

Inhibitory effects against zoonotic bacteria by *Oroxylum indicum* ointment and effects to dog wound

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ABSTRACT

A topical antimicrobial ointment was developed from the fruit extract of *Oroxylum indicum* and was evaluated for its antibacterial and wound-healing effects and acute toxicity in animal models. *O. indicum* fruit and seed extracts exhibited antibacterial activities against clinically isolated bacteria and showed *in vitro* antioxidant activities. To develop a topical antimicrobial ointment from the fruit extract of *O. indicum* and evaluate for its antibacterial and wound-healing effects. *O. indicum* fruit extract ointment was prepared and qualitatively controlled. Acute toxicity of the extract was evaluated in the animal model. Antibacterial effects and healing effects of the ointment to the dog wound were investigated. The results are expressed as mean \pm standard deviation. The ointment exhibited *in vitro* antibacterial effects. A single daily application of the ointment to a dog's wound exhibited a wound-healing effect with complete epithelialization within 7 days while the wound was completely healed with the removal of the scabs, the size was decreased to 14% of the original size within 12 days. The ointment was found no acute toxicity in the animal model. *O. indicum* ointment promoted *in vitro* antibacterial activity and wound-healing effect in dogs with no acute toxicity.

Key words: Antibacterial, ointment, *Oroxylum indicum*, wound healing

INTRODUCTION

Previous studies showed that the ethanol extracts of *Oroxylum indicum* showed antioxidant and antibacterial activities.^[1,2] The aim of the study was to develop an ointment with the fruit extract of *O. indicum* and test for its *in vitro* antibacterial effects. Moreover, acute toxicity tests and the case report of this *O. indicum* ointment on a dog bite wound were also investigated.

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MATERIALS AND METHODS

Preparation of plant material

O. indicum fruits were obtained from provinces in Thailand in 2019 and were identified according to Flora of Thailand.^[3]

Preparation of plant extract

O. indicum fruits were extracted by maceration with 95% ethanol using an electric flask shaker. The extraction solutions were evaporated using a rotary evaporator and a water bath.

Quality control of plant extract

O. indicum fruit extract was quantitatively analyzed for total phenolic, total flavonoid, and flavone contents using previous reported methods.^[2,4]

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Acute toxicity test in animal model

To evaluate the safety of the extract, acute toxicity test was conducted in the animal model. According to OECD guidelines for testing of chemicals number 423, 2001,^[5] 8-week-old female Sprague Dawley rats weighing 195–250 g were used.

Preparation of bacteria and culture media

Clinical isolates of selected bacteria including β -hemolytic *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus intermedius*, *Pseudomonas aeruginosa*, and *Streptococcus suis* were obtained from the Faculty of Veterinary Medicine, Kasetsart University, Nakhon Pathom, Thailand. *S. Suis*, *P. aeruginosa*, β -hemolytic *E. coli*, *S. intermedius*, and *S. aureus* were isolated and characterized using the method from Baron et al.^[6]

In vitro antibacterial activity determination using a disc diffusion method

The Kirby–Bauer disc diffusion method was used.^[7,8]

Preparation of *Oroxylum indicum* fruit extract ointment

The extract was incorporated into the ointment base using a fusion method to obtain a topical ointment containing 1%–10% w/w of *O. indicum* fruit extract. A physical stability study of the ointment was performed at 25°C, 75% relative humidity (RH), and 2°C–8°C, 75% RH for 6 weeks. The ointment formulation was stored at 4°C–8°C in refrigerator until it was used.

Case report: History and observation

An 8-year-old mixed breed male dog weighing 20 kg presented with a dog bite injury on the right foreleg was subjected to the experiment. The wound size was 1.4 cm × 1.4 cm with a depth of around 0.5 cm. The wound was generally cleaned and no bleeding remained. The dog was kept in a separate area in a standard environment. The dog was given free access to water and was given maintenance rations once daily. The treatment and observation were approved by the Kasetsart University Animal Committee (ID number: ACKU65-VET-063).

The entire surface of the wound was applied with the *O. indicum* fruit extract ointment (0.5 g) and covered with sterilized gauze and an adhesive medical bandage wrap. The ointment was applied and the sterilized gauze was changed once daily at 8.00 a.m. for 12 days. The wound characterization including the physical appearance and size was assessed each time. The percentages of wound size and wound contraction were calculated using the following equation:^[9]

Percentage of wound size at day (x) = $(Ax \text{ (mm}^2)/A0 \text{ (mm}^2)) \times 100$

Percentage of wound contraction at day (x) = 100–percentage of wound size at day (x)

Percentage of epithelialization at day (x) = $(Ae \text{ (mm}^2)/Ax \text{ (mm}^2)) \times 100$

Where A0 = the original wound area, Ax = the wound area on the day of imaging, and Ae = the epithelialization area on the day of imaging.

Statistical analysis

All experiments were done in triplicate. The results are expressed as mean ± standard deviation.

