Journal of Forensic Chemistry and Toxicology
Volume 2 Number 1, January - June 2016
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.2116.6

Review Article

Forensic and Pharmacognostic Studies of Jatropha Curcas

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Abstract

Jatropha curcas is a multipurpose plant with many attributes and considerable potential. It is a tropical plant that can be grown in low or high rainfall areas and can be used to reclaim land, as a hedge or as a commercial crop. Now days the cases of Jatropha poisoning are rare, but due to its high toxic value in the presence of toxins like phorbol esters, the case studies in homicidal, suicidal or accidental cases become significant. For identification of *Jatropha curcas* constituents, an attempt has been made in the present study to identify phytochemicals, amino acid, saponins, phyto sterols, glycosides, anthraquinones, flavonoids and tanins. These were examined by various chemical colour tests and phorbol ester was examined by thin layer chromatography.

The extracted oil from the leaves and seed part was studied along with standard oil using two different solvents and their Rf values were also studied. For visualization of the spots, the plates were sprayed with sulphuric acid to develop coloured spots, which were then studied by UV in the range of 254-365 nm.

Keywords: Forensic; Pharmacognostic Study; Phyto-Chemicals; TLC; Phorbol Ester.

Introduction

Jatropha curcas (pinion of India) commonly called as 'Ratanjyot' in India is a drought resistant deciduous shrub which belongs to the family *Euphorbiaceae*. *Jatropha curcas* is native in tropical America, but is now found abundantly in many tropical and sub-tropical regions throughout Africa and Asia. Its strength as a crop comes from its ability to grow on very poor and dry sites. *Jatropha curcas* is a resilient plant that can adopt too many ecological conditions in any rural or urban areas of the country (Figure 1).

Due to the toxicity of its leaves, *Jatropha curcas* is not browsed and therefore traditionally used in protecting hedges around arable land and housing. The wonder plant produces seeds with an oil content of 37%. The oil can be combusted as fuel without being refined. Also due to its toxicity, *Jatropha curcas* oil is not edible and is traditionally used for manufacturing soap and medicinal applications.

Symptoms are largely those associated with gastro-intestinal irritation. There is acute abdominal pain and a burning sensation in the throat about half an hour after ingestion of the seeds followed by nausea, vomiting and profuse watery diarrhea. In severe poisoning, these symptoms progress to hemorrhagic gastroenteritis and dehydration. Polydipsia can be extreme. Salivation and sweating may occur. There may be skeletal muscle spasm. Intense hyperpnoea or a quick panting respiration is seen together with hypotension and electro cardio graphic abnormalities. There may be CNS and cardiovascular

depression, children are more susceptible. This may be either a direct effect of toxins or secondary to dehydration.

Fig. 1: *Jatropha curcas Plant*

The toxicity of *Jatropha curcas* is based on several components (phorbol esters, curcains, trypsin inhibitors and others). There are possibilities of its consumption knowingly or accidentally. Due to its high toxic value, mainly in presence of toxins like phorbol esters, the case studies in homicidal, suicidal or accidental cases become significant.

Phytochemicals of Jatropha Curcus

The following phytochemicals have been detected in *Jatropha curcus*

Curcin

Curcin is a toxic albumin belonging to a group of proteins called ribosome-inactivating proteins (RIP), which inhibit prokaryotic and eukaryotic ribosome by specific modification of the larger rRNA. Based on physical properties, RIPs are classified into-

- *Type 1 RIP* which are single-chain (approximately 30 kD) proteins having enzymatic activity and inhibiting cell-free protein synthesis *in vitro*, but are relatively non-toxic to cells and animals.
- *Type 2 RIP* which are heterodimeric proteins (approximately 60 kD) consisting of A chain (similar to type 1 RIP) attached to sugar-binding B chain (lectin) by a disulfide bond. Type 2RIP is highly toxic compared to type 1 RIP, which are relatively nontoxic *in vivo* due to the absence of the sugar-binding chain.

