*Artículo de Investigación. Revista Ciencia e Ingeniería. Vol. 41, No. 1 pp. 75-80, diciembre-marzo, 2020.*
*ISSN 1316-7081. ISSN Elect. 2244-8780 Universidad de los Andes (ULA)*

# Phytochemical screening of *Jatropha curcas* L. leaves and roots collected from Mérida-Venezuela

# Tamizaje fitoquímico de hojas y raíces de *Jatropha curcas* L. colectadas en Mérida-Venezuela

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**Abstract**

*Jatropha curcas* L. is a perennial herb, belonging to the family Euphorbiaceae, found in countries such as Madagascar, India, Mexico and Venezuela. This plant is widely used in tropical countries as antibacterial, antifungal, anti-inflammatory and analgesic. Present investigation aims to describe the secondary metabolites profile of *J. curcas* leaves and roots. Phytochemical analysis carried out with different chemical reactions revealed the presence of alkaloids, steroids, triterpenoids, tannins, coumarins, flavonoids and phenolic compounds. These results indicate that *J. curcas* might be considered as secondary metabolites source, which supports its applications both traditional and laboratory level.

**Keywords**: *Jatropha curcas,* phytochemical screening, alkaloids, flavonoids, terpenoids, phenolic nuclei.

**Resumen**

*Jatropha curcas* L. es un arbusto perenne, perteneciente a la familia Euforbiaceae y se encuentra en países como Madagascar, India, México y Venezuela. Esta planta es ampliamente utilizada en países tropicales como antibacteriano, antifúngico, antiinflamatorio y analgésico. La presente investigación tiene como objetivo describir el perfil de metabolitos secundarios presentes en las hojas y raíces de *J. curcas*. El análisis fitoquímico llevado a cabo con diferentes reacciones químicas reveló la presencia de alcaloides, esteroides, triterpenoides, taninos, cumarinas, flavonoides y compuestos fenólicos. Estos resultados indican que *J. curcas* puede ser considerada como fuente de metabolitos secundarios, lo cual apoya sus aplicaciones tanto a nivel tradicional como de laboratorio.

**Palabras claves**: *Jatropha curcas,* phytochemical screening, alkaloids, flavonoids, terpenoids, phenolic nuclei.

## 1 Introducción

*Jatropha curcas* L. belongs to the Euphorbiaceae family. In Venezuela is commonly known as Pringamosa or Guaritoto. This species has been extensively studied due to the presence of a wide range of secondary metabolites and also for industrial and medical applications (Oskoueian y

metabolites such as diterpens, triterpens, coumarins, lignans, flavonoids, alkaloid, glucosides, among others (Aiyelaagbe y col., 2007a, 2007b, Uche y col., 2008, Igbinosa y col., 2009, Dhale y col., 2010, Orantes 2010, Ahirrao y col., 2011, Arekemase y col., 2011, Nwokocha y col., 2011, Bravesh y col., 2011, Méndez y col., 2012). In addition, different biological activities have also been proved in extracts as well as metabolites isolated from *J. curcas* around the world, being abortive, diuretic, hemostatic, insecticide, cytotoxic and jaundice the most commonly reported (Igbinosa y col., 2009, Dhale y col., 2010, Méndez y col., 2012).

According to previous investigations, flavonoids and phenolic nuclei are known to possess antioxidant, anti-allergic, anti-inflammatory, antibacterial and antifungal activities. Steroids have shown analgesic and anti-inflammatory effects and triterpenoids have exhibited analgesic activity and may also decrease blood sugar levels in some experimental animals (Mujumdar ycol., 2003, Aiyelaagbe y col., 2007a, 2007b; Uche y col., 2008, Sangeetha y col., 2009, Kalimithu y col., 2010, Mishra y col., 2010, Arekemase y col., 2011, Bravesh y col., 2011, Prasad y col., 2012).

Preliminary phytochemical screening was performed *in J. curcas* leaves and roots in present investigation in order to determine either the presence or absence of secondary metabolites such as alkaloids, coumarins, glycosides, flavonoids, steroids, triterpenos, quinones, saponins, tanins and phenolic compounds. This information might be useful for further researches dealing with substances biologically active.

