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A PRELIMINARY SCREENING OF THE MEDICINAL PLANT *DESMODIUM GYRANS* (LINN.F) DC FOR ITS ANTIMICROBIAL, PHYTOCHEMICAL AND WOUND HEALING PROPERTIES

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ABSTRACT

In the present study methanol and aqueous extract of the medicinal plant *Desmodium gyrans* were tested for the antimicrobial and wound healing properties. The antimicrobial activity of this plant was tested against the clinical pathogens such as *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Vibrio cholera*e and *Klebsiella pnemoniae*. The methanol extract of this plant showed efficient antimicrobial activity against clinical pathogens such as *Escherichia coli*, *Vibrio cholerae* and *Staphylococcus aureus*. The aqueous extract of this plant was found to be effective against *Klebsiella pnemoniae* and *Staphylococcus aureus*. The phytochemical screening of this plant revealed the presence of the phytoconstituents such as alkaloids, steroids, tannins and saponins. The aqueous extract of this plant was found to be effective in healing the wounds when it was tested in rabbits of *Orychtolacus sp* and this was compared with standard antibiotic cream Neomycin.

INTRODUCTION: Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost [1]. Hence investigation on some active principles from traditional medicinal plants has become more important [2].

The world health organization [3] has also recommended the evaluation of the effectiveness of plants in condition where we lack safe modern drugs [4]. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microorganisms, animals and plants. As there is no report on the antimicrobial, phytochemical and wound healing potential of this plant, investigation was made in this medicinal plant *Desmodium gyrans*. *Desmodium gyrans* is one of the medicinal plants which are commonly known as “Tholukanni”. It belongs to the family Fabaceae. It is a tropical Asian shrub. It is usually growing at more than 3000 meter elevation higher from the ground level.

WHOLE PLANT OF DESMODIUM GYRANS OBSERVED AT 2000mtr HEIGHT IN WESTERN GHATS

The dancing plant *Desmodium gyrans* has a terminal leaflet and one or two lateral leaflets. The terminal leaflet is ca.3-7cm long and the lateral leaflet is approximately 1cm long. The terminal leaflets orient themselves horizontally or pointing upwards during the day and hanging down during the night. These up and down movements of the terminal leaflets occur with a periodicity approximately a day and the period is temperature compensated (The period does not changed with changes in temperature). Whereas the lateral leaflets carryout up and down movements with a periodicity of 3-5 times (Ultradian) and is independent of temperature [5].

To date so many research have been carried out in the movement of this plant leaf based on temperature, electricity and magnetic power rather than the medicinal point of view. In order to find out the medicinal value of this plant we have attempted to traceout the antimicrobial, phytochemical and wound healing properties of the medicinal plant *Desmodium gyrans*.

Plant Collection: *Desmodium gyrans* was collected from Kani tribal settlement near Karayar during the month of March 2010, which is located in Tirunelveli district, Tamil Nadu. This plant was then identified by referring to the book "The flora of Presidency of Madras [6]. Voucher specimens of this plant were deposited in the Dept. of Biology, Sri Paramakalyani College, Alwarkurichi. The leaves of this plant were brought to the laboratory and shade dried for five days.

Crude extract preparation:

1. **Methanol extracts preparation:** The shade dried leaves of *Desmodium gyrans* were ground well in a mixer grinder and made into coarse powder (about 50g). 30grams of the powdered leaves were extracted with methanol in soxhlet apparatus. Then, the extract was evaporated in a rotary vacuum evaporator at 40^{o}C under reduced pressure and crude extract of about 4 g was obtained which is relatively equivalent to 13.33 % of total extraction. One gram of the crude extract was diluted with 10 ml of sterile distilled water and subjected to antimicrobial and phytochemical screening.
2. **Preparation of aqueous extract:** The aqueous extract for wound healing assay was prepared by mixing 10 g of powdered leaves of this plant with sterile distilled water and boiled to slow heat for 2h. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and the procedure was repeated twice. The extracted supernatant was concentrated to make the final volume one-fourth of the original volume [7]. It was then autoclaved at 121^{o}Cand at 15 lbs pressure and stored at 4^{o}C.

