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An *In-vitro* study of the antibacterial and anti-inflammatory activity of alkaloid extract from *Desmodium gangeticum* leaves

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Abstract
This study was designed to determine whether the alkaloid extract from the leaves of *Desmodium gangeticum* has antibacterial and/or anti-inflammatory activity *in vitro* study to investigate the mechanism of antibacterial and anti-inflammatory activity. Additionally, this study sought to define the chemical composition of *D. gangeticum*. Results indicated that alkaloid extract from the leaves of *D. gangeticum* showed significant antibacterial activity *in vitro* efficacy. Moreover, alkaloid extract from the leaves of *D. gangeticum* significantly inhibit lipoxygenase and albumin denaturation. In conclusion, data demonstrate that alkaloid extract from the leaves of *D. gangeticum* has antibacterial and anti-inflammatory activities *in vitro* condition. The anti-inflammatory effect appears to occur, at least in part, through the inhibition of lipoxygenase. Moreover alkaloids are probably the active components in *Desmodium gangeticum* that utilize antibacterial and anti-inflammatory activities.

Keywords: antibacterial; anti-inflammatory; alkaloid extract; *D. gangeticum*

Introduction

Secondary metabolites in plants have long been thought of as extravaganzas that provide no apparent biological use for the plants that make them. Their physiological function are yet to be fully understood. Yet, it is becoming obvious that numerous plant secondary products have a role in the plant's association with its environment in order to adapt with various stress factors, and so the level of these phytochemicals is mostly regulated by ecological parameters. A wide range of environmental stimuli can cause changes in the plant cell, which in turn activate a chain of processes that lead to the creation and accumulation of secondary metabolites that assist the plant cope with stress. Antimicrobial agents are abundant in medicinal plants. A broad range of medicinal components are employed to produce phytochemicals with various therapeutic effects against various microorganisms. Hundreds of plant species have been examined for antimicrobial activities, however the most have not been sufficiently studied. Given the great potential of plants as antimicrobial drug sources, the current study is focused on an analysis of such plants (Ara *et al*., 2009).

Alkaloids are synthesised by a wide range of organisms, including plants, bacteria, fungi, and animals, and have historically been utilised in medicine. They have antimalarial, antiasthmatic, vasodilator, anti-hypertensive, anti-tumor, and anti-arrhythmic properties, among others. Alkaloids can also have psychotropic and stimulant properties, as well as an analgesic effect, as seen in morphine. Finally, alkaloids have antibacterial and antivirulence properties. Many studies have demonstrated that alkaloids have antibacterial activity, and that most of these compounds may be useful in the treatment of a variety of infectious diseases (Donald *et al*., 2016) [4]. Sanguinarine is an alkaloid that has antimicrobial properties. Sanguinarine, a benzophenanthridine alkaloid produced from the rhizomes of Sanguinariacanadensis, has long been known to have antibacterial and anti-inflammatory properties (Kelley *et al*., 2012) [6].

The use of cardinal signs to diagnose inflammation is outdated and inadequate for guiding appropriate therapeutic methods. Additionally, the current inflammatory process theory concerning vascular phenomena that are required for the development of cardinal signs is flawed and unable to account for well-established empirical facts, such as the amount of osmotic pressure and temperature variability in inflamed tissue (Das *et al*., 2008) [3]. There is just one particular macroscopic symptom of inflammation, localised edema, out of the five cardinal indicators. Moreover, the driving force for the deposition of tissue fluid is defined in biochemical terms, and as such is used to define the inflammatory process (Amel *et al*., 2012) [1]. Inflammation is a degenerative process that causes local accumulation of low molecular weight catabolic products, which in turn raises tissue osmotic pressure, attracting extra fluid, with or without heat release sufficient to raise tissue temperature significantly. Since protein denaturation and the LOX proteinases enzymes are most commonly linked with pro-inflammatory processes, it's crucial to note that both enzymes also create factors that help to prevent or cure inflammation and promote tissue healing (including the prostacyclins and lipoxins), The transition of proteinases and LOX enzymes from pro- to anti-inflammatory activity is essential for the progression of a better and healthier inflammatory response (Chinenye *et al*., 2013) [2].

