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Wound healing activity studies of *Wrightia arborea Phytosome* in rats

S. Lakshmi Devi [1*] and Madhu.C. Divakar[2]

1. Department of Pharmacognosy, Sri Ramakrishna College of Pharmacy, SRIPMS, Coimbatore, Tamil Nadu -, 641 044, India.
2. Crescent College of Pharmaceutical Sciences, Payangadi R.S. Post, Kannur, Kerala-670 358, India.

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Abstract

Plan: *The study was aimed to evaluate the wound healing activity of 70% ethanolic extract (WAET) of the leaves of Wrightia arborea (Apocynaceae) and the phytosome (WAP) prepared out from WAET using incision and excision wound models on wistar albino rats.*

Methodology*: Wound contraction and epithelization period were assessed in excision wound model whereas wound tensile strength was determined in the case of incision wound model.*

Outcome: *The Wrightia arborea phytosome (WAP 4%) exhibited significant wound healing potential when compared to WAET and standard 0.2% Nitrofurazone Ointment.*

Keywords*: Wrightia arborea leaves, Incision and Excision wounds, Tensile strength, Epithelization period, WAP, WAET*

1. Introduction

Wrightia arborea (Dennst.) Mabb (Apocynaceae) leaves is a traditional herbal remedy has a long history of use, distributed in plains and slopes of the Shevaroy Hills which is commonly known as kudagupalai. It is well known for its medicinal effects and is being traditionally used for the treatment of various ailments such as to relieve tooth ache when chewed, believed to be used as an antidiarrhoeal, bark is useful in menstrual and renal complaints[1].

The stem bark and root bark are believed to be useful in snake bite and scorpion-stings[2]. Wound healing potential of *Wrightia arborea* leaves has not been experimentally evaluated so far, hence the present investigation was undertaken to study the wound healing property of methanolic and 70% ethanolic extracts of leaves of *Wrightia arborea* on excision and incision wound models[3].

Experimental

2.1. Animals

Albino rats of *Wistar* strain of either sex weighing about 150-200 g were used. Pregnant animals were excluded. They were housed in standard cages at room temperature (25±2°C) and provided with food and water *ad libitum*.

For correspondence: ldevijp@yahoo.co.in
Contact: mobile*: 0091 9047034536*
researcherid.com/rid/C-3285-2012

The animals were deprived of food for 24 h before experimentation, but had free access to drinking water. The study was conducted after obtaining institutional Animals ethical committee clearance bearing the number CPCSEA/265/09-11.

2.2. Plant material

Wrightia arborea leaves were collected from Shevaroy Hills at Salem district and it was identified and authenticated (BSI/SC/5/23/08-09/TECH) taxonomically by Professor P. Satyanarayana, Deputy Director, Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore.

2.3. Preparation of Extracts

The leaves of *Wrightia arborea* were collected and dried in shade. The leaves were then powdered and extracted with 70% ethanol for a period of 36 hr in a Soxhlet extractor[4-6]. The extract was then concentrated, dried. The percentage yield obtained was 14.5 % w/w. The ethanolic extract was designated as WAET (Wrightia *arborea* ethanolic extract).

2.4. Preliminary Phytochemical studies

Preliminary Phytochemical studies indicated the presence of tannins, flavonoides, triterpenoides, alkaloids, and steroids in the extract.

2.4.1. Preparation of Wrightia arborea phytosome

Wrightia arborea 70% ethanolic leaf extract (WAET) was made into a Phytosome (WAP 2% and WAP 4%) by reacting 3-2 moles of phosphatidyl choline with one part of WAET with an aprotic solvent such as acetone, in 1:2 ratio. The liposome drug complex thus formed were dried and formulated as Phytosome Emulsion with adequate quantity of distilled water [9-13]. The physical properties of the Wrightia phytosome obtained are tabulated in Table.1 and Table .2

