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Antimicrobial and Cytotoxic Activities of *Jatropha curcas* Linn. Seedcake from Biofuel Production

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Antimicrobial activity of *Jatropha curcas* Linn. seedcake from biofuel production has been studied. For safety evaluation, mutagenic and cytotoxic test were carried out. The seedcake was extracted by maceration method with methanol. The antimicrobial activity, cytotoxicity test, and mutagenic effect were determined by disc diffusion, Brine Shrimp Lethality Test (BSLT), and Ames test method, respectively. The methanolic extract of *J. curcas* seedcake and fractions extract showed potent antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans* at minimum inhibitory concentration (MIC) of ≤ 1 mg/mL. The cytotoxicity value (LC_{50}) of methanolic extract of *J. curcas* seedcake was 22.12 μg/mL and had no mutagenicity effect against *Salmonella typhimurium* TA 1535. From this investigation it is evident that *J. curcas* seedacake from biofuel production might have promising biological activities including antimicrobial and cytotoxic properties. Therefore it could be a good source of natural medicine.

Key Words: Antimicrobial, Cytotoxicity, *Jatropha curcas* seedcake, Mutagenic effect.

Jatropha curcas Linn., a plant with many attributes, and a bio-diesel plant known for various medicinal uses in folklore has been evaluated for few pharmacological aspects. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being (Igbinosa *et al.,* 2009). Medicinally, it has been reported that the sap or latex of *J. curcas* contained jatrophine, which is used in the treatment of cough, skin diseases and rheumatism. The latex is also known to heal wound and possessed antimicrobial properties. Its roots are known to serve as an antidote for snake bike and the extract from its leaves has an external application for piles (Ejelonu *et al*., 2010). Others studies have reported that the plant exhibits bioactive activities for fever, mouth infections, jaundice, guinea worm sores and joint rheumatism (Openshaw 2000; Rug and Ruppel 2000). Beyioku *et al*. (1998) investigated and reported the anti-parasitic activity of the latex and crushed leaves of *J. curcas*. The water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity (Matsuse *et al*., 1999).

Previous works have shown that many *Jatropha* species possess antimicrobial activity (Aiyelaagbe *et al*., 2000; 2007). Methanolic extract of the leaves of *J. curcas* has antibacterial activity against *S. aureus*, but failed to inhibit *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeroginosa*, *Salmonella typhi*, and *Streptocccus mutans* (Muanza *et al.*, 1994). In contrast, ethanol extract of *J. curcas* leaves showed a broad-spectrum of antimicrobial activity (Sharma *et al.*, 2010). Moreover, ethanolic extract of *J. curcas* has

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effectiveness on inactivation of some microorganisms i.e. *E. coli, P. fluorescens, P. aeruginosa, S. aureus* and *B. subtilis* (Sharma *et al*., 2010). The sap (latex) has antimicrobial properties against *Staphylococcus* and *Streptococcus* sp. and *E. coli*. Latex is used to dress sores, ulcers and inflamed tongues. Latex from the stem is used to arrest bleeding of wounds. Seeds are used for dropsy, gout, paralysis and skin ailments7. The latex of *J. curcas* inhibits *Staphylococcus, Bacillus, Micrococcus* and has strong inhibitory effect on normal larval growth of mosquito (Beyioku *et al*., 1998).

However, there is insufficient information regarding the antimicrobial activities of *J. curcas* Linn. Whatever limited information available on the medicinal properties of *J. curcas* is mostly on the seed extracts of the plant. The seeds of *Jatropha* contain viscous oil, which can be used for manufacture of candles and soap, in cosmetics industry, as a diesel/paraffin substitute or extender (Akbar *et al*., 2009). Biofuel production of *J. curcas* seed left seedcake from mechanical press process. The antimicrobial activity of *J. curcas* seedcake from biofuel production has not been studied yet. In this paper, the antimicrobial property of *J. curcas* seedcake from biofuel production including its cytotoxicity and mutagenic effect of the seedcake has been studied as part of the exploration for new and novel bio-active compounds.

MATERIALS AND METHODS

The seedcakes of *J. curcas* were collected in May 2009 from Research Center for Science and Technology (Puspiptek) Serpong, West Java, Indonesia.

