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# Comparative phytochemical investigations and *in-vitro* pharmacological studies of two medicinal species of *Jatropha* from South India

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**ABSTRACT**

*In the present study, comparative phytochemical screening was conducted between the ethanolic and methanolic extracts of Jatropha curcas and Jatropha gossypifolia in order to detect the presence of major class of chemical compounds. Comparative antioxidant activity of both the plants was performed using nitric oxide scavenging as well as reducing power assays. An attempt has been made to review the In-vitro cytotoxicity of medicinal plants Jatropha curcas and Jatropha gossypifolia on HeLa cell lines which will be certainly useful for the prevention and treatment of cancers.*

**Keywords**: *Jatropha curcas*, *Jatropha gossypifolia*, in-vitro studies, Nitric oxide scavenging.

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## INTRODUCTION

From the earliest times, herbs have been prized for their pain-relieving and healing abilities and today we still rely largely on the curative properties of plants. According to World Health Organization, 80 % of the people living in rural areas depend on medicinal herbs as primary healthcare system [1].Traditional medicines have been used for many centuries by a substantial proportion of the population of India[2]**.** The genus Jatropha has 175 known species of the plant belonging to the family Euphorbiaceae [3]. Jatropha is known for its use as purgative/laxative, and other medicinal uses. All parts of the plant, including seeds, leaves and bark, fresh or as a decoction, are used in traditional and folk medicine and veterinary purposes[4].

## MATERIALS AND METHODS

### Collection of plant material

*Jatropha curcas* and *Jatropha gossypifolia* were collected from Tamilnadu Agricultural University, Mettupalayam. The voucher specimen has been archived in the herbarium of R V S Arts and science college, Coimbatore.

**Preparation of plant extract**

Stems were collected from the plant .They were air dried for one week (in shade and was grinded). The grinded stem particles were soaked in 97% ethanol for 72 hours at room temperature. This ethanol extract was evaporated to remove ethanol and leaf extract was obtained as a lyophilized powder.

*Jatropha curcas*

*Jatropha gossypifolia*

**Comparative phytochemical analysis of *Jatropha curcas and Jatropha gossypifolia***

**Test for Alkaloids**

**a) Dragendroff's test**

8g of $Bi(NO_3)_3.5H_2O$ was dissolved in 20 ml of $HNO_3$ and 2.72g of KI in 400ml of water. These were mixed and allow to stand when $KNO_3$ crystals out. The supernatant was decanted off and made up to 100ml with distilled water. The alkaloids were regenerated from the precipitate by treating with sodium carbonate followed by extraction of the liberated base with ether. To 50ml of alcoholic solution of extract was added to 2ml of HCl. To this acidic medium 1ml of reagent was added. An orange red precipitate produces immediately indicates the presence of alkaloids.

**b) Wagner's test (Iodine- Potassium iodide solution)**

1.2 gm of Iodine and 2gm of $H_2SO_4$ and the solution was diluted to 100ml. 10ml of alcoholic extract was acidified by adding 1.5% v/v of HCl and a few drops of Wagner's reagent. Formation of yellow or brown precipitate confirmed the presence of alkaloids.

**Test for Glycosides**

A small amount of alcoholic extract was dissolved in 1ml of water and the aqueous NaOH solution was dissolved in 1ml of water and the aqueous NaOH solution was added. Formation of yellow colour indicates the presence of glycosides.

**Test for Tannins**

**a) Ferric chloride test**

To 1-2 ml of aqueous extract few drops of 5% aqueous $FeCl_2$ solution was added. A bluish black colour which disappears on addition of a few ml of $H_2SO_4$, there is no formation of the yellow brown precipitate.

**Test for Flavanoids**
In a test tube containing 0.5ml of alcoholic extract, 5-10 drops of dilute HCl and small piece of ZnCl or magnesium were added and the solution was boiled for a few minutes. In the presence of flavonoids, reddish pink or dirty brown colour was produced.

**Test for Saponins**
In a test tube containing 0.5ml of aqueous extract a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 30 minutes. A honey comb like froth was formed and it showed the presence of saponins.

**Test for Steroids**
**a) Salkowski test**
To 2ml of chloroform extract, 1ml of concentrated sulphuric acid was added carefully along the sides of the test tubes. A red colour was produced in the chloroform layer in the presence of steroids.

**Test for Phenols**
**a) Ferric chloride test**
To 10ml of alcoholic solution of extract, 2ml of distilled water followed by drops of 10% aqueous FeCl3 solution were added. Formation of blue or green indicates the presence of phenols

**Test for Carbohydrates**
**a) Molischs test**
2ml of the filtrate was added with two drops of alcoholic solution of alpha naphthol. The mixture was shaken well and 1 ml of concentrated sulphuric acid was added along the sides of the test tubes and allowed to stand. A violet or purple ring indicates the presence of starch [6].

