AJVR AVMA

Evaluation of in vitro activity of herbal formula *Di Er You* and herb Coptis against bacteria isolated from dogs with otitis externa

Jacquelyn N. B. Olson, DVM, MS[1,2*]; Deng-Shan Shiau, PhD[2]; Aituan Ma, DVM, PhD[3]

[1]Animal Hospital of Dunedin, Dunedin, FL
[2]Chi University, Reddick, FL
[3]College of Veterinary Medicine, Hebei Agricultural University, Baoding, Hebei Province, China

*Corresponding author: Dr. Olson (jacqui.olson.dvm@gmail.com)

Received April 7, 2023
Accepted July 5, 2023

doi.org/10.2460/ajvr.23.03.0065

OBJECTIVE
To determine the in vitro activity of the herbal formula *Di Er You* (DEY) and the single-herb Coptis against bacteria cultured from dogs with otitis externa

ANIMALS
32 client-owned dogs diagnosed with otitis externa.

METHODS
A sample of otic debris from each patient was collected and plated onto a fresh Sheep's Blood Agar plate in the hospital. After bacterial growth was confirmed, 4 wells were created, numbered randomly, and treated with saline (placebo), DEY, Coptis, and Zymox Otic Enzymatic Solution with 1% Hydrocortisone (Zymox). After 24 hours of incubation, the diameter of the zone of inhibition (dZOI) of each treatment was measured and recorded, and compared among treatments. A sample of the bacterial colonies grown was submitted to an outside lab for bacterial identification.

RESULTS
The mean ± SD dZOI values for saline, DEY, Coptis, and Zymox treated wells were 0.25 ± 1.41, 12.47 ± 3.92, 14.25 ± 7.12, and 3.22 ± 5.12, respectively. Post hoc multiple comparisons test revealed that (1) saline-treated wells had significantly smaller dZOI values than the other 3 groups (all $P < .001$), (2) Zymox treated wells had significantly smaller dZOI values than either herbal treated groups (both $P < .001$), and (3) DEY treated wells had significantly smaller dZOI values than those treated with Coptis ($P = .0042$).

CLINICAL RELEVANCE
The results from this in vitro study suggested that both DEY and Coptis could be effective treatments in inhibiting the growth of bacteria in dogs with otitis externa. Prospective randomized controlled clinical trials are warranted to confirm these findings.

Keywords: dogs, otitis externa, herbal formula, Coptis, Zone of Inhibition

Canine otitis externa is 1 of the most common conditions seen in a general practice setting.[1] It is characterized by the inflammation of the ear canal distal to the tympanic membrane and may also include the pinna. The disease is diagnosed based on history, palpation of the ear, otoscopic examination, and cytological examination of otic exudate. The most commonly isolated bacteria are *Staphylococcus spp*.[2,3] Other bacteria frequently associated with otitis externa include *Pseudomonas spp*, *Proteus spp*, *Enterococcus spp*, *Streptococcus spp*, and *Corynebacterium spp*. The most commonly found fungal isolate is *Malassezia spp*.[4]

Based on the results of the cytological evaluation, most patients are treated with a combination of ear cleaners and topical preparations including antibiotics, antifungals, and anti-inflammatories.[5,6] While these treatments are very effective at managing otitis externa, there are shortcomings to conventional therapies. The overuse and inappropriate use of antibiotics across the world is leading to a surge of multidrug resistant (MDR) organisms. A 2008 study found antimicrobial resistance to at least 1 antibiotic in 56% of *Staphylococcus* strains and resistance to more than 2 in 45% of isolates.[3] Alternative antimicrobials to antibiotics would be beneficial in combating these MDR organisms and minimizing their expansion. Furthermore, many of the current therapies include irritating agents such as acidic ingredients, astringents, or potent cerumenolytic agents, which can damage the ear epithelium in sensitive patients and result in becoming a predisposing factor.[5] A more natural approach to treatment rather than these harsh chemicals can offer a gentle alternative for patients.