Extraction of the seedcakes

The collected seedcakes were dried and grounded. The ground (4500 g) were extracted by maceration method at room temperature for 5 days. The extract then was concentrated by using rotary evaporator. The extract was fractionated by liquid-liquid extraction method using n-hexane and chloroform and formed 3 fraction : n-hexane, ethyl acetate, and methanol fraction.

Antimicrobial test

The strain of microorganisms employed in this study were *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus.* The antimicrobial assay was performed by agar disc diffusion technique. Briefly, fifty microliter (50 µL) of extract solution was applied on plate that contains 5 mg/L of crude extract. To compare the activity with standard antibiotics, tetracyclin (50 µg/disc) for antibacterial agent and ketokonazol (0.5 µg/disc) for antifungal were used. Disc containing 50 µL DMSO was used as a negative control. The discs were then incubated at 37°C for 20 to 24 hours to allow bacterial growth, after which the zones of inhibition of desired growth could be easily measured. The zone of inhibition was considered as an indicator for the antimicrobial activity. At the end of the incubation period the antibacterial activity was evaluated by measuring the inhibition zones in mm.

Cytotoxicity assay

The eggs of the brine shrimp were collected from an aquarium shop (Bandung, Indonesia) and hatched in artificial seawater (3,8% w/v NaCl solution) for 48 h to mature shrimp called nauplii. The citotoxic activity assay was performed on brine shrimp nauplii using Meyer method (Meyer *et al*., 1982). The test samples (extracts) were prepared by dissolving them in methanol and gives concentration of 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0 µg/mL. A 100 µL of the solution extract was added to vial and the solvents were evaporated and then added with 5 ml artificial seawater. Ten nauplii were incubated in known concentration of the extract for 24 h. All tests were conducted in triplicate. The number of died nauplii after 24 h were counted. The data were transformed to probity analysis for the determination of LC_{50} values of the extract.

Mutagenicity assay

Mutagenic effect of the extract was evaluated by Ames test using plate incorporation technique on *Salmonella typhimurium* TA 1535. This strain was provided and biologically identified by Microbiology Laboratorium, School of Pharmacy, Bandung Institute of Technology, Indonesia. The extract was used at three concentrations (10, 100, 1000 ppm) in both the presence and absence of S9 mixture. A 100 µL of the extract solution and 100 µL of bacterial culture incubated overnight were mixed with 2 ml of molten top agar in glass tubes. The contents were vortexed and poured on to the minimal glucose agar plates and allowed to solidify. Following incubation at

37°C for 48 h, the revertant colonies were counted. Vehicle control (DMSO) and positive control (sodium azide) was also tested under similar conditions. The identification of mutagens and no mutagens was based on the ratio of the number of revertants in the treatment groups to that in the negative control groups. If the ratio was ≥2 and dose dependent, the extract had mutagenicity (Jin *et al*., 2009).

RESULTS AND DISCUSSION

The interest in the scientific investigation of antibacterial, cytotoxic, and mutagenic activity of *J. curcas* seedcake from biofuel ptoduction is based on the claims of its effective use for the treatment of many diseases. Moreover, the search for plants with antimicrobial activity has gained increasing importance in recent years, due to a growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms or multi-resistant microbes (Bassam *et al*., 2006). Several studies have been conducted with the extracts of various *J. curcas* plant parts such as stem bark (Igbinosa *et al*., 2009; Gupta *et al.*, 2010; Obasi *et al*., 2011), leaves (Sangeetha *et al*., 2010; Kalimuthu *et al*., 2010; Sharma *et al.*, 2010;), latex or sap (Oyi *et al*., 2007), roots (Aiyelaagbe *et al*., 2007; 2011) and seed oil (Ejelonu *et al*., 2010) for antimicrobial activity as well as for the discovery of new antimicrobial compounds. In this study, we investigated the antimicrobial activity of *J. curcas* seedcake from biofuel production against a Gram-negative bacterium, *E. coli*, two Gram-positive bacteria, *B. subtilis* and *S. aureus,* and a fungus, *C. albicans*.

