

# Studies on Phytochemistry and Wound Healing Activity of Leaf Extract Ointment of *Clerodendrum infortunatum* in Canines

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## ABSTRACT

The present study was carried out to evaluate phytochemistry and the efficacy of ointments containing leaf extract of *Clerodendrum infortunatum* for treating wounds in canines in clinical cases. The 50% ethanolic and methanolic extract of the plant was subjected to preliminary phytochemistry and GC-MS analysis to assess the presence of active phyto-constituents. The wound healing was assessed with percent wound contraction on 0, 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup>, 16<sup>th</sup>, and 20<sup>th</sup> day and epithelization period in 30 canines presented with a history of wound at VCC, private clinics, and dog shelters run by NGOs. The 10% w/w ointment of both the extract was used and it was compared with commercially available Povidone Iodine ointment USP 5% (Betadine®). The preliminary phytochemical analysis of the extracts showed presence of glycosides, alkaloids, carbohydrates, amino acids, phytosterol, phenolic compounds and flavonoids. Whereas, GC-MS analysis revealed the presence of 21 compounds in ethanolic extract and 24 compounds in methanolic extract. The wound contraction in the methanolic extract ointment treated group was most significant and also demonstrated an epithelization period of  $17.5 \pm 1.210$  days as compared to  $22.8 \pm 0.525$  and  $20.9 \pm 0.669$  days in Povidone Iodine ointment and ethanolic extract ointment treated groups, respectively. The finding of the study suggests that, the ointment made from the methanolic extract of *Clerodendrum infortunatum* leaves possesses promising wound healing activity.

**Key words:** *Clerodendrum infortunatum*, Extract ointment, GS-MS analysis, Phytochemical analysis, Wound healing activity.

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## INTRODUCTION

The skin serves a variety of purposes and is the body's biggest organ which anatomically and functionally comprised with epidermis or outer layer and dermis or middle layer (Paul and Sharma, 2004). A variety of skin-related disorders are frequently treated with preparations made from traditional medicinal plants. Herbal wound treatment includes debridement, cleaning and the creation of an environment that promotes the healing process naturally and have a strong potential for treating minor wounds effectively (Maver *et al.*, 2015). Following skin injuries, a complicated chain of processes known as wound healing takes place that involves the extracellular matrix, local cells, and the vasculature. Cytokines and growth factors are necessary for the beginning of the healing process as well as to direct and maintain healing over time. Even while it is believed that, the wound of dogs and cats restores in comparable stages and with comparable healing mediators, a lot of this material has not been thoroughly studied in these species (Lux, 2022).

The plant *Clerodendrum infortunatum* Linn belong to family Verbenaceae and have been used in Indian folk medicine in the treatment of bronchitis, asthma, fever, burning sensation, disease of blood, inflammation, diabetes,

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wound etc (Das *et al.*, 2010). Despite of many folklore claims, there is a dearth of data about clinical studies on wound healing activity of *C. infortunatum*. Therefore, the present study was aimed to assess the phytochemistry of extracts of *C. infortunatum* and wound healing activity of ointments prepared from the extract in clinical cases in dogs.

## MATERIALS AND METHODS

### Plant Material, Extraction and Preparation of Ointments

The leaves of *Clerodendrum infortunatum* were procured from the outskirts of Nagpur city. The plant was duly authenticated by the expert botanists of the University Department of Botany; RTM Nagpur University (Voucher specimen no. 084, 28/11/2022). The clean, dried leaves were subjected to 50% ethanolic and methanolic extracts in Soxhlet's apparatus after defatting with n-Hexane (Sheel *et al.*, 2014).

The ointments of extracts were prepared with simple ointment base as per the method described by Panigrahi *et al.* (2018). The melted uniform mixture of 90% soft white paraffin, 5% lanolin and 5% hard paraffin was allowed to cool at room temperature to obtain a semisolid ointment base. The 10% extract ointment (w/w) was prepared by uniformly mixing 10 gm of extract in 90 gm of ointment base. The tight capped bottles containing 25 gm of ointment were packed, labelled and stored in a cool and dark place for further use in animals.

