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Exploration of medicinal properties of *Jatropha gossypiifolia* latex.

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ABSTRACT

This study evaluates the antimicrobial potential of *Jatropha gossypiifolia* latex, a medicinal plant, against various bacterial and fungal pathogens. The latex showed promising inhibitory effects against Gram-positive and Gram-negative bacteria and selected fungal strains, indicating its potential as a natural antimicrobial agent. The latex showed significant antioxidant properties through ferric reducing antioxidant power (FRAP) assay. It also showed potent anti-inflammatory effects. The study reveals the anti-diabetic potential of *Jatropha gossypiifolia* latex, which inhibits key carbohydrate metabolism enzymes. It could regulate blood glucose levels, making it a promising natural alternative for diabetes management.

Keywords: *Jatropha gossypiifolia*, latex, antimicrobial, antioxidant, anti-inflammatory, anti-diabetic

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INTRODUCTION

The Euphorbiaceae family, one of the largest angiosperm families, comprises 7,800 species across 300 genera and 5 subfamilies, primarily found in tropical and subtropical regions. *Jatropha*, a genus of medicinal plants, includes *Jatropha curcas*, *Jatropha eliptica*, *Jatropha gossypiifolia*, and *Jatropha mollissima*, known for their medicinal properties, chemical constituents, and biological activities. *Jatropha gossypiifolia* L, also known as "bellyache bush", is a versatile plant used for treating various ailments, including eczema, itching, mouth blisters, wounds, and swellings, due to its purgative effects of their seed oil. Recent studies on *Jatropha* species reveal promising biological effects, with *J. gossypiifolia* showing promising effects, including antiviral, anti-inflammatory, antibacterial, and wound-healing properties; Antibacterial activity [1], Antiviral activities [2], Antidiarrheal activity [3], Antivenom activity [2], Anti-Protozoal activities [1], Contraceptive Activity [2], Antineoplastic activity [3]. Anticholinesterase activity [2], Antihypertensive activity [1], Iron Chelating Activity [3], Antioxidant Activity [3], Anti-inflammatory/Analgesic Action [1], Antidiabetic Activity [1], Healing action [2].

*J. gossypiifoli*a leaves, have anti-inflammatory, antimicrobial, and anticoagulant properties, and are used in various preparations. Root extracts, offers direct topical application to the skin, potentially promoting faster wound healing and providing quicker relief from pain, swelling, and inflammation. Latex has hemostatic and wound-healing properties, promoting natural wound healing through flavonoids and saponins. It can precipitate proteins, making it useful in biochemical analysis. Latex is traditionally used for treating Insects bites, and diarrhea. Hence due to easy availability of latex over roots and more benefits the latex is unique for its hemostatic and wound-healing potential. Latex was used for evaluating the antimicrobial potential of *Jatropha gossypiifolia* latex, a medicinal plant, against various bacterial and fungal pathogens.

MATERIAL AND METHODS

Latex collection:- Latex samples of *Jatropha gossypiifolia* were collected from Ranjangaon Ganpati mature plants, identifying healthy, disease-free specimens based on morphological characteristics. The latex was obtained by making small incisions on stems and petioles using a sterile scalpel, and the excluded latex was allowed to flow into sterile collection tubes.

Latex extract preparation:- 10 ml of freshly collected latex was mixed thoroughly with 90 mL of distilled water in a sterile beaker. The mixture was boiled till it was reduced to 1/4th of the original volume. The resulting solution was then subjected to centrifugation at 4000 rpm for 10 minutes to separate insoluble residues. The supernatant was stored in a refrigerator for overnight for further use.

Phytochemical analysis of the latex extract

Detection of alkaloids:- Hager's test: 1ml of plant latex were treated with few drops of Hager's reagent. A yellow precipitate was formed, indicating the presence of alkaloids

Detection of tannin:- A volume of 1 ml of 1% $FeCl_3$ was added to 1 ml plant latex and 9 ml water appearance of greenish black color confirmed the existence of tannins in the test sample.

Detection of saponin:- 1ml plant latex was added to 20 ml water and shaken vigorously. A layer of foam confirmed the existence of saponins in the test samples.

