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Original Article

Formulation and Evaluation of *Jatropha curcas* Latex Based Gel for the Treatment of Canker Sores

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ABSTRACT

Background: The recent food habits of the people causing severe health issues. One of the most relevant oral problems that disturb the daily life of the people is canker sores. The latex of *Jatropha curcas* can be used as a promising agent to treat canker sore. **Objectives:** The latex of *Jatropha curcas* was found to hold the capability to boost the speedy curing of the canker sores in the mouth. **Materials and Methods:** The activity of *Jatropha curcas* was compared with *Solanum nigrum*, which was already proven to be used as an ingredient in the formulation of various oral related drugs products. The activity of latex of *Jatropha curcas* in terms of anti-bacterial, antioxidant and anti-inflammatory assays have been analyzed and compared with leaves of *Solanum nigrum*. **Results:** Six different formulations of *Jatropha curcas* latex based gel was prepared and subjected to evaluation of physicochemical properties. **Conclusion:** The cytotoxicity studies of the formulations were performed to assess the cellular growth in the presence and absence of active ingredients in the formulations. The IC_{50} values were also determined to find the minimal concentration to inhibit 50% of cell population.

Keywords: *Jatropha curcas*, Latex, Anti-oxidant Assay, Anti-Inflammatory, Cytotoxicity Activity.

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INTRODUCTION

Canker sore is a small shallow ulcer that appears in the origin of the mouth, which is medically known as Recurrent Aphthous Stomatitis (RAS). These canker sores, which are one of the most common types of oral lesion in the world, usually occur over the inner sides of the cheeks, lips, either side of tongue and throat. These sores are considered as an illness as these sores are found to be more painful to the people and disturb the normal eating practice. *Jatropha curcas* belongs to the family: Euphorbiaceae is a small tree or a bush that ranges upto 5 m height. It holds many medicinal uses ranging from insecticidal, molluscicidal, skin irritation, anti-inflammatory, anti-parasitic and haemagglutination properties.[1] *Jatropha curcas* latex was found to decrease blood clotting time further the latex demonstrated significant wound healing activity and these formulations may be solid or liquid based on its applications.[2] *Solanum nigrum* is a short-lived perennial herb or shrub belonging to the Solanaceae family which reaches an average height of 30 to 120 cm.[3] Its medicinal uses are numerous that includes anti-hepatic, anti-inflammatory, antipyretic, anti-diarrheal and anti-hyperlipidemic activity.[4] The present research aims to compare the bioactivity of *Jatropha curcas* latex and *Solanum nigrum* leaves in terms of anti-microbial, anti-oxidant and anti-inflammatory properties further to formulate and evaluate *Jatropha curcas* latex based gel to find a solution for the treatment of canker sores.

MATERIALS AND METHODS

Collection of Raw Materials

The leaves of *Solanum nigrum* and the latex from *Jatropha curcas* were collected from Aval Poondurai, Erode District, Tamil Nadu. The samples were allowed for shade dry and powdered. It was then extracted using water as a solvent for the further study.

Phytochemical Analysis

To test the presence of the phytochemical constituents in the extracts, the methods designed by[5] were used to detect for the presence of tannins, alkaloids and saponins. The presence of steroids and glycosides were evaluated using Lieberman Burchad reaction[6] and Salkowski test respectively.

In vitro Anti-Bacterial Activity

The pathogenic bacterial cultures such as *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus salivarius* were used to test the

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efficiency of the sample extract with concentration of 100 µg/mL using Agar well diffusion method.[7,8]

Antioxidant assay

DPPH Assay

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay was used to determine the free radical scavenging activity of the test samples. The extract was prepared with different concentrations ranging from (20, 40, 60, 80 and 100 µg/mL). The experiment proceedings were carried out according to the procedure.[9] The following formula was used to calculate the percentage inhibition.

$$\%\ Inhibition = \left[\frac{A_0 - A_1}{A_0}\right] * 100$$

Where A_0 - Absorbance of the Control and A_1 - Absorbance of the Sample.

