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Research Article

Antioxidant and wound healing studies on different extracts of *Stereospermum colais* leaf

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ABSTRACT

Stereospermum colais (Bignoniaceae) leaves were extracted successively with n-hexane, chloroform, ethyl acetate and ethanol by continuous hot percolation process and aqueous extract by cold maceration process. All the extracts were subjected to anti oxidant activity. Chloroform extract showed maximum antioxidant activity with an IC_{50} value of 36µg/ml. Chloroform, ethanol and aqueous extracts were taken for the screening of wound healing activity by excision model. Chloroform and ethanol extract showed significant activity when compared with control and standard. Percentage of wound contraction on 15^{th} day was found to be 96.34 ± 1.64, 95.15 ± 1.54 and 16.6 ± 0.33, 17 ± 0.25. The observations confirmed that chloroform and ethanol extracts showed significant wound healing activity.

Keywords: *Stereospermum colais;* anti oxidant; wound healing activity.

1. INTRODUCTION

Stereospermum colais is a large straight stemmed deciduous tree 18-30 m in height and 2.8 m in girth found throughout in moist regions of India up to an altitude of about 1200 m, chiefly in deciduous forests. It is known as Yellow snake tree in English, Padri in Hindi and Pathiri in Tamil (Parrota, 2001). The leaves are useful in otalgia, odantalgia, rheumatalgia, malarial fever and wounds. The juice of the leaves mixed with lime juice is used in maniacal cases. Decoction of the leaves is used for treating chronic dyspepsia and also has anti pyretic properties. The root of this plant is used as an ingredient of the reputed Dasamula an Ayurvedic formulation. The roots are bitter, astringent, acrid, anodyne, appetiser, constipating, diuretic, Lithotropic, expectorant, cardio tonic, aphrodisiac, anti-inflammatory, anti bacterial, febrifuge and tonic, anti emetic, anti pyretic. The decoction of root is used in asthma and cough (Warrier, 2002).

Wounds are inescapable events in life. Wound may arise due to physical, chemical or microbial agents. The four phases of normal wound healing include Haemostasis, Inflammation, Proliferation and Remodeling. Wound healing processes are well organized biochemical and cellular events leading to the growth and regeneration of wounded tissue in a special manner. Healing of wounds involves the activity of an intricate net work of blood cells, cytokines and growth factors which ultimately leads to the restoration to normal condition of the injured skin or tissue (Clark, 1991). Antioxidants counter the excess proteases and reactive oxygen species (ROS) often formed by neutrophil accumulation in the wounded area and protect protease inhibitors from oxidative damage. Fibroblasts and other cells may be killed by excess ROS and skin lipids will be made less flexible, so antioxidant substances will reduce the possibility of these adverse events occurring. Because of these several factors, overall antioxidant effects appear to be important in the successful treatment of wounds (Houghton *et al*., 2005). The present study attempts to bring out the hitherto unearthed antioxidant and wound healing potentials of the plant.

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2. MATERIALS AND METHODS

2.1. Plant material

Fresh leaves of *Stereospermum colais* were collected from Javadhi hills in the month of May 2008 and were authenticated by Botanist Dr. P. Jayaraman, PARC, Chennai (voucher specimen no. PARC/2008/200) and then shade dried and powdered.

2.2. Successive solvent extraction

The powdered leaves of the plant material were extracted successively with n-hexane, chloroform, ethyl acetate, ethanol in a Soxhlet's apparatus for 20 hrs and finally macerated with water for 48 hrs. Each extract was concentrated by distillation of the solvent and then evaporated to dryness on water bath. The % yield of each extract was 4.11 %w/w (n-hexane), 5.07%w/w

(chloroform), 5.21%w/w (ethyl acetate), 9%w/w (ethanol), 26.48 %w/w (aqueous).

2.3. Anti oxidant activity by DPPH free radical scavenging method

Oxidation is one of the most important processes, which produce free radicals in food, chemicals and even in living systems. Free radicals have an important role in the processes of food spoilage, chemical materials degradation and also contribute to more human disorders in human beings. Active oxygen and in particular, free radicals are considered to induce oxidative damage in bio molecules and to play an important role in aging, cardiovascular diseases, cancer and inflammatory diseases (Gupta *et al.,* 2008). Anti oxidant activity of *Stereospermum colais* were studied by DPPH free radical scavenging method.