Materials and Methods

Collection of Plant Material

Desmodium gangeticum leaves were taken from the Government Siddha Medical College's Medicinal Plant Garden in Arumbakkam, Chennai 600 106, an acknowledged institution of the Department of AYUSH, Government of India.

Phytochemical Analysis

Desmodium gangeticum aqueous extract was freshly obtained and separated into different test tubes, and phyto constituents were determined using procedures proposed by Trease and Evans (1989).

Extraction of Alkaloid

Extraction was carried out using Manosalva *et al*. (2014) [8] previously reported procedure. In a nutshell, oven-dried and powdered *Desmodium gangeticum* leaves (200 g) were extracted progressively with methanol at room temperature for 48 hours. The leftover residues were agitated with 100 mL of 10% HCl for 1 hour, incubated for 12 hours at 10°C, and filtered after the pooled methanolic extract of plant tissue was evaporated in vacuum at 40°C. $CHCl_3$ was used to wash the filtrates (5X80 mL). Evaporation of the $CHCl_3$ washings generated brown non-alkaloidal extracts, which were not examined. With NH_4OH, the aqueous phases were adjusted to pH 10 and extracted with $CHCl_3$ (5X80 mL). To acquire the alkaloid extracts of the stems, the solvent was evaporated.

Thin layer chromatography

Isolated alkaloid extraction from *Desmodium gangeticum* was loaded over pre-coated TLC (60 F_2 54) and introduced with a 1:0.5:0.1 ratio solvent system (Hexane, Chloroform, and Methanol). Visible and non-visible spots generated, which is fluorescent with Ultraviolet rays at 360nm.

Antibacterial activity

Donald *et al*. (2016) [5] used the disc diffusion method to test the antibacterial activity of the alkaloid-rich portion from the leaves of *D. gangeticum*, which is followed here.

Leakage of the Membrane in Pathogenic Bacteria

Different quantities of MH medium, antibacterial substance, and pathogenic bacteria cells were introduced to 10 ml cultures with final concentration 100 g/ml antibacterial compound and 10^9cfu/ml pathogenic bacteria to identify the leakage of reducing sugars and proteins via membrane. Experiments without the antibacterial ingredient were carried out as a control. The cultures were incubated at 37±2°C with 150 rpm shaking. When antibacterial compounds were added to cultures and they were treated for 4 hours, one millilitre of culture was taken. The sample was centrifuged at 12,000 rpm, the liquid supernatant was promptly frozen at -30 °C, and the quantities of reducing sugars and proteins were calculated as soon as feasible (Bradford 1976; Miller, 1959).

Lipoxygenase inhibition assay

With minor modifications, a spectrophotometric assay for assessing LOX activity was utilised as described (Kemal *et al*., 1987) [7]. The assay was performed with soybean lipoxygenase (LOX). The loss of soybean LOX activity (5g) was measured in inhibition experiments using 0.2 μM linoleic acid (Sigma) as the substrate produced in a solubilized condition (Kemal *et al*., 1987) [7] in 0.2 M borate buffer (pH 9.0). Using a UV-Vis spectrophotometer, inhibition experiments in the presence of various doses of extracts (5, 10, 15, 20 μg /mL) and a reference compound *viz*., quercetin, were recorded at 234 nm (Beckman Coulter, DU 730 Life Sciences). The extract's inhibitory impact was also calculated as a percentage of enzyme activity inhibition. The IC_{50}, which represents the concentration needed to inhibit 50% of LOX activity, was also estimated.

Inhibition of Protein Denaturation Activity

The approach utilised to assess *Desmodium gangeticum* alkaloid extract's *in-vitro* anti-arthritic activity was "inhibition of protein denaturation" (Chippada *et al*., 2011), with diclofenac sodium as a reference. 0.45 ml bovine serum albumin (5 percent w/v aqueous solution) and 0.05 ml test solution end up making the test solution (0.5 ml). 0.45 ml bovine serum albumin (5 percent w/v aqueous solution) and 0.05 ml distilled water make up the test control solution (0.5 ml). 0.45 mL distilled water and 0.05 mL test solution make up the product control (0.5 mL). 0.45 ml bovine serum albumin (5 percent w/v aqueous solution) and 0.05 ml diclofenac sodium makes up the standard solution (0.5 ml). The samples were incubated at 37°C for 20 minutes before increasing the temperature to 57°C for 3 minutes. 2.5 mL of phosphate buffer was added to the prior solutions after they had cooled. At 416 nm, the absorbance was read using a UV-Visible spectrophotometer.

