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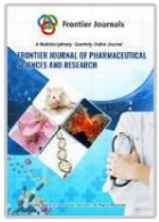

Original Research Paper

CLERODENDRON SPLENDEN -A POTENT AGENT FOR WOUND HEALING

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ABSTRACT

Clerodendron splendens is a well-known plant in folklore medicine for treating various skin infections and in wound healing. Based on its traditional use, this plant was evaluated for its antimicrobial, wound healing and antioxidant properties. Juice expressed from the fresh leaves and ethanol, acetone and chloroform extracts of the dried leaves, stem and root, all exhibited various degrees of antimicrobial activity against the test microorganisms with minimum inhibitory concentrations ranging from 0.15 to 7mg/ml. The time-kill kinetics studies conducted suggested microbistatic activity of the various extracts against these test microorganisms employed.

The extracts were topically applied twelve hourly to excision wounds created on the back of rats. Two more groups serving as the controls were also treated with 1%^{w}/w Silver sulphurdiazine cream and the vehicle (normal saline + tween80). The extracts showed faster rate of wound contraction in the first 5 days than the controls. Higher breaking strengths were recorded, especially in the 300mg/ml treated group, suggesting the possibility of higher collagen content of the repaired tissues than in the control groups. Thus, this study provides a scientific rationale for the traditional use of this plant in the management of wounds and other infectious conditions.

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1. INTRODUCTION

Herbal medical practice has been the main form of treatment especially among indigenous populations around the world particularly in developing countries, and the World Health Organization has also estimated that 80% of people worldwide rely on herbal medicines for some aspect of their primary healthcare. Currently even the industrialized nations are developing interest in the use of herbs. In the United States, increasing public dissatisfaction with conventional medicine and its increased cost, combined with an interest in returning to natural or organic remedies with the mistaken impression that natural products are safe, has led to an increase in the use of herbal medicines.[1]

Wound healing is the body's natural process of regenerating dermal and epidermal tissues in a wounded area. When an individual is wounded, a set of complex biochemical events takes place. These events may be categorized into the following stages: the inflammatory, proliferative, and remodeling phases. Compounds which are known to promote these stages can be therapeutically employed to accelerate the wound-healing process.[2]

Topical antimicrobial agents, including both antiseptics (Iodine, Ethanol) and antibiotics, are currently helpful in reducing the risk of infection. Molecular iodine and silver are potent broad-spectrum antimicrobial agents that promote healing in microbially compromised wounds .[3]

Triphala which is a traditional ayurvedic herbal formulation consisting of three medicinal plants, *Terminalia chebula*, *Terminalia bellirica*, and *Phyllanthus emblica*, have been reported to possess numerous biological and pharmacological activities when applied to wounds.[3]

Antimicrobial agents from plant sources are gaining renewed interest and usage because they have a lower propensity to induce bacterial resistance than antibiotics and highly capable of enhancing wound healing. Therefore, this study seeks to investigate the wound healing properties of *Clerodendron splendens,* which may justify the traditional use of this plant. This investigation was done by Determining the wound healing properties of *Clerodendron splendens* in vivo.

It is hoped that results of this tests will aid in the proper use of this plant and probably promote further investigations leading to the detection and isolation of compound(s) that will be invaluable in wound healing.[4]

2.PLANT PROFILE

Clerodendron splendens known as the flaming glory bower belongs to the family Verbenaceae. The leaves and flowers have been reported to contain carbohydrates, glycosides, unsaturated sterols, triterpinoids, flavonoids and volatile oils. The major sterol detected was 24ß-etylcholesta-5, 22E, 25(27)-trien-3ß-ol (also known as 25(27)-dehydroporiferasterol). Trace of its 22-dihydro derivative, clerosterol (also known as 25(27)-dehydroclinonasterol) was also found. The dominant n-alkane present was C29 (n-nonacosane) and the dominant n-alkanol was C28 (n-octacosanol).[4]Traditionally *Clerodendron splendens* used as remedy in burns, in various skin diseases. The plant parts also used as Anti-pyretic and anti-inflammatory.