The free radical scavenging activity of the extracts of *Stereospermum colais* was measured using the modified method of Bios (1985). One ml of each extract and the standard in various concentrations (10, 20, 40, 60, 80 & 100 µg/ml) was added to one ml of 0.1 mM solution of Diphenyl- 2 –picryl hydrazyl (DPPH) in methanol. The mixture was vortexed and then incubated in a dark chamber for 30 minutes after which the absorbance was measured at 517 nm, using a spectrophotometer (UV-1650 pc, Shimadzu) against a DPPH control containing only 1 ml of methanol in place of the extract. DPPH is a purple coloured stable free radical. When reduced it becomes the yellow colored Diphenyl picryl hydrazine. Ascorbic acid was used as a standard (James *et al*., 2008 and Ramnik *et al*., 2008). Percent inhibition was calculated using the following expression

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

C_{50} value was calculated. IC_{50} values denotes the concentration of test, which is required to scavenge 50 % of DPPH free radicals

2.4. Wound Healing Activity

Excision wound model was used to evaluate the wound healing activity.

2.4.1. Experimental animals

Healthy Wistar albino rats of either sex and of approximately the same age, weighing between 150-250 g were used for the study. They were individually housed, maintained in clean polypropylene cages containing paddy husk bedding and fed with standard Diet and water *ad libitum*.

2.4.2. Experimental procedure

Excision wound was inflicted on the rats according to methods described by Morton and Malone (1972) under light ether anesthesia. The dorsal fur of the animals was shaved with an electric clipper. Full skin thickness was excised from the marked area to get a wound measuring about 300 mm^2 by using toothed forceps and pointed scissors (Nayak *et al.,* 2007).

The animals were divided into 5 groups of 6 animals each. Group I- Control group with wound and treated with Ointment base; Group II- Standard group with wound and treated with Ont. Framycetin 1 % w/w; Group III- Test group with wound and treated with Chloroform extract of 10 % w/w *Stereospermum colais* ointment; Group IV - Test group with wound and treated with Ethanolic extract of 10 % w/w *Stereospermum colais* ointment; Group V- Test group with wound and treated with Aqueous extract of 10 % w/w *Stereospermum colais* ointment. All the formulations were applied once a day till the complete epithelialization starting from the day of wounding.

Wound healing property was evaluated by wound contraction percentage and wound closure time. The wound surface area was measured immediately by placing a transparent paper over the wound and tracing it out, area of this impression was calculated using the graph sheet. The same procedure is employed every third day until healing was complete (Srinivas *et al.,* 2008 and Manjunatha *et al.,* 2006).

The parameters observed in the study were as follows:

Epithelialization Period: It was monitored by noting the number of days required for the scar to fall off from the wound surface without leaving a raw wound behind.

Wound Contraction: It was noted by following the progressive changes in wound area planimetrically, excluding the day of the wounding. The evaluated surface area was then employed to calculate the percentage of wound contraction, taking the initial size of the wound, 300 mm^2 as 100 % by using the following equation:

$$\% \text{ of wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

Histopathological study: For histopathological examination tissues are collected from the completely healed wound when the scar is removed and fixed in 10 % formalin. After the usual processing, 5µm-thick sections were cut and stained with haematoxylin and eosin. Then the tissues are observed under microscope to study different histopathological phenomenon (Mukherjee, 2002).

2.4.3. Statistical Analysis

The data were subjected to One way ANOVA followed by Bonferroni test and the values of $P< 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSIONS

DPPH is relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents as a result of which

Table 1: Effect of successive extracts of *Stereospermum colais* on DPPH free radical scavenging method

Drug	Percentage Inhibition						IC_{50} (µg/ml)
	10 µg/ml	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	
Ascorbic acid	27.72 ± 0.23	49.84 ± 0.88	69.78± 0.31	85.98 ± 0.43	94.08± 0.13	98.44± 0.31	22
n-Hexane	5.91 ± 0.12	8.72 ± 0.17	19.62 ± 0.17	43.30 ± 0.06	52.02± 0.14	58.87± 0.20	76
Chloroform	19.00 ± 0.10	37.07 ± 0.56	52.33± 0.62	72.58 ± 0.54	78.19± 0.21	86.91± 0.08	36
Ethyl acetate	9.03 ± 0.06	18.06 ± 0.23	31.77 ± 0.42	50.77 ± 0.20	57.63± 0.12	66.35± 0.13	58
Ethanol	12.14 ± 0.24	28.66 ± 0.31	48.90 ± 0.39	61.68 ± 0.26	72.89± 0.21	76.63± 0.20	42
Aqueous	10.90 ± 0.24	22.42 ± 0.17	47.66 ± 0.14	54.20 ± 0.08	66.35± 0.27	71.96± 0.39	48

