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Comparative Phytochemical Screening of *Jatropha* L. Species in the Niger Delta

Nwokocha, A. Blessing, I.O. Agbagwa and B.E. Okoli
Department of Plant Science and Biotechnology, Faculty of Sciences, University of Port Harcourt, Nigeria

Corresponding Author: Dr. I.O. Agbagwa, Department of Plant Science and Biotechnology, Faculty of Sciences, University of Port Harcourt, Nigeria

ABSTRACT

Phytochemical studies were carried out on the leaf, stem, root and seed of four *Jatropha* L. species (*J. curcas* L., *J. gossypifolia* L., *J. multifida* L. and *J. podagrica* Hook) found in some parts of the Niger Delta, with a view to ascertain the inclusion of these morphologically distinct species into a genus. Qualitative and quantitative analysis of five secondary metabolites (alkaloids, tannins, saponins, flavonoids and phenols) were undertaken. The result showed that all secondary metabolites analyzed were present in all tissues studied but at different concentrations. The concentration of tannins in the leaves of the species in descending order was observed to be 7.43% for *J. curcas,* 6.79% for *J. podagrica*, 5.16% for *J. multifida* and 5.14% for *J. gossypifolia*. Concentration of saponins in the leaf and seed of the species were: *J. curcas* (4.89, 2.33%); *J. gossypifolia* (4.15, 2.37%); *J. multifida* (3.15, 2.44%); *J. podagrica* (3.15, 2.44%). Saponin concentration in the leaf and seed of *J. multifida* and *J. podagrica* was observed to be the same suggesting close affinity. Generally, tannins were the most abundant in plant parts, followed by saponins, alkaloids, flavonoids and then phenols. Results obtained confirm the relatedness of these species and spotlight these important phytochemicals in the species. However, variations observed in their concentrations confer individuality on the species.

Key words: Euphorbiaceae, *Jatropha,* Niger Delta, phytochemicals, secondary metabolites

INTRODUCTION

Jatropha L. (Euphorbiaceae) is a very diverse subtropical and tropical genus which includes succulents, caudiciform species, herbaceous perennials and woody trees. Four of the eight species recorded in West Tropical Africa and described by Hutchinson and Dalziel (1958) occur in the Niger Delta. These are *J. curcas* L., *J. gossypifolia* L., *J. multifida* L. and *J. podagrica* Hook. Ratha and Paramathma (2009) described 12 species of *Jatropha* in India, using morphological characters. A number of *Jatropha* species especially *J. curcas*, yield oils of biofuel value (Agarwal and Agarwal, 2007; Fairless, 2007; Akbar *et al.,* 2009), a fact which has brought much attention to the genus particularly in India. The oil is also used for manufacture of candles and soap and in cosmetics industry. *J. curcas* can be used to prevent and/or control erosion, to reclaim land, grown as a live fence especially to contain or exclude farm animals and be planted as a commercial crop (Kumar and Sharma, 2008; Openshaw, 2000; Martinez-Herrera *et al.*, 2006). These characteristics along with its versatility make *Jatropha* of vital importance to developing countries. Apart from the earlier morphological description of on the West African species and comparison based on epidermal features (Olowokudejo, 1993), much is not known on the common *Jatropha* species in the Niger Delta.

Secondary metabolites are phytochemicals produced as by-products of primary metabolism (Bako and Aguh, 2007) and are less widespread in plants. It is of course this restricted occurrence among plants that renders them valuable and useful in taxonomic delimitation of species. The groups of compounds mostly utilized for this purpose include alkaloids, phenolics, glucosinolates, amino acids, terpenoids, oils and waxes, carbohydrates and crystals. Recent investigations and reports of the usefulness of these compounds either for medicinal/therapeutic purposes or taxonomic elucidation of species include Edeoga *et al.* (2005), Hassan *et al.* (2007), Ibrahim *et al.* (2007), Mallikharjuna *et al.* (2007), Irshad *et al.* (2010), Nyananyo *et al.* (2010), Rasool *et al.* (2010) and Ganesh and Vennila (2011). The only phytochemical report known to the authors on *Jatropha* species occurring in the Niger Delta is that of Burkill (1994). This simply reported the occurrence of tannins in the leaves of *J. gossypifolia* and saponin in the leaf of *J. multifida*. In view of the importance of these species as highlighted and the seemingly lack of systematic information on the species in the Niger Delta, the present study subjected the species to qualitative and quantitative phytochemical screening. This is with a view to verify the presence or absence of these classes of secondary metabolites in the species as well as ascertain their relatedness or not. The study is a precursor aimed at stimulating interest on members of this genus.