## 2 Materiales y Métodos

*2.1 Plant material*

*J. curcas* leaves were collected from El Chamicero "A", El Quebradón area, Montalbán Parish, Campo Elías Municipality, Ejido City, Mérida State, Venezuela, at an altitude of 1147 m.s.n.m, in May 2012. This species was identified by Eng. Pablo Meléndez, Herbarium MERF-ULA. A voucher specimen (code JR-53) was deposited in the Herbarium of the Faculty of Pharmacy and Bioanalysis.

*2.2 Extraction*

Leaves and roots were dried at 40°C for 7 days in an air circulation oven. Powdered leaves were extracted by maceration using hexane (LH) and methanol (LM). Separately, roots were grounded and extracted with methanol (LR). This procedure was preformed three times to complete an exhaustive process. All extracts were concentrated under reduce pressure using a rotaevaporator equipment. Liquid-liquid fractionation was also conducted on LH and LM obtained from initial extraction, using solvents of different polarities [hexane (LM-H), dichloromethane (LM-D) and ethyl acetate (LM-A)]. These extractions were carried out by same procedure described before. All extracts were kept at 4 °C until performance of phytochemical screening analysis.

*2.3 Phytochemical qualitative screening*

*J. curcas* extracts were qualitatively evaluated using a variety of phytochemical analysis to determine either the presence or absence of chemical constituents. Chemical reactions conducted are described below:

*2.3.1 Testing for Alkaloids*: each extract (10 mg) was dissolved in 2 ml of hydrochloric acid 5%, after mixing and filtered, three aliquots were taken. Drops of Wagner, Mayer and Dragendorff reagents were added to each. Red-brown precipitate (Wagner), or yellowish white precipitate (Mayer) and red-orange precipitate (Dragendorff) indicated the presence of such metabolites (Domínguez 1979).

*2.3.2 Testing for Coumarins*: 10 mg of each sample were added to 0.5 mL of ethanol along with 2 drops of concentrated ammonium hydroxide. If examination under UV light at a wavelength of 365 nm shows the presence of blue or green fluorescence might be indicative of a positive result (Domínguez, 1979).

*2.3.3 Testing for Glycosides*: 10 mg of each extract were dissolved in 1 mL of distilled water followed by 5 drops of aqueous sodium hydroxide. A yellow colour indicated the presence of glycosides in the extract (Rajesh y col., 2010).

*2.3.4 Testing for Cardiotonic glycosides*: Reaction with 3,5- dinitrobenzoic acid, Keller-Killiani and Legal assays were performed to determine the presence of these metabolites.

- *Keller - Killiani reaction*: 10 mg of each extract were dissolved in Keller's reagent and then 5 drops of concentrated sulfuric acid were added. The occurrence of brown ring between the two phases formed is indicative of deoxy glycosides nuclei presence (Domínguez 1979).

- *Legal Reaction*: 3 drops of pyridine, 1 drop of sodium nitroprusside solution (aqueous) 5% and 3 drops of 2N sodium hydroxide were added to 10 mg of each extract. Intense red coloration indicated the presence of cardenolides or $\alpha,\beta$-unsaturated lactones nuclei (Domínguez 1979).

- *Reaction with 3,5- dinitrobenzoic acid*: 5 drops of a freshly prepared 1:1 potassium hydroxide (0.5 N) and 3,5-dinitrobenzoic acid (2%) mixture was added to 10 mg of each sample. Blue or violet coloration was indicative of cardiotonic glycosides presence (Marcano y Hasegawa, 2002).

*2.3.5 Testing for Flavonoids*: Shinoda, Pew, 10 % sodium hydroxide and 2-aminoethyl diphenyl borate assays were performed in order to determine the presence of these metabolites.

