

Phytochemical Evaluation and Antioxidant Study of *Jatropha curcas* Seeds

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ABSTRACT

Jatropha curcas L. is a soft-wooded shrub found both in wild and cultivated conditions across the country. The seeds of *J. curcas* have been used as a purgative, antihelminthic, abortifacient as well as for treating ascites, gout, paralysis, and skin diseases. Present study includes physicochemical, phytochemical, antioxidant and HPTLC analysis of *J. curcas* seeds. Ethanolic, aqueous and hydroalcoholic extracts from *J. curcas* were screened for antioxidant evaluation using DPPH free radical scavenging activity. Protocatechuic and Gallic acid, two potential antioxidants present in this species, has been studied through HPTLC which may be utilized for the proper standardization of the drug.

In *J. curcas*, IC_{50} of ethanolic, aqueous and hydroalcoholic extract were found to be $46 \pm 3.46 \mu\text{g/ml}$, $36.66 \pm 0.57 \mu\text{g/ml}$ and $32.66 \pm 1 \mu\text{g/ml}$ respectively. Ascorbic acid is used as standard which showed IC_{50} of $2.66 \pm 0.11 \mu\text{g/ml}$. HPTLC studies showed the presence of protocatechuic acid and gallic acid at R_f 0.69 and 0.32 with r^2 of 0.998 and 0.994 respectively with mobile phase toluene: ethyl acetate : formic acid (5:5:0.5).

J. curcas seed extract showed significant in vitro antioxidant activity, 50% hydroalcoholic extract showed the most potent activity. Quantification of protocatechuic and Gallic acid in 50% hydroalcoholic extract of *J. curcas* has been performed and was found to be 0.146% and 0.092% respectively.

Key words: Antioxidant, DPPH, Gallic acid, *J. curcas*, Protocatechuic acid

INTRODUCTION

Jatropha curcas L. is a drought-resistant shrub belonging to the Family Euphorbiaceae, widely found all across the country and is also cultivated in Central and South America, south-east Asia and Africa^[1]. Some of the ethnomedical uses of the extracts of *J. curcas* leaves and roots include a remedy for cancer, as an abortifacient, antiseptic, diuretic, purgative and haemostatic^[2]. The seeds of *J. curcas* have been used as a purgative, antihelminthic, abortifacient as well as for treating ascites, gout, paralysis, and skin diseases. The seed oil has been used as an ingredient in the treatment of rheumatic conditions, itch and parasitic skin diseases, fever, jaundice and gonorrhoea, as a diuretic agent, and a mouth-

wash. The seed is known to contain purgative oil, curcin, a phytotoxin known to cause dehydration, cardiovascular collapse as a result of haemorrhagic gastro-enteritis, and central nervous system depression^[5]. Several cases of *J. curcas* nut poisoning in humans after accidental consumption of the seeds have been reported with symptoms of giddiness, vomiting and diarrhoea and in the extreme condition even death has been recorded^[6].

It is also used traditionally for the treatment of sciatica, dropsy, paralysis, piles, snake bites, rheumatism, dysentery, diarrhoea and certain skin diseases^[7-14]. The plant is known famous for its bio-diesel^[15]. The levels of essential amino acids except lysine in *J. curcas* meal protein are higher than those of the FAO reference protein for a growing child of 2-5 years^[16].

METHODS AND MATERIALS

Plant material

The plant specimen i.e. seeds of *Jatropha curcas* L. were collected from Banthara, Lucknow, India in 2010. The plant was authenticated by Dr. AKS Rawat, NBRI. A voucher specimen (262541) has been submitted in institute's herbarium.