MATERIALS AND METHODS

The plant materials viz., leaf, stem, seed and root of the *Jatropha* species were collected from different localities in the Niger Delta between September 2009 and March 2010. The research was conducted from March to October 2010. The plants were identified with the help of The Flora of West Tropical Africa (Hutchinson and Dalziel, 1958) and are deposited in the Herbarium, Department of Plant Science and Biotechnology, University of Port Harcourt.

Qualitative and quantitative determination of secondary metabolites in *Jatropha* species: Extracts of samples of leaf, stem, seed and root from each species were subjected to preliminary screening for the presence of secondary metabolites (alkaloids, tannins, flavonoids, phenols and saponins) using standard procedures (Trease and Evans, 1989) with some modifications. The test procedure for each compound is presented below.

Alkaloids: Five milliliter of 2% HCl was added to 2 mL of each plant extract in a test tube placed on a steam bath and warmed. It was filtered and divided into two parts for the following tests:

- A few drops of Wagner's Reagent (Potassium-iodine solution) were added to one part of the filtrate in a test tube. A reddish brown precipitate was observed
- A few drops of Meyer's Reagent (Potassium mercuric iodine solution) were added to the other part of the filtrate in a test tube. A cream coloured precipitate was observed

In both cases, the formation of precipitation indicated the presence of alkaloids.

Tannins: Five milliliter of the extract was treated with 2 mL of HCl and boiled for 5 min. The presence of red precipitate confirmed the presence of tannins.

Flavonoids: One milliliter of the extract was treated with 1 mL of dilute NaOH. The presence of a cloudy precipitate confirmed the presence of flavonoids.

Saponins: One milliliter of the extract was added to 4 mL of distilled water and shaken. A stable frothing or foaming indicated the presence of saponins.

Phenols: One milliliter of the extract was added to 1 mL of 10% $FeCl_2$ and mixed together. The presence of blue precipitate confirmed the presence of phenols.

After preliminary analysis to determine presence of these phytochemicals, the samples were further subjected to quantitative analysis to determine the percentage of each of these secondary metabolites in each plant part of each species. The following procedures were adopted:

- Quantitative analysis of alkaloids by the gravimetric method of Harbone (1993)

  Percentage alkaloids were computed as follows:

$$\text{Alkaloids (\%)} = \frac{(W_2 - W_1)}{\text{Weight of Sample}}$$

Where:
$(W_2\text{-}W_1)$ = Weight of residue.

- Tannin determination by the Van Burden and Robinson (1981)

  Tannins were computed thus:

$$\text{Tannins (mg/100 mL)} = \frac{\text{X-blank}}{\text{Standard-blank}}$$

Where:
X = Absorbance.

- Flavonoid determination by the hydrolysis gravimetric method of Harbone (1993)

Percentage Flavonoid was calculated by:

$$\text{Flavonoids (\%)} = \frac{(W_2 - W_1)}{\text{Weight of Sample}}$$

Where:
$(W_2\text{-}W_1)$ = Weight of residue.

- Phenol analysis using the follin- ciocaltean colorimetric method of Harbone (1993):

$$\text{Phenols (\%)} = \frac{100}{W} \times \frac{C}{1000} \times \frac{VF}{VA} \times \frac{D}{1}$$

Where:
W = Weight of sample analyzed
C = Concentration of standard in mg/ml
VF = Total filtrate volume
VA = Volume of filtrate analyzed
D = Dilution factor where applicable

- Saponin in plant sample was determined by the combined solvent extraction method

  Saponin concentration was computed thus:

$$\text{Saponins mg/100 mL} = \frac{100 \times 100\ (W_2 - W_1)}{\text{Weight of sample}}$$

Where:
$(W_2\text{-}W_1)$ = Average weight of residue.

The experiments were performed in triplicates (or three times) for each parameter and the resultant percentages were summed up to deduce the average percentage for comparison of means for each parameter.

RESULTS

Alkaloids Qualitative assessment (Table 1) revealed that alkaloids were observed to be deeply present (++) in the leaves and present (+) in the root and stem of all four species. However, the quantitative analysis results (Table 2) show alkaloid distribution in the different plants to be in the following decreasing order: leaf (*J. curcas* 4.54%; *J. multifida* 2.85%; *J. gossypifolia* 2.81%; *J. podagrica* 0.33%), stem (*J. gossypifolia* 2.16%; *J. curcas* 1.94%; *J. multifida* 1.72%; *J. podagrica* 0.15%), seed (*J. curcas* 2.85%; *J. multifida* 2.63%; *J. gossypifolia* 2.36%; *J. podagrica* 0.18%) and root (*J. curcas* 1.83%; *J. multifida* 1.68%; *J. gossypifolia* 1.60%; *J. podagrica* 0.26%). A similar order was observed in the leaf and root.