**Comparative antioxidant activity of *Jatropha curcas and Jatropha gossypifolia***
**1. Nitric oxide radical scavenging assay**
Sodium nitro prusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with $O_2$ to produce nitrite ions, which can be measured at 540 nm spectrophotometrically in the presence of Griess reagent.

Procedure: 5mg of extract was dissolved and mad up to 10ml with methanol. The sample was completely soluble. 50µl of 10mM sodium nitro prusside and 50µl test solution of various concentrations are illuminated using fluorescence light at room temperature for 150 minutes. Following incubation, 125µl of Griess reagent was added and incubated for 30 minutes at room temperature. The absorbance was measured at 546nm[7].
(Griess reagent: 1% sulphanilic acid, 2% phosphoric acid and 0.1% N-1- napthyl ethylene diamine dihydrochloride)

**2. Reducing power**
The sample solution was mixed with 2.5ml of 0.2M of phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricynaide. The mixture was incubated at $50^0$ C for 20 minutes and aliquot (2.5ml) of 10% TCA was added to mixture followed by centrifugation at 3000rpm at 10 minutes. The upper layer of solution was mixed with 2.5ml of distilled water and 2.5ml of 0.1% $FeCl_3$ and absorbance at 700nm. Increased absorbance shows increase in reducing power [8].

***invitro* cytotoxicity of *Jatropha curcas* and *Jatropha gossypifolia* extract on HELA cell lines**
HeLa cell lines were used as the cancer cell lines for determining the anti proliferative effects of *Jatropha curcas* and *Jatropha gossypifolia* alkaloids extacts.MTT and Neutral red method were used to access the total cell viability[9].

## RESULTS

**Table 1: Comparative preliminary phytochemical screening between *Jatropha curcas* and *Jatropha gossypifolia***

| TEST | *Jatropha curcas* | | *Jatropha gossypifolia* | |
|---|---|---|---|---|
| | **Methanol** | **Ethanol** | **Methanol** | **Ethanol** |
| Alkaloids | + | _ | + | + |
| Flavonoids | _ | _ | _ | _ |
| Saponins | _ | _ | _ | _ |
| Phenols | + | + | _ | + |
| Carbohydrates | _ | _ | _ | _ |
| Tannins | + | _ | _ | _ |
| Steroids | + | + | + | + |
| Glycosides | + | _ | + | _ |