Diterpenes (Phorbol Esters)

The term 'phorbol esters' is used today to describe naturally occurring group of compounds mainly distributed in plant species of the Euphorbiaceae family. Phorbol esters are esters of phorbol, a tetra cyclic diterpenoid with a tigliane skeletal structure. Terpenoids are classified according to the number of carbon atoms they carry. Monoterpenoids have 10 carbons; diterpenoids have 20, and so on. (Fig 2)

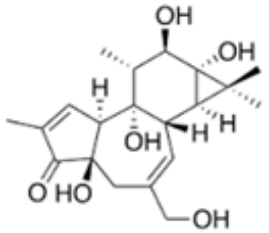

Fig. 2: Phorbol esters

Diterpenes are believed to be the most potent compounds synthesized by *Jatropha* species. There are at least 20 diterpenes reported from *Jatropha* species. Among the diterpenes, a group of compounds having tigliane skeleton called phorbol esters are the most toxic molecules in *Jatropha* species. Six phorbol esters are identified in *Jatropha curcas*. The concentration of phorbol esters varies from 2 to 3 mg/g kernel meal and from 2 to 4 mg/g oil in different provenances of *Jatropha curcas*. The phorbol esters are lipophilic, present mainly in oil, and when present in oil or kernel not affected by heat. In *Jatropha curcas* the phorbol esters (mg/g dry matter) are present in kernels, leaves, stems flowers, buds, roots, bark(outer brown skin) bark(inner green skin) and wood, but not in latex.

Other Compounds

Tannins

Tannins are the phenolic substances associated with toxic and impaired nutrient absorption and anti-nutritional effects including reduced food/feed intake, growth retardation. Tannins possess multiple phenolic hydroxyl groups leading to the formation of complexes primarily with proteins and to a lesser extent with metal ions, amino acids, and polysaccharides.

Saponins

These are steroid or triterpene glycoside compounds which are present in a variety of plants.

In plants, saponins may serve as anti-feed ants or help in protecting the plant against microbes and fungi. However, saponins are often bitter in taste, and thus, when present in high concentrations would reduce plant palatability in livestock.

Phytates

Phytic acid (known as inositol hexakisphosphate IP_6 or phytate when in the salt form) is the principle storage form of phosphorus in most plant seeds. Inositol penta- (IP_5), tetra-(IP_4), and triphosphate (IP_3) are also termed phytates. Phosphorus in phytate form is, in general, not bioavailable to non-ruminant animals because these animals lack the digestive enzyme phytase, which is required to separate phosphorus from the phytate molecule. Phytates also form sparingly digestible phytate–protein complexes, thus reducing the availability of dietary protein.

Lectins

Lectins are carbohydrate-binding (glyco) proteins and are ubiquitous in nature. Plant lectins when consumed by animals survive digestion in the Gastro Intestinal Tract and bind to membrane glycosyl groups of the cells lining.

Experimental Procedure

Materials

The samples were collected from the wild patch of *Jatropha curcas* plants grown at the Ajmer city of Rajasthan (N: 26° 26' 59.6256'', E: 74° 38' 23.6940'') in the month of February. Fresh leaves and seeds has been collected from the same plants and stored at 4°C in aluminium foil.

Methods

Macroscopic and Microscopic Study

Fig. 3: *Jatropha curcas Leaf*

Macroscopic Examination

Leaves: *Jathropha curcas* has green leaves with a length and width of 6 to 15 cm, with 5 to 7 shallow lobes. The leaves are arranged alternately with apetiole 3 to 20 cm long and broadly ovate in outline (Figure 3).