The use of alternative therapies is one avenue for additional options for the treatment of otitis externa. One such therapy is photobiomodulation, which, through the use of low-level laser therapy, has already been proven helpful in reducing inflammation, stimulating tissue healing, and reducing pain.[7,8] Traditional Chinese veterinary medicine (TCVM), which includes acupuncture and herbal medicine, is rapidly becoming a common adjunctive or alternative option to conventional Western treatments for many diseases, including canine otitis externa. Acupuncture and herbal medications have also been shown to have anti-inflammatory effects.[9,10] These effects occur through several mechanisms such as vagus nerve activation, toll-like receptor 4 (TLR4)/NF-κB signaling, macrophage polarization, mitogen-activated protein kinase (MAPK) signaling pathway, cholinergic anti-inflammatory pathway, and reduction of inflammatory indicators, such as IL-6 and TNF-α.[9,10]

In TCVM, before determining a proper treatment, the practitioner would first make a diagnosis of patterns of imbalances in the body and blockages in the meridian system.[11] A pattern named Gallbaldder Damp Heat is considered a common cause of otitis.[11] One herbal formula that can be used topically to treat this condition is called *Di Er You* (DEY).[11] The herbs in this formula, which is comprised of Coptis (*Huang Lian*), Alum (*Bai Fan*), Borneol (*Bing Pian*), and Glycerol (*Gan You*), aim to treat this pattern by clearing Heat, draining Damp, releasing toxins, opening the orifices, and alleviating itching.[12] Coptis (*Huang Lian*) is derived from the rhizome and root of Coptis Chinensis. Alum (*Bai Fan*) is potassium aluminum sulfate, and Borneol (*Bing Pian*) is the crystal derived from the sap of the plant Dryobalanops aromatica.[12] Percentages of the ingredients of this formula are provided **(Table 1)**. May conducted a pilot study in 19 dogs with otitis externa to investigate the effects of DEY in improving the infection degree (in yeast, cocci, and rods) and clinical signs by comparing with conventional topical ear medications (Surolan, Mometamax, Posatex, and Gemish).[13] The study showed that both DEY and conventional treatment groups had significant improvements in both infection degree and clinical sign improvements and that DEY was at least as effective as the conventional treatments in improving rod bacterial infections and also at least as effective in improving clinical sign scores (erythema, inflammation, amount of exudate, and pruritus).[13]

The herb Coptis, the primary herb in DEY, has been shown in vitro to produce a substantial Zone of Inhibition (ZOI) when used against various strains of bacteria isolated from canine pyoderma.[14] With bacteria cultured from 31 cases of canine pyoderma, the in vitro study reported by Bartholomew demonstrated that both Coptis and the formula Golden Yellow Powder herbal medicines had more effective antibacterial activity compared with 2 conventional antibiotic topical medications (Zymox and Animax).[14] The bacterial isolates included several species of *Staphylococcus*, which are also frequently found in cases of canine otitis externa.[2]

The objective of this in vitro study was to explore the potential of herbal formula DEY and single-herb Coptis as effective alternatives to treat canine otitis externa. The study hypothesized that DEY and Coptis have antibacterial effects against naturally occurring bacterial pathogens cultured from canine subjects with otitis externa and that their effects would be greater than those of conventional topical treatments.

Methods

Study subjects

The subject population for this study consisted of client-owned dogs that had been diagnosed with otitis externa based on the physical exam findings of erythematous ear canals, otic debris, and the microscopic finding of greater than 10 bacteria (cocci or rod) per high power field on ear swab cytology. Subjects were recruited from the routine patient population from general practice in Florida. Patients with the following conditions were excluded: (1) recent use of either oral or topical antibiotics or steroids within 1 week of presentation, (2) a perforated ear drum, or (3) recent treatment with acupuncture or laser therapy within 1 week of presentation. For each qualified subject, after the owner's consent, samples of these naturally occurring infections were obtained for use in the study.

Experimental procedure

Once admitted to the study, a sterile swab was used to collect otic debris from the vertical ear canal of the patient. This debris was then plated onto a Sheep's Blood Agar plate and incubated at 79.5 °F

Table 1—Components of *Di Er You*.

Ingredient	Latin	Part used	Percentage	Preparation
Huang Lian	Coptis chinensis	Rhizome	24.00%	Powder made from ground raw herb
Bai Fan	Alum	Mineral	9.00%	Powder made from ground mineral
Bing Pian	Borneol	Crystalized sap	0.06%	Powder made from ground crystalized sap
Gan You	Glycerol		67.00%	

for 24 hours until bacterial growth was noted. Then, using a Copan swab a sample of this isolate was sent to an outside lab for identification. The sample was then plated onto a fresh Sheep's Blood Agar plate. Each sample was treated with saline (placebo), DEY, Coptis, and Zymox Otic Enzymatic Solution with 1% Hydrocortisone (Zymox).

