

EVALUATION OF ANALGESIC AND ANTIMICROBIAL ACTIVITY OF STEM BARK EXTRACT OF *JATROPHA CURCAS*

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ABSTRACT

The analgesic *in vitro* antimicrobial activity of methanol: acetone: water (70:20:10) extract of the stem bark of *Jatropha curcas* L. were evaluated. Analgesic activity of JCE were determined by help of three different models name Acetic acid induced writhing, tail clip, tail flick. JCE showed significant activity in these models by inhibition of writhing and increase latency compare to control and Standard (Aspirin). Antimicrobial activity was performed on bacteria: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Enterococcus faecalis*; Fungi: *Alternaria alternata* and *fusarium solani*. JCE Extract showed the presence of antimicrobial activity. This indicates the JCE extract have analgesic and Antimicrobial activity.

KEY WORDS: *Jatropha Curcas*, Analgesic, Antimicrobial

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INTRODUCTION

Herbs have always been principal forms of medicine in India and presently they are becoming popular throughout developing countries, as people strive to stay healthy in face of chronic stress and pollution and to treat illness with medicine that work in concert with body's own defense. *Jatropha curcas* L. or physic nut, is a bush or small tree (up to 5 m height) and belongs to the Euphorbiaceae family and contains approximately 170 known species.¹ *Jatropha*, a drought-resistant shrub or tree, which is widely distributed in the wild or semi-cultivated areas in Central and South America, Africa, India and South East Asia.² It is a multipurpose, drought resistant, perennial plant gaining lot of importance for the production of biodiesel. It has thick glorious branches. The tree has a straight trunk and grey or reddish bark, masked by large white patches. It has green leaves with a length and width of 6 to 15 cm, with 5 to 7 shallow lobes. The branches contain whitish latex, which causes brown stains. Inflorescences are formed terminally on branches. The plant flowers are unisexual.^{3,4} After pollination, a tri-ocular ellipsoidal fruit is formed. The seeds are black and in the average 18 mm long and 10 mm wide ripe *Jatropha* fruits.⁵ It is a multipurpose species with many attributes and considerable potential. The wood and fruit of *Jatropha* can be used for numerous purposes including fuel. It is

used against mucosal diseases, arthritis, gout, jaundice, Toothache, gum inflammation, gum bleeding. Plant extract used to treat Allergies, burns, cuts and wounds, inflammation, leprosy, leukoderma, scabies and small pox. Water extract of branches used in HIV, tumor, Wound healing.² The plant contains Organic acids, Cyclic tri-terpenes stigmasterol,⁶ Curcacycline A, Curcin,⁷ a lectin Phorbol esters Esterases, sitosterol and its d-glucoside.⁸ Stem bark contains amyryl and taraxerol. Previous works have shown that many *Jatropha* species possess antimicrobial activity. Several studies have confirmed the antimicrobial efficacy of different *Jatropha* species; however, there is insufficient information regarding the antimicrobial activities of *J. curcas* L. In this paper, the antimicrobial property of crude extract of the stem bark of *J. curcas* L. has been studied as part of the exploration for new and novel bio-active compounds. Herbs and medications share a common history, as most of our well-known medications were derived from plants. Herbal remedies are marketed commonly as pills, capsules, tinctures or dietary supplements and are largely unregulated. According to US federal legislation that was enacted in 1994, herbs and other dietary supplements can be marketed without testing for safety or effectiveness. Broad and vague claims are allowed, and the US FDA does not have to approve packaging or sales information before a product

reaches the market⁹. Analgesic compounds available in the market still present a wide range of undesired effects, leaving an open door for new and better compounds¹⁰. Thus, a study was made on the analgesic effects of the plant *J. curcus*.

MATERIALS AND METHODS

Plant materials and preparation of extract

Fresh stem bark of *Jatropha curcas* L. was collected from a local area of Jaipur were identified in the department of botany, Rajasthan University, Jaipur. A voucher specimen no. RUBL 20844 was deposited in the department. The fresh stem bark was air-dried to constant weight, pulverized and stored in an air-tight container for further use. 200 g powder of dried stem bark was subjected to soxhlet extraction with methanol: acetone: water (70:20:10). The extract was then filtered and the filtrate was concentrated to dryness. The extract was subjected to phytochemical tests for tannins, saponins, steroids, alkaloids and glycosides, flavonoids, carbohydrates, proteins and amino acid using reported methods.^{11,12}

Animal and microorganisms

Animal for Analgesic activity

Albino rats of either sex (150–200 g) were used for the experimental study. The animals were maintained under standard husbandry conditions in polypropylene cages and provided with food and water *ad libitum*. The animals were kept on fasting overnight prior to the experimentation and all the procedures used in these studies were approved by the Institutional Animal Ethics Committee.