The antimicrobial activities of the methanolic extract and fractions extract of the *J. curcas* seedcake from biofuel production tested by disc diffusion method were shown in Table 1. The data indicated that 5 mg/mL of methanolic extract, n-hexane fraction, ethyl acetate fraction, and methanol fraction possess antimicrobial activity against all tested microorganisms. Meanwhile, n-hexane fraction has no antimicrobial activity against all tested microorganisms. *S. aureus* was the most sensitive strain of those tested

Table 1. Antimicrobial activities of methanolic extract and fraction extracts of *Jatropha curcas* seedcake from biofuel production

Microorganism	Diameter of inhibition (mm) of 5 mg/mL of methanolic extract or fractions extract[a]				
	Methanolic extract	n-Hexane fraction	Ethyl acetate fraction	Methanol fraction	Positive control
E. coli	9.90±0.97	-	19.56±0.67	11.85±0.41	8.17±0.13
B. subtilis	10.17±0.81	-	15.68±0.43	13.91±0.52	9.63±0.85
S. aureus	21.07±1.31	-	10.33±0.67	17.17±1.01	15.47±1.86
C. albicans	9.56±0.89	-	11.65±0.78	9.9±0.28	13.12±0.13

a: Results are means of three different experiments

Table 2. Minimum Inhibitory Concentration (MIC) of methanolic extract and fractions extract of *Jatropha curcas* seedcake from biofuel production

Microorganism	Minimum Inhibitory Concentration (MIC) (mg/mL)			
	Methanolic extract	n-hexane fraction	Ethyl acetate fraction	Methanol fraction
E. coli	1	-	0.01	0.01
B. subtilis	0.1	-	0.001	0.1
S. aureus	1	-	1	1
C. albicans	1	-	1	0.1

Table 3. Determination of LC_{50} in brine shrimp lethality bioassay for methanolic extract of *Jatropha curcas* seedcake from biofuel production.

Concentration (µg/ml)	% Mortality of brine shrimp			Mean	LC_{50} (µg/ml)
	Expt. 1	Expt. 2	Expt. 3		
400	100	100	100	100	
200	100	90	100	90	
100	80	70	90	80	
50	70	60	60	70	
25	60	60	50	60	
					22.12
12.5	50	40		40	
6.25	40	40	30	30	
3.13	30	20	20	20	
1.56	20	10	20	20	
0	0	0	0	0	

Table 4. Mutagenicity of methanolic extract of Jatropha curcas seedcake using TA 1535 strain of *S. typhimurium* TA 1535

(±) S9	Treatment	Conc. (ppm)	His^+ revertants/plate				Treatment/ K-
			Plate I	Plate II	Plate III	Mean	
(-) S9	Negative control	0	212	161	UC	186.50	-
	Positive control	100	654	536	771	653.67	3.50
	Extract	10	264	276	163	234.33	1.26
	Extract	100	245	323	313	293.67	1.57
	Extract	1000	219	245	317	260.33	1.40
(+) S9	Negative control	0	219	308	221	249.33	-
	Positive control	100	687	938	728	784.33	3.15
	Extract	10	432	446	470	449.33	1.80
	Extract	100	394	429	446	423.00	1.70
	Extract	1000	495	415	266	392.00	1.57

UC : Uncounted;
Negative control: dimethylsulphoxide;
Positive control: sodium azide (NaN_3)

microorganisms with the methanolic extract and methanol fraction of seedcake of *J. curcas*, with the strongest inhibition zone of 21.07 mm and 17.17 mm, respectively. The methanolic extract also exhibited moderate antimicrobial activity against *E. coli*, *B. subtilis*, and *C. albicans*, with inhibition zone of 9.90 mm, 10.17 mm, and 9.6 mm, respectively. The ethyl acetate fraction was found most effective against *E. coli*, with inhibition zone of 19.6 mm. The inhibition zone of ethyl acetate fraction observed for *B. subtilis*, *S. aureus*, and *C. albicans* was 15.68 mm, 10.37 mm, and 11.60 mm, respectively. Moreover, the inhibition zone of methanol fraction was found to be 11.85 mm, 13.91 mm, and 9.99 mm for *E. coli*, *B. subtilis*, and *C. albicans*, respectively (Table 1). The minimum inhibitory concentrations (MICs) of methanolic extract and fractions extract are summarized in the Table 2. The methanolic extract and fractions extract showed potent antimicrobial activity against the all tested microorganisms at MIC of ≤ 1 mg/mL. Ethyl acetate in particular displayed MIC of as low as 0.001 mg/mL against *B. subtilis*.