Tannins: Tannin was present (+) in the stem of the four species investigated. It was very deeply present (+++) in the leaf of *J. curcas* and *J. multifida* and deeply present (++) in the seed of *J. curcas* and *J. gossypifolia* and root of *J. multifida* (Table 1). A decreasing order of the observed results of quantitative analysis of tannin concentration in the leaves and seeds of the species follows thus: leaf (*J. curcas* 7.43%; *J. podagrica* 6.79%; *J. multifida* 5.16%; *J. gossypifolia* 5.14%), seed (*J. curcas* 4.23%; *J. multifida* and *J. podagrica* 3.89% each; *J. gossypifolia* 3.52%). Also, the same tannin concentration of 2.73% (*J. gossypifolia* and *J. podagrica*) and 1.21% (*J. multifida* and *J. podagrica*) were recorded in the root and stem of the species respectively (Table 2).

Flavonoids: Flavonoids were observed to be very deeply present (+++) in the leaves of *J. curcas* and *J. multifida* and deeply present (++) in that of *J. gossypifolia*. They were observed to be present (+) in all other plant parts of the species (Table 1). The highest flavonoid concentration of 3.25% was observed in the leaf of *J. podagrica* (Table 2). Flavonoid was 2.76% in *J. curcas* leaf, 2.41% in *J. gossypifolia* and 2.18% in *J. multifida*. The same concentration of 2.14% (seed) and 1.28% (stem) were observed in *J. multifida* and *J. podagrica*. The least flavonoid concentration of 1.20% was observed stem of *J. gossypifolia*.

Table 1: Preliminary (qualitative) screening of secondary metabolites in *Jatropha* species

Taxa	Plant part	Alkaloids	Tannins	Flavonoids	Saponins	Phenols
J. curcas	Leaf	++	+++	+++	++	++
	Root	+	+	+	+	+
	Seed	++	++	+	+	+
	Stem	+	+	+	+	+
J. gossypifolia	Leaf	++	++	++	++	+
	Root	+	+	+	+	+
	Seed	++	++	+	+	+
	Stem	+	+	+	+	+
J. multifida	Leaf	++	+++	+++	++	++
	Root	+	++	+	+	+
	Seed	+	+	+	+	+
	Stem	+	+	+	+	+
J. podagrica	Leaf	++	++	+	++	+
	Root	+	+	+	+	+
	Seed	++	+	+	++	+
	Stem	+	+	+	+	+

+: Present, ++: Deeply present, +++: Very deeply present

Table 2: Concentration of secondary metabolites present in *Jatropha* species

Taxa	Plant part	Alkaloids (%)	Tannins (%)	Flavonoids (%)	Saponins (%)	Phenols (%)
J. curcas	Leaf	4.54	7.43	2.76	4.89	0.38
	Root	1.83	2.83	2.23	3.62	0.20
	Seed	2.85	4.23	2.57	2.33	0.26
	Stem	1.94	1.47	1.61	2.27	0.59
J. gossypifolia	Leaf	2.81	5.14	2.41	4.15	0.26
	Root	1.60	2.73	1.75	2.83	0.24
	Seed	2.36	3.52	2.26	2.37	0.18
	Stem	2.16	1.36	1.20	2.18	0.13
J. multifida	Leaf	2.85	5.16	2.18	3.15	0.26
	Root	1.68	2.14	2.10	2.16	0.23
	Seed	2.63	3.89	2.14	2.44	0.18
	Stem	1.72	1.21	1.28	2.73	0.16
J. podagrica	Leaf	0.33	6.79	3.25	3.15	0.33
	Root	0.26	2.73	2.06	3.25	0.26
	Seed	0.18	3.89	2.14	2.44	0.18
	Stem	0.15	1.21	1.28	2.77	0.15

Saponins: The saponins were deeply present (++) in the leaf of the four species and seed of *J. podagrica*. They were observed to be present (+) in all other plant parts of the species. A descending order of the concentration of saponins in the leaf and stem of the species as revealed from the quantitative analysis follows thus: leaf (*J. curcas* 4.89%; *J. gossypifolia* 4.15%; *J. multifida* and *J. podagrica* 3.15% each), stem (*J. podagrica* 2.77%; *J. multifida* 2.73%; *J. curcas* 2.27%; *J. gossypifolia* 2.18%). The least saponin concentration of 2.16% was observed in the root of *J. multifida* while the highest concentration of 4.89% was in the leaf of *J. curcas*.