FRAP Assay

The reducing potential was analyzed by Ferric Reducing Antioxidant Power (FRAP) assay. The extract was prepared with different concentrations ranging from (20, 40, 60 80 and 100 µg/mL). The procedure given by Benzie and Strain was used to detect the antioxidant property.[10]

In vitro Anti-Inflammatory Activity

Human Red Blood Cell Assay

The estimation of *in vitro* anti-inflammatory activity was done by Human Red Blood Cell (HRBC) assay.[11] The experimental procedure involved the collection of blood sample and mixed with the exact volume of the contaminant free Alsever's solution. The mixture was then centrifuged at 3000 rpm for 10 min. The pellet was washed with isosaline solution and 10% v/v suspension was made for the further analysis. Various concentrations of samples and control was prepared with 1 mL of Phosphate buffer, 2 mL of Hyposaline, 0.5 mL of HRBC suspension. The assay mixture was incubated at 37°C for 30 min. This mixture was centrifuged at 3000 rpm for 10 min and the hemoglobin content was analyzed at 560 nm under UV spectrometry.

$$\%\ Hemolysis = \left(\frac{A_1}{A_0}\right) * 100$$

$$\%\ Inhibition = \left[\frac{A_0 - A_1}{A_0}\right] * 100$$

Where A_0 is the Absorbance of the Control and A_1 is the Absorbance of the Sample.

Protein (Trypsin) Inhibitory Method

The method was carried according to the experimental protocol.[13,14] 2 mL of reaction mixture with 0.06 g trypsin, 1 mL of 20 mM Tris HCl buffer of pH 7.4 and 1 mL of test sample of various concentrations ranging from (20, 40, 60 80 and 100 µg/mL). The reaction mixture was then incubated at 37°C for 5 min. Then 1 mL of 0.8% (w/v) casein was added and incubated for 20 min. 2 mL of 70% perchloric acid was added to terminate the reaction and the absorbance was measured at 210 nm.

$$\%\ \text{Inhibition of Proteinase} = [(A_\circ - A1)/A_\circ]100$$

Where A_0 is the Absorbance of the Control and A_1 is the Absorbance of the Sample.

Bioformulation of *Jatropha curcas* based Gel

The formulation of *Jatropha curcas* latex based gel was prepared as per the standard protocol described by Abolfazl.[14] Six formulations of latex based gels were prepared and tabulated (Table 2).

Evaluation of Physicochemical Properties of Gel

Microscopic and Macroscopic Tests: The formulated gels were subjected to microscopic tests where a small drop of formulated gel was taken and analyzed for the uniformity and gel texture. Macroscopic tests included the inspection for the lumps, colour and transparency.

Centrifuge Tests: The formulated gels were centrifuged at 2000 rpm for different time intervals and they were analyzed for gel stability and sedimentation.

pH Stability Test: Each formulation out 1 g of was taken and mixed with water and pH of each solution was analyzed at different time intervals.

Cytotoxicity Study

The growth of cells in the presence and absence of the active agents in the formulations were studied using MTT-Microculture Tetrazolium Assay. The human lymphocyte was isolated from human lymphocyte peripheral blood according to the procedure.[15] The isolated lymphocyte cells were counted by Tryphan blue method and dissolved into RPMI media which contains 10% Foetal Bovine Serum (FBS) to get 10^6 cells mL^{-1}.

RESULTS

Phytochemical Evaluation of *Solanum nigrum* and *Jatropha curcas*

The leaves of *Solanum nigrum* and the latex from *Jatropha curcas* were collected, dried, powdered and extracted using water as a solvent. The phytochemical investigation shows the presence of tannins, alkaloids, saponins and glycosides in both water extracts of *Solanum nigrum* leaf and *Jatropha curcas* latex.

In vitro Anti-Microbial Activity

The antibacterial activity of water extract was tested against *Staphylococcus aureus, Escherichia coli* and *Streptococcus salivarius* (Figure 1). The samples with 100 µg/mL concentration were loaded and the zone of inhibition was determined as tabulated