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Extraction of plant material

The fresh seeds were collected, thoroughly washed with water to remove all debris. The seeds were shade dried. The dried seeds were powdered by using electric grinder at 100 mesh size. Extraction was performed by soxhlation process in two steps. Firstly the powdered seeds are defatted under soxhlet assembly using 250ml of 98% petroleum ether for 6 hours. This is followed by 9 hours soxhlation of defatted seeds powder by using 250 ml of ethanol, aqueous and 50% hydroalcoholic solvent separately. The final extracts obtained were passed through Whatman No. 1 filter paper. The filtrates obtained were concentrated under vacuum in a rotary evaporator at 40°C and stored at 4°C for further use. The crude extracts were obtained by dissolving a known amount of dry extract in 98% ethanol to obtain a stock solution of 1000 µg/ml. The stock solutions of all three extracts were serially diluted with methanol to obtain lower dilutions (10, 15, 20, 25, 30, 35, 40, 45, 50 and 60 µg/ml).

Physicochemical and Phytochemical Studies

Physicochemical and Phytochemical studies were performed according to the methods^[17,18,19], such as extractive values, total ash, acid insoluble ash, total sugar, starch, tannin, and phenols on shade-dried powdered material.

In vitro antioxidant Activity

Antioxidant activity of the plant extracts and standard was assessed on the basis of the radical scavenging effect of the stable DPPH free radical.^[20] The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as the standard in solutions ranging from 1 to 10 µg/ml. 0.002% DPPH solution in methanol was prepared. Then 2 ml of this solution was mixed with 2 ml of sample solution and the standard solution to be tested separately. These solution mixtures were kept in the dark for 20 min and optical density was measured at 517 nm using a Shimadzu spectrophotometer against methanol. The blank used was 2 ml of methanol with 2 ml of DPPH solution (0.002%).

The optical density was recorded and percent of inhibition was calculated according to the methods^[21] using the formula given below:

% of inhibition of DPPH activity = $(A-B/A) \times 100$, where, A is optical density of the blank and B is optical density of the sample.

HPTLC Studies

Reagents used were from Merck (Germany) and standard Gallic and protocatechuic acid was procured from Sigma-Aldrich (Steinheim). Air dried (45-55°C) powdered seeds of *J. curcas* (2.0g) in triplicate were extracted separately with 3 × 10 ml 50% hydroalcoholic solvent. Extracts were concentrated

under vacuum and redissolved in methanol, filtered and finally made up to 100 ml with methanol prior to HPTLC analysis.

Chromatographic Conditions

Chromatography was performed on Merck HPTLC precoated silica gel 60GF₂₅₄ (20X20 cm) plates. Methanolic solutions of samples and standard compounds gallic and protocatechuic acid of known concentrations were applied to the layers as 6 mm-wide bands positioned 15 mm from the bottom and 15 mm from side of the plate, using Camag Linomat V automated TLC applicator with nitrogen flow providing a delivery speed of 150 nl/s from application syringe. These conditions were kept constant throughout the analysis of samples.

Detection and Quantification of Gallic and Protocatechuic acid

Following sample application, layers were developed in a Camag twin trough glass chamber that had been pre-saturated with mobile phase of toluene : ethyl acetate : formic acid (5:5:0.5) till proper separation of bands up to 8 cm height. After development, layers were dried with an air dryer and gallic and protocatechuic acid was simultaneously quantified using Camag TLC scanner model 3 equipped with Camag Wincats IV software. Following scan conditions were applied: slit width 5 mm x 0.45 mm; wavelength 310 nm for gallic acid and 280 nm for protocatechuic acid and absorption-reflection mode. In order to prepare calibration curves, stock solution of gallic and protocatechuic acid (1 mg/ml) was prepared and various volumes of these solutions were analyzed through HPTLC, calibration curves of peak area Vs. concentration were prepared for quantification.

RESULTS AND DISCUSSION

Phytochemical screening

Hydroalcoholic extract of *J. curcas* seeds showed positive test for flavonoids, proteins, carbohydrates, glycosides, phenolic compounds and saponins.

Physicochemical Studies

Parameters such as Extractive values (Water, alcohol and petroleum ether soluble), total ash and acid insoluble ash values, total Sugar, total starch, total tannins and total phenolics were determined. Results are shown in Fig 1.