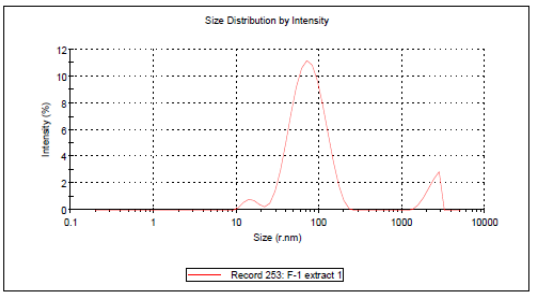
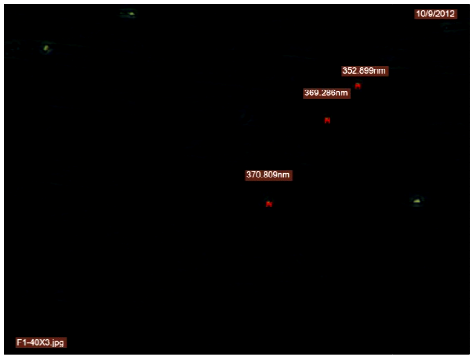


Fig -1 : Particle size distribition intensity of *Wrightia arborea* phytosome

Table-1: Particle Size Distribution data by intensity of *Wrightia arborea* phytosome

Peak	*Size r.nm*	*Size nm*	*% intensity*	*Width r.nm*	*Width nm*
1	79.44	158.88	88.6	35.06	70.12
2	2345	4684.00	8.3	398.0	796.0
3	15.46	30.92	3.1	3.129	6.258



Wrightia arborea Phytosome WAP (40 x)

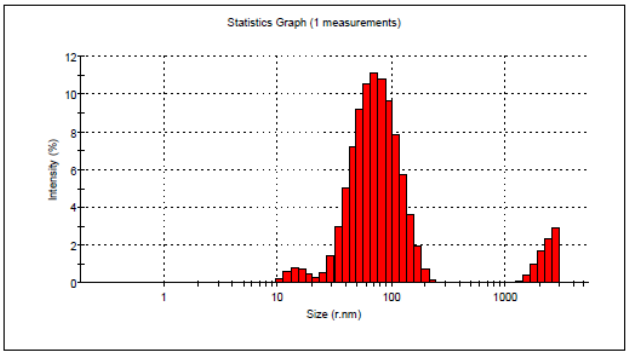


Fig - 2: Particle Size statistics intensity of *Wrightia arborea* phytosome (WAP)

Table-2. Particle Size statistics data by intensity of *Wrightia arborea* phytosome

Size r.nm	Size (nm) Mean ± S.D	Mean Intensity %	Zeta potential	Poly Dispersity Index (PDI)
10.52	21.45 ± 0.81	0.2		
12.18	25.47 ± 0.15	0.6		
14.11	28.57 ± 0.13	0.8		
16.34	32.68 ± 0.16	0.7		
18.92	37.36 ± 0.37	0.5		
21.91	43.74 ± 0.40	0.3		
25.37	50.16 ± 0.62	0.5		
29.39	58.77 ± 0.34	1.4		
34.03	68.88 ± 06	3.0		
39.41	78.72 ± 0.55	5.0		
45.64	91.44 ± 0.16	7.2		
52.85	105.54 ± 0.32	9.2		
61.21	122.24 ± 0.61	10.6		
70.89	141.40 ± 0.17	11.1	27.1±0.33	0.336±0.15
82.09	164.31 ± 0.82	10.8		
95.07	190.88 ± 0.19	9.6		
110.01	220.47 ± 0.32	7.8		
127.5	255.21 ± 0.62	5.7		
147.7	295.55 ± 0.45	3.7		
171.0	342.31 ± 0.44	1.9		
198.0	396.87 ± 0.33	0.7		
229.3	458.12 ± 0.16	0.1		
1335	2670.71 ± 0.33	0.1		
1545	3090.42 ± 0.51	0.4		
1790	3580.51 ± 0.17	0.9		
2073	4146.61 ± 0.42	1.6		
2400	4800.77 ± 0.45	2.4		
2780	5560.64 ± 0.44	2.9		