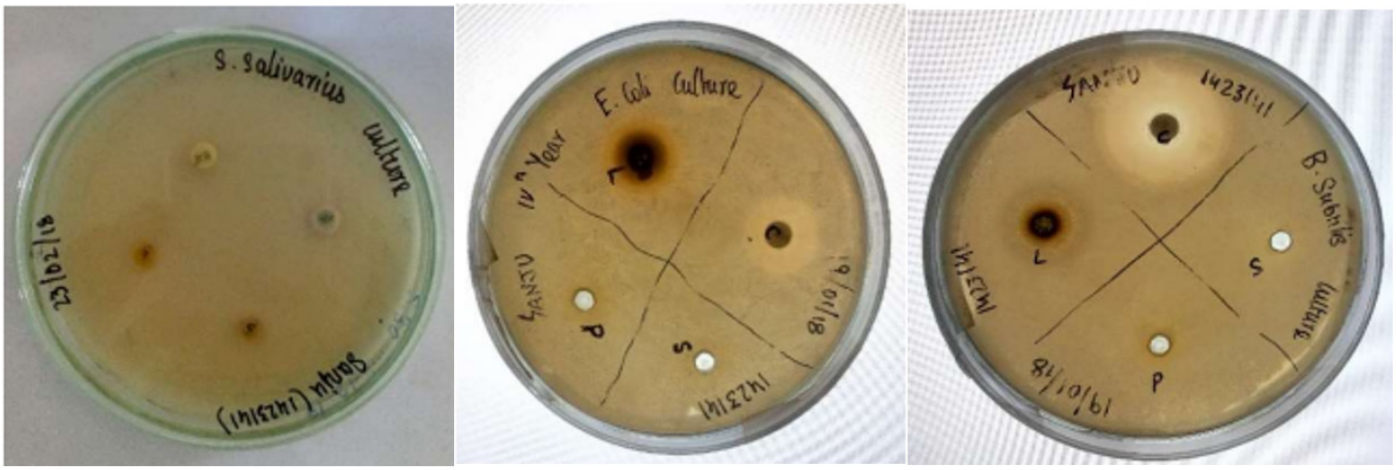

Figure 1: Zone of clearance in *Streptococcus salivarius, Escherichia coli* and *Bacillus subtilis* cultures.

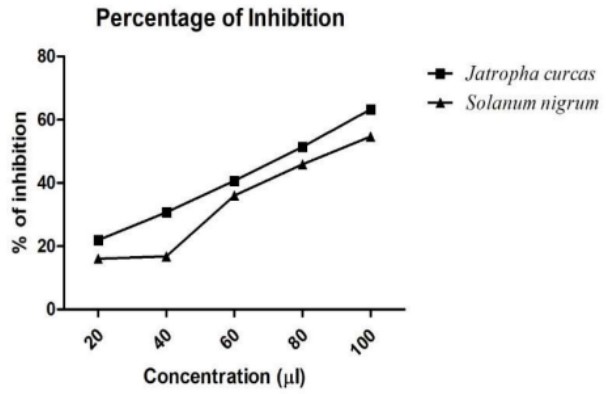

Figure 2: DPPH assay result for the percentage of inhibition of the latex of *Jatropha curcas* and the leaf extract of *Solanum nigrum.*

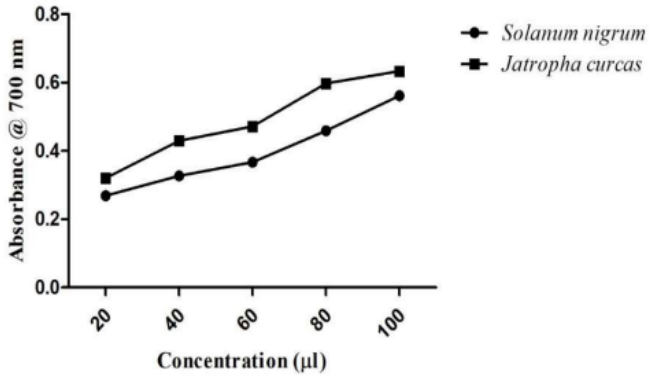

Figure 3: Absorbance values of the latex of Jatropha curcas and the leaf extract of *Solanum nigrum* from FRAP Assay.

(Table 1). The maximum activity against *Staphylococcus aureus* with the inhibition of 24.0±0.05 mm with concentration of 100 µg/mL of *Jatropha curcas* latex extract. When tested against the oral origin strain, *Streptococcus salivarius*, the experimental results revealed the better inhibition of 7.0±0.09 mm for 100 µg/mL of *Jatropha curcas* latex extract concentration. The results concluded that *Jatropha curcas* latex extract had highest antibacterial activity when compared with activity showed by *Solanum nigrum* leaf extracts.

Antioxidant Activity

DPPH Assay

The radical scavenging potential of the samples was tested as per Brand-method[9] and the absorbance was read at 517 nm. The inhibition percentage was calculated (Figure 2). From the obtained results, it was clear that the inhibition percentage increases with increase in concentration of the test sample. The water extract of *Jatropha curcas* latex showed significant radical scavenging activity ranging from 22.98% to 64.02%. The inhibitory activity of *Jatropha curcas* latex extract was found to be higher than *Solanum nigrum* leaf extract.