Antioxidant activity

In vitro antioxidant study of *J. curcas* seeds was performed using three different seed extracts viz. ethanolic extract, aqueous extract and hydroalcoholic extract. Ascorbic acid is used as standard. In this study hydroalcoholic extract was found to be most potent among all three extracts which showed the least IC₅₀ value of 32.66 ± 1 µg/ml, whereas

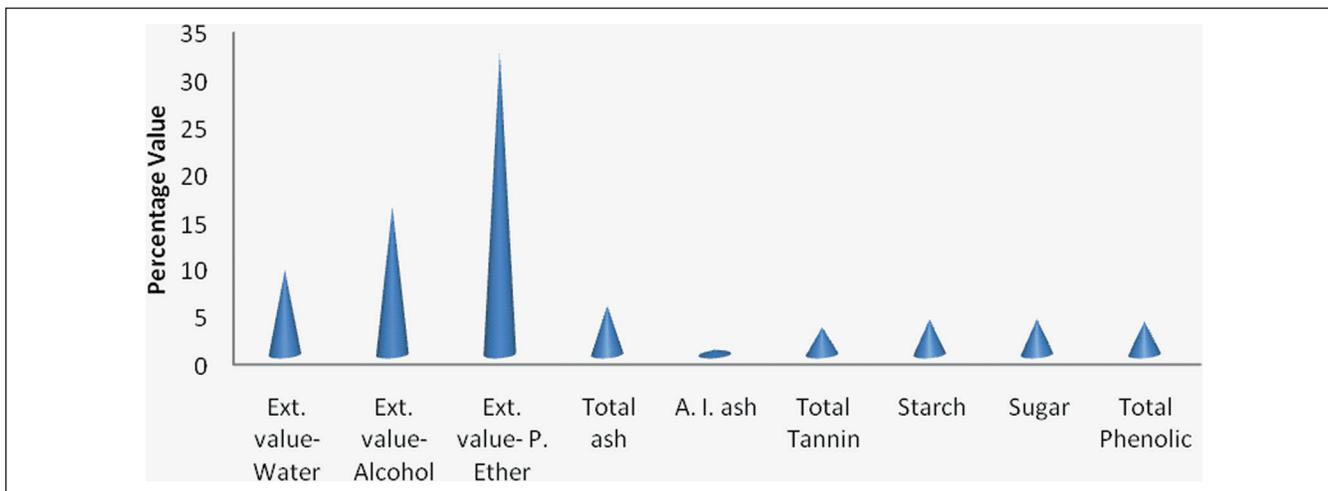


Figure 1: Physico-chemical values of *J. curcas* seed

Table 1: Antioxidant Activity

S. No.	Samples	IC ₅₀ (µg/ml)
1	Ascorbic acid	2.66 ± 0.11
2	<i>J. curcas</i> ethanolic extract	46 ± 3.46
3	<i>J. curcas</i> aqueous extract	36.66 ± 0.57
4	<i>J. curcas</i> hydroalcoholic	32.66 ± 1