- *Shinoda test*: 1 mL of absolute ethanol and 3 drops of concentrated hydrochloric acid were added to 10 drops of the

diluted extract in main solvent. Formation of red color indicated the presence of aurones and chalcones. In cases where no colour change was observed, pieces of metallic magnesium were placed. The formation of orange, red or magenta coloration indicated the presence of flavones, flavonols and flavonones, respectively. (Domínguez 1979).

- *Pew's test*: 2 mg of zinc powder and 5 drops of 5N hydrochloric acid were added to 1 mL of each diluted extract in the source solvent. The presence of red, pink or coffee color indicated the existence of dihyflavones, flavonones, and dihydrochalcones, respectively (Domínguez, 1979).

- *Test with 10% sodium hydroxide*: 3 drops of sodium hydroxide 10% were added to 1 mL of diluted extracts in main solvent. Formation of yellow-red, coffee-orange, purple-red or blue coloration indicated the presence of xanthones and/or flavones, flavonols, chalcones and anthocyanins, respectively (Domínguez 1979).

- *Test with 2-aminoethyl diphenylborate*: Methanolic solutions of each extract were analyzed by thin layer chromatography (TLC) on silica gel 60 F254 plates, at room temperature. Solvent system was chloroform-methanol-formic acid-water (3:6:1:1). Visualization was performed under UV-Vis light (350nm) before and after staining with 1% methanolic 2-aminoethyl diphenylborate reagent. Appearance of yellow fluorenscence, after spraying with reagent was indicative of flavonoids nuclei (Marcano y col., 2002, Garrido y col., 2013).

*2.3.6 Testing for steroids and/or triterpenoids*: To determine the presence of these metabolites Salkowski, Rosenthaler and Lieberman-Bouchard assays were performed (Domínguez 1979).

- *Salkowski test*: 2 mL of chloroform and 1 mL of concentrated sulfuric acid were slowly added to 10 drops of the extract dissolved in the source solvent until double phase formation. The presence of a reddish-brown color in the interface was indicative of steroidal ring.

- *Rosenthaler test*: 3 drops of Rosenthaler reagent and 2 drops of concentrated sulfuric acid were added to 2 mL of the extract dissolved in the source solvent. Formation of Violet color in the middle layer was indicative of the presence of triterpenoids.

- *Lieberman-Bouchard test*: 1 mL of anhydrous acetic acid and 3 drops of concentrated sulfuric acid were added to 2 mL of the extract dissolved in the source solvent. After 5 minutes a blue-green color middle layer was indicative of sterols. Pink, red, magenta or violet color revealed the presence of triterpenoids.

*2.3.7 Testing for Quinones and Anthraquinones*: To determine the presence of these metabolites Borntraguer, ammonium hydroxide, concentrated sulfuric acid (Domínguez, 1979) and benzene assays (Rajesh y col., 2010) were performed.

- *Borntraguer test*: 10 mg of each extract were dissolved in 3 mL of distilled water and filtrated. After filtration 3 mL of 5% potassium hydroxide solution were added to each. The mixture was heated to boiling for 3 minutes. Alkaline solution was allowed to cool down and then extracted with 3 ml of chloroform. Organic phase was separated and shaken with 2 mL of 5% potassium hydroxide solution. Occurrence of red color in alkaline phase indicated the presence of quinones. Those samples showing yellow colour with green fluorescence where treated with one drop of 6% hydrogen peroxide, formation of red colour was considered positive for anthrone derivatives.

- *Test with ammonium hydroxide*: one drop of concentrated ammonium hydroxide was added to 10 mg of each sample, previously dissolved in the source solvent. After two minutes, formation of red colour indicated the presence of anthraquinones.

- *Test with sulfuric acid*: a drop of concentrated sulfuric acid was added to 10 mg of each extract dissolved in the original solvent. Red color indicated the presence of quinones.

- *Test with benzene*: 1 mL of benzene was added to 10 mg of each extract dissolved in isopropyl alcohol, followed by stirring and filtration. 0.5 mL of 10% ammonia solution was added to the filtrate. This mixture was shaken; formation of pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of anthraquinones.