Result and Discussion

Phytochemical Screening

Desmodium gangeticum phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, terpenoids, and phenols (Table -1). Glycosides and tannin are not present. Antimicrobial activity has been reported for these substances in a variety of ways.

Table 1: Phytochemical screening of aqueous extracts from the leaves of *Desmodium gangeticum*

S.No.	Constituents	*Desmodium gangeticum* extract
1.	Alkaloids - Dragendroffs reagent - Mayer's test	 + +
2.	Flavonoids - Alkali test - Lead acetate test	 + +
3.	Polyphenols -Ferrozine test	 +
4.	Terpenoids -Salkowski test	 +
5.	Tannins -$Fecl_3$ Test	 -
6.	Glycosides -Keller-Killani test	 +
7.	Saponins -Froth test	 +

-- = Negative (absent); + = Positive (present)

TLC finger print profile

The alkaloid extract of *Desmodium gangeticum* leaves was loaded onto pre-coated TLC plates (60 F2 54 Merck) and developed using a 9.5:2.5:0.4 ratio solvent solution comprising toluene, tetahydrofuran, and acetic acid. UV 240nm and 360nm were used to examine the developed plate (Fig-1)

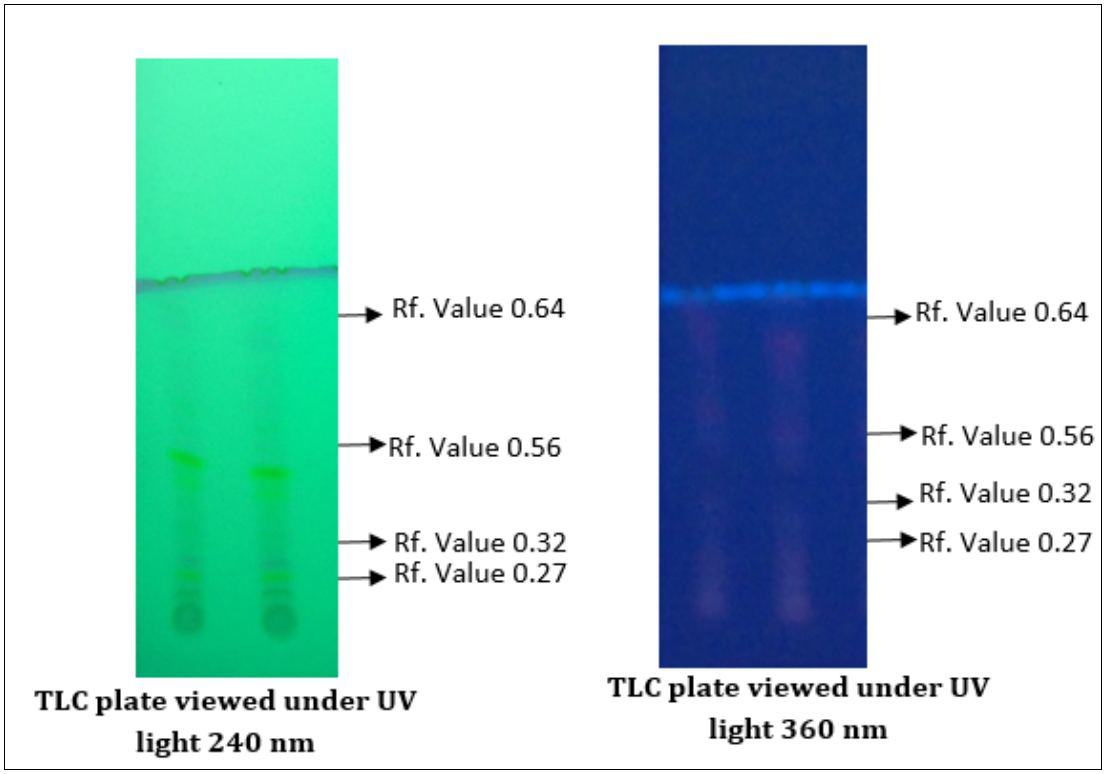


Fig 1

Antibacterial activity

The disc diffusion test revealed that *Desmodium gangeticum* alkaloid extract was efficacious against all microorganisms tested, including Gram-positive and Gram-negative bacteria. In the ranges of extract concentrations of 5, 10, 15, and 