Microbes and Media: The clinical bacterial isolates such as *E.coli, Salmonella typhi, Klebsiella pneumoniae, Vibrio cholerae and Staphylococcus aureus* were used. The bacterial isolates were obtained from Asan memorial college, Chennai and the slants were maintained in nutrient agar, which were stored at 4^{0}C until further use.

Antimicrobial activity: The extracts were screened for their antimicrobial activity invitro by plate hole diffusion assay as described by [8, 9]. The clinical pathogens such as *E. coli, Salmonella typhi, Klebsiella pneumoniae, Vibrio cholerae and Staphylococcus aureus* were inoculated in nutrient broth and kept for incubation at 37^{o}C for 24 h after incubation the suspension was checked for 0.7 McFarland value and was used as the seed culture.

For screening Muller-Hinton agar was prepared and seeded with 0.5 ml of respective bacterial pathogens. Then the holes were made by using a sterile cork-borer and is added with different volumes (25µl, 50µl, 75µl and 100µl) of the crude methanol and aqueous extract of *Desmodium gyrans* (1g/10 ml D.W) and kept for incubation at 37^{o}C for 24h.

After incubation for 24h the results were recorded for the zone of inhibition and Streptomycin (10µg/5ml) was used as positive control for this study.

Preliminary Phytochemical Screening: The methanol and aqueous extract of *Desmodium gyrans* was subjected to various bio chemical tests to detect the presence of different phytoconstituents [10].

Wound Healing Assay:

1. **Acclimatization of Rabbit:** The aqueous extracts of this plant *Desmodium gyrans* was tested for wound healing potential. The wound healing assay was performed in *Orchtolacus sp*. of rabbits. Rabbits were procured from the rabbit farm in Tenkasi and brought about to the laboratory, where the rabbits were reared up in rabbit cage and the temperature was maintained at 27^{o}Cand the relative humidity was maintained in the range of about 70-80 % for the acclimatization purpose. The rabbits were fed up with cabbage and carrot and provided with water.

2. **Wound Excision and Dressing:** From the acclimatized stock seven rabbits were recruited for the experiments. One was kept as a control; three rabbits were treated with the aqueous extract of *Desmodium gyrans* (1g) three rabbits were treated with the standard antibiotic neomycin (0.5g). The wound of about 14mm^2 was made in the thigh region of each test rabbits by using a sterile scissors and the rabbits were numbered.

   The extract was made into a paste in distilled water. In the incised area of three rabbits, 0.5gram of the extract paste was applied gently using a cotton swab. Another three rabbits were treated with the commercial antibiotic neomycin (0.5 g). The treatment was subsequently followed for about 7 days.

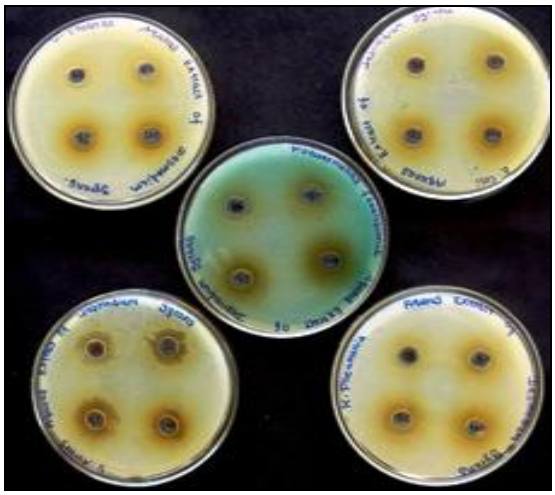
Measurement of Wound healing: The wound healing activity was measured by outlining the wound as accurately as possible on a super imposed sheet of sterile cellophane. This outline was then transferred directly to a sheet of tracing paper and the area within it determined with a radial plantimeter. Subsiquent measurements of the wound were made at regularly spaced intervals as healing progressed [11].