**Comparative antioxidant assay of *Jatropha curcas* and *Jatropha gossypifolia***

**Figure: 1. Nitric oxide scavenging assay**

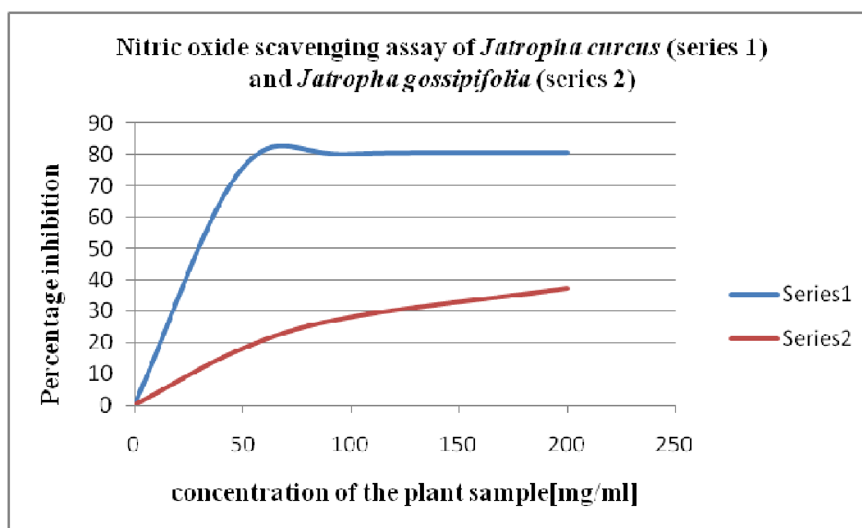


**Figure: 2. Reducing power assay**

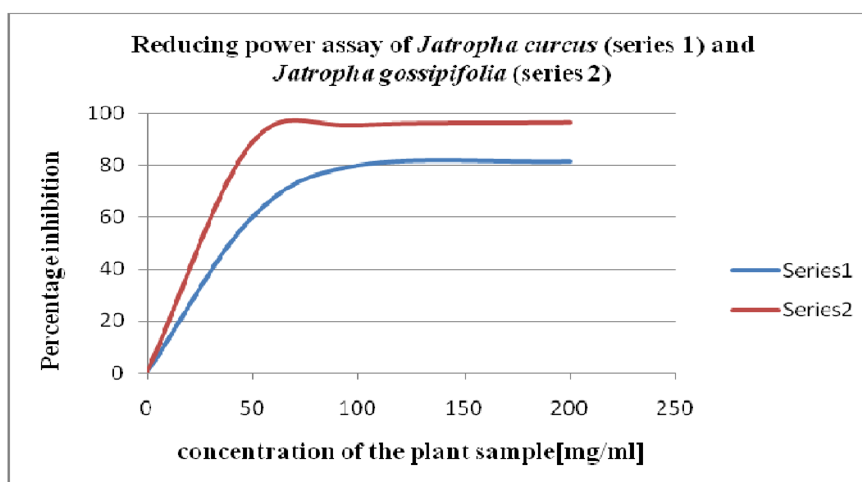

**Table: 2. Nitric oxide scavenging assay**

| Sample | Volume of the sample [$mgml^{-1}$] | Percentage Inhibition | $IC_{50}$ |
|---|---|---|---|
| *Jatropha curcas* | 50<br>100<br>200 | 75.54%<br>80.04%<br>80.55% | 37.20 $mgml^{-1}$ |
| *Jatropha gossypifolia* | 50<br>100<br>200 | 18.89%<br>28.34%<br>37.12% | >250$mgml^{-1}$ |

**Table: 3. Reducing Power Assay**

| Sample | Volume of the sample [$mgml^{-1}$] | Percentage Inhibition | $IC_{50}$ |
|---|---|---|---|
| *Jatropha curcas* | 50<br>100<br>200 | 60.02%<br>80.04%<br>81.57% | 41.20 $mgml^{-1}$ |
| *Jatropha gossypifolia* | 50<br>100<br>200 | 88.66%<br>95.40%<br>96.54% | 25.33$mgml^{-1}$ |

**Table: 4. *Invitro* cytotoxicity of *Jatropha curcas* extract on HELA cell lines**

| Sample Volume (µl) | MTT Assay | Neutral red assay |
|---|---|---|
| Control | 93.75% | 88.40% |
| 10 | 52.56% | 46.15% |
| 100 | 33.33% | 51.7% |
| 200 | 26.92% | 20% |
| 400 | 17.95% | 44% |

**Table: 5. *Invitro* cytotoxicity of *Jatropha gossypifolia* extract on HELA cell lines**

| Sample Volume (µl) | MTT Assay | Neutral red assay |
|---|---|---|
| Control | 52.56% | 51.38% |
| 10 | 33.33% | 20.53% |
| 100 | 26.92% | 44.87% |
| 200 | 17.95% | 58.82% |
| 400 | 12.69% | 62.15% |

## DISCUSSION

Preliminary phytochemical screening of the plant revealed the major class of compounds present in both methanolic and ethanolic extracts of *J. curcas* and *J. gossypifolia.* Methanolic extract of *J.curcas* had Alkaloids, Phenols, Tannins, Steroids and Glycosides in major amounts. Ethanolic extract of *J.curcas* contains Phenols and Steroids in major quantity. Methanolic extract of *J. gossypifolia* gave the presence of Alkaloids, Steroids and glycosides. Ethanolic extract *of J. gossypifolia* gave Alkaloids, Steroids and Phenols presence **[Table 1].**

Comparative antioxidant assay of *Jatropha curcas* and *Jatropha gossypifolia* was carried out to check the antioxidant potential of the plants using Nitric oxide scavenging assay and reducing power assay. $IC_{50}$ value was calculated for both the plants. Nitric oxide scavenging assay gave $IC_{50}$ of 37.20 $mgml^{-1}$ for *Jatropha curcas* and >250$mgml^{-1}$ for *Jatropha gossypifolia***[Figure 1,Table 2].** Reducing power assay gave $IC_{50}$ of 41.20 $mgml^{-1}$ for *Jatropha curcas* and 25.33 $mgml^{-1}$ for *Jatropha gossypifolia* **[Figure 1,Table 2].** Both the plants *J. curcas* **[Table4]** and *J. gossypifolia* **[Table 5]** showed significant cytotoxic activity.

## CONCLUSION

From the present work we conclude that the two species of Jatropha are highly potential in biological activity. The preliminary screening of the samples revealed the presences of high value class of compound like phenolic group as

the major content in the plants. In terms of the nitric oxide scavenging activity the Jatropha curcas possess higher activity than the other species while it shows higher reducing power than the earlier.

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