Ejelonu *et al.* (2010) reported that 10 mg/mL of seed oil of *J. curcas* has antibacterial activity against *E. coli* and *Streptococcus pyogenes* with

inhibition zone of 15.0 mm and 8.0 mm, respectively. In this research we found that 5 mg/mL of methanolic extract of *J. curcas* seedcake has strong activity against *E. coli* with an inhibition zone of 9.09 mm. The anti-*E. coli* of methanolic extract of *J. curcas* seedcake might be stronger than that of seed oil. A 10 mg/mL of methanolic extract of *J. curcas* stem bark has antimicrobial activity against *E. coli*, *B. subtilis* and *S. aureus* with inhibition zone of 14.0 mm, 14.0 mm. and 20.0 mm, respectvively (Igbinosa *et al.*, 2009). Whereas, Obani *et al.* (2011) reported that 10 mg/mL of methanolic extract of *J. curcas* stem bark has antimicrobial activity against *E. coli*, *B. sutilis*, and *S. aureus* with inhibition zone of 13.17 mm, 14.50 mm, and 12.00 mm, respectively. Our results showed that inhibition zone of 5 mg/mL of seedcake methanolic extract against *E. coli*, *B. subtilis* and *S. aureus* was 9.90 mm, 10.17 mm, and 21.07 mm, respectively. These results suggested that antimicrobial activity of seedcake methanolic extract against those microorganisms also might be stronger than those of stem bark extract. Sharma *et al.* (2010) found that 10 mg/mL of ethanolic leaf extract of *J. curcas* inhibit the growth of *E. coli* and *S. aures* with inhibition zone of 11.0 mm and 10.0 mm, respectively. Moreover, 10 mg/mL of ethanolic leaf extract of *J. curcas* has no antimicrobial activity against *B. subtilis* (Sharma *et al.*, 2010). Sangeetha *et al*. (2010) investigated that 10 mg/mL of ethanolic extract of *J. curcas* leaf has antimicrobial activity against *E. coli* and *B. subtilis* with inhibition zone of 10.0 mm. A 10 mg/mL of methanolic extract of *J. curcas* leaf shows antimicrobial activity against *E. coli* and *S. aureus* with inhibition zone of 6.00 mm and 12.0 mm, respectively. In contrast, inhibition zone of 5 mg/mL of seedcake methanolic extract against *E. coli*, *B. subtilis* and *S. aureus* was 9.90 mm, 10.17 mm, and 21.07 mm, respectively. Interestingly, 10 mg/mL of methanolic extract of *J. curcas* stem bark has no anti-*Candida albicans* activity (Obasi *et*

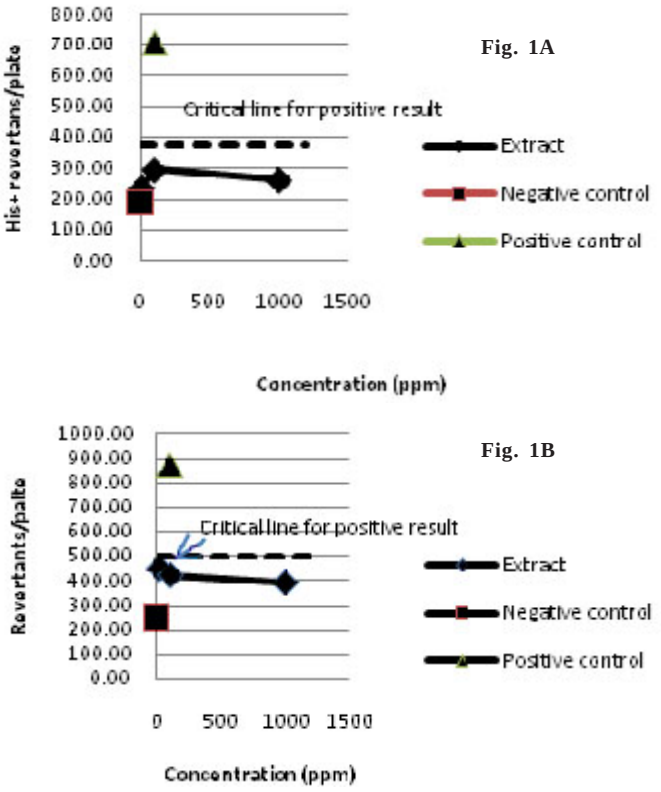

Fig. 1. Methanolic extract of *J. curcas* seedcake from biofuel production showed a negative response in the Ames test.