20µg/ml, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Proteus vulgaris were tested (Table-2). With inhibition zones of 16.3±1.4 mm and 15.4±1.3 mm, respectively, alkaloid extract demonstrated excellent activity against Gram-positive and Gram-negative bacteria *Escherichia coli* and *Staphylococcus aureus*.

The alkaloid extract, on the other hand, inhibited only one Gram negative bacterial strain, Proteus vulgaris (13.6±1.8). Plant stems, roots, leaves, bark, flowers, and fruits can all contain antimicrobial compounds (Borchardt *et al*., 2008). Natural plant products, such as quinine from cinchona, have played an important role in the hunt for therapeutic medications.

Table 2: Antibacterial activity of the alkaloid extract of *Desmodium gangeticum* on the growth of Pathogenic bacteria

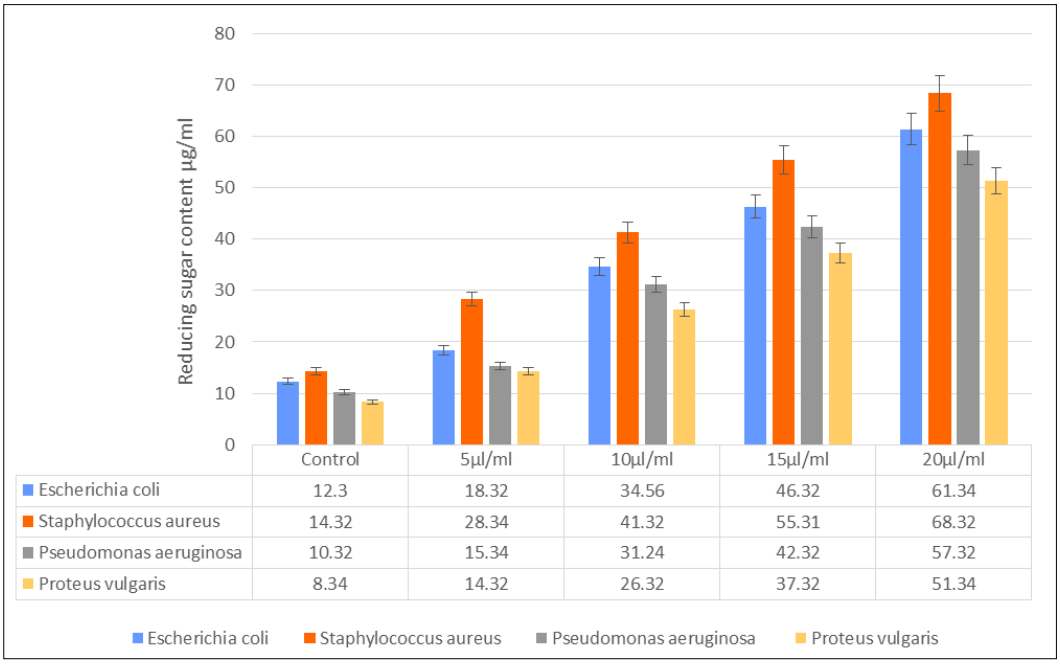
Pathogenic bacteria	Alkaloid extract exhibited the Zone of inhibition (mm)[a]				
	Positive control 10 µl Ampicillin	Different concentrations Crude extract (µl/ml)			
		5 µl	**10** µl	**15** µl	**20** µl
Staphylococcus aureus	13mm	8.3±1.6	10.7±1.4	12.3±1.3	15.4±1.3
Escherichia coli	15mm	9.8±1.5	12.9±1.3	15.6±1.7	16.3±1.4
Pseudomonas aeruginosa	14mm	7.6±1.8	9.8±1.7	11.4±1.6	14.2±1.2
Proteus vulgaris	14mm	6.3±1.3	8.4±1.6	10.3±1.3	13.8±2.1

*Calipers were used to measure the inhibitory diameter. The mean values of all the assays were reported after they were duplicated.