Detection of carbohydrates:- Benedict's test: few drops of Benedict's reagent were mixed with plant latex followed by boiling water bath. Reddish brown precipitate formation indicated the existence of carbohydrate in the test samples.

Detection of terpenoids:- 1 ml chloroform was added to 2.5 ml of plant latex followed by careful addition of 1.5mL concentrated sulphuric acid along the walls of the tube. Reddish brown color indicated the existence of terpenoids in the test samples [4].

Detection of phenol:- 1ml sample was dissolved in 3 ml distilled water and few drops of neutral 5% $FeCl_3$. The presence of dark green color indicates the presence of phenol compounds [5].

Antioxidant potential of plant latex by ferric reducing antioxidant power assay:

The plant latex (100 µl, 300 µl, 500 µl) were added to 2.5ml Phosphate buffer solution (PBS) with 0.2 M, pH 6.6, 2.5ml of Potassium Ferricynide (1%) after 20 min. Incubation at 50°C of the mixtures, 1.5 ml trichloroacetic acid (10%) was added. Centrifuged at 3000 rpm for 10 minutes. 2.5ml supernatant was taken and again mixed with 2.5ml water and 0.5ml of 0.5% $FeCl_3$. The absorbance of the mixture was measured at 700nm by using spectrophotometer. Ascorbic acid used as positive control and distilled water used as blank instead of plant latex [6].

Antibacterial activity of plant latex by agar well diffusion method

The study evaluated the antimicrobial activity of plant latex using sterile Mueller-Hinton agar (MHA) plates for bacteria. Wells were filled with 100 µl of the plant extract or standard antibiotic, and the extract's antimicrobial activity was compared with a standard antibiotic Amoxicillin. Antibiotic stocks were prepared at 100 mg/ml concentration [7].

Anti-inflammatory activity of plant latex

Protein denaturation assay

A mixture of 1% bovine albumin serum (BSA) (1ml) and 0.2 ml latex was prepared. 2% Aspirin was used as a standard anti-inflammatory drug. Positive control was similarly prepared by adding aspirin to BSA solution. The mixtures were incubated at room temperature for 10 minutes then heated at 51ºC for 20 min, cooled down at room temperature. Absorbance was taken at 660nm [6].

The percentage inhibition of protein denaturation was calculated as follows

$$\text{Formula: Inhibition } \% = 100 - \frac{AI \times 100}{AC}$$

Where: AI is the measured absorbance in the presence of an inhibitor and AC is the absorbance of the control

Heat-induced hemolysis

Fresh human blood was collected in an anticoagulant tube to prepare 10% RBC solution. 2ml blood in 13 ml PBS (pH 7.4, 10mM) was centrifuged at 2000 rpm for 5 minutes. The supernatant was discarded, and the packed red blood cells were washed till clear PBS was obtained. Supernatant were discarded and added 4.5 ml PBS to the pellet and gently mixed to get 10% RBC solution. The reaction mixture consisted of 1ml of 10% RBC suspension and 1ml of plant latex. A negative control consisted of distilled water instead of plant latex while the positive control consisted of Aspirin (2%) as the known anti-inflammatory drug. The mixtures were incubated at 54°C for 30 minutes in water bath, followed by centrifugation at 2500 rpm for 10 minutes. The supernatant collected, 1ml water was added and absorbance of the supernatant was measured at 560nm using UV spectrophotometer [6].

The percentage inhibition of hemolysis was calculated using the formula:

$$\text{Inhibition [\%]} = 100 - \frac{\text{AI} \times 100}{\text{AC}}$$

Where: AI is the measured absorbance in the presence of an inhibitor and AC is the absorbance of the control.

Protease inhibition assay

The reaction mixture was prepared containing 0.1ml bovine albumin serum (1%) and 0.1ml of plant latex, blank sample was prepared using distilled water instead of plant latex, positive control used Aspirin (2%) as the anti-inflammatory drug. The mixtures were incubated at room temperature for 5 minutes followed by 250µL of trypsin. The mixture was centrifuged at 2500 rpm for 5 minutes and 1ml of

water was added to the collected supernatant. The absorbance of the sample was measured at 300nm using UV spectrophotometer.