2.5. Acute Dermal toxicity studies

A safe dermal dose of the extract was determined by Acute Dermal toxic class method of organization of Economic Co-Operation and Development (OECD) as per 434 Guidelines. Female albino rats of *Wistar* strain weighing between 150 – 200g were used for the study. They were caged individually and the room temperature was maintained at 22^0C ($\pm 3^0$) and the relative humidity at 30-70%. The lightings were artificial, the sequence being 12 hours light and 12 hours dark cycle. The animals were fed with conventional laboratory diets and drinking water *ad libitum.* A total of ten animals consisting of five animals in each group were used. Approximately 24 h before the study, fur was removed from the dorsal area of the trunk of the animals by shaving. Care must be taken to avoid abrading the skin, which could alter its permeability. 10% of the body surface area was cleared for the application of the test substance.

The test substance (200mg/kg) of *Wrightia* arborea methanolic extract Phytosome (AMP) and *Wrightia arborea* 70% ethanolic extract Phytosome (A70EP) was applied as a thin and uniform film over the dorsal area of the trunk which was approximately 10% of the total body surface area. The substance was held in contact with skin by porous gauze dressing for 24 hours exposure period. Animals were observed immediately after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4h, and daily thereafter for a period of 14 days[7]. LD_{50} was done as per OECD guidelines-434 for fixing the dose for biological evaluation. The LD_{50} of the extracts as per OECD guidelines falls under class II with no signs and symptoms of acute toxicity at a dose of 200 mg/ kg b.w. The doses selected were 10 and 20 mg per application for WAET

2.6. Wound healing studies

Wound healing property of 70% ethanolic extract(WAET) and WAP were studied on excision and incision wounds using Wistar strain rats of either sex. Percentage of wound contraction and period of epithelization were measured in excision wounds, whereas tensile strength of healed wounds was measured in incision wounds[8]. Animals were divided into 6 groups of 6 each. Group I was served as the control (treated with plain phytosome without drug extract), and Group II & III were treated with WAP 2% and WAP 4% respectively. Group IV was served as standard and treated with 0.2% w/w Nitrofurazone ointment for comparing the wound healing potential of the extract. All treatments were made by topical application of WAP (0.5g) twice daily.

2.7..Excision wound model [14,15]

Hairs were removed from the dorsal plane of thoracolumbar region of the animals using a depilator. A round seal of $500mm^2$ were impressed on dorsal plane were impressed on dorsal plane of thoracolumbar region, 5 cm away from the ears on the depilated part of skin and extending to a depth of 0.2 cm from the demarcated area was excised to get a wound, under mild anesthesia. Drugs were topically applied twice daily till complete epithelization, starting from day of excision. Area of wounds were measured by tracing the wounds on to tracing paper, on day of wounding (0 day) and subsequently on 4^{th}, 8^{th}, 12^{th} and 20^{th} days of post wounding. Number of days required for falling of scab without any residual raw wound, gave the period of epithelization[16,17].

Percentage wound contraction were calculated on 4th The percentage of wound reduction was calculated using the formula 8th,12th,16th and 20th days of post wounding (Table -1). The percentage of wound reduction was calculated using the formula.

$$\% \text{ wound reduction} = \frac{\text{Wound area day 0 - Wound area on respective day}}{\text{Wound area day 0}} \times 100$$

2.7.1. Incision wound model

Hairs were removed from back portion using a depilator. Each animal was secured to operation table in its natural position under light ether anaesthesia, straight full thickness paravertebral incision of 6 cm length was made including the cutaneous muscle of the depilated back of each rat, with the help of sterilized sharp blade.

The incisions were sutured using silk threads as interruped sutures with the help of straight round bodied needle[18]. The test extracts (WAP 2% and WAP 4%), standard drug and control were applied twice daily for 10 days. On the 8th post wounding day, sutures were removed and the breaking strength was determined on 10th post wounding day by 'continuous constant water flour technique' of Lee[19].

2.7.2. Measurement of wound Breaking Strength of incised wound

Measurement of wound breaking strength was performed following the method of Lee[21] with certain modifications. A board was placed on a raised platform on the table, on which the anesthetized animal was made to lie on its abdomen. Two clamps were clamped on either sides of healed wound at a distance of 0.5 cm. The left clamp was fastened tightly to a stand by means of a thread. The right clamp was connected to a leak proof polyethylene container through a pulley, by means of a thread.