For each sample, once the bacterial growth was confirmed 24 hours later, a second plate was inoculated with the primary colony and a 6 mm biopsy punch was used to make wells for applying the treatments. Agar wells were filled to the top with each test treatment.

Each well was numbered (1, 2, 3, and 4) within each sample **(Figure 1)**. To avoid potential measuring bias, the assigned treatment for each well was randomized. The assignment key was kept with the investigator and was blinded to the observer who performed the treatment outcome measurements. After the treatments were applied, the plate was incubated at 79.5 °F for 24 hours. The diameter of the zone of inhibition (dZOI) was measured 24 hours after plating in millimeters across the diameter at the widest point for each well. An independent observer blinded to the treatment conducted all the measurements.

Treatment materials and preparation

***Di Er You* (DEY)**—Agar well was filled to the top with full strength formula. DEY is a liquid formulation of Coptis (*Huang Lian*), Alum (*Bai Fan*), Borneol (*Bing Pian*), and Glycerol (*Gan You*). It was refrigerated until use. The DEY drops used in this study were obtained from Jing Tang Herbal in Ocala, FL.

Coptis—Paste solution was made at a concentration of 10 g to 10 mL of boiling tap water and then stored in a cool, dry location until use. Agar well was filled to the top using this paste. The Coptis powder used in this study was obtained from Jing Tang Herbal in Ocala, FL.

Zymox Otic Enzymatic Solution with 1% Hydrocortisone—Agar well was filled to the top with full strength formula. Zymox is a topical otic formulation produced by Pet King Brand Animal Health and is comprised of Hydrocortisone (1%), Glycerin, Deionized Water, Hydroxy Propyl Cellulose, Benzyl Alcohol, Potassium Iodide, Dextrose, Propylene Glycol, Glucose Oxidase, Lysozyme, Lactoperoxidase, and Lactoferrin. Zymox was stored in a cool, dry location until use.

Saline—Sterile saline was filled to the top of the well to serve as the control. Sterile saline was drawn aseptically from a 500 mL IV bag of 0.9% saline. The bags were stored in a cool, dry location until use.

Statistical analysis and sample size

Based on the observed dZOI values, the study tested the statistical hypothesis (null) H_0: The mean dZOI has no difference among the 4 treatments; vs the (alternative) H_A: There exists a difference in mean dZOI among the 4 treatments. As the study design was a randomized complete block trial where each sample (subject) received all 4 test treatments, including the saline control, without assuming normality, Friedman's test, a nonparametric version of the 2-way ANOVA, was used to test the above hypotheses. The null hypothesis would be rejected (ie, a difference exists among treatments) when the *P*-value from the Friedman's test was less than 0.05 (ie, significance level = 0.05). When that was the case, pairwise comparisons using Conover's multiple comparisons test was followed to determine which pairs of treatments had significant difference.