3. MATERIAL AND METHODS

The leaves of the *Clerodendron splendens* were collected from Southerner hill area in the Salem in Nov 2018. The plant was authenticated by government botanist. A herbarium specimen (number FP 08/08/1) is being kept.

The fresh leaves of *Clerodendron splendens* were thoroughly washed with tap water, to get rid of dirt and soil particles. The washed leaves were weighed (112.8g) and by means of porcelain mortar and pestle triturated into a fine pulp. The pulp was then strained with white calico and 27.4ml of fresh leaves extract of Density 1.067g/ml was obtained. This was kept in an amber coloured bottle for antimicrobial testing. Ten milliliter of this extract was dried at 40^0C and the dried weight in the extract determined to be 1.9g.[5]

Three hundred (300) grams of powdered leaves was weighed and 1.5L of 70% Ethanol added. The mixture was shaken and left to stand at room temperature with intermittent shaking for 72 hours. The mixture was subsequently filtered through Whatman filter papers (number 10) and poured into porcelain crucibles. The filtrate was evaporated to dryness using a Hot Air Oven at 37°C to a constant mass. The extract obtained was labelled and kept in a desiccator for use. A similar procedure was used to obtain extracts from the stem and roots. Extraction was also done with Acetone and Chloroform using the same procedure and the yields are as recorded in.[5]

4.EXPERIMENTAL SETUP

Animals

Albino wistar rats, weighing 90–140 g obtained from the CPCSEA approved animal house. They were housed in metal cages and maintained on Normal Commercial Pellet Diet. The animals were given water (ad libitum) and maintained under laboratory conditions (room temperature, 24-28°C, relative humidity of 60-70% and 12-hour light–dark cycle).[6]

Excision wound model

The animals were anaesthetized with diethyl ether prior to and during creation of the wounds. The dorsal fur of the animals was shaved to a circular diameter of about 45mm by means of razor blades and the anticipated area of the wound to be created was outlined on the shaved skin of the animals with Ammonium oxalate crystal violet paint (with a cork of diameter 25mm, hence an approximate area of 490mm^2). The area was cleaned with ethanol before the rats were inflicted with excision wounds. A full thickness of the skin with circular diameter of 25 mm and an approximate area of 490mm^2 was created along the markings using toothed forceps, surgical blades and pointed scissors. The entire wound was left open and the animals divided into seven groups of seven animals each.

Group 1 animals were topically treated with 1%^{w}/w Silver Sulphadiazine Ointment.

Group 2 were treated with vehicle (Tween 80 and Normal saline) only, as the control.

Group 3 Animals were treated with the fresh leaves extract of concentration 190mg/ml.

Groups 4, 5, 6 and 7 were topically treated with 3, 30,300 and 1000mg/ml concentrations of *Clerodendron splendens* ethanol extracts respectively.[8]

Wound treatment was commenced on the 2nd day of wound creation. The drugs were then topically applied to the wounds 12 hourly for 19 days. During the course of treatment, scaled photographs of the wound areas were taken (by means of a high-resolution Digital Camera, *Nikon* CoolPix L11 L10) alongside a millimeter scale every 48h starting from the 1st day of wound treatment. The wound areas were then determined with the aid of a computer programme (Sigma Pro Scan 5).[8]

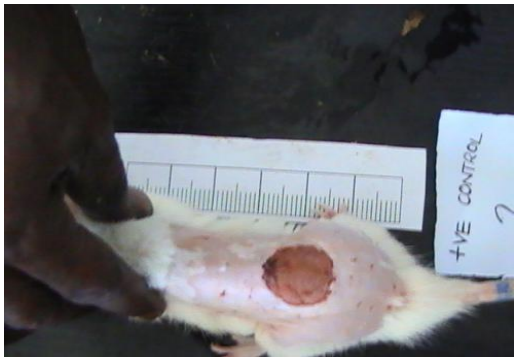

Fig. 1 Measurement of wound area

Statistical Analysis

Each result is presented as mean ± SEM (standard error of mean).