Microorganisms for Anti-microbial activity

The test microorganisms used in this study (bacteria: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Enterococcus faecalis*; Fungi: *Alternaria alternata* and *fusarium solani*).

Acute toxicity study

The acute toxicity was performed according to [OECD 423, 2001]¹³. The selected female albino rats were used to determine the dose. The animals were divided into four groups of three in each. The animals were fasted overnight prior to the acute experimental procedure. Distilled water was used as vehicle to suspend the extracts and administered orally as following doses – 100, 300, 1000 and 2000 mg/kg body wt. immediately after dosing, the animals were observed continuously for first four hours for behavioral changes and for mortality at the end of 24hrs and daily for 14 days respectively. No mortality was reported even after 14 days. This indicates that the extract is safe up to a single dose of 2000 mg/kg body weight.

Analgesic activity

Writhing test

Albino rats of either sex weighing (150-200) g were selected and divided into 4 groups of 6 each. The total number of writhing, following intra-peritoneal (i.p.) administration of 0.6% (10 ml/kg) acetic acid, was recorded for 20 min after injection of acetic acid. Rats place into the glass jar to correct observation of writhing. The animals were pretreated with test Extract JCE 100 and 300 mg/kg body weight and standard aspirin 100 mg/kg b. wt. orally 60 min before injection of acetic acid¹⁴.

Tail clip test

Albino rats of either sex fasted over night with water given *ad libitum*, maintained at room temperature and irresponsive rats were separated by testing all rats with tail-clip. Rats that did not commence continuous efforts to remove the clip within 10 seconds were rejected. Responsive rats were tested again before the administration of aqueous suspension of standard drug or drug extract. The rats were divided into 4 groups, each containing six rats. The first group of rats served as control receive vehicle. Second group of rats were administered with standard drug Aspirin at a dose of 100 mg/kg body weight, orally. And the remaining groups were treated with the different doses 100 and 300 mg/kg body weight, orally of JCE extract of test drug. Analgesic activity was measured 0, 30, 60, 90, 120 and 180 min after administration of JCE extract, Aspirin and distilled water¹⁵.

Tail-flick test

Albino rats of either sex weighing (150-200) g were selected and divided into 4 groups of 6 each. For each dose of the drug, a separate group of animals was used. The tail of the rat was placed on the nichrome wire of an analgesiometer (maintain space 2-4 mm between nichrome wire and tail) and the time taken by the animals to withdraw (flick) its tail from the hot wire was taken as the reaction time. Irresponsive rats were separated by testing all rats with tail-flick Rats that did not commence efforts to flick the tail within 10 seconds were rejected. Test JCE doses of 100 and 300 mg/kg and standard Aspirin 100 mg/kg. Distilled water served as control. Analgesic activity was measured 0, 30, 60, 90, 120 and 180 min after administration of JCE extract, Aspirin and distilled water¹⁶.

Anti-microbial activity

In-vitro antibacterial activity

The determination of the inhibitory effect of the JCE on test bacteria was carried out by agar well diffusion method¹⁷. Bacterial cultures were grown at 37⁰ C for 24 h in Nutrient Broth. The culture suspensions were adjusted

by comparing against 0.5 McFarland Standard $2-3 \times 10^8$ CFU. Petri dishes with 10 ml of Nutrient Agar were prepared, previously inoculated with 100 μ L of the culture suspension. The wells (7.0 mm) were made and the JCE and reference (ciprofloxacin, 5mcg) which is dissolved in DMSO was added to wells (100 μ L) and same volume (100 μ L) of DMSO was used as a control. The inoculated plates were incubated for 24 h. After incubation, the diameter of the inhibition zone was measured with calipers. The measurements were done basically from the edge of the zone to the edge of the well.

In-vitro antifungal susceptibility

The procedure for the PDA method was as follows [NCCLS, 1992]¹⁸. PDA powder was dissolved in distilled water to a final concentration of 39g/liter and then sterilized at 121°C for 15 min. The sterilized PDA solution was placed in a water bath, and the temperature was cooled to and maintained at 55 to 60°C. The antifungal agent stock solutions were mixed with the PDA solution to produce a series of different final concentrations as 2%, 6% and 8%. Drug-free agar containing only 1% DMSO was used as a positive control and without inoculation one plate used as negative control. The mixtures of antifungal agent and PDA solutions were poured directly into the plates. After the plates were cooled to room temperature, freshly made fungal suspension (5×10^3 to 2×10^4 /ml) was inoculated onto the agar plate. The plates were incubated aerobically at 35°C until the 7 days and then measure the growth of the fungi on the plate.