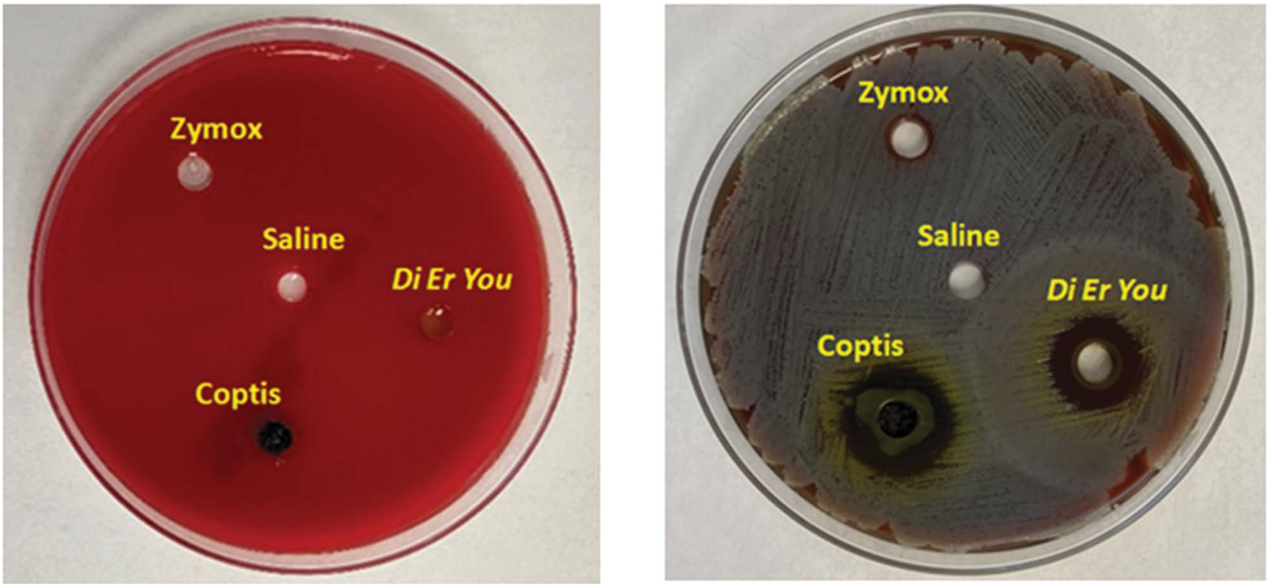

Figure 1—An example of a test sample prepared in a Sheep's Blood Agar plate pictured pretreatment (left panel) and after 24 hours of the treatments (right panel). For blinding, the randomly assigned treatments on the 4 wells were labeled in numbers during the study.

To ensure a 95% power of a 4-treatment 2-way ANOVA test with a 0.05 significance level, the required sample size will be 19 subjects, assuming that the maximal mean difference among the treatment equals to sample standard deviation. With the use of nonparametric Friedman's test, according to Williamson, 15% more samples should be planned, which means 22 subjects would be required. The study was able to include a total of 32 subjects; hence, the power of this study was over 95% under the above statistical considerations and assumptions.

Results

A total of 39 canine patients diagnosed with otitis externa were recruited for participation, and all owners consented. Seven subjects were excluded from the data collection and analysis: 6 were excluded due to not satisfying inclusion and exclusion criteria (eg, recently treated with antibiotics or steroids, acupuncture, or herbal medications) after review by the primary investigator; 1 sample was submitted for culture but not tested against the treatments due to human error (the sample was mishandled and did not yield any bacterial colonies) and was excluded from the study. Therefore, data from 32 subjects were included in the analysis.

There were a variety of breeds (>10) among these 32 subjects, among which 17 (53.1%) were female and 15 (46.9%) were males. The mean age among subjects was 7.7 years old, ranging from 1 to 17 years old (median = 8.5, SD = 3.9), and the mean body weight was 50.0 lbs. (or 22.7 kgs), ranging from 7.4 to 118.0 lbs. (or 3.3 to 53.6 kgs). The median body weight was 46.5 lbs (or 21.1 kgs); SD = 32.4 lbs (or 14.7 kgs). Demographic statistics including sex proportions, summary statistics of age and weight, respectively, and breed distribution of subjects are listed **(Table 2)**.

Bacterial identification was performed on all study samples. Fourteen samples had multiple bacterial colonies. In total, 16 separate bacterial isolates were identified from the samples, as shown **(Table 3)**. The species of bacteria identified were as follows: *Staphylococcus pseudintermedius* (9), Coagulase negative *Staphylococcus species* (8), *Staphylococcus schleiferi subsp coagulans* (5), *Escherichia coli* (4), *Enterococcus species* (4), *Proteus mirabilis* (3), Methicillin resistant *Staphylococcus schleiferi subsp coagulans* (2), *Pseudomonas aeruginosa* (2), Methicillin resistant coagulase negative *Staphylococcus species* (2), *Bacillus species* (2), Beta hemolytic *streptococci* (2), *Diptheroids* (2), Methicillin resistant *Staphylococcus pseudintermedius* (2), *Staphylococcus schleiferi subsp schleiferi* (1), *Pseudomonas species* (1), and *Micrococcus luteus* (1). Various strains of *Staphylococcus* species represented more than half of the bacterial colonies identified (29/50, 58%).