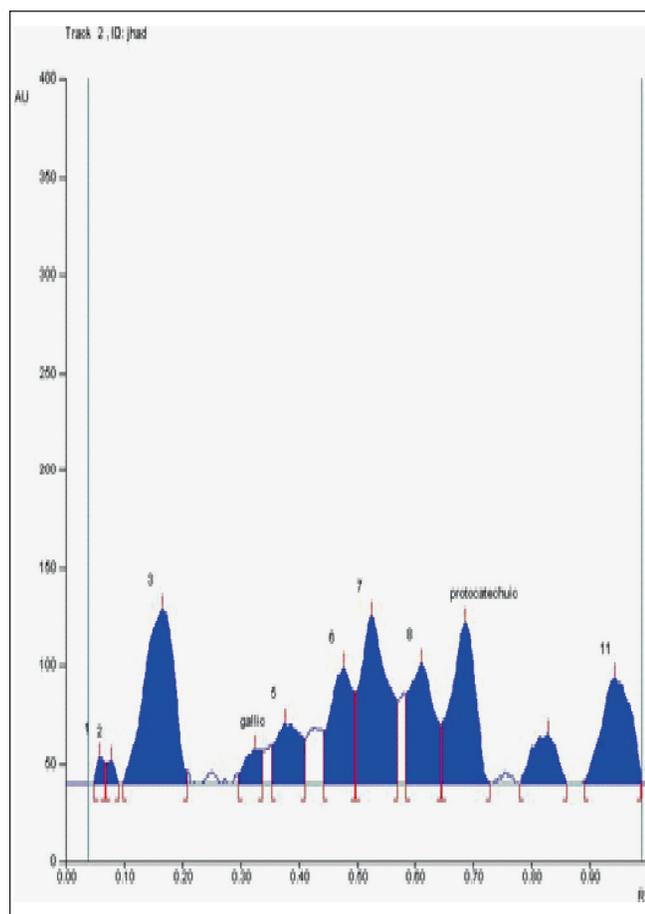


Figure 2: Densitogram obtained from extract, showing gallic and protocatechuic acid

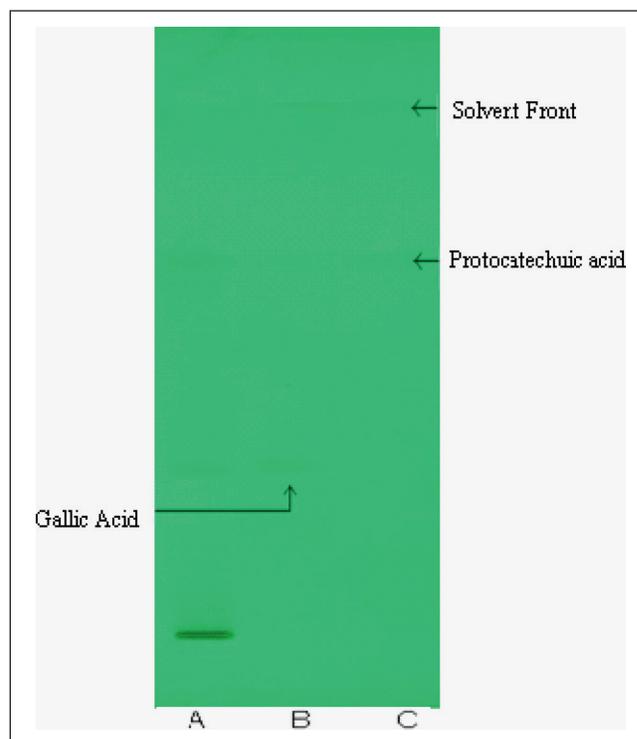


Figure 3: Banding pattern of *J. curcas* hydroalcoholic Extract, Gallic and Protocatechuic acid

A. *J. curcas* hydroalcoholic extract
 B. Gallic acid; C. Protocatechuic acid

aqueous and ethanolic extracts showed IC₅₀ value of 36.66 ± 0.57 µg/ml and 46 ± 3.46 µg/ml respectively. Standard ascorbic acid showed IC₅₀ value of 2.66 ± 0.11 µg/ml. Results are shown in Table 1.

Results of HPTLC

The HPTLC of hydro-alcoholic extract of *J. curcas* seeds was performed with different phenolic marker compounds

at six point calibration curve in which gallic acid and protocatechuic acid were observed and quantified. A Densitogram and Banding pattern obtained from extract shows gallic and protocatechuic acid, Fig 2 & 4.

Gallic acid was estimated up to 0.092% in hydroalcoholic extract, $r^2 = 0.994$, $y = 70.572 + 11.934 * x$, $R_f = 0.32$, Fig 3. In same extract protocatechuic acid was quantified up to 0.146%, $r^2 = 0.998$, calibration equation: $y = 701.4 + 9.283 * x$, $R_f = 0.69$, Fig 5.