### Qualitative Phytochemistry & GC-MS Analysis

To determine the presence or absence of various phytoconstituents, the extracts were subjected to a preliminary qualitative phytochemical examination using the techniques outlined by Ghosh *et al.* (2015). The GC-MS analysis of extracts was carried out using SHIMADZU GCMS-QP2020 MS detector. Compounds were identified in terms of RT values and mass spectra with those obtained from the NIST search library. The obtained compounds were searched for detailed information.

### Wound Healing Activity

The wound healing activity of the extract ointments was evaluated in total 30 dogs presented to Veterinary Clinical Complex (VCC) of Nagpur Veterinary College (11 cases), Private clinic (4 cases) and Dog shelter run by NGOs (15 Cases). The cases were divided in to three groups comprising 10 dogs each and received conventional treatment that consists of oral administration of Amoxicillin @ 20 mg/kg b.wt. for 3 to 5 days and Meloxicam @ 0.2 mg/kg b.wt. for 3 days. The three treatment groups (I, II and III) were treated topically with commercial Povidone Iodine ointment (Betadine®), ethanolic extract ointment and methanolic extract ointment, respectively.

The initial and subsequent average wound area (in sq. mm) was measured by using Digital Vernier Calliper and also by tracing the wound manually on transparent sheet and photographed on 0, 4<sup>th</sup>, 8<sup>th</sup>, 14<sup>th</sup>, 16<sup>th</sup> and 20<sup>th</sup> day and if required until complete healing (Ferrari *et al.*, 2015). The wound area contraction in percent was calculated using the following formula:

$$\% \text{ Wound contraction} = \frac{(\text{Initial wound area}) - (\text{Specific day area})}{(\text{Initial wound area})} \times 100$$

### Statistical Analysis

The data was analyzed through one-way and two-way analysis of variance and represented as Mean  $\pm$  SEs. The data was analyzed using WASP software and Microsoft excel, and P value less than 5% was considered significant.

## RESULTS AND DISCUSSION

### Qualitative Phytochemistry & GC-MS Analysis

The preliminary phytochemical analysis of both 50% ethanolic and 50% methanolic extracts of leaves of *C. infortunatum* revealed presence of alkaloids, carbohydrates, glycosides, amino acids, phytosterol, phenolic compounds, tannins, resins and flavonoids. The presence of alkaloids, carbohydrates, flavonoids, tannins, phytosterols and phenolic compounds was reported by Balogun *et al.* (2009) and Limsay *et al.* (2018) in ethanolic extract of *C. infortunatum*. Similarly, Ahmed *et al.* (2007) and Sheel *et al.* (2014) reported presence of alkaloids, steroids, flavonoids, carbohydrates and tannins in methanolic extract of *C. infortunatum*.

The GC-MS analysis of ethanolic and methanolic extracts revealed presence of 21 and 24 bioactive phytoconstituents, respectively, and their details are given in Table 1 and 2.

Many phytoconstituents found in GC-MS analysis of extracts of *C. infortunatum* in present study were earlier reported for various pharmacological activities such as; 1,2 cyclopentanedione possesses potent antioxidant activity (Kim *et al.*, 2002), 2-methoxy-4-vinylphenol reduces inflammation by inhibiting NF-B and MAPK (Mitogen Activated Protein Kinase) activation in wound healing (Jeong *et al.*, 2011), furans are attributed for anticancer, analgesic, anti-inflammatory, antimicrobial, anti-histaminic etc. and are involved in many pharmaceutical products with wide spectrum of biological activity (Banerjee *et al.*, (2015), 5-hydroxy-methyl-ester is reported to have antimicrobial, anti-inflammatory and antioxidant activity against Gram-negative bacterial strains found in burn wound (Ahmed and Othman, 2013) and 1,3,5-Triazine-2,4,6-triamine showed antifungal and anti-bacterial activity (Makwana *et al.*, 2016), Heneicosane possesses significant antibacterial and antifungal activity (Vanitha *et al.*, 2020).