Table 3—Number of subject samples identified with each bacterial population.

Staphylococcus pseudintermedius	9
Coagulase negative *Staphylococcus* species	8
Staphylococcus schleiferi subsp Coagulans	5
Escherichia coli	4
Enterococcus species	4
Proteus mirabilis	3
Methicillin resistant *Staphylococcus schleiferi subsp coagulans*	2
Pseudomonas aeruginosa	2
Methicillin resistant coagulase negative *Staphylococcus species*	2
Bacillus species	2
Beta hemolytic streptococci	2
Diptheroids	2
Methicillin resistant *Staphylococcus pseudintermedius*	2
Staphylococcus schleiferi subsp Schleiferi	1
Pseudomonas species	1
Micrococcus luteus	1

The mean (with standard error) dZOI from each of the 4 treatment well groups is shown **(Figure 2)**, and the distribution (via boxplot) of each group's dZOI is depicted **(Figure 3)**. The dZOI values in the saline-treated group were all zeros except for 1 well, which had a value of 8, and the group mean ± SD was 0.25 ± 1.41 mm (median = 0 mm). In the Zymox treated

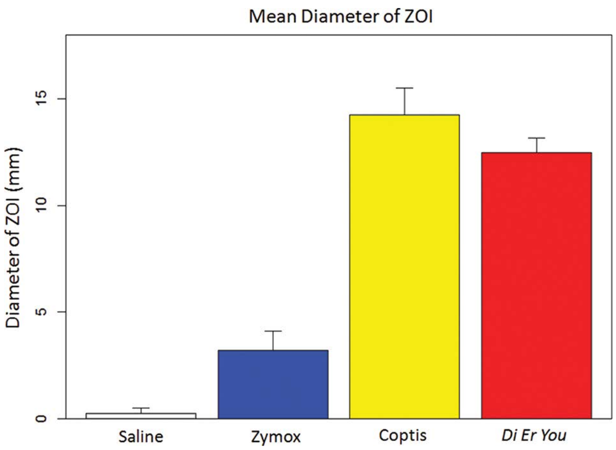

Figure 2—Mean diameter of zone of inhibition (dZOI; in millimeters) within each treatment group of wells.

Table 2—Subject's demographic statistics.

Number of subjects	**32**
Sex	Female: 53.1% (17) male: 46.9% (15)
Age (years)	Range = 1–17; mean = 7.7; median = 8.5; SD = 3.9
Weight (lbs)	Range = 7.4–118.0; mean = 50.0; median = 46.5; SD = 32.4
Breed	Bulldog (4), Chihuahua (1), Cocker Spaniel (2), German Shepherd (2), Golden Doodle (2), Great Dane (1), Greyhound (1), Labrador Retriever (2), Lhasa Apso (1), Maltese (2), Pitbull mix (1), Pug (1), Rottweiler (1), Shar Pei (2), Shih Tzu (1), Toy Poodle (2), Terrier (4), and 2 mixed

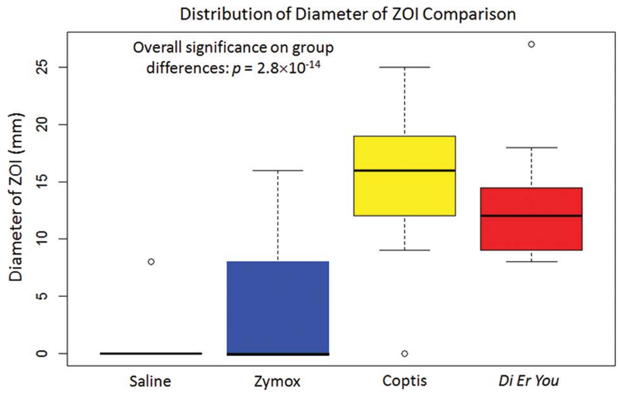

Figure 3—Distribution of diameter of zone of inhibition (dZOI) within each treatment group of wells, depicted by box plots.

group, the dZOI ranged from 0 to 16 mm, with the group mean ± SD = 3.22 ± 5.12 mm (median = 0 mm as 22/32 had dZOI = 0 mm). For the Coptis-treated wells, the dZOI ranged from 0 to 25 mm (5/32 had dZOI = 0 mm), with the group mean ± SD = 14.25 ± 7.12 mm (median = 16 mm). For the wells treated with DEY, the range of dZOI was from 8 to 27 mm (32/32 > 0 mm), with the group mean ± SD = 12.47 ± 3.92 mm (median = 12 mm). These group statistics are summarized **(Table 4)**. Based on Friedman's test, there exists a significant difference among the 4 well groups ($P = 2.8 \times 10^{-14}$).