RESULTS

Preparation and quality control of *Oroxylum indicum* extract

O. indicum fruit extract appeared as a dark brown semisolid. *O. indicum* fruit extract contained total phenolic and total flavonoid contents of 4.96 ± 0.37 g GAE/100 g extract and 5.16 ± 0.6 g QE/100 g extract, respectively. Baicalin, baicalein, and chrysin contents in *O. indicum* fruit extract analyzed by HPLC were found to be 0.40 ± 0.00 , 0.36 ± 0.00 , and 1.12 ± 0.00 g in 100 g extract, respectively.

Preparation of *Oroxylum indicum* fruit extract ointment

O. indicum fruit extract ointment appeared as brown semisolid ointment with a smooth texture and a specific odor. A physical stability study showed that after *O. indicum* fruit extract ointment was stored at 25°C, 75% RH, and 2°C–8°C, 75% RH for 6 weeks, no physical change in color, texture, and odor was found. The results suggested the physical stability of the ointment for 6 weeks when it was kept at both room temperature and in a refrigerator.

Acute toxicity test in animal model

An acute toxicity test of *O. indicum* fruit extract or *O. indicum* fruit extract ointment in rats showed no toxicity ($LD_{50} > 5$ g/kg rat body weight) by oral administration. No abnormal signs were found in visceral organs and there was no difference in average body weight. According to the Globally Harmonized System of Classification and Labeling of Chemicals, *O. indicum* fruit extract and *O. indicum* fruit extract ointment could be classified into category 5 or unclassified with an LD_{50} value > 5 g/kg body weight.

In vitro antibacterial activity determination using a disc diffusion test

At a concentration of 1000 μ g/disc, *O. indicum* extract showed antibacterial activities against all tested bacteria with the zone of inhibition in the range of 10–14 mm [Table 1]. *O. indicum* fruit extract ointment (concentration of extract = 500 μ g/disc) also showed antibacterial activities

Table 1: Antibacterial effects of *Oroxylum indicum* fruit extract and ointment

Sample	Zone of inhibition (mm)				
	<i>Staphylococcus aureus</i>	<i>Staphylococcus intermedius</i>	<i>Streptococcus suis</i>	β - hemolytic <i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
OIF*	11.33±1.26	12.83±0.76	10.42±2.13	13.00±1.41	10.25±1.06
OIF ointment**	10.00±1.00	10.00±1.32	9.50±0.71	7.87±0.18	7.67±0.29
Ointment base	0	0	0	0	0
AMC30	18.50±0.00	18.67±0.31	26.33±1.26	21.17±0.57	0
DO30	16.67±0.58	15.83±0.76	17.17±1.61	24.33±1.04	14.83±0.76
SXT25	0	0	0	27.50±0.87	15.13±1.15

*Concentration of extract 1000 µg/disc, **Concentration of extract 500 µg/disc. OIF: *Oroxylum indicum* fruit extract, AMC30: Amoxicillin/clavulanic acid 30 µg, DO30: Doxycycline 30 µg and SXT25: Sulfamethoxazole-trimethoprim 25 µg

against all tested bacteria, while the ointment base showed no activity.

Case report

Application of *O. indicum* fruit extract ointment promoted a wound-healing effect within 12 days. The original wound (day 0) was deep, pale white, and covered with white mucous. The wound area was 19,600 mm², which was 100% of the wound size and no epithelialization was found. As shown in Table 2, the wound area continuously decreased after the application of the *O. indicum* fruit extract ointment. The wound contraction reached around 50% by day 5. The epithelialization also reached a high level on day 2, and the wound was completely healed (100% epithelialization) by day 7. On day 12, the wound was completely healed with the removal of the scab, and the size was decreased to 14% of the original size. No signs of infection such as swelling, redness, or pus forming were found during the observation. Signs of skin irritation including a rash, scabbing, red spots, dry or flaky skin were not found during the observation. The wound characteristics during the 12 days of observation after the application of *O. indicum* fruit extract ointment are shown in Figure 1.

DISCUSSION

O. indicum ointment was applied to the bite wound in the dog case report once daily and exhibited wound-healing effects and the complete epithelialization of the wound within 7 days. Wound bites from animals and humans can lead to serious infections.^[10-12] The biter's oral cavity and the victim's skin flora can originate the recovered microorganisms.^[13] Various aerobic and anaerobic microorganisms or polymicrobial situations can be found in human and animal bite infections.^[14] In human bites, *Streptococcus* spp. including *Streptococcus pyogenes* are usually found while *Pasteurella* spp. including *Pasteurella multocida* are the most common in dog and cat bites.^[11,15] More specifically, in a dog bite wound, *Capnocytophaga* spp., *Neisseria weaveri*, *Weeksella zoohelcum*, *Neisseria canis*, and *S. intermedius* can be found.^[14]