The percentage inhibition of protein denaturation was calculated using the formula:

$$\text{Inhibition [\%]} = 100 - \frac{\text{AI} \times 100}{\text{AC}}$$

Where: AC Where, AI is the measured absorbance in the presence of an inhibitor and AC is the absorbance of the control [6].

Anti-diabetic activity of plant latex

Alpha amylase inhibition assay

The ability of the plant latex extract to inhibit alpha amylase activity was evaluated using a modified dinitrosalicylic acid method. A test reaction mixture containing 0.2ml alpha amylase (Aristozyme) and 0.2ml plant latex, negative control was prepared using distilled water instead of plant latex, positive control used 2% acarbose which is the antidiabetic drug. The mixture was incubated for 10 min at 30°C. After incubation, 1% starch solution was added and further incubated for 3 minutes. The reaction was stopped by adding 0.2 ml DNSA reagent followed by heating for 10 minutes at 85-90°C. The mixture were cooled to ambient temperature and diluted with 5ml distilled water. The absorbance was measured at 540nm using UV spectrophotometer. The inhibition of alpha amylase was expressed as percentage of inhibition and was calculated by the following equation:

$$\textbf{Inhibition [\%]} = \frac{[(AC^{+} - AC^{-}) - (AS - AB)]}{(AC^{+} - AC^{-})} \times 100$$

Where AC is the absorbance of the control and AS is the absorbance of sample [6].

RESULTS

Characterization of plant latex by phytochemical test:- The phytochemical analysis confirmed the presence of alkaloids, tannin, saponins, carbohydrate, terpenoids, phenol in the plant latex.

Table 1: Phytochemical analysis

Phytochemical constituents	Test performed	Observation	Result
Alkaloids	Hager's test	Yellow coloration	+
Tannin	Ferric chloride test	Blue black coloration	+
Saponins	Froth formation test	Stable froth formation	+
Carbohydrate	Benedict's test	Brown coloration	+
Terpenoids	Liberman- Burchard test	Reddish-brown coloration	+
Phenol	Ferric chloride test	Dark blue grayish coloration	+

Antioxidant potential of plant latex by ferric reducing antioxidant power assay

The antioxidant activity of *Jatropha gossypiiifolia* latex extract increased with concentration, indicating a dose dependent response. The observed antioxidant activity of *Jatropha gossypiifolia* latex is comparable to standard antioxidants like ascorbic acid.

Table 2: Antioxidant potential of crude latex

Concentration (µg/mL)	Absorbance at 700 nm	Positive Control (ascorbic acid)
100	1.105	0.428
300	2.56	1.903
500	3.141	1.3

Antibacterial Assay of Plant latex

The crude latex and latex extract of *Jatropha gossypiifolia* demonstrated broad spectrum antimicrobial activity, larger inhibition zone against ***Enterococcus sp.*** and ***Klebsiella sp.*** were observed by latex extract**.** The results indicating the antibacterial activity of both extracts of *Vigna aconitifolia* against all test organisms are tabulated in (Table 3).

Table 3: Antimicrobial Assay of crude latex and latex extract

Microorganisms	Zone of Inhibition (mm)	
	Klebsiella sp.	***Enterococcus sp***
Latex crude	14	15
Latex Extract	15	16
Amoxicillin	18	20

Anti-inflammatory activity plant latex

Inhibition of protein denaturation assay

Protein denaturation was maximally inhibited by 28.31% in the extract, compared to 28.03% in the crude extract. The extract effectively prevented the denaturation of albumin caused by heat. The percentage of protein denaturation inhibitions is listed in the (Table 4). At 100µg/ml, aspirin, a proven positive control, demonstrated the highest level of inhibition (61.44%). The extract's ability to lessen heat-induced protein denaturation may be a role in its anti-inflammatory properties.

Heat induced hemolysis

The latex extract demonstrated a maximum of 79.37% prevention of heat-induced hemolysis, compared to 54.37% for the crude latex. Table 4 lists the percentages of heat-induced hemolysis inhibitions. The highest inhibition of aspirin was 86.57% at 100µg/ml. Inflammation is known to be caused by protein denaturation. As a result, the diluted extract from sprouts prevented heat-induced hemolysis and shown the ability to stabilize membranes, which is one of the anti-inflammatory processes.