Seeds- Seeds of *Jatropha curcas* are ellipsoid, 1 to 2 cm long, mottled black and coarsely pitted. The seeds become mature when the capsules change from green to yellow after two to four months. *Jatropha curcas* growth rate is variable and produce seeds after approximately two years depending upon many factors as rainfall, etc. Three months after sowing, mean height growth of seedlings produced in greenhouse conditions ranged between 15 cm and 33 cm and showed variability among seeds. The seed yield up to 31-37% of valuable oil (Figure 4).

Fig. 4: *Jatropha curcas Seeds*

Microscopic Examination

Fresh leaves of *Jatropha curcas* were considered for the microscopic examination. For the purpose trinocular microscope was used. Different sectional views were taken from surface view of epidermis and lamina (Table 1).

Table 1: Characteristics of jatropha curcas leaf

Properties	Observation
Colour	Dark Green and Light Green
Shape	Broadly Ovate
Size	10-15 cm. x 7.5-12.5 cm
Apex	Acute
Venation	Palmate
Margin	Cordate
Taste	Characteristic, Bitter

In microscopic examination of the fresh leaf, midrib showed 5-7 layered thick walls, closely packed collenchymatous cells on both the surfaces with spongy and vascular bundles. Upper epidermis was covered by thin cuticle. Both the epidermis showed anomocytic stomata. Simple covering trichomes were very rare on both epidermises. Transverse view of lamina showed single layer of closely packed

palisade cells below the upper epidermis layer of the leaf (Figure 5).

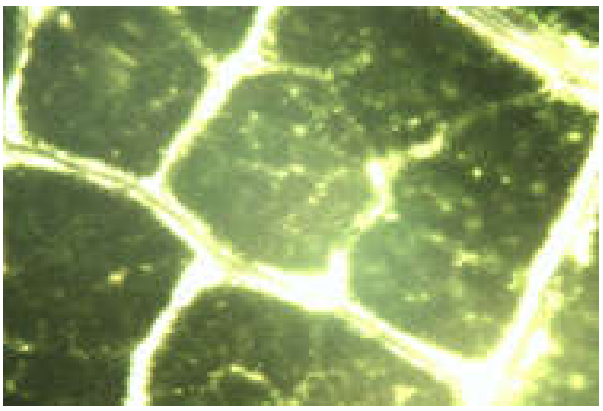

Fig. 5: Microscopic view of leaf

Qualitative Screening of Phytochemicals

N-hexane and butanol extract of leaves of *Jatropha curcas* were screened for the presence of alkaloids, amino acids, anthraquinones, flavonoids, glycosides, phytosterol and saponins by using standard protocols.

Preparation of the Extract

Jatropha curcas leaves fully dried at room temperature were grinded into powder and for it, ten grams was weighted accurately and extracted with n-hexane in a Soxhlet extraction apparatus. At the end of the extraction process, the flask containing n-hexane extract was removed and n-hexane was evaporated by using a rotary evaporator. The weight of the residual extract was measured and precent yield was calculated. The same procedure was used to prepare butanol extract. These extracts were kept in refrigerator for phytochemical study.

Extract yield% = $W_1/W_2 \times 100$

Where W_1 = net wt. of powder in grams after extraction.

W_2 = net wt. of powder in grams taken for extraction.

Screening Test

One gram of ethyl acetate, acetone: water (7: 3) was dissolved in 100 ml of its own mother solvents (in n-hexane as well as in butanol extract) to obtain a stock of concentration 1% (V/V) for the screening test.

Chemical tests were carried out on the extracts using standard procedures to identify the constituents by characteristic colour changes as described by Sofowara (1993), Obebedy and Sofowara (1978) (Table 2).

Test for Alkaloids

In 5ml stock extract 2 ml HCl was added. It made acidic medium and then 1ml of dragendroff's reagent was added, which gave orange or red color precipitate. It indicated the presence of alkaloids.

Test for Amino Acid

In 2 ml stock extract 40% NaOH solution was added in a test tube. After that one drop of 1% $CuSO_4$ solution was added in it. A blue color appeared which showed the presence of amino acids in the extract.