Post hoc pairwise comparisons using Conover's multiple comparisons test revealed that all pairs of well groups had statistically significant differences in their dZOI values. Statistical significance outcomes (*P*-values) are summarized **(Table 5)** and concluded the following: (1) Each of the well groups treated with medicine (Zymox, Coptis, or DEY) had significantly greater dZOI values than the saline well group, (2) Well groups treated with either herbal medicine (Coptis, or DEY) had significantly greater dZOI values than the Zymox treated well group, and (3) The well group treated with Coptis had significantly greater dZOI values than the well group treated with DEY.

Table 4—Statistics of dZOI change for each treatment.

Treatment	Range	Mean ± SD	Median	# dZOI = 0
Saline	0–8	0.25 ± 1.41	0	31
Zymox	0–16	3.22 ± 5.12	0	22
Coptis	0–25	14.25 ± 7.12	16	5
Di Er You	8–27	12.47 ± 3.92	12	0

Table 5—Post hoc pairwise group comparison between agents tested.

Agents tested	*P*-value
Zymox vs saline	8.3×10^{-7}
Coptis vs saline	$< 2.0 \times 10^{-16}$
Di Er You vs saline	$< 2.0 \times 10^{-16}$
Coptis vs Zymox	$< 2.0 \times 10^{-16}$
Di Er You vs Zymox	$< 2.0 \times 10^{-16}$
Coptis vs *Di Er You*	0.0042

Discussion

The study presented sought to determine the antibacterial effectiveness of the herbal formula DEY and the single-herb Coptis in comparison to the commonly used topical treatment Zymox against the growth of infections in vitro cultured from canine otitis externa. Coptis has already been shown to have antibacterial effects against *S pseudintermedius* and methicillin resistant species.[14] Research into the mechanism of action show that the 2 primary alkaloids in Coptis, berberine and coptisine, have demonstrated effectiveness as anticancer, anti-inflammatory, and antibacterial drugs through regulating the signaling transduction of pathways such as NF-κB, MAPK, PI3K/Akt, NLRP3 inflammasome, RANKL/RANK, and Beclin 1/Sirt1.[15,16] Additionally, several in vitro studies have demonstrated antibacterial activity of Copits (or berberine) against various strains of *Staphylococccus spp*, the most common bacteria in otitis. For example, Wojtyczka et al found noticeable antibacterial effects of berberine chloride against *S haemolyticus* ATCC 29970, *S epidermidis* ATCC 12228, *S capitis subsp capitis* ATCC 35661, *S galinarium* ATCC 700401, *S hominis subsp hominis* ATCC 27844, *S intermedius* ATCC 29663, and *S lugdunensis* ATCC 49576.[17] Their study also noticed significant synergistic effects of berberine in combination with antibiotics linezolid, cefoxitin, and erythromycin, which suggested potential application of their compound combinations as an effective solution for antibiotic-resistant bacterial infections. The study by Kim et al screened 239 ethanolic Chinese medicinal plants (TCMP) extracts for their potential antibacterial activity against multidrug-resistant *S aureus* strains and identified Coptis chinensis as one of the most promising antibacterial TCMP extracts with low cytotoxicity.[18] Using the disc diffusion method to evaluate antimicrobial effects, Leach's study also found that Coptis chinensis was comparably effective to the antibiotic vancomycin against coagulase-negative *staphylococcus* and *S aureus*.[19]

Zymox was selected for comparison due to its known reduction in bacterial colony counts and its common use in the clinical setting.[20] Fujimura showed that ZYMOX-P (ZYMOX Plus Otic) had a bactericidal effect against Pseudomonas aeruginosa in vitro and is beneficial when used for the management of canine otitis externa.[20] The current study was not intended to show that the herbals are better than antibiotics, just that they have antimicrobial properties, and could be an option for treating canine otitis externa. Future studies would be needed to investigate superiority when tested against antibiotics, especially with MDR organisms.