### Wound Healing Activity

The initial and subsequent wound area was traced manually (Square mm) and the data obtained was further used to calculate the percent wound contraction as per the formula and depicted in Table 3. The most significant wound contraction was demonstrated by methanolic extract ointment and the results were superior to Povidone Iodine ointment. The healing rate in methanolic extract ointment



**Table 1:** Details of the active principles found in GC-MS analysis of the 50% ethanolic extract of *C. infortunatum* leaves

Sr. No.	Compound identified	Formula	Peak Height	RT (Min.)	Percent Area
1	Octadecanoic acid, 2-propenyl ester	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub>	13464856	29.257	2.10%
2	Neophytadiene	C <sub>20</sub> H <sub>38</sub>	8696911	24.462	2.54%
3	Heneicosane	C <sub>21</sub> H <sub>44</sub>	4185153	13.574	0.61%
4	Hexadecane	C <sub>16</sub> H <sub>34</sub>	6391972	11.434	0.98%
5	Heptadecane	C <sub>17</sub> H <sub>36</sub>	3164715	10.214	0.46%
6	Hexadecane, 1-iodo-	C <sub>16</sub> H <sub>33</sub> I	10599148	9.339	2.25%
7	-Nitrobenzyl iodide	263.03	18281161	8.740	2.90%
8	Cyclohexen-1-one, 3-methyl	C <sub>6</sub> H <sub>7</sub> N	4865070	8.366	0.60%
9	2-Methoxy-4-vinylphenol	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	10130515	7.388	1.02%
10	Fenobucarb	C <sub>12</sub> H <sub>17</sub> NO <sub>2</sub>	4768198	7.304	0.64%
11	6-Oxo-Heptanoic acid	C <sub>7</sub> H <sub>12</sub> O <sub>3</sub>	11113219	7.008	2.78%
12	2,6,10-Trimethyltridecane	C <sub>15</sub> H <sub>32</sub>	7462774	6.846	1.09%
13	1,2,3-Propanetriol, 1-acetate	C <sub>5</sub> H <sub>10</sub> O <sub>4</sub>	13854782	6.716	2.86%
14	Benzofuran, 2,3-dihydro-	C <sub>8</sub> H <sub>8</sub> O	11424856	6.534	2.03%
15	Catechol	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	22303625	6.338	3.64%
16	Naphthalene	C <sub>10</sub> H <sub>8</sub>	5272060	6.183	1.34%
17	Undecane	C <sub>11</sub> H <sub>24</sub>	12164722	5.621	0.81%
18	1,3,5-Triazine-2,4,6-triamine	C <sub>3</sub> H <sub>12</sub> N <sub>6</sub>	9031121	5.401	0.80%
19	4-Propyl[1,3]oxathiane 3-oxide	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub> S	3387886	5.354	0.47%
20	3-(Furan-2-yl)propan-1-aminexx	C <sub>7</sub> H <sub>11</sub> NO	2320357	5.101	0.24%
21	1,2 cyclopentanedione	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	21238944	4.110	1.97%

**Table 2:** Details of the active principles found in GC-MS analysis of the 50% methanolic extract of *C. infortunatum* leaves

Sr. No.	Compound identified	Formula	Peak Height	RT (Min.)	% Area
1	Bromomethyl methyl ether	C <sub>2</sub> H <sub>5</sub> BrO	2235418	73.267	1.35%
2	Methyl propyl ether	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	2048686	73.188	0.98%
3	Propanoic acid	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	2339212	69.746	0.39%
4	2-hydroxy- methylester	C <sub>6</sub> H <sub>12</sub> O <sub>3</sub>	2339212	69.746	0.39%
4	Hydrazine	H <sub>4</sub> N <sub>2</sub>	2371007	68.405	0.59%
5	Propanoic acid, 2-hydroxy-, ethyl ester	C <sub>5</sub> H <sub>10</sub> O <sub>3</sub>	1765918	61.765	0.90%
6	L-Lactic acid	C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>	1481031	26.026	0.57%
7	1-Methoxy-3-(2-hydroxyethyl)nonane	C <sub>12</sub> H <sub>26</sub> O <sub>2</sub>	3114918	16.898	1.17%
8	Heneicosane	C <sub>21</sub> H <sub>44</sub>	3150998	11.251	0.70%
9	Diethyl Phthalate	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	4265544	8.564	0.68%
10	Pentadecane	C <sub>15</sub> H <sub>32</sub>	4251468	7.184	0.47%
11	Eicosane	C <sub>20</sub> H <sub>42</sub>	2583774	7.030	0.34%
12	Cycloheptasiloxane, tetradecamethyl-	C <sub>14</sub> H <sub>42</sub> O <sub>7</sub> Si <sub>7</sub>	5729649	6.606	1.09%
13	Tetradecane	C <sub>14</sub> H <sub>30</sub>	3380053	6.09	0.28%
14	4-Hydroxy-3-methylacetophenone	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	4082000	5.453	0.41%
15	Isosorbide Dinitrate	C <sub>6</sub> H <sub>8</sub> N <sub>2</sub> O <sub>8</sub>	2597400	5.152	0.28%
16	1,2,3-Propanetriol, 1-acetate	C <sub>5</sub> H <sub>10</sub> O <sub>4</sub>	6067203	4.931	0.70%
17	Benzofuran	C <sub>8</sub> H <sub>6</sub> O	4009137	4.785	0.38%
18	Catechol	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	7776531	4.630	0.87%