A reservoir containing water was placed at a suitable height and connected to a polyethylene bag by means of a rubber tube. The position of the board was adjusted so that, the polyethylene bag was hanging freely. Water was added to polyethylene bag rapidly at a constant rate from the reservoir until the wound opened Amount of water in polyethylene bag was measured in ml and was considered as tensile strength of the would (Table-2).

2.8. Statistical Analysis

All results were expressed as mean ± SEM. Significance of difference between control and drug treated groups were determined by one - way analysis of variance (ANOVA) followed by Dunnett's test. P value <0.01 was considered statistically significant.

3. Results

LD_{50} studies showed that the animals were safe up to a maximum dose of 200 mg/ kg body weight. There were no changes in normal behavior pattern and no signs and symptoms of toxicity and mortality were observed. *Wrightia arborea* Phytosome (WAP4%) showed significant wound healing activity compared to WAET and WAP 2% (Tables 3and 4).

In excision wounds, the wound area when measured after a period of 20 days a significant decrease in the wound area was observed in WAP 4%, WAET 4% and standard (Nitrofurazone) treated groups as 90.40%; 65.63% and 99.37% respectively on 20^{th} day (Table-3).

Significant wound contraction was also observed on 20^{th} day for all treated groups ($P<0.01$), in comparison with the control group. Time for complete epithelization was significantly short in animals, treated with extract of WAP 4%, WAET 4% and standard treated groups ($P<0.01$).

In incision wound model, significant increase in tensile strength of healed wounds were observed in WAP 2% (301.5±4.8), 4% (360.5±3.9[a]); WAET 2% (296.2±2.5); 4% (302.2±4.2) treated groups and standard (587.6±5.2) were compared with the control (286±3.4). The results were significant at $P<0.01$ (Table-4).

4. Discussion

Wound healing involves different phases such as contraction, epithelization, granulation, collagenation and so on[20]. It normally involves an initial inflammatory phase followed by fibroblast proliferation, formation of collegen fibres and shrinking, occurring concurrently but independent of one another [21]. Several plants like *Leucas hirta*[20], *Ocimum sanctum*[22], *Ocimum gratissimum*[23], *Curcain*[24], *Centella asiatica*[25], *Chandanadi yamak*[26], *Moringa oleifera* and *Aegle murmelos*[27]possess wound healing potential. Phytoconstituents such as flavonoids, tannins and triterpenoids are reported to be responsible for wound healing property[20-27]. Presence of triterpenoides and flavonoides in extracts of leaves of *Wrightia arborea* may be responsible for its wound healing activity.

Wound healing effect is also attributed to free radical scavenging activity of flavonoids[27]. Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing onset of cell necrosis, but also by improving vascularity.

Lipid peroxidation is an important process in several types of injuries like burns, infected wound and skin ulcers. Hence any drug that inhibits lipid peroxidation is believed to increase the strength of collagen fibers, by increasing circulation or by preventing cell damage or by promoting DNA synthesis. Flavonoides and triterpenoides are known to promote wound healing process mainly by their astringent and antimicrobial property[23, 30].

It is concluded that methanolic extract showed better results of this study, which support the traditional use of *Wrightia arborea* for treatment of wounds. Further research is warranted to isolate and identify the bioactive constituents responsible for this activity.