In this study, data of otic debris collected from 32 canine patients diagnosed with otitis externa suggested that both Coptis and DEY have antibacterial activity against the bacterial isolates studied. Between the 2, Coptis appears to be more potent than DEY since it produced a greater dZOI than DEY. This finding was not unexpected since Coptis is a component of DEY, which causes it to be in a lower concentration in the formulation than as a raw herb. These findings correlate with a previous study of Coptis and the topical herbal formulation *Golden Yellow Powder* when tested against canine pyoderma.[14] In that study, Coptis was also determined to be more potent than *Golden Yellow Powder*, of which Coptis is a component.[14]

Interestingly, there were several subjects where Coptis did not yield a ZOI. All of the bacteria collected from these subjects were *Staphylococcus* species, but those same species had ZOI with other subjects. This suggests that there may be strains of bacteria that are resistant to Coptis as well. However, DEY consistently created a ZOI in all subjects. This could indicate that, while Coptis may be more potent as a single herb in specific bacteria, the combination of Coptis (*Huang Lian*) along with Alum (*Bai Fan*), Borneol (*Bing Pian*), and Glycerol (*Gan You*) to create the formulation DEY may make it more effective across bacterial species. Alternatively, the Coptis paste may have been less uniform than the liquid DEY, which could also account for these differences.

In only 1 sample, saline, the negative control, demonstrated a dZOI of 8. On this same sample, the dZOI were 9, 0, and 8 for Zymox, Coptis, and DEY treatments, respectively. Possibilities for this outlier result from the saline treatment include a human error in recording, procedural error, or a bad sample. Contamination is possible but not suspected due to the performance of other wells of treatments. Since the investigators could not retrospectively confirm the reasons, it was decided to keep the data as collected. Furthermore, post hoc analysis after excluding this sample did not change the statistical conclusions, either on the overall group difference or any pairwise group comparisons.

Staphylococcus species represented more than 50% of bacterial species identified in this study. This correlates with other studies identifying this as the predominant bacterial pathogen in otitis externa.[2,3]

One additional limitation of this study was that in several subjects there were several bacterial colonies identified. Since this study was simply looking for a ZOI indicating antibacterial properties it did not confound the results. However, in future studies, it would be useful to isolate colonies to be able to comment on the effectiveness against different species.

Given the encouraging results from this study, future research is warranted. Future studies could include investigations into resistant bacterial strains as well as testing against commonly used Western medications. Studies specifically looking into multidrug resistant strains of some of the isolates in this study could provide evidence that Coptis and DEY are useful for these infections. If these in vitro studies continue to yield positive results, then in vivo studies could be conducted to test if the efficacy is similar in canine ears. May's pilot study showed that both DEY and conventional treatment groups had significant improvements in both infection degree and clinical sign improvements and that DEY was at least as effective as the conventional treatments in improving rod bacterial infections and at least as effective in improving clinical sign scores (erythema, inflammation, amount of exudate, and pruritus).[13] Additionally, no adverse events were reported by either the owners or clinicians of any dog in the study.[13] Results of more in vivo studies with larger sample sizes could yield additional evidence-based TCVM therapeutics for use against canine otitis externa more specifically MDR bacterial causes of otitis externa.

In conclusion, Coptis and DEY have more effective antibacterial efficacy in vitro and significantly larger dZOI than Zymox. Although Coptis was not effective against some bacteria, DEY was effective against all bacterial samples and more effective than Zymox in all but 1 sample. Pending further studies, DEY may be an effective additional treatment option for dogs with otitis externa.

Acknowledgments

The authors would like to thank Dr. Xie for his teaching and tireless work to advance TCVM, Dr. Allison Schnoke for helping enroll study subjects, Dr. Michael Bartholomew for providing guidance with the study, Dr. Gregory Todd for his mentorship, and Sydney Roberts for being an excellent veterinary assistant and blinded result recorder.

Disclosures

The authors have nothing to disclose. No AI-assisted technologies were used in the generation of this manuscript.

Funding

Funding for this study was provided by a grant made possible by the WATCVM and reduced rates on cultures provided by Antech and Veterinary Practice Partners.

