. Figure 1 illustrates the % inhibition of H_2O_2, DPPH, and superoxide anion radicals. It was observed from the results that 0.1% HCl methanolic extracts showed a significantly ($p < 0.05$) higher % inhibition in both H_2O_2, DPPH, and superoxide anion scavenging abilities as compared with the 0.1% HCl methanolic leaf extracts of *Justicia secunda.*

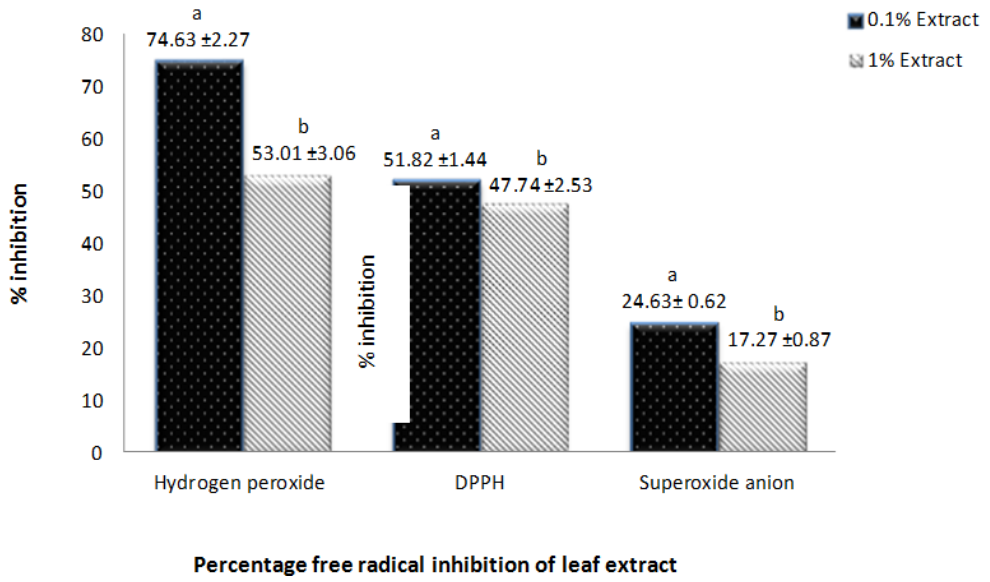

Figure 1. Hydrogen peroxide scavengig ability, DPPH radical scavengig ablity and superoxide anion scavengig ability of methanolic leaf extracts of *J. secunda* (Values are expressed as Mean ± SD % inhibition of triplicate determinations; Group bars having the same alphabet does not differ significantly at P < 0.05).

DISCUSSION

In this present study, the qualitative phytochemical screening of the acidified methanolic leaf extract of *J. secunda confirmed* the presence of flavonoids, alkaloids, phenols, saponins, tannins, steroids, anthocyanins, and phlobatannins; but the absence of carbohydrates. The protective properties ascribed to phytochemicals are as a result of their ability to either react with free radicals or inhibit the process of oxidation in cells. In other words, phytochemicals act as either antiradical or antioxidant compounds (Chanda and Dave, 2009; Samatha et al., 2012; Suprava et al., 2013).

Phenols according to Shahidi et al. (1992) are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their ability to chelate radicals, inhibit lipoxygenase and scavenge free radicals. The results from this study indicate high phenolic content in 0.1% HCl methanolic leaf extract. Flavonoid concentration was also high in the experimental plant. Flavonoids represent one of the most important sources of natural phenols. They possess a broad spectrum of chemical and biological activities including radical scavenging properties. Flavonoids has also been associated with possible role in the prevention of several chronic diseases involving oxidative stress as well as their protective effect against low – density lipoprotein oxidation (Das and Pereira, 1990; Shanaz et al., 2011)

Generally, acidified alcoholic solvents are the choice solvents for the extraction of anthocyanins (Devi et al., 2011; Kang et al., 2013; Puertolas et al., 2013). The acid contributes to stabilize anthocyanins but it may also transform the original form of the pigment in the tissue by breaking associations with metals, co-pigments or other factors (Strack and Wray, 1994).

Higher ($p < 0.05$) anthocyanin content observed in the 0.1% HCl methanol leaf extract as compared with the 1% HCl methanolic leaf extract in this study could be due to minimal anthocyanin degradation during concentration procedures in solvent recovering (Jackman et al., 1987). The high anthocyanin content observed could enhance the plant's potential as a good source for food colourant production and natural antioxidant because anthocyanins are responsible for colouration in plants; and their consumption has been linked as protective agent against many chronic diseases (Welch et al., 2008)

Assay of the *in vitro* antioxidant scavenging abilities in this study revealed that *J. secunda* may possess free radical scavenging activities. Hydrogen peroxide, DPPH and

Superoxide anion scavenging radical abilities has been previously employed to assess the *in vitro* antioxidant scavenging properties of plants (Malik et al., 2011; Spreena et al., 2011; Singh et al., 2012; Nwaoguikpe et al., 2014 and Seo et al., 2014).

Results of this study showed significantly higher scavenging properties (that is, H_2O_2, DPPH, Superoxide anion radical scavenging properties). The observed high antioxidant scavenging properties in *J. secunda* leaf extract could be attributed to the higher concentrations of flavonoids, phenols, and anthocyanins in the leaf extract of the plant (Table 2). Together, the phytochemicals contained in the leaf of *J. secunda* coupled with the antioxidant activities as exhibited by the plant's ability to scavenge H_2O_2, DPPH, and superoxide anion radical could justify its use locally for medicinal purposes.

Conclusively, the leaves of *J. secunda* may serve as a significant source of natural antioxidants which may be helpful in preventing the progress of various oxidative stresses. However, the components responsible for the anti-oxidative activity are currently unclear. Therefore, further investigation is needed to isolate and identify the antioxidant components present in the plant leaf extract. Also, *in vivo* anti-oxidant of the leaf extracts need to be assessed prior to clinical use.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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