Table 2: Effect of *Stereospermum colais* on wound contraction and epithelialization period

Group	Percentage of wound contraction (original wound area 300 mm^2)					Period of epithelialization in days
	Day 3	Day 6	Day 9	Day 12	Day 15	
Control	17.80 ± 1.4	30.92 ± 2.2	49.99 ± 2.4	60.64 ± 3.6	76.23 ± 1.2	20 ± 0.86
Standard	23.75 ± 2.3[a*]	54.69 ± 1.6[a*]	67.72 ± 2.0[a*]	83.39 ± 1.3[a*]	100 ± 0.0 [a*]	15 ± 0.06 [a*]
Chloroform	21.70 ± 0.2 [a*]	54.12 ± 1.9 [a*]	66.21 ± 1.7 [a*]	82.03 ± 1.8 [a*]	96.34 ± 1.6 [a*]	16.6 ± 0.33[a*]
Ethanol	21.47 ± 1.8 [a*]	48.33 ± 3.0 [a*]	64.76 ± 2.3 [a*]	79.39 ± 2.4 [a*]	95.15 ± 1.5 [a*]	17 ± 0.25 [a*]
Aqueous	20.00 ±1.1[a*]	46.52 ± 1.8 [a*]	63.21 ± 1.7 [a*]	72.94 ± 2.1 [a*]	90.56 ± 2.4 [a*b*]	18 ± 0.36[a*b*]
One-way ANOVA F	1.972	18.70	11.82	14.73	34.52	27.10

Values are expressed as mean ± SE; n = 6 in each group; [a*] P <0.05 Vs control, [a*b*] P <0.05 Vs std

the electrons become paired off forming the corresponding hydrazine. The solution therefore loses colour stoichometrically depending on the number of electrons taken up (Mangathayaru *et al.,* 2007).

Successive extracts were tested for the antioxidant activity in various concentrations ranging from 10 – 100 µg/ml by DPPH method. The results show the effect of these extracts to scavenge free radicals. It was observed that free radicals were scavenged by the extracts in a concentration dependent manner. The maximum percentage inhibition of DPPH for n-hexane, chloroform, ethylacetate, ethanol, and aqueous extracts was 58.87 %, 86.91 %, 66.35 %, 76.63 %and 71.96 % respectively at 100 µg concentration. Standard drug ascorbic acid showed 98.84 % inhibition of the DPPH radical at 100 µg concentration. IC_{50} value of standard, n-hexane, chloroform, ethylacetate, ethanol, and aqueous extracts are given in Table 1.

In wound healing activity, significant promotion of wound healing activity was observed in both chloroform and ethanol extracts. The mean percentage contraction of wound area was calculated on 3, 6, 9, 12 and 15 post wounding days in all the groups. On 3rd day there is no significant difference between the groups. A very rapid contraction of the wound was shown in standard, chloroform and ethanol treated groups between 6 and 9 days post surgery (p<0.05) when compared with remaining groups. On day 12 chloroform and ethanol extract shows significant similar to that of the standard group treated with Framycetin 1 % w/w. On the day 15, mean wound area of control, chloroform, ethanol and aqueous groups were 76.23 ± 1.27, 96.34 ± 1.64, 95.15 ± 1.54, 90.56 ± 2.40 and respectively where as standard group shows 100 ± 0, indicating that chloroform and ethanol extract group shows significant wound healing property comparable to that of standard group and highly significant with control group. Aqueous group is less significant when compared with standard (Table 2).

The time required for complete epithelialization of the excision wound is an important parameter to assess the wound healing process. Complete epithelialization was noticed on the 15th day and also found that the mean time taken for complete epithelialization in chloroform, ethanol treated group was less than the animals treated with aqueous, control and more or less similar to the values of standard drug treated group. The period of complete epithelialization was delayed by 2 days.

