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Establishment of Callus Derived from *Jatropha curcas* L. Petiole Explants and Phytochemical Screening

K. Nyembo[1*], N. Mbaya[1], N. Muambi[1] and C. Ilunga[2]

[1]*Department of Phytobiology, Division of Life Science, General Atomic Energy Commission, Regional Center of Nuclear Studies of Kinshasa XI, University of Kinshasa, DR, Congo.*
[2]*Department of Biotechnology, Division of Life Science, General Atomic Energy Commission, Regional Center of Nuclear Studies of Kinshasa, XI, University of Kinshasa, DR, Congo.*

Authors' contributions

This work was carried out in collaboration between all authors. Author KN designed the study, wrote the protocol and the first draft of the manuscript. Author NM performed critical reviews of the manuscript. Authors NM and CI conducted the experiments of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The focus of this study was to assess the production of secondary metabolites of callus derived from *Jatropha curcas* L. petiole explants.
Study Design: Laboratory experimental tests; Tissue culture, Lyophilisation, Phytochemical Analysis, Determination of tannins by Folin-Denis method.
Place and Duration of the Study: Department of Phytobiology, Department of Biotechnology. General Atomic Energy Commission. Regional Center of Nuclear Studies of Kinshasa P.O BOX 868 Kin.XI DR Congo during March and June 2013.
Methodology: *In vitro* callus cultures were initiated from petiole explants of *Jatropha curcas* L. on

**Corresponding author: Email: baudyne@yahoo.fr;*

Murashige & Skoog (1962) basal medium supplemented with growth regulator formulations 4.44 µM of 6-benzylaminopurine (BAP), 4.92 µM of Indole-3-butyric acid (IBA), and 100 ml L^{-1} coconut milk. Callus was lyophilized. The extracts were subjected to phytochemical tests for the presence of plant secondary metabolites. The amount of tannins was estimated by Folin-Denis method.

Results: Excellent growth of callus was obtained. Callus was soft, friable, lush green in color and grew fast from 8 to 30 days of culture then stabilized at low growth rate. The results obtained by phyto-chemical tests revealed the presence of saponins, tannins, alkaloids, steroids and flavonoids. The tannin contents *in vitro* proliferated callus, leaves, stem barks, root barks, roots and latex of *J. curcas* were found to be 161, 173, 220, 214, 43 and 245 µg tannic acid equivalent/g of dry weight respectively.

Conclusion: The results of this study have revealed that *in vitro* induced callus from *J. curcas* petiole explants was able to produce secondary metabolites. The pharmacological activity of *J. curcas* reported by various researchers can be attributed to the presence of these phytochemicals.

Keywords: Callus; in vitro culture; phytochemical; tannin; secondary metabolites.

1. INTRODUCTION

Jatropha curcas L. (*J. curcas)* belongs to *Euphorbiaceae* family and grows in tropical and sub-tropical regions. This crop has gained lot of importance due to property to produce biodiesel [1]. Furthermore, *J. curcas* has been used as traditional medicine to cure various infections and extracts from various parts of the plant, such as seeds, stem barks, roots and leaves have been shown fungicidal [2,3] and bactericidal properties [4-7].

In vitro culture techniques for *J. curcas* have been researched and developed in many laboratories for potential mass propagation [8-12]. Cell suspension culture is considered one of the best approaches for studying the biosynthesis of natural products, and callus is the richest sources of cell mass when establishing such cultures [13]. The past few decades have seen increasing scientific interest in both growth of modern plant tissue culture and the commercial development of this technology as means of producing valuable phyto chemicals [14].

Phenolic compounds which include tannins, phenolic acids and flavonoids are considered to be an important class of phytochemicals and were found in high concentrations in a wide variety of plant. These compounds possess many biological effects which are mainly attributed to their strong antioxidant properties in scavenging free radicals inhibition of peroxidation and chelating transition metals [15]. Free radicals can be harmful to the organism if not properly regulated and thus may cause several degenerative diseases, such as diabetes, aging, cancer, cardiovascular diseases, metabolic syndrome and arthrosclerosis [16]. Antioxidants can protect the human body from frees radicals, reactive oxygen species effects and reduce the risk of cardiovascular and neurodegenerative disease [17-19].