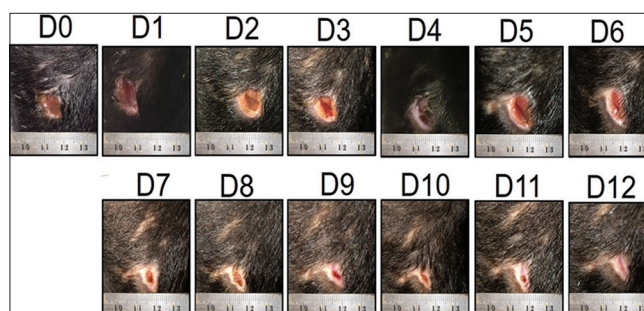


Figure 1: The wound characteristics in the case report. The different numbers after the letter 'D' indicate the day of observation after the application of the *Oroxylum indicum* fruit extract ointment

Extracts from various parts of *O. indicum* were reported to promote antibacterial effects.^[2] In this study, *O. indicum* ointment also showed *in vitro* antibacterial effects. Previous mechanisms of action to inhibit bacteria were causing cell morphological damage and aggregation, cell disruption, and lysis.^[2] The antibacterial effects of the ointment, therefore, should have a similar mechanism of action to the extract.

Dog bite wounds normally do not cause immediate symptoms aside from the tearing injury.^[13] Signs and symptoms can be developed within 24–72 h due to the direct transfer of skin and oral microorganisms into the wound.^[13] The indications of infection are such as swelling, redness, and discharge.^[13] Moreover, the enlargement of adjacent lymph nodes could be found and the motion of a limb may be reduced.^[13] Wound management depends on the stage of wound healing including irrigation, mechanical and chemical debridement, the use of antiseptics and antimicrobials, adherent and nonadherent dressings, and topical applications. The goal of a topical antimicrobial application in the treatment of wounds is to control microbial colonization and proliferation and promote the healing of the wounds.^[16,17]

O. indicum ointment also showed a healing effect within 12 days and promoted the complete epithelialization of the tissue within 7 days in the dog wound case with no signs of infection or skin irritation. It was reported

Table 2: Wound characteristics, wound contraction (%), and epithelialization (%) of case report after *Oroxylum indicum* ointment application

Day	Wound characteristic	Wound size (mm ²)	Percentage wound size	Percentage wound contraction	Epithelialization area (mm ²)	Percentage epithelialization
0	Deep, pale white, covered with white mucous	19,600.00	100.00	0	0	0
1	Formation of red tissue in the middle of the wound, formation of white tissue at the edge of the wound	19,600.00	100.00	0	6,600.00	33.67
2	The wound looked shallower, forming more white tissue at the edge of the wound which had a yellow flesh color	15,600.00	79.59	20.41	13,800.00	88.46
3	The wound looked shallower, forming more yellow flesh-colored tissue at the edge of the wound with red tissue in the middle	13,650.00	69.64	30.36	7,825.00	70.69
4	The wound looked shallower, forming more yellow tissue at the edge of the wound, the red tissue in the middle looked narrower	11,200.00	57.14	42.86	8,500.00	75.89
5	The wound looked shallower, forming more yellow tissue at the edge of the wound, the red tissue in the middle looked even narrower	9,428.57	48.10	51.90	8,628.00	91.51
6	The wound looked shallower, the yellow tissue at the edge continued the healing effect toward the center of the wound	7,700.00	39.29	60.71	7,010.00	91.03
7	The wound was dry and the tissue was completely healed	7,400.00	37.76	62.24	7,400.00	100.00
8	The dried wound was scabbed showing the tissue was completely healed	4,650.00	23.72	76.28	4,650.00	100.00
9	The dry scabbed wound was narrowed and the tissue completely healed	4,200.00	21.43	78.57	4,200.00	100.00
10	The dried scabbed wound narrowed further and the tissue was completely healed	4,000.00	20.41	79.59	4,000.00	100.00
11	The dried scabbed wound was narrower still	3,142.86	16.04	83.96	3,142.86	100.00
12	All the scabs had fallen off	2,750.00	14.03	85.97	2,750.00	100.00

that flavones are the major compounds in fruit extract of *O. indicum*, in which baicalin, baicalein, and chrysin are most abundant.^[2] Flavones exhibited antimicrobial, antioxidant, anti-inflammatory, and anticarcinogenic effects.^[18] Moreover, baicalin was found to exhibit anti-inflammatory and antioxidative properties by modulating nuclear factor kappa-light-chain-enhancer of activated B cells, cyclooxygenase-1, and inducible nitric oxide synthase activity.^[19] It also inhibited the ultraviolet-induced generation of reactive oxygen species in fibroblasts and prevented the activation of transcription factors responsible for collagen degradation.^[20] The results suggest that *O. indicum* ointment could be used for the treatment of animal wounds for its antibacterial activity and wound-healing effects. Flavones, especially baicalin, baicalein, and chrysin, could be responsible for these effects.

CONCLUSION

It was the first time to demonstrate the *in vitro* antibacterial effects of *O. indicum* ointment. Application of the ointment once daily exhibited a wound-healing effect in the dog case.

Further study in the animal model should be conducted to confirm the pharmacological activities along with the study of the mechanisms of action. Stability of the pharmaceutical formulation should be also performed.

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Conflicts of interest

There are no conflicts of interest.

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