Statistical analysis of the data were performed with one-way analysis of variance (ANOVA) followed by the Tukey post hoc comparison test. Dose dependent effects were evaluated using correlation coefficient. Significant differences were set at p- values lower than 0.05.[4]

5.RESULTS AND DISCUSSION

The effect of *C. splendens* extracts on wounds inflicted on rats was monitored by measuring the size of the wound with time and these are recorded in the table no. 1- 5.[6]

Rate of wound contraction

The fresh leaves extracts showed more percentage of wound healing than the control group during the first few days (Days3-5). By the 7th day the control had contracted to the same extent as the fresh leaves. Complete healing was recorded at about day 15 for almost all the groups.

The degree of wound contraction was calculated from the formula below:

Degree of wound contraction =

[1-(wound area on corresponding day/wound area on day zero)]× 100%.[11]

TABLE 1 Effect of *C. splendens* fresh leaves extract on wound size with time

	Time (days)									
Rats	**1**	**3**	**5**	**7**	**9**	**11**	**13**	**15**	**17**	**19**
1	218.28	94.09	109.16	75.73	28.67	11.48	3.2	1.58	0.56	0.02
2	227.72	109.49	104.9	76.46	24.47	13.85	5.18	3.38	1.35	1.42
3	200.5	107.17	127.99	65.68	35.87	20.19	14.64	11.09	2.97	2.3
4	230.72	127.37	161.5	78.84	47.99	33.55	18.41	15.35	3.12	10.2
5	197.87	124.96	162.32	83.39	29.81	20.73	14.18	5.63	4.77	0.45
6	207.76	100.69	145.53	80.89	36.75	19.45	8.83	4.37	1.69	1.44

TABLE 2 Effect of 300mg/ml extract on wound size with time

	Time (days)									
Rats	**1**	**3**	**5**	**7**	**9**	**11**	**13**	**15**	**17**	**19**
1	185.87	120.7	105.4	54.9	23.04	15.82	8.82	6.98	4.59	0.87
2	155.31	91.45	152.74	44.06	13.82	10.45	8	1.16	0.42	0.03
3	186.62	130.53	150.4	58.91	37.84	25.34	10.23	8.66	7.62	4.07
4	209.18	128.21	120.36	60.76	29.1	19.16	10.69	11.06	5.17	2.44
5	187.07	132.48	140.85	65.41	31.02	13.6	7.42	0.77	0.68	1.68
6	274.98	145.22	141.55	78.92	41.15	29.38	22.26	5.02	7.42	1.86

TABLE 3 Effect of 600mg/ml extract on wound size with time

	Time (days)									
Rats	1	3	5	7	9	11	13	15	17	19
1	153.44	127.48	114.08	92.65	50.77	35.21	8.82	2.65	0.34	0.65
2	240.77	176.5	239.63	60.08	30.6	17.12	8	1.26	0.15	0.42
3	161.93	142.34	171.45	77.51	30.44	11.33	10.23	2.75	0.8	0.17
4	186.17	154.48	150.54	54.79	25.6	17.5	10.69	5.55	2.58	0.83
5	229.56	154.92	77.95	79.01	45.19	24.46	18.54	8.69	6.81	0.72
6	181.67	133.33	160.79	71.95	42.7	29.76	24.58	8.8	8.46	7.13

TABLE 4 Effect of vehicle on wound size with time

	Time (days)									
Rats	**1**	**3**	**5**	**7**	**9**	**11**	**13**	**15**	**17**	**19**
1	200.35	98.43	112.66	67.28	43.61	19.56	12.58	4.28	2.4	1.8
2	213.94	133.23	156.19	77.78	44.65	23.29	21.48	9.79	3.16	1.06
3	139.03	111.05	128.71	67.52	46.22	25.72	14.31	7.98	5.24	2.01
4	159.87	109.78	127.43	69.05	33.08	22.82	9.11	6.38	1.95	0.35
5	168.66	91.07	98.59	45.68	24.84	6.78	4.98	2.41	0.52	0.94
6	181.99	117.42	118.08	57.98	33.2	20.12	13.95	3.79	2.27	1.61