The specific objective of this tissue culture study was to induce callus from petiole explants of *J. curcas* and to assess the production of secondary metabolites.

2. MATERIALS and METHODS

2.1 Collection of Plant Material

Fresh leaves, petioles, roots, root barks, stem barks, latex of *J. curcas* were collected from experimental garden of CREN-K /Kinshasa RD-Congo.

2.2 Initiation of Callus and Maintenance

Starting materials of 150 petioles were used as initial explants for callus production. Under the laminar airflow cabinet, we cut them of 3-4 mm and then they were sterilized with 0.25% mercuric chloride solution for 4 minutes followed by 6 rinses in sterile distilled water to remove the traces of the sterilizing substance. Five petiole explants were transferred aseptically on the surface of a solidified medium in each Petri dish containing 25 ml of macro and micro salts Murashige & Skoog, (MS) [20] supplemented with 4.44 µM Benzylaminopurine (BAP), 4.92 µM Indole-3-butyric acid (IBA) and 100 ml/L coconut milk for initiation of callus. The pH of the medium was adjusted to 5.8 using NaOH 0.1N and Difco agar (0.8% w/v) was used as solidifying agent before autoclaving at 120ºC for 20 minutes.

Cultured Petri dishes were incubated for callus formation in a growth room at 27±0, 1ºC under a 16 hours photoperiod with a light intensity of 2000 lux. Calli were sub-cultured after 14 days interval on fresh medium for further proliferation period of two months.

2.3 Lyophilization

The process of freeze-drying was done using the freeze-dryer 7670530 LABCONCO. This method can protect biological activity of samples. Callus and latex were frozen in vials at a low temperature (-30ºC). The water was then extracted via vacuum during 24 hours, resulting in a porous dry "lyocake". Lyophilized callus and latex were pulverized and sieved to 0.2 mm mesh. These materials can be easily stored.

2.4 Preparation of Extracts for Phytochemical Analysis

The dried samples were pulverized to fine powder using an electric grinder. Five grams of each powdered plant material were submitted to cold extraction in analytical grade methanol and kept at room temperature for 24 hrs. Extracts were recovered by filtration using Whatman N° 1 filter paper. Excess of methanol was evaporated in hot-air oven at 45ºC and later the condensed extract was weighed and reconstituted in minimum volume of methanol and stored at 4ºC for further analysis.

2.5 Phytochemical Analysis of the Plant Extract

The methanolic extracts were subjected to preliminary qualitative phytochemical screening for the presence of plant secondary metabolites such as alkaloids, saponins, steroids, tannins and flavonoids using methods described by Harborne [21] and Evans [22].

2.6 Determination of tannins by Folin-Denis Method

The amount of tannins was estimated by Folin-Denis method. Tannic acid was taken as the standard for estimating the total amount of tannins in the methanolic extract. The tannin contents were calculated as tannic acid equivalent from the calibration curve of standard tannic acid by plotting the absorbance versus concentration. Different dilutions of standard tannic acid were prepared ranging (0 g/l, 0.5 g/l, 1 g/l, 1.5 g/l, and 2 g/l). This was then transferred to two sets of tubes. These tubes were then incubated at 80ºC in a water bath during one hour. The absorbance was measured with a spectrophotometer at 760 nm wavelength. The tannin content was expressed in terms of tannic acid equivalents/g dry weight (TAE/g DW).

3. RESULTS AND DISCUSSION

3.1 Callus Formation

Eighty five per hundred of the total petiole explants cultured attained the stage of callus formation approximately 8 days after incubation. According to our visual observations, callus arising from the edges of explants was soft and friable. The callogenesis rate was fast from 8 until 30 days of culture, and then stabilized at a low growth rate (Figs. 1 and 2). Green points were observed on the surface of this callus which was maintained by transferring on the same fresh medium after every two weeks during two months.