CONCLUSION

J. curcas seed extract showed significant in vitro antioxidant activity, hydroalcoholic extract showed the most potent

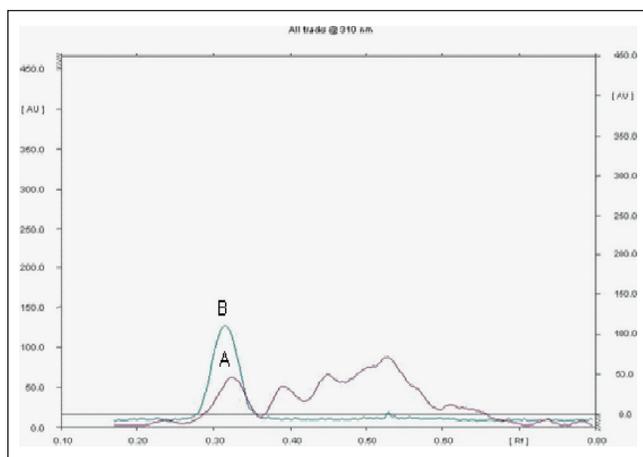


Figure 4: TLC chromatogram of extract and standard (Gallic acid)
A. *J. curcas* hydroalcoholic extract B. Gallic acid

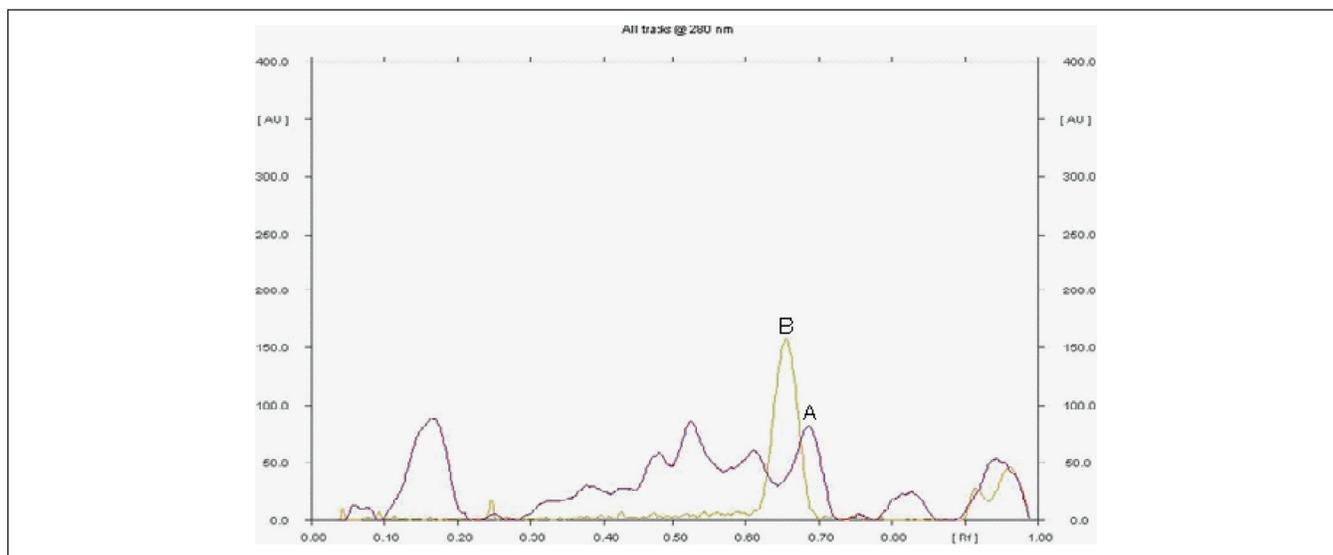


Figure 5: TLC chromatogram of extract and standard (Protocatechuic acid)
A. *J. curcas* hydroalcoholic extract
B. Protocatechuic acid

activity that could protect against oxidant and free radical injuries, in addition to having their medicinal properties. Thus, the effective source of *J. curcas* seeds could be employed in all medicinal preparations to combat myriad diseases associated with oxidative stress and related disorders. Presence of phenolic compounds in all three extracts suggests that the antioxidant activity may be due to the polyphenolic content. The presence of protocatechuic and gallic acid quantified in this species may be utilized for the proper standardization of the drug.

