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Research Article

Evaluation of Wound Healing Potency of *Dipteracanthus patulus* (*Jacq*) Leaf Extracts Using Chick Embryo Wound Model

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ABSTRACT

INTRODUCTION:
Wound healing is a critical process in tissue repair, and traditional medicinal plants offer promising therapeutic potential. Dipteracanthus patulus (Jacq) is a plant with known medicinal properties, but its wound healing effects is not well studied. This study evaluates the wound healing potency of its leaf extracts using the chick embryo wound model.

MATERIALS AND METHOD:
D.patulus leaf collected was dried at room temperature under shade 15 days and coarsely powdered. The powdered materials were extracted with ethanol and chloroform. The last traces of the solvent were removed and concentrated to dryness under vacuum using a rotary evaporator. The dried extract was weighed and then kept at -4ºC until ready for use. The yield of the extract was 66.42 % (w/w) and 33.68 % (w/w). In each experiment, the extract was diluted with water to desired concentration.

RESULT:
In Chorioallantoic membrane Assay model, the result was tabulated by counting the number of blood vessels in various treatments. Ethanolic and chloroform extract, positive control promoted an increase in number of blood vessels compared to negative control saline. Treated 500µg/ml. Ethanolic and chloroform extract group and 50µg/ml Diclofenac sodium positive control treated group was observed formation of new blood vessels.

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CONCLUSION:

Dipteracanthus patulus both extract stimulated angiogenesis as proved by CAM model showed a faster wound contraction. By comparing both the extracts, ethanolic extract show better activity than the chloroform extract. Dipteracanthus patulus leaf extracts was found to possess angiogenesis.

INTRODUCTION

Medicinal plants are considered as rich resources of ingredients which can be used in drug development and synthesis of medicines. Plants play a vital role in the development of human cultures around the whole world. Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of future studies. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other was used for medicinal purposes [1]. Wound healing is a complex and dynamic process, with the wound environment changing with the shifting health status of an individual. Knowledge of the physiology of the normal wound healing trajectory through the phases of hemostasis, inflammation, granulation and maturation provides a framework for understanding the basic principles of wound healing. Through this understanding, the health care professional can develop the skills required to care for a wound and the patient can be helped with the complex task of tissue repair [2]. Wound healing in mammalian tissues is a natural process of restoring the cellular structures and tissue layers, comprising three distinct phases: the inflammatory phase, the proliferation phase, and the remodeling phase; the main events being chemo-taxis, phagocytosis, neocollagenesis, collagen deposition, angiogenesis and re-epithelialization. These phases and their bio physiological functions must occur at the right time in proper sequence and continue for a specific duration at an optimal intensity. However, angiogenesis and re-epithelialization play a major role in the successful completion of the process. In spite of the fact that many mammalian animal models have been widely used in wound healing studies, several limitations for routine use of such models render them non-viable. Most important being, ethical constraints, cost and time consumption for preliminary screening and quantitative assessments of the crude extracts and phytoconstituents. Also, the molecular mechanism behind wound healing process is difficult to explore due to the lack of proper models. These issues can be overcome by using models like chick embryo, zebra fish, and drosophila, among others which serve as alternatives to the animal models. In the present study chick embryo has been used as a model to screen the wound healing potential of Dipteracanthus patulus leaf extracts [3, 4].

MATERIAL AND METHODS

Collection and authentication of plant and the plant parts:

The plant materials used in this study were leaves of Dipteracanthus patulus (Jacq). (Family - Acanthaceae) is collected from the sengundrapuram, (Virudhunagar Dist, Tamil Nadu, India.). The plant was authenticated by Dr.Stephen, Department of Botany, American College, Madurai, India.

Preparation extracts:

D.patulus leaf collected was dried at room temperature under shade 15 days and coarsely powdered. The powdered materials were extracted with ethanol and chloroform. The last traces of the solvent were removed and concentrated to dryness

under vacuum using a rotary evaporator. The dried extract was weighed and then kept at -4ºC until ready for use. The yield of the extract was 66.42 % (w/w) and 33.68 % (w/w). In each experiment, the extract was diluted with water to desired concentration [5].

D.patulus leaves extracts were subjected to qualitative testing to determine the presence of various plant bioactive ingredients such as alkaloids, flavonoids, tannins, saponins, polysaccharides, glycosides, and other compounds by using the following standard methods [6, 7].