Table 3: Wound Area Measurement Data- Excision Wound Model

Group	*Treatment*	*Wound Area Measurement (mm^2) and Percentage of Wound Contraction*					
		0 day	*4th day*	*8th day*	*12th day*	*16th day*	*20th day*
I	control	506.5±4.61 (0%)	455.4±5.71 (10.08%)	402.3±7.1 (20.57%)	325.6±4.2 (35.71%)	281.2±3.3 (44.48%)	235.5±4.0 (53.50%)
II	WAP 2%	505.5±4.9^{ns} (0%)	444.3±3.4^{ns} (12.10%)	387.5±2.4^{ns} (23.34%)	270.4±3.3^{a} (46.50%)	155.6±4.9^{a} (69.21%)	125.8±1.9^{a} (75.11%)
III	WAP 4%	498.4±3.4^{ns} (0%)	420.5±2.4^{a} (15.63%)	340.3±3.5^{a} (31.72%)	238.5±2.6^{a} (52.14%)	86.4±1.7^{a} (82.66%)	47.8±1.2^{a} (90.40%)
IV	WAET 2%	502.5±2.2^{ns} (0%)	448.4±1.2^{ns} (10.76%)	395.5±4.2^{ns} (21.29%)	320.2±5.1^{ns} (36.27%)	265.7±3.5^{b} (47.12%)	210.6±4.2^{a} (58.08%)
V	WAET 4%	496.5±4.4^{ns} (0%)	440.7±2.2^{b} (11.23%)	385.4 ±1.2^{b} (22.38%)	252.6±2.4^{a} (49.12%)	215.6±5.4^{a} (56.57%)	170.6±1.6^{a} (65.63%)
VI	Standard 0.2% w/w	510.5±5.1^{ns} (0%)	352.6±4.9^{a} (30.93%)	246.3±5.0^{a} (51.75%)	93.2±3.4^{a} (81.75%)	11.1±1.4^{a} (97.80%)	3.2±0.1^{a} (99.37%)

WAP: *Wrightia arborea* Phytosome
WAET: *Wrightia aborea* 70% ethanolic Extract
Standard: Nitrofurazone ointment
Control: Simple Ointment
All values are expressed as Mean ± SEM (n=6 animals in each group). $^{a}P<0.01$ Vs Control; $^{b}P<0.05$ Vs Control; $^{ns}P>0.05$ Vs Control. One way ANOVA followed by Dunnett's test.

Table: 4. Effect of *Wrightia arborea* leaf extract on Incision Wound Model

Group	*Drug treatment*	*Tensile strength (Mean±SD)*
1.	Control	286±3.4
2.	WAP 2%	301±4.8^{b}
3.	WAP 4%	360.5±3.9^{a}
4.	WAET 2%	296.2±2.5
5.	WAET 4%	302.2±4.2^{b}
6.	Standard Nitrofurazone	587.6±5.2^{a}

n=6 $^{a}P<0.01$ Vs Control; $^{b}P<0.05$ Vs Control. One way ANOVA followed by Dunnett's test

WAP: *Wrightia arborea* Phytosome
WAET: *Wrightia aborea* 70% ethanolic Extract
Standard: Nitrofurazone ointment
Control: Simple Ointment