Fig. 1. Initiation of callus formation from petiole explants

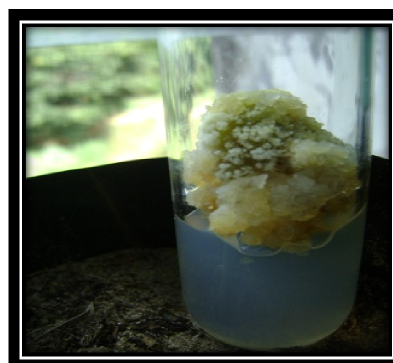

Fig. 2. Proliferated callus from petiole explants

Hypocotyl explants of *J. curcas* cultured in MS medium supplemented with 2.26 µM 2.4-D alone and with 2% v/v coconut milk proved to be more

effective for establishment of callus on a large scale in short period of time [23]. Although, it has been reported that 2.4-D in low concentration is the most commonly used auxin for good callogenesis, in the present study best callus growth was found when petiole explants were placed on full strength MS medium supplemented with a 1:1 concentration ratio of BAP and IBA with 100ml/L coconut milk. Gawri and Upadhy [24] reported that tannins, alkaloids, glycosides, flavonoids, and phlobatannis were present in the petioles of fresh leaves of *Jatropha curcas* as well as the cultured callus but the concentration of alkaloids and glycosides were higher in the callus. It is well known that, *in vitro* cultures are able to produce secondary metabolites in quantities more than that of original parts of the plants [25].

3.2 Phytochemical Analysis

The qualitative estimation of five secondary metabolites (saponins, tannins, alkaloids, steroids and flavonoids) was undertaken. The phytochemical investigations showed that all secondary metabolites analyzed were present in the plant parts as well as the callus (Table 1). Using qualitative analysis, Igbinosa et al. [4], Sudha et al. [7] and Asobara et al. [26] have reported the presence of the same compounds in all tissues of *J. curcas* studied: stem bark, leaves, roots and seeds extracts. All the phytochemical components detected were known to support bioactive activities in medicinal plants [27,28]. These bioactive molecules are produced in plants in less quantity, which is largely influenced by environmental factors. This has led to looking for alternate resources like callus and suspension cultures where the synthesis of bioactive molecules can be enhanced either with PGRs or elicitors [29].

3.3 Determination of Phenolic Compounds

The presence of tannins was determined by Folin-Denis method. This is based on the reduction of phosphomolybdic acid and tungstic in alkaline medium in the presence of tannins to give a blue color whose intensity is measured at 760 nm. In Table 2 is reported that the tannin contents of callus, leaves, stem barks, root barks, roots and latex were found to be 161, 173, 220, 214, 43 and 245 µg TAE/g DW respectively. In the current study, highest and lowest tannin contents of 245 and 43µg TAE/g DW were recorded in latex and roots methanolic extract respectively. Previous studies have reported the presence of tannins in *J. curcas* latex [30,31]. Igbinosa et al. [32] have found a high polyphenolic content in *J. curcas* stem bark. The tannins possess anticancer activity and they can be used in cancer prevention [33]. Tannins found in the plant are useful in the treatment of inflamed or ulcerated tissues. They have also been used for treating intestinal disorders such as diarrhea and dysentery [34]. The presence of tannins in *J. curcas* supports the traditional medicinal use of this plant in the treatment of different human ailments.

Table 1. Qualitative analysis of methanolic extracts of *J. curcas*

Compounds	*J. curcas*		
	Callus	Leaves	Root barks
Saponins	+	+	+
Tannins	+	+	+
Alcaloïds	+	+	+
Stéroïds	+	+	+
Flavonoïds	+	+	+

Note: '+' indicates presence

Table 2. The tannin contents of the methanolic extracts of *J. curcas*

J. curcas	Concentrationµg TAE/g DW
Callus	161
Leaves	173
Stem barks	220
Root barks	214
Roots	43
Latex	245

4. CONCLUSION

The results of this study have revealed that *in vitro* induced callus from *Jatropha curcas* L. petiole explants was able to produce secondary metabolites. Petioles were suitable explant material. The concentration of tannin in callus was observed to be 161 µg TAE/g DW. *In vitro* culture should be used to increase the production of secondary metabolites. The presence of bioactive compounds in the plant parts of *J. curcas* such as saponins, tannins, alkaloids, steroids and flavonoids indicates the medicinal values of this plant. Therefore, further research is needed for the isolation, identification and quantification of other biopharmaceuticals in the *J. curcas* extracts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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