Results and Discussion: Preliminary phytochemical screening of the extracts of *D.gyrans* showed the presence of Alkaloids, Steroids and Saponins, Xanthoproteins and Tannins (**Table 1 and Figure 2**). The methanol extract of *D. gyrans* was found to be more effective against *V. cholerae* and *E. coli* (**Table 2 and Figure 1**).

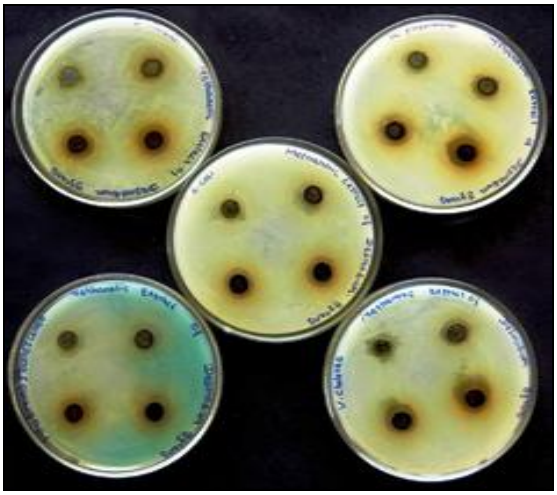
The aqueous extracts of the plant were found to be effective against multidrug resistant *Staphylococcus aureus*. Both methanol and aqueous extracts of the plant *D. gyrans* showed the presence of Alkaloids and steroids, and there phytochemicals were the reason for its antimicrobial activity in the extract. The standard antibiotic Streptomycin showed zone of inhibition of about 22mm.

The wound healing assay showed the aqueous extract of *Desmodium gyrans* have the potential to heal wounds. The wound incised in the rabbit got cured in 8 days. In case of rabbits treated with commercial antibiotic neomycin, complete curing took ten days (**Table 3 and Figure 3**).

The usage of the plant *Desmodium gyrans* by Kani Tribes of Western Ghats to treat wounds had been well proved by the experimental studies with the extract. Hence there is a great scope for developing a wound healing drug from *Desmodium gyrans.*



(A)



(B)

FIG. 1: (A) ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACT OF *DESMODIUM GYRANS* AGAINST PATHOGENIC BACTERIAL SPECIES; (B) ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF *DESMODIUM GYRANS* AGAINST PATHOGENIC BACTERIAL SPECIES

TABLE: 1 PHYTOCHEMICAL SCREENING OF DIFFERENT EXTRACTS OF *DESMODIUM GYRANS*

Name of the Phytoconstituent	Methanol extract	Aqueous extract
Alkaloid	+	+
Steroid	+	+
Flavanoid	-	-
Terpenes	-	-
Saponins	+	+
Tannins	+	+
Xanthoproteins	-	-

+ indicates the presence, - indicates the absence

TABLE 2: SHOWING THE ANTIMICROBIAL ACTIVITY OF DIFFERENT EXTRACTS OF THE PLANT *DESMODIUM GYRANS*

Bacterial culture	Diameter of Zone of inhibition of different concentration of extracts (mm)							
	Methanol				Aqueous			
	25 µl	50 µl	75 µl	100 µl	25 µl	50 µl	75 µl	100µl
Escherichia coli	12.00±0.57	13.00±1.00	13.33±0.66	14.66±0.33	12.33±0.33	13.66±0.88	14.00±0.57	15.00±0.00
Vibrio cholerae	11.33±0.33	12.33±1.20	13.33±1.20	15.00±0.57	12.66±0.33	13.33±0.66	14.00±0.00	14.66±0.33
Pseudomonas fluorescence	11.00±0.57	12.33±0.33	13.00±0.57	14.00±0.57	11.00±0.00	12.66±0.33	12.66±0.66	14.33±0.33
Klebsiella pneumoniae	12.00±0.00	13.00±0.00	13.66±0.33	14.33±0.33	11.33±0.33	12.33±0.33	13.00±0.57	13.66±0.33
Staphylococcus aureus	11.67±0.33	12.66±0.33	13.66±0.33	14.33±0.66	11.00±0.57	12.00±0.00	14.33±0.33	15.33±0.33