TABLE 5 Effect of standard drug on wound size with time

	Time (days)									
Rats	**1**	**3**	**5**	**7**	**9**	**11**	**13**	**15**	**17**	**19**
1	201.85	166.92	178.72	74.48	32.94	22.21	13.13	14.47	6.12	2
2	314.33	209	173.58	62.88	28.71	14.37	5.54	1.25	0.56	0.29
3	195.28	139.58	122.3	46.39	25.6	14.21	5.4	2.51	0.75	0.26
4	132.78	112.77	74.73	37.02	20	8.39	2.54	1.7	0.39	0.07
5	172.69	144.2	133.72	45.89	24.49	17.1	2.5	2.2	0.00	0.00
6	186.28	136.83	163.57	48.86	28.81	16.41	7.51	4.94	1.15	0.28

TABLE 6 Rate of wound contraction

Rats	Fresh Leaves	300mg/ml	600mg/ml	Vehicle	Standard
1	314.14	451.14	501.14	351.14	551.14
2	281.14	476.14	621.14	431.14	421.14
3	397.00	471.14	481.14	441.14	546.14
4	466.14	576.14	401.14	401.14	461.14
5	421.14	461.14	341.14	459.6	411.14
6	501.14	581.14	441.14	541.14	461.14
7	393.81	331.14	464.49	591.14	391.14

Mean tensile strength determination

An increase in concentration of the extract showed an increase in the breaking strength of the wound. The 300mg/ml group exhibited a slightly higher breaking strength than the standard drug.

TABLE 7 Effect of topical treatment on tensile strength of wounds

Animal Groups	Fresh leaves	300mg/ml 478.3	600mg/ml 464.5	Vehicle 459.5	Standard 463.3
Tensile strength(g)	396.3 ±34.88	±31.96 300mg/ml	±39.13 600mg/ml	±36.64 Vehicle	±24.05 Standard

Values are mean ± S.E. (standard error), $n = 7$ mice (in each group).

6. CONCLUSION

In conclusion, this study confirms the promising wound healing activity, of *Clerodendron splendens.* Results indicated that the leaves extract stimulated wound contraction, increased the breaking strength of the repaired tissue and possibly reduced the incidence of wound infection. Wound healing involves a chain of well-orchestrated biochemical and cellular events leading to the growth and regeneration of wounded tissue in a specific manner.

The breaking strength of the formed tissue, which may be due to the increase in collagen concentration, increased with increase in concentration of the extract. The 300mg/ml extract had breaking strength higher than the standard as well as the vehicle treated wounds.

The extract of the leaves, used in the wound healing study also had antioxidant activity and very likely protected the cells against oxidative stress. The IC50 of the extracts was estimated to be 5.61±0.040 mg/ml. That of L- ascorbic under the same conditions was 0.4873 ± 0.0122 mg/ml. Thus, the antioxidant activity of the ascorbic acid is about 11.5 times that of the extract. This DPPH radical scavenging activity of the extract suggests that external application of the extracts on the wounds entrapped free radicals liberated from the wound surrounding cells which have the ability to protect cells from microbes.

The preliminary phytochemical analysis of the plant showed the presence of tannins, phytosterols, terpenoids, flavonoids and traces of alkaloids.

Some plant extracts also promote wound healing due to their antioxidant effect by altering the redox environment of the wound and hence reducing the concentration of oxygen radicals, thereby reducing their damage to cell membranes. This antioxidant effect could be due to the phenolic constituents, like flavonoids. Flavonoids are also known to reduce lipid peroxidation, prevent the onset of cell necrosis and also improve vascularity, hence enhancing the supply of blood to the area. Any drug that inhibits lipid peroxidation like flavonoids is also believed to increase the viability of collagen fibres and promote DNA synthesis these factors all enhance wound healing.

7.CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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