Test for Anthraquinones

2M HCl was added to the sample and the mixture was heated on a hot water bath for 15 minutes, then it was cooled and filtered. The filtrate was extracted with chloroform layer which was later on separated and shaken with 10% KOH and it became pink-red which showed the presence of anthraquinones in the extract.

Test for Flavonoids

Dilute NaOH (1N) was added to one ml extract, then yellow color in plant extract appeared, and soon it became colorless, when few drops of acid (10%H_2SO_4) were added to it. It indicated the presence of flavonoids in the extract.

Test for Glycosides

On water bath, 1ml of extract hydrolysed with HCl for few hours and cooled at room temperature. Then to it 1 ml pyridine was added with a few drops of sodium nitropruside solution, which further made alkaline with NaOH solution. Pink to red color appeared, which indicated the presence of glycosides in the extract.

Test for Phytosterol

The extract was refluxed with solution of alcoholic KOH till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for presence of phytosterol. The residue was dissolved in few drops of dilute acidic acid; 3ml acetic anhydride was added followed by few drops of conc. H_2SO_4. Bluish green color appearance

indicated the presence of phytosterol in the extract.

Test for Saponins

Extract was diluted with 20 ml of distilled water and agitated in a graduated cylinder for 15 minutes continuously. A formation of layer (1cm) of foam showed the presence of saponins in the extract.

Table 2: Phyto-chemical analysis

S. No.	Chemical Tests	N-hexane Extract	Butanol Extract
1.	Alkaloids	+	+
2.	Amino acid	-	+
3.	Anthraquinones	-	-
4.	Flavonoids	+	-
5.	Glycosides	-	-
6.	Phytosterol	-	+
7.	Saponins	-	+

(Note: +=present, -=absent)

Identification of Phorbol Ester by TLC

A standard glass TLC plate was coated with the slurry of silica gel G in water to a uniform thickness of 0.25 mm. After that the plate was activated by heating in an oven at 110^0C for about one hour. The extracted material is then spotted on silica gel G spotting plate with the help of micro capillary tube, and then transferred to the saturated chromatographic chamber in which appropriate amount of solvent was added and allowed to developed till the solvent front move up to 7 cm with the developing solvent to a fixed demarcated finishing line and then plates were taken out and allowed to dry in open. The whole process was repeated for two different solvent systems. For visualization of the spots, the plates were sprayed with sulphuric acid to develop coloured spots, which were then studied by UV in the range of 254-365 nm (Figure 6).

The rf value of phorbol esters in hexane: ethyl acetate: glacial acetic acid (30:60:10) was 0.39 (reported standard 0.40) whereas in dichloromethane: acetone (90:10) was 0.79 (reported standard 0.80).

Fig. 6: TLC photographs showing the movement of extracted compound (left side) from spotting point with the standard compound (right side) in each TLC plate when viewed under UV chamber

Results and Discussion

In the present study an attempt has been made to study *Jatropha curcas* plant by Macro and Microscopic, Phytochemical and TLC examination. These examinations are found to be very useful tools for the identification and characterization of *Jatropha curcas* leaves and seeds. A simple, accurate and precise analytical method is used for the analysis of leaves and seeds of *Jatropha curcas*, which could be useful in future forensic identification of unknown plant material. It has been observed that Macro and Microscopic, Phytochemical and TLC examination are very useful tools for the identification of *Jatropha curcas* leaves and seeds. Phytochemical studies were carried out for the identification of *Jatropha* leaves with standard plant leaves. Thin layer chromatography studies showed the presence of active principles of *Jatropha curcas*. This is further suggested that the proposed methods are simple, sensitive, reproducible, and economical and requires very less equipment. These can be employed for qualitative evaluation of *Jatropha curcas* leaves and also for the routine forensic analysis of *Jatropha curcas*. Therefore, this could be a method of choice for official monographs in Forensic Toxicology.

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