al., 2011), meanwhile, 5 mg/mL of methanolic extract of *J. curcas* seedcake shows anti-*C. albicans* activity with inhibition zone of 9.56 mm. The latex or sap of *J. curcas* in concentration of 10 mg/mL has stronger antimicrobial activity against *E. coli*, *B. subtilis*, *S. aureus*, and *C. albicans* with inhibition zone of 25 mm, 26 mm, 21 mm, and 24 mm, respectively, than those of 5 mg/mL of methanolic extract of *J. curcas* seedcake. Generally, the antimicrobial activity of methanolic extract of *J. curcas* seedcake was relatively stronger than those of stem bark methanolic extract, seed oils, leaf methanolic or ethanolis extract of *J. curcas.*

In the brine shrimp lethality bioassay the methamolic extract of *J. curcas* seedcake from biofuel production exhibited citotoxicity toward brine shrimp (Table 3). The mortality rate of brine shrimp was found to be increased with the increase of concentration. The percent mortality of the brine shrimp nauplii was calculated for every concentration. A plot of log concentration of the sample versus percent of mortality showed an approximate linear correlation between them. The LC_{50} value of the methamolic extract of *J. curcas* seedcake from biofuel production was 22.12 µg/mL. This result comparable the methanolic extract of *J. curcas* stem bark which has LC50 of 19.95 µg/mL (Gupta *et al*., 2010).

The mutagenicity assay was carried out due to ensure that the methanolic extract of *J. curcas* seedcake from biofuel production had no mutagenic effect. The seed of *J. curcas* contains phorbol ester that has toxic effect to human. The mutagenicity assay result showed that all the ratios of the number of His^+ revertans per plate in the extract group compared to that in the negative control group were >2, either in the presence or absence of S9 mix (Table 4). It can be concluded that methanolic extract of *J. curcas* seedcake from biofulel production has no mutagenicity effect against *S. typhimurium* TA 1535 with or without metabolic activation (S9 mix). Figure 1A and B show that the curve of methanolic extract and negative control with S9 (A) and without S9 (B) was always under critical line for positive control. These results suggest that the extract used in this study has no mutagenicity effect.

Overall, to our knowledge, this might be the first report regarding antimicrobial activity of methanolic extract of *J. curcas* seedcake from biofuel production. From this investigation it is evident that this seedcake of *J. curcas* has no mutagenic effect and might have promising biological activities including antibacterial, antifungal and cytotoxic properties. Therefore it could be a good source of natural medicine.