*2.3.8 Testing for Saponins*: To determine the presence of these metabolites Foam assays with and without sodium bicarbonate were performed.

- *Test Foam height (without sodium bicarbonate):* 1 mL of distilled water was added to 10 drops of the extract dissolved in methanol (20 mg/mL) in a test-tube, shaken vigorously to froth, then allowed to stand for 30 minutes. Saponin content was measured as follows: no froth (absence); froth less than 3 mm high (poor); froth 6mm high (moderate) and froth greater than 8 mm high (abundant). (Domínguez, 1979).

- *Foam Test (with sodium bicarbonate):* 1 mL of distilled water and 1 drop of sodium bicarbonate saturated solution were added to 5 drops of the extract dissolved in solvent source (20 mg/mL) in a test-tube and shaken vigorously during 3 minutes. Formation of honeycomb shaped foam indicated the presence of saponins.

*2.3.9 Testing for Tannins*: 100 mg of each extract were dissolved in 10 mL of ethanol, and extracted with 25 mL of distilled water in boiling during 15 minutes. Once allowed to fresh at room temperature, 0.2 mL of 10% sodium chloride solution were added to mixture and filtered. The filtrate was divided into four equal portions in test tubes. 5 drops of 1% gelatin solution were added to first portion, 5 drops of gelatin - salt solution (1%gelatin + 10% salt) were added to second portion, 4 drops of 10% ferric chloride solution were added to third portion, and 3 drops of 1% potassium ferricyanide solution were added to fourth portion. Precipitation observed after addition of either second or third reagent was indicative of the presence of tannins. Those samples showing grayish-black or black-blue colour after addition of third reagent indicated the presence of tannins with catechol or pyrogallol nuclei, respectively. Samples turning to blue colour after fourth reagent revealed the presence of phenolic compounds

(Rajesh y col., 2010).

*2.3.10 Testing for Phenols*: 10 mg of each extract were dissolved in 1mL of ethanol, then 2 mL of distilled water was added followed by 4 drops of ferric chloride aqueous solution 10 % w/v. Formation of a blue or green color indicated the presence of phenols (Orantes 2010).

## 3 Results and Discusion

Phytochemical screening conducted in several extracts obtained from leaves and roots of *J. curcas* revealed the presence of triterpenoids, steroids, alkaloids, coumarins, flavonoids and tannins. According to results, LM-D, LM-A and LA extracts showed the widest variety of secondary metabolites (Table 1). Triterpenes and steroids were abundantly observed in LM-D and LM-A extracts while moderate presence was detected in LM-H and LR. Triterpene and steroidal compounds are known to play an important role in nature since they have a close relationship with several hormones involved in plant biosynthesis. Furthermore, previous investigations have revealed that anti-inflammatory, antibacterial, analgesic and antiviral properties might be related to this type of components (Aiyelaagbe y col., 2007a, Uche y col., 2008, Igbinosa y col., 2009, Seth y col., 2010).

Alkaloids were barely observed in LM, however after fractionation of this extract a more abundant presence was detected in LM-A, possible due to the purification process. It is well documented that alkaloids are mainly biosynthesized by some plants to prevent or protect from predators, however, a wide variety of medical uses have been studied for researchers where they have related these metabolites to analgesic, anticancer, stimulant activity, among others (Aiyelaagbe y col., 2007a, Igbinosa y col., 2009).

Abundant presence of flavonoids was observed in LA while moderate was detected in LR, LM-D, LM-A and low concentrations in LM. Furthermore, phenolic nuclei were present in LR, LM-D, LM-A and LA extract. This indicates that phenolic compounds found in *J. curcas* are of polar nature possibly present in coumarins, flavonoids and/or tannins highly hydroxylated. Coumarins and tannins were observed in large proportions in LA supporting the theory that compounds with phenolic nuclei have many hydroxyl substituents. In addition, phenolic and flavonoid compounds are known to possess antimicrobial, anti-allergic, anti-inflammatory, antiviral and anticancer properties. These compounds have also been related to free radical scavenging activity, thus may act as primary antioxidants (Uche y col., 2008, Seth y col.,2010, Shanmugan y col., 2010, Oskoueian y col., 2011).