REFERENCES

- Schmook, B & Seralta-Peraza, L. *J. curcas*: distribution and uses in the Yucatan Peninsula of Mexico. In: Gubitz M, Mittelbach M, & Trabi M, editors. GM. Biofuels and industrial products from *Jatropha curcas*. DBV Graz, p. 53–57; 1997.
- Dalziel, J.M., The Useful Plants of West-Tropical Africa. Crown Agents for Oversea Governments and Administration, London, p. 147; 1955.
- Adam SEI. Toxic effects of *Jatropha curcas* in mice. *Toxicology*. 1974; 2(1): 67-76.
- Ahmed OMM, Adam SEI. Effects of *Jatropha curcas* on Calves. *Veterinary Pathology*. 1979; 16(4): 476-482.
- Abdu-Aguye I, Sannusi A, Alafiya-Tayo RA, Bhusnurmath SR. Acute Toxicity Studies with *Jatropha curcas* L. *Human Toxicology*. 1986; 4: 269-274.
- Becker, K & Makkar, H. P. S. Effects of phorbol esters in carp (*Cyprinus carpio* L.). *Veterinary Human Toxicology*. 1998; 40: 82-86.
- Anonymous. The wealth of India. Raw materials, Vol V. CSIR, New Delhi, p. 293–297; 1959.
- Dymock, C., Warden, C.J.H., Hooper, D., (Reprinted). *Pharmacographica Indica—A History of the Principal drugs of Vegetable origin*. Bishen Singh Mahindra Pal Singh. Dehra-Dun, p. 274; 1976.
- Nadkarni, A.K. Dr. K.M. Nakarni's Indian Material Medica Vol.1 Popular prakashan, Bombay, p. 705; 1976.
- Agarwal, V.S. *Economic Plants of India*. Kailash Prakashan, Calcutta, p. 196; 1986.

11. Ambasta, S.P.(Editor-in-Chief).The Useful Plants of India.Publication and Information Directorate, CSIR, New Delhi, p. 302; 1986.
12. Chakre OJ, Asolker LV, Kakkar KK. Supplement to glossary of Indian medicinal plants. Part-I (A-K).National Institute of Science Communication, New Delhi, p. 535-536; 2000.
13. Ambasta SP, The useful Plants of India, National Institute of Science Communication, New Delhi, p. 75-76; 1998.
14. Okujagu, T.F; Etatuvie S.O; Eze I; Jimoh B; Nweke C; Mboji C. Medicinal Plants of Nigeria; South-west Nigeria. In collaboration with Lagos state Traditional Medicine Board and Botany Department, University of Lagos. 1st ed. 1: 20; 2006.
15. Krithikar KR, Basu DB, Indian Medicinal Plants, Ed. 2, Vol 2, Dehradun Oriental Enterprises, p. 1570-1579; 2000.
16. Makkar HPS, Becker, K. Potential of *Jatropha* seed cake as a protein supplement in livestock feed and constraints to its utilization. In: Proceedings of *Jatropha* 97: International Symposium on Biofuel and Industrial Products from *Jatropha curcas* and other Tropical Oil Seed Plants, February 23-27, Managua, Nicaragua, 1997.
17. Peach, K and Tracy, M.V., *Modern Methods of Plant Analysis*. Vol III and IV Springer, Heidelberg, p. 258-261; 1955.
18. Anonymous, *Indian Pharmacopoeia*, 2nd edn (Govt. of India, New Delhi). p. 38-40; 1965.
19. Anonymous, *Official methods of Analysis* (AOAC), 4th edn. Association of Official Chemists, Inc. U.S.A., p. 187-188; 1984.
20. Khalaf, N.A., Shakya, A.K., A. Al-Othman, Z. El-Agbar and Farah, H. Antioxidant activity of some common plants. *Turk. J. Biol.* 2008; 32: 51-55.
21. Al-Nooman S. A., Ela-Segaey O., Ab-Allah A., Experimental study of antioxidant and hepatoprotective effects of clove and cardamom in ethanol induced hepatotoxicity. *Tanta Medical Sciences Journal*. 2007; 2(1): 27-36.