Statistical analysis

Results are expressed as Mean \pm S.E.M. Statistical significance was determined by using the one way analysis of variance (ANOVA) and repeated Measures (ANOVA) followed by Dunnett's multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULT AND DISCUSSION

Analgesic activity

Writhing test

The results presented in Table 1 show the number of abdominal writhing episodes evoked by the intraperitoneal injection of acetic acid in rat and the Analgesic effect of JCE. The treatment with JCE (100 and 300 mg/kg p.o.) reduced significantly the number of writhing by JCE 100 mg/kg – 52.43% and JCE 300 mg/kg – 66.11% compared to control animals. The standard drug Aspirin showed 57.6% inhibition. However, the JCE shown significant effect compare to control group at all doses (100 and 300).

Tail-clip test

Tail-clip test shows that administration of JCE (100 and 300 mg/kg p.o.) significantly increased the reaction time at 30, 60, 90 and 120 min compared to control. JCE 300 mg/kg p.o. showed more significant activity than Aspirin.

Tail-flick test

Tail-flick test shows that administration of JCE (100 and 300 mg/kg p.o.) and aspirin significantly increased the reaction time at 30, 60, 90 and 120 min compared to control. JCE 300 mg/kg p.o. showed more significant activity than Aspirin.

Antibacterial activity

This extract of the plant tested showed varying degree of antibacterial activities against the test bacterial species (**Table 1**). The antibacterial activities of the extract compared favorably with that of standard antibiotics ciprofloxacin and have appeared to be broad spectrum as its activities. The inhibition zone for *Pseudomonas aeruginosa* and *Enterococcus faecalis* was less (0 - 30 mm) as compared to other bacteria.

The inhibitory effect of the extract of *J. curcas* against pathogenic bacterial strains can introduce the plant as a potential candidate for drug development for the treatment of ailments caused by these pathogens.

Antifungal activity

These JCE extract showed broad antifungal activity against the tested fungal (**Table 5**).

CONCLUSION

The present study on antimicrobial activity and phytochemical screening of stem bark extract of *Jatropha curcas* L. provides the useful information about Analgesic and antimicrobial activity of methanol: acetone: water (70:20:10) extract of the stem bark of *Jatropha curcas* L. further study in this field may helpful to find out more effective analgesic and Antimicrobial agents.

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Table 1: Analgesic effect of JCE by Acetic acid induced writhing test

s.no.	Drug	Dose (mg/kg,P.o)	Number of writhings (in 20 min)	Inhibition (%)
1.	Control	-	151 ± 10.74	-
2.	Aspirin	100	64 ± 8.85***	57.6
3.	JCE	100	71.83 ± 5.49***	52.43
4.	JCE	300	51.16 ± 4.18 ***	66.11

Values are expressed as mean±S.D.; n = 6; significance at P < 0.05*, 0.01** and 0.001*** as compared to the control.

Table 2: Analgesic effect of JCE with tail-clip test

s. no	Drug	Dose (mg/kg, P.o)	Reaction time after administration of control/standard/test					
			0 min	30 min	60 min	90 min	120 min	180 min
1	Control	-	5.57±0.48	5.76±0.48	6.86±0.46	6.84±0.56	7.21±0.33	6.45±0.41
2	Aspirin	100	5.42±0.30	8.38±0.54	13.15±0.45	15.19±0.60	18.76±0.38*	9.82±0.53
3	JCE	100	5.11±0.20	8.02±0.46	10.97±0.27	13.11±0.61	16.88±0.55	8.29±0.59
4	JCE	300	5.22±0.20	9.17±0.44	12.29±0.89	19.78±1.24	25.69±0.70***	13.51±1.15

Values are expressed as mean±S.D.; n = 6; significance at P < 0.05*, 0.01** and 0.001*** as compared to the control.