According to different assays performed in present investigation, there is no presence of quinones, saponins, glycosides or mucilages in any of *J. curcas* extracts analyzed.

## 4 Conclusions

Phytochemical screening of *J. curcas* extracts exhibit a similar secondary metabolites profile as reported in literature for *Jatropha* genus. Presence of triterpenoids, steroids, coumarins, flavonoids, alkaloids and phenolic compounds indicate that this species might be a source of secondary metabolites with potential biological activities.

**Acknowledgements**

Authors wish to acknowledge MSc. Libia Alarcón, Lic. Gabriela Rodríguez, Lic. Patricia Pérez, MSc. Haydee Calles, Dra. Irama Ramírez, for donation of chemical reagents, Natural Products Laboratories A and B, Research Institute, Faculty of Pharmacy and Bioanalysis, University of Los Andes. Authors also like to express gratitude to Mr. Diego Peña Toloza, who translated present manuscript.

**Table 1.** Phytochemical qualitative screening of leaves and roots extracts from *J. curcas*

| **Metabolites** | **Assays** | **LH** | **LR** | **LM** | **LM-H** | **LM-D** | **LM-A** | **LA** |
|---|---|---|---|---|---|---|---|---|
| Alkaloids | Wagner reaction | - | - | + | - | - | +++ | - |
| | Mayer reaction | - | - | + | - | - | +++ | - |
| | Dragendorff reaction | - | - | + | - | - | +++ | - |
| | Hager reaction | - | - | + | - | - | +++ | - |
| Coumarins | Fluorescence at 365nm | - | +++ | + | - | - | + | +++ |
| Glucosides | NaOH *(ac)* | - | - | - | - | - | - | - |
| Cardiotonics Glucosides | Keller – Killiani reaction | - | - | - | - | - | - | - |
| | Legal reaction | - | - | - | - | - | - | - |
| Flavonoids | Shinoda | - | + | + | - | + | - | +++ |
| | NaOH 10% | - | ++ | + | - | ++ | ++ | +++ |
| | CCF: 2-aminoetil diphenyl borate | - | - | - | - | - | - | - |
| | Pew | - | ++ | + | - | - | - | +++ |
| Esteroids and/or Triterpenoids | Lieberman Bouchard | ++ | - | - | ++ | ++ | +++ | - |
| | Rosenthaler | - | ++ | - | - | +++ | - | - |
| | Salkowskí | - | + | - | - | +++ | ++ | - |
| Mucílagos | Cooling a 0°C | - | - | - | - | - | - | - |
| Phenolic compounds | $FeCl_3$ | - | ++ | - | - | ++ | ++ | +++ |
| Quinones | $C_6H_6$ *(conc.)* | - | - | - | - | - | - | - |
| | $NH_4OH$ *(conc.)* | - | - | - | - | - | - | - |
| | Borntraguer | - | - | - | - | - | - | - |
| | $H_2SO_4$ *(conc.)* | - | - | - | - | - | - | - |
| Saponins | Foam height | - | - | - | - | - | - | - |
| | Sodium Bicarbonate | - | - | - | - | - | - | - |
| Tannins | Gelatine 1% | - | - | - | - | - | - | - |
| | Gelatine (1%) – Salt (10%) | - | - | - | - | - | - | - |
| | $FeCl_3$ 10% | - | - | - | - | - | - | - |
| | $KFe(CN)_6$ 1% | - | - | - | - | - | - | +++ |

Foot note: - Absence, + Low, ++ Moderate, +++ Abundant, LH hexane extract of leaves, LR methanol extract of roots, LM methanol extract of leaves, LM-H methanol-hexane extract of leaves, LM-D methanol-dichloromethane extract of leaves, LM-A methanol-ethyl acetate extract of leaves, LA methanol-water extract of leaves.

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***Recibido:****10 de agosto de 2019*

***Aceptado:*** *15 de octubre de 2019*

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