References

1. Burns J. "Banfield reports on state of pet health". *Journal of the American Veterinary Medical Association* 238.12 2011;1520–1549. doi:10.2460/javma.238.12.1520
2. Korbelik J, Singh A, Rousseau J, Weese J. Characterization of the otic bacterial microbiota in dogs with otitis externa compared to healthy individuals. *Vet Dermatol.* 2019; 30:228–e270. doi:10.1111/vde.12734
3. Oliveira LC, Leite CA, Brilhante RS, Carvalho CB. Comparative study of the microbial profile from bilateral canine otitis externa. *Can Vet J.* 2008;49:785–788.
4. Korbelik J, Singh A, Rousseau J, Weese S. Analysis of the otic mycobiota in dogs with otitis externa compared to healthy individuals. *Vet Dermatol.* 2018;29:417–e138. doi:10.1111/vde.12665
5. Bajwa J. Canine otitis externa - treatment and complications. *Can Vet J.* 2019;60(1):97–99.
6. Paterson S. Topical ear treatment - options, indications and limitations of current therapy. *J Small Anim Pract.* 2016;57:668–678. doi:10.1111/jsap.12583
7. Avci P, Gupta A, Sadasivam M, et al. Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring. *Semin Cutan Med Surg.* 2013;32(1):41–52.
8. Hamblin M. Photobiomodulation or low-level laser therapy. *J Biophotonics.* 2016;9(11–12):1122–11224. doi:10.1002/jbio.201670113
9. Oh J, Kim S. Anti-inflammatory effects of acupuncture at ST36 point: a literature review in animal

studies. *Front Immunol.* 2022;12:813748. doi:10.3389/fimmu.2021.813748
10. Hu S, Wang J, Liu E, et al. Protective effect of berberine in diabetic nephropathy: a systematic review and meta-analysis revealing the mechanism of action. *Pharmacol Res.* 2022;185:106481. doi:10.1016/j.phrs.2022.106481
11. Xie H, Preast V. *Traditional Chinese Veterinary Medicine Fundamental Principals.* 2nd edition. Chi Institute Press; 2013:111–112.
12. Xie H, Preast V. *Xie's Chinese Veterinary Herbology.* Wiley-Blackwell. 2010:132.
13. May P. *Efficacy of Di Er You Herbal Ear Drops For The Treatment Of Otitis Externa In Dogs*, Master's thesis, Chi University, Reddick, FL, 2019.
14. Bartholomew M. A controlled in-vitro study comparing efficacy of two commercial antibiotic topical compounds and two Chinese herbal medicine topicals against bacteria cultured from 31 canine pyoderma cases. *Am J Trad Chin Vet Med* 2017;12(2):17–28.
15. Wu J, Luo Y, Deng D, et al. Coptisine from Coptis chinensis exerts diverse beneficial properties: a concise review. *J Cell Mol Med.* 2019;23(12):7946–7960. doi:10.1111/jcmm.14725
16. Zhang G-B, Maddili SK, Tangadanchu V, et al. Discovery of natural berberine-derived nitroimidazoles as potentially multi-targeting agents against drug-resistant *Escherichia coli*. *Sci China Chem.* 2017;61(5):557–568. doi:10.1007/s11426-017-9169-4
17. Wojtyczka RD, Dziedzic A, Kępa M, et al. Berberine enhances the antibacterial activity of selected antibiotics against coagulase-negative Staphylococcus strains in vitro. *Molecules.* 2014; 19(5):6583–6596. doi:10.3390/molecules19056583
18. Kim G, Gan R-Y, Zhang G, et al. Large-scale screening of 239 traditional Chinese medicinal plant extracts for their antibacterial activities against multidrug-resistant Staphylococcus aureus and cytotoxic activities. *Pathogens.* 2020;9(3):185. doi:10.3390/pathogens9030185
19. Leach F. Anti-microbial properties of Scutellaria baicalensis and Coptis chinensis, two traditional Chinese medicines. *Bioscience Horizons.* 2011;4(2):119–127. doi:10.1093/biohorizons/hzr014
20. Fujimura M. Effects of an enzyme agent containing mutanase and dextranase for treatment of biofilms in bacteria- and yeast-infected canine otitis externa. *Pol J Vet Sci.* 2022;25(3):383–389.