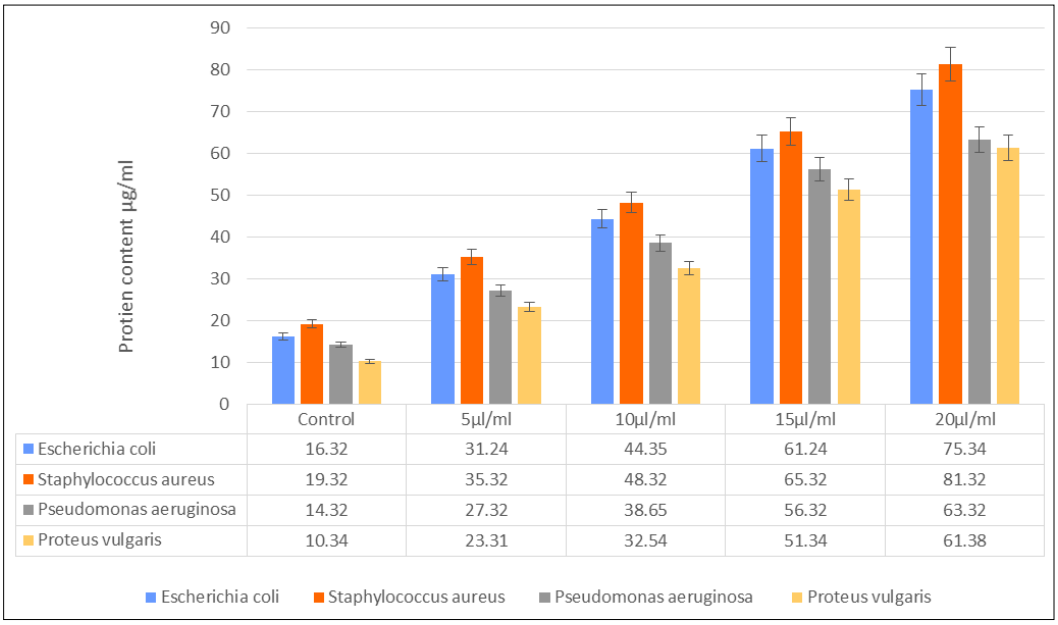
Effect of *Desmodium gangeticum* alkaloid extract on pathogenic bacteria membrane leakage

Estimating the reducing sugar in bacterial cultures treated with *Desmodium gangeticum* alkaloid extract revealed membrane permeability. After 18th hours, the amount of reducing sugar estimated in alkaloid extract of *D. gangeticum* treated with pathogenic bacterial cultures ranged from 42.15 to 88.89 µg/mg of bacterial dry weight in *S. aureus*, 37.44 to 72.49 µg/mg in Escherichia coli, 24.59 to 52.14 µg/mg in Proteus vulgaris, and 36.1 to 63.17 µg/mg in *P. aeruginosa* (Graph-1). The amount of protein in the alkaloid extract from *D. gangeticum*-treated broth cultures was calculated, and the OD value was compared to a standard BSA graph. The protein estimation value was higher than the control, implying that the alkaloid extract of *D. gangeticum* was effective against the pathogen even at an early stage.

Protein concentrations in *Staphylococcus aureus* ranged from 23.47 to 53.26 µg/mg, 19.27 to 51.78 µg/mg in Escherichia coli, 17.56 to 48.36 µg/mg in *P. aeruginosa*, and 8.76 to 15.49 to 43.29 µg/mg in Proteus vulgaris of *D. gangeticum* alkaloid extract at 18th hour (Graph-2). The primary alkaloid, cryptolepine, produces cell lysis and morphological alterations in S. aureus, according to Sawer *et al*. (2005) [11], however the antimicrobial actions of the alkaloid may be due to a different mechanism.