Table 3: Analgesic effect of JCE with tail-flick test

s. no	Drug	Dose (mg/kg, P.o)	Reaction time after administration of control/standard/test					
			0 min	30 min	60 min	90 min	120 min	180 min
1	Control	-	3.98±0.38	4.41±0.33	4.56±0.16	5.11±0.17	4.82±0.33	5.25±0.40
2	Aspirin	100	4.60±0.30	8.63±0.83	12.72±0.85	15.47±0.91	17.25±0.81***	10.94±0.45
3	JCE	100	4.53±0.62	6.77±0.39	9.23±0.20	12.16±0.44	13.97±0.50*	7.88±0.51
4	JCE	300	4.57±0.60	11.1±0.66	15.41±0.72	18.28±0.80	21.56±0.83***	15.69±0.69

Values are expressed as mean±S.D.; n = 6; significance at P < 0.05*, 0.01** and 0.001*** as compared to the control.

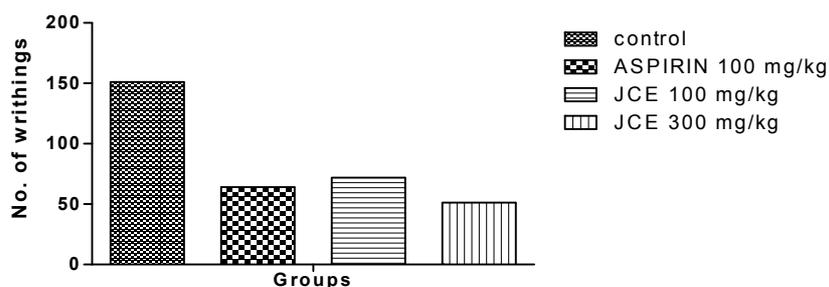
Table 4: Antibacterial activities profile of JCE extract by well diffusion model

S. No.	Bacteria	Zone of inhibition (In mm)				
		JCE 2mg/ml	JCE 4mg/ml	JCE 6mg/ml	JCE 8mg/ml	STD Ciprofloxacin (5 mcg)
1.	<i>Escherichia coli</i>	2	5	9	12	32
2.	<i>Staphylococcus aureus</i>	9	13	20	22	34
3.	<i>Pseudomonas aeruginosa</i>	0	5	12	16	28
4.	<i>Bacillus cereus</i>	6	8	10	16	30
5.	<i>Enterococcus faecalis</i>	0	3	7	8	28

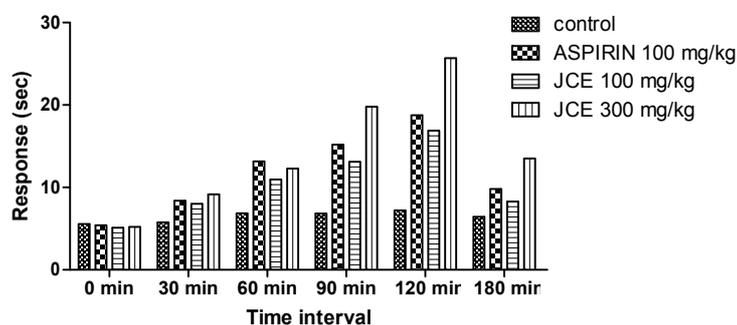
Table 5: Antifungal activity profile of JCE extract by in-vitro fungal susceptibility testing

S. No.	Fungus	Zone of growth (In mm)			
		Positive control	JCE 2mg/ml	JCE 6mg/ml	JCE 8mg/ml
1.	<i>Alternaria alternata</i>	83	40	25	20
2.	<i>Fusarium solani</i>	82	25	22	10

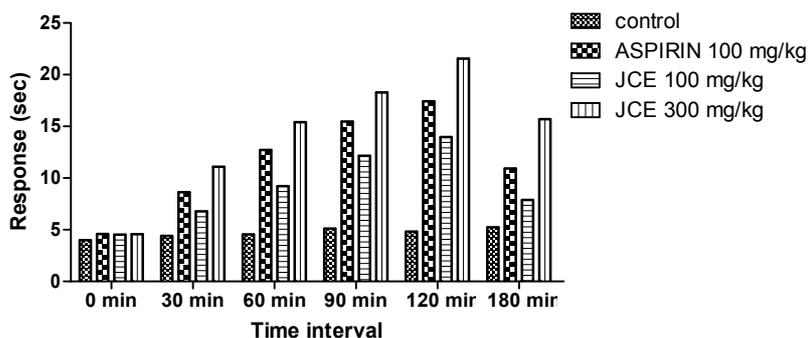
Analgesic effect of JCE by writhing model (Figure 1)



Analgesic activity of JCE by Tail-clip method (Figure 2)



Analgesic activity of JCE by Tail-flick method (Figure 3)



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