3.1. Histopathological evaluation

The photographs of histopathological observations are presented in Fig 1. The wound sections from the skin of the normal animal shows adnexia and sub epithelial collagen where as animal in control group shows regenerating epithelium which is less significant when compared to other groups. Standard shows complete healing with dense collagenation. The sections of the tissues of 10% w/w chloroform shows significant collagenation and 10% w/w ethanol extract shows significant collagenation with dilated vessels, when compared with standard and 10 % w/w aqueous extract shows exudates and it is less significant. This shows that treatment of rat wound with chloroform and ethanol extract ointment has led to reduction in scar formation, enhanced fibroblast proliferation, angioge-

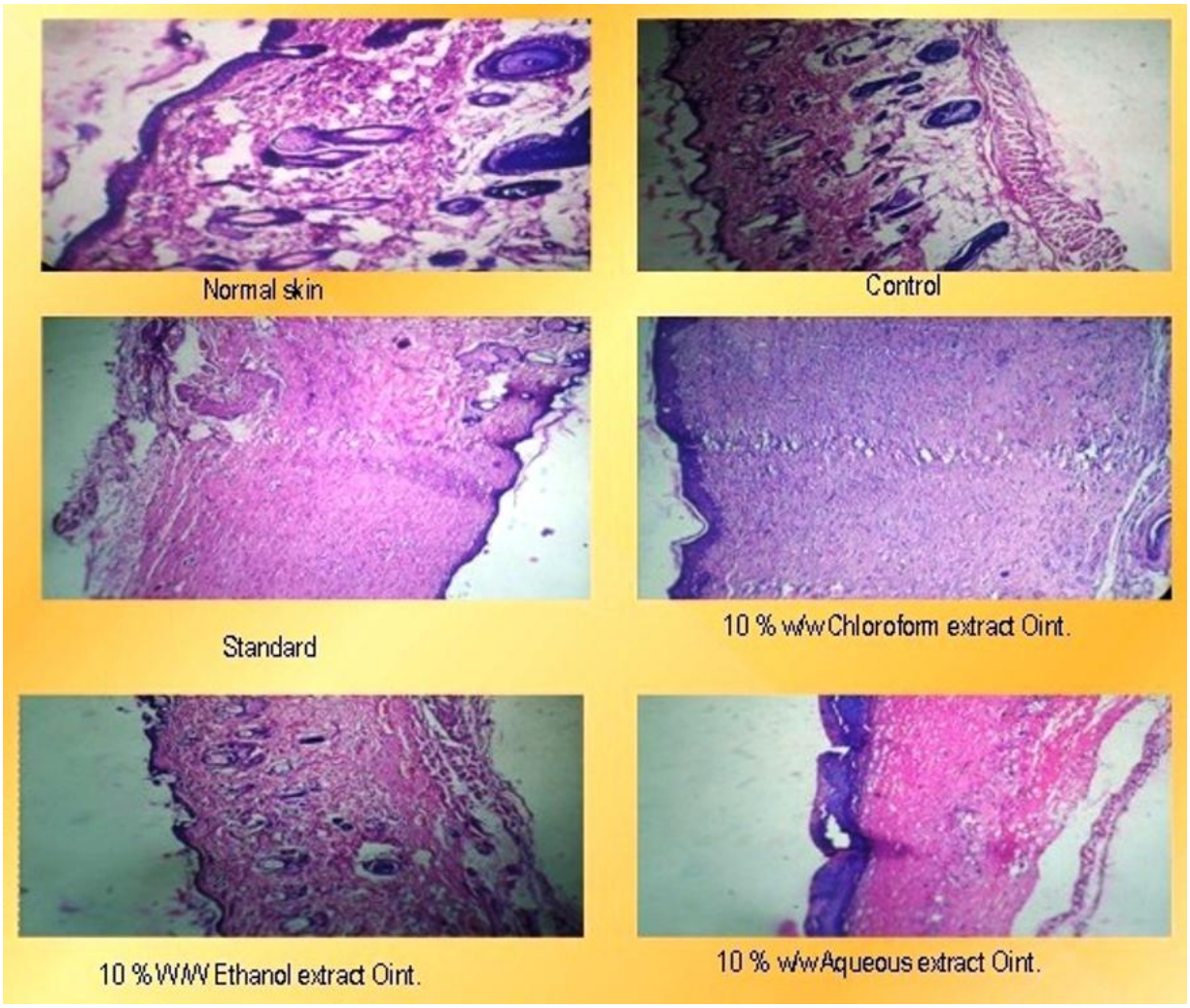

Figure 1: Effect of various extracts of *Stereospermum colais* on wound healing

nesis, keratinazation and epithelisation which confirms the healing action.

4. CONCLUSIONS

Chloroform and ethanol extract shows significant wound healing property comparable to that of standard group. The external application of these extracts on the wound prevented the microbes to invade through the wound, resulting in protection of wound against the infections of the various microorganisms. At the same time, external application of the extracts entrapped the free radicals liberated from the wound surrounding cells, which are having inherent machinery to protect the cells from the microbes. Therefore, it can be assumed that the Synergistic effect of both anti microbial and anti oxidant activity accelerated the wound-healing process.

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