Protease inhibition assay

The extract exhibited a maximum of 38.76% protease inhibition when compared to. Table 4 lists the protease inhibition assay's percentage inhibitions. The highest inhibition of aspirin was 73.35% at 100µg/ml. There was notable antiproteinase activity in these extracts. The suppression of neutrophil lysosomal content release at the site of inflammation may be the cause of this effect. The fact that leukocyte proteinases cause tissue injury in inflammatory areas is widely established. Therefore, in these circumstances, proteinase inhibition offers a substantial level of protection.

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Table 4: Results of protein denaturation, heat-induced hemolysis, protease inhibition assay

Extract	% inhibition of protein denaturation (albumin denaturation)	% inhibition of heat-induced hemolysis (membrane stabilization)	% inhibition of protease inhibition (proteinase inhibition)
Latex crude	28.03	54.37	25.59
Latex extract	28.31	79.37	38.76
Positive control (aspirin)	61.44	86.57	73.35

Antidiabetic activity of plant latex

α-amylase is one of the enzymes that hydrolyze carbohydrates, breaking down oligosaccharides and digesting starch to create glucose. Restricting α-amylase activity is one of the primary methods used to manage hyperglycemia in diabetic individuals. Acarbose is the enzyme inhibitor that is most frequently administered. Latex extract showed a greater ability to inhibit α-amylase (Table 5) than the crude latex. The percentage inhibition of latex was found to be almost half to that of acarbose, the higher concentrations of latex can be regarded as a possible inhibitor of α-amylase.

Table 5: Anti-diabetic activity of plant latex

Latex	% inhibition
Latex Extract	53.37
Latex crude	49.94
Positive Control (Acarbose)	89.56

DISCUSSION

The study explores the medicinal properties of *Jatropha gossypiifolia* latex, focusing on its antioxidant, anti-inflammatory, anti-diabetic, and antimicrobial activities. It involves collection of latex and characterization of its bioactive compounds, including flavonoids, tannins, alkaloids, and terpenoids, which were also obtained in the stem bark of same plant genus by a different study [8]. The leaf extract of *J. curcas* demonstrated significant anti-inflammatory and analgesic effects in rodent models, as confirmed by protein denaturation and membrane stabilization methods, which was also shown by the *J. gossypiifolia* latex in the current study [9]. Latex was found to possess polyphenols, a known marker for inflammation that can inhibit protein denaturation efficacy. In a different study, Cedrus deodar wood oil showed excellent membrane-stabilizing activity, using inhibition of heat-induced hemolysis, mimicking lysosomal membrane stabilization observed *in vivo*, a key mechanism of anti-inflammatory effects [10]. Present study also exhibited the similar activity with latex extract. The extract demonstrated significant protease inhibition activity, with 38.76% inhibition, suggesting potential therapeutic applications in inflammation and tissue degradation conditions, similar to *Jatropha curcus*' anti-inflammatory and analgesic effects [9]. Another research showed significant inhibition of alpha- amylase and alpha-glucosidase enzymes in diabetic rats, possibly due to phytochemicals like saponins and flavonoids [11]. In a similar study, Alpha-Amylase inhibition by six Allium species was demonstrated [12]. The ability of the latex to inhibit alpha-amylase enzyme activity was evaluated using the DNSA method. The extract showed higher inhibition than the crude latex. Anti-hyperglycemic effect of *J. gossypiifolia* root extracts in diabetic rats, attributing this effect to the presence of flavonoids and saponins, which were also shown to be present in the latex in this current study [11]. FRAP (Ferric Reducing Antioxidant Power) assay measures the ability of antioxidants to reduce Fe3+ to Fe2+ [14]. The present study demonstrated significant radical scavenging activity of latex extract, comparable to that reported by [13].

CONCLUSION

The medicinal properties of *Jatropha gossypiifolia* latex revealed its potential as a natural therapeutic agent. It exhibits antioxidant, antimicrobial, anti-diabetic, and anti-inflammatory properties,

supporting its traditional use in folk medicine. Bioactive compounds like flavonoids, alkaloids, tannins, and saponins contribute to its pharmacological effects. This research reinforces traditional medicinal use and opens new possibilities for plant-based treatments.

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