Preliminary Phytochemical Screening

TABLE 1: Tests for Phytochemical Screening

Tests for Phytochemical Screening		
Test	**Procedure**	**Observations (Indicating Positive Test)**
Test for flavonoids		
Shinoda test	Plant extract + few bits of magnesium ribbon + few drops of conc. HCl	The pink scarlet colour appeared
Alkaline reagent test	1 mL Plant extract + 2 mL of 2% NaOH solution	An intense yellow colour
Test for Alkaloids		
Dragendroff's/ Kraut's test	Few ml plant extract + 1-2 mL *Dragendorff's reagents*	A reddish-brown precipitate
Hager's test	Few mL plant extract + 1-2 mL *Hager's reagents*	A creamy white precipitate
Test for Glycosides		
Borntrager's test	2 mL plant extract + 3 mL Chloroform + shaken well + chloroform layer is separated + 10% Ammonia solution	A pink coloured solution
Legal's test	Dissolve 50 gm plant extract in pyridine + Sodium nitroprusside + 10% Sodium hydroxide	A pink coloured solution
Test for Cardiac Glycosides		
Keller-Killani test	1 mL plant extract + 1.5 mL glacial acetic acid + 1 drop of 5% ferric chloride + conc. H_2SO_4	A reddish-brown layer formed
Bromine water test	Plant extract + a few mL of bromine water	A yellow precipitate
Test for Carbohydrates		
Barfoed's test	1 mL plant extract + 1 mL *Barfoed's reagent* + Heated for 2 min.	A red precipitate (monosaccharides)
Molish's test	2 mL plant extract + 2 drops of alcoholic α–naphthol + 1 mL conc. H_2SO_4	A violet ring
Test for Reducing sugars		
Benedict's test	0.5 mL plant extract + 0.5 mL *Benedict's reagent* + Boiled for 2 min.	Green/yellow/red colour

Fehling's test	1 mL each of *Fehling's solution A* & *B* + 1 mL plant extract + boiled in water bath	A red precipitate
Test for Proteins and Amino acids		
Biuret test	2 mL plant extract + 1 drop of 2% copper sulphate sol. + 1 mL of 95% ethanol + KOH pellets	A pink coloured solution
Millon's test	2 mL plant extract + few drops of *Millon's reagent*	A white precipitate
Test for Phenolic compounds		
Iodine test	1 mL plant extract + few drops of dil. Iodine sol.	A transient red colour
Ferric chloride test	Plant extract + few drops 5% ferric chloride sol.	Dark green/bluish black colour
Test for Tannins		
Braymer's test	1 mL plant extract + 3mL distilled water + 3 drops 10% Ferric chloride solution	Blue-green colour
10% NaOH test	0.4 mL plant extract + 4 mL 10% NaOH + shaken well	Formation of emulsion
Test for Phlobatannins		
HCl test	2 mL plant extract + 2 mL 1% HCl (boiled)	A red precipitate
Test for Saponins		
Foam test	0.5 gm plant extract + 2 mL water (vigorously shaken)	Persistent foam for 10 min
$NaHCO_3$ test	Plant extract + few mL sodium bicarbonate solution + distilled water (vigorously shaken)	Stable honeycomb like froth
Test for Terpinoides		
	2 ml chloroform + 5 mL plant extract, (evaporated on water bath) + 3 mL conc. H_2SO_4 (boiled on water bath)	A grey coloured solution
Test for Triterpinoides		
Salkowski's test	plant extract + few drops of conc. H_2SO_4 (Shaken well and allowed to stand)	Golden yellow layer
Test for Diterpenes		
Copper acetate test	Plant extract is dissolved in distilled water + 3-4 drops of copper acetate solution	Emerald green colour
Test for steroids		
Libermann Burchard Test	50 gm plant extract is dissolved in 2 mL acetic anhydride + 1-2 drops of conc. H_2SO_4 (along the side of test tube)	A green colour appearance
Test for Phytosterols		

Hesse's response	5 mL plant extract + 2 mL chloroform + 2mL conc. H_2SO_4	Pink ring / Red colour (in lower chloroform layer)
Sulphur test	Plant extract solution + pinch of sulphur powder	Sulphur sinks to the bottom
Test for Cholesterol		
	2 mL plant extract + 2 mL chloroform + 10 drops of acetic anhydride + 2-3 drops of conc. H_2SO_4	A red-rose colour
Test for Coumarins		
NaOH paper test	0.5 gm moistened extract is taken in test tube, mouth of test tube is covered with 1N NaOH treated filter paper, heated for few min. in water bath	Yellow fluorescence from paper under the UV light
Test for Fixed Oils and Fat		
Spot test/ Stain test	Little quantity of plant extract is pressed in between to filter papers	Oil stain on the paper
Test for Gums and Mucilages		
Alcohol test	Dissolve 100mg plant extract in 10mL distilled water + 25mL absolute alcohol (constant stirring)	White or cloudy precipitate

Embryo collection

Fertilized white shell eggs were purchased from Tamilnadu Veterinary College And Research Institute, Tirunelveli. The outer surface of the embryos were cleaned with 75% ethanol and incubated at 37°C throughout the study.

Preparation of saturated filter disk for wound assay [8]

Whatman No.1 filter paper was Small disks were generated using a standard 5 mm whole puncher, sterilized by auto-claving and stored for further use. The pre-sterilized filter disks were saturated with add D.patulus extracts, from 500 µg/ml, Diclofenac sodium 50 µg/ml and sterile saline were used as positive and negative controls respectively. Embryonated chicken eggs (9 days old) were selected then divided into four groups and a small window, (1cm2) was made in the shell follows

GROUP I : Negative Control saline.