FRAP Assay

Through ferric reducing assay, the optical density of the reaction mixture was observed at 595 nm and the activity increased significantly with increase in concentration from 20 to 100 µg/mL of the extract (Figure 3). The reduction of Fe^{3+} ions to Fe^{2+} increases with the increase in the concentration, where *Jatropha curcas* latex extract exhibited more antioxidant activity when compared with *Solanum nigrum* leaf extract.

In vitro Anti-Inflammatory Activity

Human Red Blood Cell assay

The anti-inflammatory activity of the sample was studied by the procedure demonstrated[11] and the absorbance was read at 560 nm. The results showed that the addition of anti-inflammatory agents inhibits the process of cell lysis or disrupts the cell membrane of human red blood cells. The percentage hemolysis by *Jatropha curcas* latex extract decreases from 35.05% to 12.30%

Table 1: Zone of Inhibition by Sample Extracts.

Sample	Zone of Inhibition (mm)		
	Escherichia coli	*Staphylococcus aureus*	*Streptococcus salivarius*
Ampicillin	19.0±0.01	33.5±0.01	10.5±0.04
Solanum nigrum (Leaf extract)	10.5±0.02	7.5±0.04	4.0±0.01
Jatropha curcas (Latex extract)	16.0±0.04	24.0±0.05	7.0±0.09

Table 2: Compositions of Different Formulations.

Ingredients (g)	Formulations					
	F_1	F_2	F_3	F_4	F_5	F_6
Carbopol 940	0.5	1.0	-	-	-	-
Sodium CMC	-	-	0.5	1.0	-	-
HPMC K_4M	-	-	-	-	0.5	1.0
PEG 4000	1.0	1.0	1.0	1.0	1.0	1.0
Ascorbic acid	0.1	0.1	0.1	0.1	0.1	0.1
Extract	1.0	1.0	1.0	1.0	1.0	1.0
Water	100	100	100	100	100	100

Table 3: pH Study of the Formulations for a Period of 0-14 days.

Formulations	pH values							
	Storage period (Days)							
	0	2	4	6	8	10	12	14
F_1	4.51	4.56	4.55	4.50	4.48	4.53	4.59	4.57
F_2	4.78	4.86	4.85	4.72	4.89	4.77	4.80	4.84
F_3	4.43	4.40	4.46	4.38	4.39	4.43	4.51	4.48

with increase in concentration from 20 to 100 µg/mL respectively. The results also clearly indicated that *Jatropha curcas* latex extract showed more inhibitory action than *Solanum nigrum* leaf extracts. Hence, *Jatropha curcas* sample exhibited more anti-inflammatory activity than *Solanum nigrum* sample.

Protein (Trypsin) Inhibitory Method

The procedures for the protein inhibitory method were carried out using the trypsin protein according to the experimental procedure[12,13] and the absorbance of the mixture was recorded at 210 nm. The inhibition percentage for the samples was calculated and presented in Figure 4. The results showed that the value of inhibition increases with the decrease in the content of trypsin molecule with percentage inhibition from 73.03% to 74.84% for *Jatropha curcas* latex extract. These results also showed that *Jatropha curcas* latex sample exhibited higher inhibition than *Solanum nigrum* leaves.

Bioformulation of *Jatropha curcas* based Gel

As mentioned, six different formulations[14] (F_1 to F_6) were prepared as tabulated (Table 2) using the gelling agents like Carbopol 940, SCMC and HPMC were collected and stored (Figure 5).

Evaluation of Physicochemical Properties of Gel

Microscopic and Macroscopic Tests: Microscopic and macroscopic tests showed that the formulations F_2, F_3 and F_6 were found to be better in the colour and texture when compared to F_1, F_4, F_5 were not much clearer in their colour.

Centrifuge Test: After the centrifugation process, the tubes were checked for the gel stability and sedimentation. The formulations F_4, F_5 and F_6 got some sedimentation at the bottom, but the formulations F_1 and F_2 some changes and the formulation F_3 showed better results with was consistent uniformity of texture.

pH Test: Formulations F_1, F_2 and F_3 were found to have stable pH as tabulated (Table 3). It has been concluded that the formulated

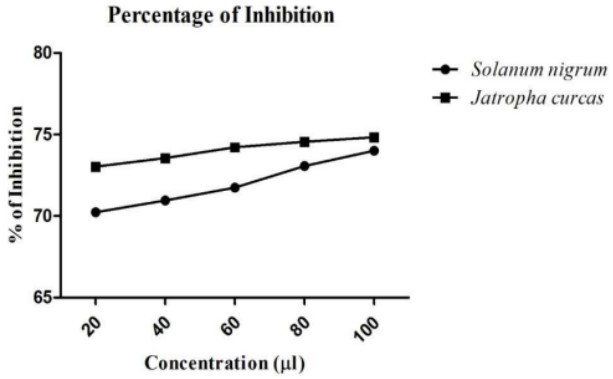

Figure 4: Percentage of inhibition of *Solanum nigrum* and *Jatropha curcas* against Protein (trypsin).