Graph 1: Effect of alkaloid extract of *Desmodium gangeticum* on leakage of membrane of pathogenic bacteria (Reducing sugar content µg/ml)



Graph 2: Effect of alkaloid extract of *Desmodium gangeticum* on leakage of membrane of pathogenic bacteria (Protien content µg/ml)

Lipoxygenase activity inhibition by an alkaloid extract from *D. gangeticum*.

The anti-inflammatory efficacy of the alkaloid extract of *D. gangeticum* was tested by inhibiting LOX using linoleic acid as a substrate. The *D. gangeticum* alkaloid extract at a concentration of 20 µl/ml inhibited more than the other concentrations. At 20 µl/ml, the inhibition percentage was higher than 72.31 percent (Table-3). At 20 µg/mL, the standard diclofenac sodium showed 65.32 percent inhibition. The inhibitory action of the *D. gangeticum* alkaloid extract was higher than that of the positive control. The addition of molecular oxygen to fatty acids with a cis, cis-1, 4-pentadiene system is catalysed by lipoxygenase. Unsaturated fatty acid hydroperoxides are formed as a result of this process. These products are then transformed into others that play an important part in the inflammatory process. As a result, substances capable of inhibiting that enzyme can be classified as antioxidants and anti-inflammatory agents (Rajput *et al*., 2006) [10].

Table 3: Inhibition of lipoxygenase activity of alkaloid extract of *D. gangeticum*

Different concentration of extract	Inhibition percentage of LOX	Diclofenac sodium (+ve control)
5 µl/ml	16.32±1.56	14.32±2.78
10 µl/ml	34.56±1.37	31.45±1.67
15 µl/ml	51.32±2.87	46.34±1.38
20 µl/ml	72.31±0.89	65.32±2.48
EC_{50} Value	58.32±1.45	68.32±1.23

The results are expressed as a percentage of Lipoxygenase inhibition compared to control. The mean+SD of five experiments is shown by each value.

Inhibition of protein denaturation by alkaloid extract of *D. gangeticum*

The effect of an alkaloid extract from *D. gangeticum* with significant activity on protein denaturation inhibition was compared to that of the standard drug Diclofenac sodium. Denaturation of protein may cause the formation of auto antigen in some arthritic diseases. According to the findings of this investigation, alkaloid extract is capable of reducing auto antigen production and inhibiting protein denaturation in rheumatic disease. At a concentration of 20 µg/ml, the maximum percentage inhibition of protein denaturation was 78.56 percent, which was similar to the percentage inhibition of diclofenac sodium (75.32 percent) (Table-4). There is a growing concern around the world about developing new anti-inflammatory medications that are not only effective but also safe. The findings of protein denaturation inhibition tests highlight the importance of *D. gangeticum's* alkaloid-rich fraction as a valuable resource for the isolation and development of novel selective anti-inflammatory drugs (Mishra *et al*., 2011) [9].

Table 4: Inhibition activity of protein denaturation by alkaloid extract of *D. gangeticum*

Different concentration of extract	Inhibition percentage of protein denaturation	Diclofenac sodium (+ve control)
5 µl/ml	22.34±2.37	18.32±1.49
10 µl/ml	41.32±1.87	39.65±2.39
15 µl/ml	61.32±1.45	58.32±1.45
20 µl/ml	78.56±2.34	75.32±2.34
EC_{50} Value	52.31±2.46	55.32±1.46

The results are expressed as a percentage of protein denaturation inhibition compared to control. The mean+SD of five experiments is shown by each value.

Conclusion

Finally, the findings of this study may help to boost the standardisation process for botanicals that contain the alkaloid extract of *Desmodium gangeticum* as one of the constituents. In several cases, the compound(s) isolated from the plants do not act as drugs, but they do lead to the discovery of innovative therapeutic agents. Speedy identification of novel compounds with considerable anti-inflammatory activities from plant resources is proven to be crucial agents in the mainstream of bacterial infection and anti-inflammatory drug discovery blitz.

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