Phenols: Phenols were generally low qualitatively and quantitatively. They were observed to be deeply present (++) only in leaves of *J. curcas* and *J. gossypifolia* and present in all other plant parts investigated in the four species. The highest and least phenol concentrations of 0.59 and

0.13% were recorded in the stems of *J. curcas* and *J. gossypifolia*, respectively. While the seed of *J. podagrica, J. multifida* and *J. gossypifolia* were observed to have the same phenol concentration of 0.18%, the leaves of *J. multifida* and *J. gossypifolia* had 0.16%.

DISCUSSION

The results of the comparative qualitative and quantitative phytochemical analysis of secondary metabolites in the leaf, root, seed and stem of *Jatropha* species in the Niger Delta are presented in Tables 1 and 2. Burkill (1994) reported the presence of tannins and saponins in the leaf of *J. gossypifolia* and *J. multifida* respectively which is confirmed by this study. The qualitative screening from the presence study revealed the presence of alkaloids, tannins, saponins, flavonoids and phenols in all plant parts investigated but to varying intensities/qualities as shown in Table 1. Preliminary qualitative test according to Mallikharjuna *et al.* (2007) is useful in the detection of bioactive principles and subsequently may lead to drug discovery and development. Qualitative phytochemical screening of two species of *Avicennia* (Ganesh and Vennila, 2011) and three cucurbits (Irshad *et al.*, 2010) revealed the presence of alkaloids, flavonoids, tannins, saponins and phenols in a manner suggesting close relationship of the species studied. Similar results (Table 1) were obtained in this research work which confirm the relatedness of the investigated *Jatropha* species as well as reveal their potentials in the drug industry. Specifically, the qualitative screening revealed that tannins were very deeply present in the leaves and stems of *J. curcas* and *J. multifida* and deeply present in the roots of *J. curcas, J. gossypifolia* and *J. multifida*. Likewise, alkaloids were deeply present in the leaf of *J. multifida*, the stem of all species except in *J. multifida* and the roots of *J. curcas*, *J. gossypifolia* and *J. multifida*. Olawale-Abulude (2007) undertook phytochemical screening of leaves of twenty-eight woody species from different plants families in Nigeria and discovered the presence of tannins, alkaloids and flavonoid in all samples. Thus these secondary metabolites seem cosmopolitan in plants but to varying degrees and types. Their different degrees of occurrence in plant parts confer taxonomic usefulness on them. This is similar to the findings of Nyananyo *et al.* (2010) on some Niger Delta plants.

Among the five groups of phytochemicals investigated from the leaf, root, seed and stem of the species, tannins were found to be the most abundant followed by saponins and alkaloids. Phenols were the lowest in concentration and together with flavonoids varied across the different plant parts. Specifically, tannins were concentrated to 7.43% in the leaves of *J. curcas* with corresponding concentration of 0.38% by phenols. Similarly, saponin (the most abundant phytochemical in the root of the species) with concentration of 3.62% (the highest in all seed samples) in the root of *J. curcas* had corresponding phenol concentration of 0.20%. A similar sequence with tannins being the most abundant phytochemical in plant parts, followed by saponins, alkaloids, flavonoids and then phenols as seen in this study has been reported by Mallikharjuna *et al.* (2007). Other recent investigation showcasing presence, distribution in plant parts and importance of these phytochemicals similar with the results of this investigation include Ogunkunle and Ladejobi (2006), Sunita and Abhishek (2008), Ferreira *et al.* (2009), Kumar *et al.* (2009) and Ganesh and Vennila (2011). It is noteworthy that while all these phytochemicals are present in the different *Jatropha* species connoting taxonomic affinity, the differences in their concentrations uniquely confers individualism on each species and thus support their being treated as taxonomic species. This agrees with Irshad *et al.* (2010) where phytochemical results established closer relationship between *Lagenaria siceraria* and *Luffa cylindrical* than *Cucumis maxima*.

This study reveals the presence of the subject secondary metabolites in the different *Jatropha* species to varying concentrations which is taxonomically useful; it also brings to bare the fact that the species are potential sources of these important phytochemicals. For instance, flavonoids are one of the most popular secondary metabolites possessing a variety of biological activities at nontoxic concentrations (Irshad *et al*., 2010). Dietary flavonoids are noted to play effective roles in cancer prevention (Ren *et al*., 2003; Aggarwal and Shishodia, 2006). Flavonoids together with the other secondary metabolites identified in the *Jatropha* species have been severally reported in other plants to show curative activity against diverse pathogens, used traditionally as analgesic, antimicrobial and soothing herbs (Hassan *et al*., 2004; Faruq *et al*., 2004; Olafimihan, 2004; Singh *et al*., 2009; Thirunavukkarasu *et al*., 2010; Ganesh and Vennila, 2011).

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