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References

1. Chatterjee A, Pakrashi SC. The Treatis of Indian Medicinal Plants, National Institute of Science Communication and Information Resources, New Delhi, **2003**, 125-127.
2. Kirtikar KR, Basu BD. Indian Medicinal Plants 2nd ed., Vol.4: **1995**, 611-612.
3. Madhavan V, Yadav DK, Murali A, Yogandrasimha SN. Wound healing activity of aqueous and alcohol extracts of leaves of *Colebrookea oppositifolia* Smith, *Indian Drugs*, **2009**, 46(3), 209-213.
4. Kokate CK. Practical Pharmacology, 4th ed., Vallab Prakashan, Delhi, **1996**, 146.
5. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments, Nirali Prakashan, Pune, **2000**, 149.
6. Kokate CK, Prohit AP, Gokhale SB. Pharmacognosy, 18th ed., Nirali Prakashan, Pune, **2002**, 97.
7. OECD Guideline for testing of chemicals, Proposal for a new Draft Guideline 434: Acute Dermal toxicity. **2004**; 1-13.
8. Patil SM, Sapkale GN, Patil MB, Sompure CK. Wound Healing Activity of Roots of *Asparagus Racemosus* Wild, *Indian Drug,* **2010**, 47 (4), 61-65.
9. Marena C, Lampertico M. Preliminary clinical development of Silipide: a new complex of silybin in toxic liver disorders, *Planta Med.* **1991**, (57),A 124-125.
10. Jiang YN, Yu ZP, Yan ZM, Chen JM., Studies on preparation of herba epimedii flavonoid phytosomes and their pharmaceutics. *Zhongguo Zhong Yao Za Zhi.*. **2001**, 2,105-108.
11. Maiti K, Mukherjee K, Gantait A, Saha BP, Mukherjee PK. Enhanced therapeutic potential of Naringenin phospholipid complex in rats*, J Pharm Pharmacol*. **2006**, 58, (9), 1227-1233.
12. Bagyalakshmi J, Vamsikrishna RP, Manavalan R, Ravi TK, MannaPK. Formulation Development and *In Vitro* and *In Vivo* evaluation of Membrane-Moderated Transdermal systems of Ampicillin sodium in Ethanol: pH 4.7 Buffer solvent system**,** AAPS *Pharm Sci Tech*. **2007**, 8(1), 1-11.
13. The United states of Pharmacopeia-27, **2004**; 1357.
14. Patil KS. Wound healing activity of the seed Kernels of *Caesalpinia crista* Linn., *J Nat Rem*, **2005**, 5 (1), 26-30.
15. Divakar MC, Lakshmi Devi S, Pazhanisamy SK, Rao SB. Studies on Wound healing property of *Polyscias scutellaria* leaf saponins*. Indian J Nat Prod,* **2001**, 17 (2), 37-42.
16. Madhavan V, Tomar AS, Murli A, Yoganarasimhan SN, Wound healing and antipyretic activity of stem bark of *Wrightia tinctoria* , *J Trio Med Plants*, **2006**, 7(1), 69.
17. Vidya SM, Krishna V, Manjunatha BK, Singh SD, Mankani KL. Evaluation of wound healing activity of roots and leaf exracts of *Clerodendrum serratum* L., *Indian Drugs*, **2005**, 42 (9), 609.
18. Ghosh T, Dash GK. Bose A, Panda BR. Wound healing properties of *Argemone mexocana, Indian J Nat Prod,* **2004**, 20 (4) 3.
19. Lee KH. Studies on the mechanism of action of salicylate 11. Retardation of wound healing by aspirin. *J Pharmacol Sci,* **1968**, 1042-1044.
20. Vidyas SM, Krishna V, Manjunath BK, Singh SD, Kankani KL. Wound healing Activity of *Leucuas hirta*, *Indian J Pharm Sci,* **2006**, 380.
21. Ganachari MS, Kumar S, Patel A. Wound healing activity of *Saussurea lappa* roots, *Indian drugs*, **2005**, 42 (5), 295.
22. Shetty S, Udupa S, Udupa L and Somyasi N. Wound healing activity of *Ocimum sanctum Linn* with supportive role of antioxidant enzymes *Indian J Physiol Pharmacol*, **2006**, 50, 163.
23. Lara OO, Fakoya FA, Agbani EO and Lwalewa EO. Vascular permeability-increase effect of the leaf essential oil of *Ocimum gratissimum* Linn. as a mechanism for its wound healing property. *Afr J Trad Compl Alt Med*, **2005** (2), 253.
24. Nath LK, Dutta SK. Wound healing response of the proteolytic Enzyme Curcain. *Indian J Pharmacol*, **1992**, (24), 114-115.
25. Sunilkumar, Prasameshwaraiah S, Shivakumar HG. Evaluation of topical formulations of aqueous extract of *Centella asiatica* on open wounds in rats. *Indian J Exp Biol*, **1998**, (36).569-572.
26. Fulzele SV, Satturwar PM, Joshi SB, Dorle AK. Wound healing activity of Chandanadi Yamak in Rats. *Indian J. Pharm. Sci.,* **2003** (3) 301-304.
27. Udupa SL, Udupa AL, Kulkarni DR. Studies on the anti-inflammatory and wound healing properties of *Moringa oleifera* and *Aegle marmelos*. *Fitoterapia*, **1994**, (2), 119-123.
28. Joshni SD, Aravind MB, Ashok, Veerapur VP, Shastry CS. Wound healing activity of *Dodonaea viscosa* leaves*, Indian Drugs*, **2003**, 9, 549.