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REFERENCES

1. Akbar, E., Yaakob, Z., Kamarudin, S.K., Ismail, M., Salimon, J. Characteristic and composition of *Jatropha Curcas* oil seed from Malaysia and its potential as biodiesel feedstock. *European J. Sci. Res.*, 2009; **29**: 396-403.
2. Aiyelaagbe, O.O., Adesogan, E.K., Ekundayo, O., Adeniyi, B.A., 2000. The antimicrobial activity of roots of *Jatropha podagrica* (Hook). *Phytother. Res.*, 2000; **14**: 60–62.
3. Aiyelaagbe, O.O., Adeniyi, B.A., Fatunsin, O.F., Arimah, B.D. *In vitro* ntimicrobial activity and phytochemical analysis of *Jatropha curcas* roots. *Int. J. Pharmacol.*, 2007; **3**: 106-110.
4. Aiyelaagbe, O.O., Hamid, A.A., Fattorusso, E., Taglialatela-Scafati, O., Schr¨oder, H.C., M¨uller, E.G. Cytotoxic activity of crude extracts as well as of pure components from *Jatropha* species, slants used extensively in African traditional medicine. *Evid. Based. Compl. Alternat. Med.*, 2001; 2011: 134954.
5. Bassam, A., Ghaleb, A., Naser, J., Awni, A., Kamel, A. Antibacterial activity of four plant extracts used in Palestine in folkloric medicine against methicillin-resistant *Staphylococcus aureus*. *Turkey. J. Biol*. 2006; **30**: 195-198.
6. Beyioku, A.F., Oyibo, W.A., Anuforom, B.C. Disinfectant/antiparasitik activities of *Jatropha curcas*. *East African Med. J.* 1998; **75**: 508-511.
7. Ejelonu, B.C., Oderinde, R.A., Balogun, S.A. Chemical and biological properties of *Jatropha curcas* and *Mucuna solan* seed and seed oil. *Libyan Agri. Res. Center. J. Int.* 2010; **4**: 263-268.
8. Gupta, D.D., Haque, M.E., Islam, M.N., Mondal, M.S.I., Shibib, B.A. Antimicrobial and cytotoxic activities of *Jatropha curcas* (Euphorbiaceae). *Dhaka Univ. J. Pharm. Sci.*, 2010; **9**: 139-142.
9. Igbinosa, O.O., Igbinosa, E.O., Aiyegoro, O.A. Antimicrobial activity and phytochemical

screening of stem bark extracts from *Jatropha curcas* (Linn). *African J. Pharm. Pharmacol.*, 2009; **3**: 058-062.

10. Jin, J., Liu, B., Zhang, H., Tian, X., Cai, Y., Gao, P. Mutagenicity of Chinese traditional medicine semen *Armeniacae amarum* by two modified Ames tests. *BMC Compl. Alter. Med.,* 2009; **9**: 43.
11. Kalimuthu, K., Vijayakumar, S., Senthilkumar. Antimicrobial activity of the biodiesel plant, *Jatropha curcas* L. *Int. J. Pharm. Bio. Sci.* 2010; **1**: 1-5.
12. Matsuse, I.T., Lim, Y.A., Hattori, M., Correa, M., Gupta, M.P. A search for antiviral properties in Panamian medicinal plants: The effect on HIV and its essential enzymes. *J Ethnopharmacol.*, 1999; **64**: 15-22.
13. Muanza, D.N., Kim, B.W., Euler, K.L., Williams, L. Antibacterial and antifungal activities of nine medicinal plants from Zaire. *Inter. J. Pharmacog.* 1994; **32:** 337-345.
14. Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobson, L.B., Nichols, D.E., Mc Laughlin, J.L. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med.,* 1982; **45**: 31-34.
15. Obasi, N.L., Ejikeme, M.P., Egbuonu, C.A.C. Antimicrobial and phytochemical activity of methanolic extract and its fractions of *Jatropha curcas* Linn. (Eurphorbiaceae) stem bark. *African J. Pure. Appl. Chem.,* 2011; **5**: 92-96.
16. Openshaw, K. A review of *Jatropha curcas*: an oil plant of unfulfilled promise. *Biomass and Bioenergy.* 2000; **19**: 1-15.
17. Oyi, AR., Onaolapo, J.A., Haruna, A.K., Morah, C.O. Antimicrobial screening and stability studies of the crude extract of *Jatropha curcas* Linn latex (Euphorbiaceae). *Nigerian J. Pharm. Sci.* 2007; **6**: 14-20.
18. Rug, M., Ruppel, A. Toxic activities of the plant *Jatropha curcas* against intermediate snail hosts and larvae of Schistosomes. *Trop. Med. Int. Health.,* 2000; **5**: 423-430.
19. Sangeetha, J., Divya, K., Prashanth, M.V., Vamsikrishna, A., Rani, G,L. Antiinflammatory and antibacterial activity of *Jatropha curcas* Linn. *J. Pharm. Res. Health. Care*., 2010; **2**: 258-262.
20. Sharma, A., Saxena, S., Rani, U., Rajore, S., Batra, A. Broad-spectrum antimicrobial properties of medicinally important *Jatropha curcas* L. *Int. J. Pharm. Sci. Rev. Res.,* 2010; **4**: 11-14.