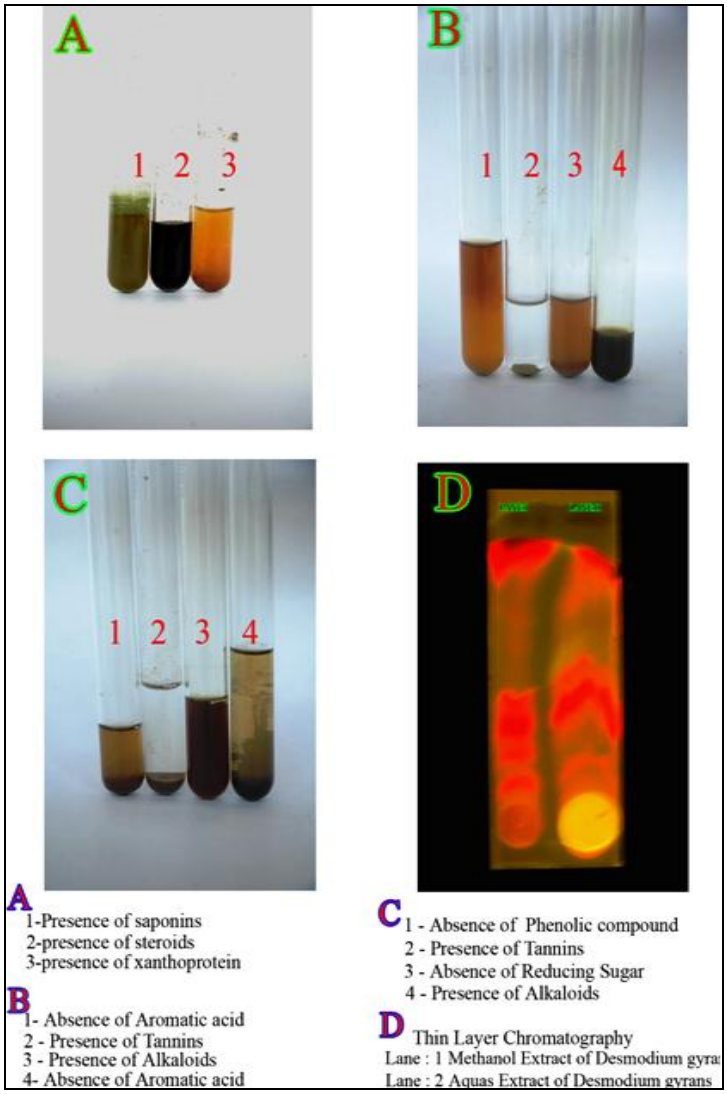


FIG. 2: SHOWING THE PRESENCE OF PHYTOCHEMICALS OF *DESMODIUM GYRANS*

TABLE 3: SHOWING THE WOUND HEALING POTENTIAL OF THE EXTRACTS OF THE PLANT *DESMODIUM GYRANS*

Number of Days	Neomycin (mm^2)	*Desmodium gyrans* (mm^2)
First day	15.33±0.66	15.33±0.66
Fifth day	14.33±0.66	13.00±1.00
Sixth day	13.33±0.66	11.66±1.66
Seventh day	12.66±0.88	10.00±0.57
Eighth day	11.33±1.33	8.33±0.88
Ninth day	10.33±1.45	8.00±0.57
Tenth day	7.66±0.88	5.33±0.33

FIG. 3: WOUND HEALING ACTIVITY

The evaluated wound healing assay clearly indicated the wound healing potential of the extracts of the plant *Desmodium gyrans* much more than the standard antibiotic neomycin. Further research is needed to isolate and characterize the bioactive principle to develop new natural drugs from this plant.

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