GROUP II : Positive Control Diclofenac sodium (50 µg/ml)

GROUP III : Ethanolic extract of D.patulus. (500 µg/ml)

GROUP IV : Chloroform extract of D.patulus. (500 µg/ml)

Wound assay

Egg shell window was closed and incubated at 370 C for 72 hrs. All dissection tools used in the assay were sterilized using 75% ethanol before use. The embryos were incubated for to allow good maturation of the chorioallantoic membrane.The window was then opened and the growth of new capillary blood vessels were observed and finally compared with the positive and negative control.

RESULTS AND DISCUSSION

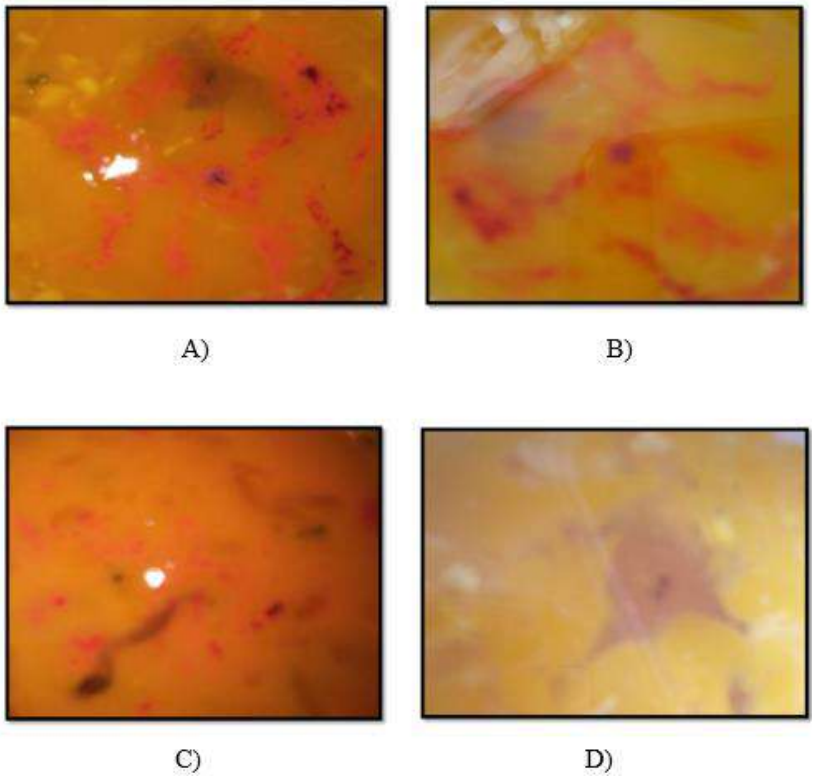
Preliminary phytochemical screening of the ethanolic and chloroform extract of D.patulus (Jacq).

Table 2: Preliminary phytochemical screening

S. No	Constituents	Ethanol extract of D.patulus	chloroform extract of D.patulus
1	Flavonoids	+	+
2	Alkaloids	+	+
3	Glycosides	+	-
4	Cardiac Glycosides	-	-
5	Carbohydrates	-	-
6	Reducing sugars	-	-
7	Proteins and Amino acids	-	-
8	Phenolic compounds	+	+
9	Tannins	+	+
10	Phlobatannins	-	-
11	Saponins	+	-
12	Terpinoides	-	+
13	Tri-terpinoides	+	+
14	Diterpenes	-	-
15	steroids	-	+
16	Phytosterols	+	-
17	Cholesterol	-	-
18	Coumarins	+	-
19	Fixed Oils and Fat	-	-
20	Gums and Mucilages	-	-

Chorioallantoic membrane assay model (CAM)

The result was tabulated by counting the number of blood vessels in various treatments. Ethanolic and chloroform extract, positive control promoted an increase in number of blood vessels compared to negative control saline. Treated 500 µg/ml Ethanolic and chloroform extract group and 50 µg/ml Diclofenac sodium positive control treated group was observed formation of new blood vessels.(Fig A, B & C) The negative control does not show the formation of blood vessels. (Fig D)



A) Treated 500 µg/ml of Ethanolic extract of D.patulus.
B) Treated 500 µg/ml of Chloroform extract of D.patulus.
C) Positive Control Diclofenac sodium-50 µg/ml.
D) Negative Control saline.

In Chorioallantoic membrane Assay model, Angiogenesis is vital in normal processes of the development of blood vessel of embryo, formation of corpus luteum and wound healing. Angiogenesis during wound repair helps the two fold function of providing the nutrients required by the healing tissue and contributing to structural repair through the formation of granulation tissue. The preliminary phytochemical analysis of the both extract revealed the presence of several components. Mainly Flavonoids have proved anti-inflammatory activities. Inflammation being the first phase of wound repair mechanism has to be controlled within a day or two when the actual healing process begins.

CONCLUSION:

The study demonstrated that Dipteracanthus patulus (Jacq) leaf extracts exhibit significant wound healing potential in the chick embryo model. The extracts were found to accelerate the wound closure process, reduce inflammation, and promote tissue regeneration, suggesting the presence of bio active compounds with therapeutic properties. These findings support the traditional use of Dipteracanthus patulus in wound management and suggest its potential for development into a natural topical agent for wound care. Further studies, including clinical trials, are recommended to validate these results and explore the underlying mechanisms of action.

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