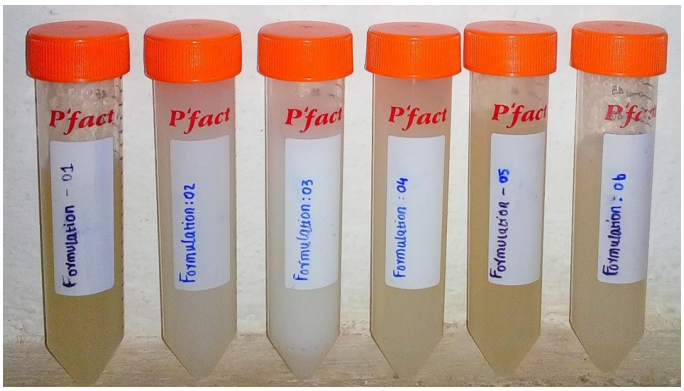

Figure 5: Formulations of *Jatropha curcas* based Gel.

Table 4: MTT Assay Results for *Jatropha curcas* based Gel Formulations.

Formulations	Content	Concentrations (µg/mL)					IC_{50} value
		20	40	60	80	100	
F_1	% Dead cells	8.15	8.72	9.51	10.08	11.01	39.09
	% Live cells	91.85	91.28	90.49	89.92	88.99	
F_2	% Dead cells	7.16	7.23	8.61	9.28	10.44	32.22
	% Live cells	92.84	92.77	91.39	90.72	89.56	
F_3	% Dead cells	5.81	6.28	7.11	8.15	8.75	36.10
	% Live cells	94.19	93.72	92.89	91.85	91.25	

gels could be used for a longer time period thus resulting in longer shelf life.

Cytotoxic Study on *Jatropha curcas* based Gel

The cellular growth with the activity of *Jatropha curcas* based gel formulation was evaluated using the methodology.[15] The formulated samples were tested using MTT assay and the absorbance were read at 540 nm and tabulated in (Table 4). The results showed that the protection of cells was found to be ranging from 88.99% to 94.19%. Formulations F_1, F_2 and F_3 were found to have the maximum capability to protect the cells when compared to other formulations. The IC_{50} value would give the minimal concentration required by the test sample to inhibit the cells. Formulation F_2 with Carbopol 940 as the gelling agent was found to have the lower IC_{50} value of 32.22 which would inhibit 50% of the cells.

CONCLUSION

Though there are several alternatives that exist to treat canker sores, the latex of *Jatropha curcas* would also act as a better alternative. In this study, the activity of *Solanum nigrum* and *Jatropha curcas* were compared and the results showed that *Jatropha curcas* latex was found to have more beneficiary activities in terms of phytochemical constituents using water as a solvent. The results of anti-bacterial activity of 100 µg/mL concentration of *Jatropha curcas* latex extract against the oral strain, *Streptococcus salivarius*, showed the inhibition of 7.0±0.09 mm. *In vitro* antioxidant assays by DPPH and FRAP revealed the maximum radical scavenging activity of 64.02% and maximum ferric reducing power with the optical density of 0.633 respectively. The better results were exhibited by *Jatropha curcas* latex when compared with *Solanum nigrum* leaves in terms of anti-inflammatory assays like HRBC assay and Protein (trypsin) inhibitory with maximum percentage inhibition of 87.61% and 74.84% respectively. *Jatropha curcas* latex based gel was formulated with Carbopol 940, SCMC or HPMC as gelling agent along with PEG 4000, Ascorbic acid, extract and water. In the evaluation of physicochemical properties of gel, the formulations F_1 and F_2 were found to given better results with minimal colour change, without sedimentation and better pH stability. The study of cytotoxicity against the chosen formulations of F_1, F_2 and F_3 showed no negative results against the cells and concluded that these formulations would cause no effects upon the lymphocytic cells. The IC_{50} values, 39.09 (F_1), 32.22 (F_2) and 36.10 (F_3) gave the minimal concentration to inhibit half population of the cells. The studies showed that the latex of *Jatropha curcas* could be used as an alternative for the treatment of canker sores, which greatly reduces the demand for several other food crops and also increases the species culture of *Jatropha curcas*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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