19	Naphthalene	C <sub>10</sub> H <sub>8</sub>	2485652	4.486	0.24%
20	Glyceraldehyde	C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>	2103074	4.315	0.50%
21	Undecane	C <sub>11</sub> H <sub>24</sub>	11025587	4.064	0.66%
22	Hexadecane	C <sub>16</sub> H <sub>34</sub>	7468309	83.948	3.37%
23	4H-Pyran-4-one	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	1290674	81.029	0.33%
24	Ethanone, 1-(2-hydroxyl-methyl-5-phenyl)	C <sub>11</sub> H <sub>12</sub> O	1381515	81.485	0.47%



**Fig. 2:** Representative photographs showing progress of wound healing in different treatment groups

**Table 3:** Estimation of percent wound contraction and epithelization period

Group	Wound Contraction (%)					CD	Epithelization period(days)
	4 <sup>th</sup> day	8 <sup>th</sup> day	12 <sup>th</sup> day	16 <sup>th</sup> day	20 <sup>th</sup> day		
I	28.25 <sup>Bd</sup> ±3.305	53.92 <sup>Bc</sup> ±3.057	77.76 <sup>Bb</sup> ±1.165	91.70 <sup>Ba</sup> ±1.165	98.45 <sup>Ba</sup> ±0.285	<b>7.204</b>	22.8 <sup>B</sup> ± 0.525
II	27.61 <sup>Bd</sup> ±3.964	58.66 <sup>Bc</sup> ±4.650	84.85 <sup>Bb</sup> ±3.603	95.22 <sup>Ba</sup> ±1.646	99.41 <sup>Aa</sup> ±0.254	<b>9.784</b>	20.9 <sup>B</sup> ± 0.699
III	41.37 <sup>Ac</sup> ±3.991	82.76 <sup>Ab</sup> ±2.558	97.51 <sup>Aa</sup> ±0.724	99.91 <sup>Aa</sup> ±0.048	99.98 <sup>Aa</sup> ±0.008	<b>6.440</b>	17.5 <sup>A</sup> ± 1.210
<b>CD</b>	<b>11.524</b>	<b>10.818</b>	<b>8.013</b>	<b>3.564</b>	<b>0.676</b>		<b>2.673</b>

treated group was observed very rapid as 97.51±0.724% wound contraction was achieved on 12<sup>th</sup> day as compared to 84.85±3.603% and 77.76±1.165% in ethanolic extract ointment and Povidone Iodine ointment treated groups, respectively. The methanolic extract ointment also demonstrated minimum epithelization period of 17.5 ± 1.210 days as compared to 20.9 ± 0.669 and 22.8 ± 0.525 days in ethanolic extract ointment and Povidone Iodine ointment treated groups, respectively (Fig. 1).

Kuluvar *et al.* (2009) observed enhanced wound contraction and lesser epithelization period in wounds treated topically with ointment of extract of *C. infortunatum* in rats. Kokate and Limsay (2022) also reported the similar observation in excised wounds in rats applied with ointment of methanolic extract of *C. infortunatum*

and the histopathological studies revealed enhanced angiogenesis in the animals treated with *C. infortunatum* extract ointment.

## CONCLUSION

The observations in the present study revealed presence of many bioactive phytoconstituents in the plant extracts of *C. infortunatum* and ointments prepared from its extract demonstrated significant healing in clinical cases of wound in canines. The methanolic extract ointment was found more efficacious than the Povidone Iodine ointment at 10% concentration and ethanolic extract ointment of *C. infortunatum*. Further studies are recommended to validate the efficacy of *C. infortunatum* extract ointment in canines as well as in other species.

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