JB&JS OPEN ACCESS

Protocatechuic Acid as a Topical Antimicrobial for Surgical Skin Antisepsis

Preclinical Investigations

Omid Jalali, MD, Molly Best, MD, Alison Wong, MD, Brett Schaeffer, MD, Brendon Bauer, MD, and Lanny Johnson, MD

Investigation performed at Loma Linda University, Loma Linda, California

Background: There is a need for novel skin antiseptic agents to combat the health-care burdens associated with surgical site infection (SSI) and bacterial resistance. The purpose of this proof-of-principle pilot study was to investigate the potential of the phenolic compound protocatechuic acid (PCA) as a topical antimicrobial for surgical skin antisepsis.

Methods: The Kirby-Bauer method of disc diffusion was used to investigate the in vitro antimicrobial activity and comparative effectiveness of PCA and 7 related compounds against SSI pathogens. To explore the in vivo efficacy of topical PCA for providing deep, penetrating skin antisepsis, living *Cutibacterium acnes* was intradermally injected into the skin of female BALB/c mice. Mice were assigned to treatment with daily applications of topical PCA at 3 doses (78, 39, and 19.5 mM) or no treatment (n = 2 mice per group). After 96 hours, infected skin samples were harvested to compare mean *C. acnes* counts by treatment.

Results: Compared with other polyphenols, PCA demonstrated the broadest spectrum of antimicrobial activity against tested SSI pathogens, including drug-resistant organisms. At 96 hours following infection, the mean *C. acnes* burden in untreated mice was 6.65 log colony-forming units (CFUs) per gram of skin. Compared with the untreated group, daily topical application of 78 mM of PCA was associated with a significantly lower *C. acnes* CFU burden in mice skin (mean, 5.51 log CFUs per gram of skin; p = 0.0295). Both lower dosages of topical PCA failed to show an effect.

Conclusions: PCA demonstrated laboratory efficacy against pathogens implicated in SSI, including drug-resistant organisms. In vivo, topical PCA demonstrated dose-dependent skin penetration and antimicrobial activity against mouse skin *C. acnes* loads. Human clinical studies exploring the antimicrobial efficacy of topical PCA for preoperative shoulder skin antisepsis are warranted.

Clinical Relevance: Topical PCA may have the potential to improve current shoulder SSI treatment and prevention protocols.

Surgical site infection (SSI) causes substantial patient morbidity[1]. Since two-thirds of SSIs are confined to the incision area, much focus for SSI prevention has been on optimizing the strategy for preoperative skin antisepsis[2]. Rises in bacterial resistance[3-5] and insufficient skin-penetration properties[6-8] have been shown to limit the efficacy of existing skin antisepsis protocols in the prevention of SSI. As a result, there is a continuing need for alternative skin antiseptic agents, with the hope of identifying reagents with a broad spectrum of utility and adequate skin penetration and without a history of overuse or resistance.

Natural substrates with antimicrobial properties have been identified as possible sources of novel antimicrobials[9]. There is mounting evidence that the phenolic compound protocatechuic acid (PCA) functions as a broad-spectrum antimicrobial agent, including activity against drug-resistant organisms[10-12], and demonstrates potent skin-penetration properties and deep dermal bioavailability when applied topically[13,14]. In exerting their antibacterial activity, phenolic compounds have been shown to enhance the generation of reactive oxygen species in bacteria[15] and disrupt bacterial cell-membrane integrity, causing the inhibition

Disclosure: The authors indicated that no external funding was received for any aspect of this work. On the **Disclosure of Potential Conflicts of Interest** forms, *which are provided with the online version of the article*, one or more of the authors checked "yes" to indicate that the author had a relevant financial relationship in the biomedical arena outside the submitted work (one author, L.J., has a website that sells the test reagent that is the subject of the study) and "yes" to indicate that the author had a patent and/or copyright, planned, pending, or issued, directly relevant to this work (http://links.lww.com/JBJSOA/A187).

and leakage of intracellular contents[16]. However, most recent investigations involving PCA have focused on plant extracts, of which the exact ingredients and dosages are unknown[17-19], limiting the feasibility for studies of potential clinical applications of the antimicrobial effects observed with this compound.

In vitro studies of natural compounds containing PCA have demonstrated its dose-dependent antimicrobial activity against several pathogens implicated in SSI, including *Cutibacterium acnes* (formerly *Propionibacterium Acnes)*[18], a gram-positive anaerobe responsible for infections following shoulder surgery[20,21]. In vivo, plant extracts containing PCA were shown to inhibit *C. acnes*-induced inflammation in mouse skin through the inhibition of lipase activity and the suppression of proinflammatory cytokines[18]. No study, to our knowledge, has examined the efficacy of topical PCA in reducing *C. acnes* bacterial loads in living skin, a particularly noteworthy consideration when evaluating its potential to prevent SSI with this pathogen.

The purpose of this proof-of-principle pilot study was to investigate the potential of PCA as a topical antimicrobial for surgical skin antisepsis. Our study aims were to (1) investigate the spectrum of antimicrobial activity of PCA, focusing on its activity against skin and wound pathogens, and (2) explore the in vivo application of PCA as a topical agent for deep, penetrating skin antisepsis using a mouse model of *C. acnes* dermal skin infection.

Materials and Methods

Antimicrobial Susceptibility Testing

The Kirby-Bauer method of disc diffusion was used for the testing of antimicrobial efficacy and specificity[22]. Standard procedures recommended by the National Committee for Clinical Laboratory Standards were followed[23]. A set of discs saturated with test or control compounds were placed onto inoculated agar plates. The plates were inoculated with gram-positive, gram-negative, aerobic, and anaerobic bacteria and the fungus *Candida albicans* (see Appendix Table 1 for a full list of organisms included in the analysis) to determine the spectrum of antimicrobial activity of the test compounds. As we sought to identify an antimicrobial agent with the potential to reduce SSI, the primary focus of our laboratory analysis was to determine the comparative effectiveness of test compounds against *Staphylococcus aureus, Pseudomonas aeruginosa, methicillin-resistant S. aureus* (MRSA), and *C. acnes*, which are clinically challenging organisms.

The primary disinfectant used for the in vitro investigation was a 100-mM PCA solution in a water vehicle. Vanillic acid, hippuric acid, delphinidin, pelargonidin, cyanidin chloride, 28% cyanidin-3-glucoside, and 2,4,6-trihydroxybenzoic acid were also tested (at the same concentration), as they are plant secondary metabolites related to PCA. The testing of related compounds was performed to determine which compound was the likely plant secondary metabolite responsible for the antibacterial effect demonstrated in previous plant-based therapies and studies[18,19]. Amoxicillin served as a positive control.

Depending on the microorganism tested, plates were incubated for 18, 24, or 48 hours. The diameters of the zones of inhibition were measured using a sliding caliper and interpreted as follows: no inhibition (NI) of growth under the test sample, inhibition (I) of growth under the test sample, no zone (NZ) of inhibition surrounding the test sample, or a clear zone (CZ) of inhibition surrounding the test sample, with the width of the clear zone (in millimeters) recorded.

Testing Against C. acnes in Mouse Skin

All experimental procedures were carried out at an independent laboratory (TransPharm Preclinical Solutions), and were performed in compliance with the laws, regulations, and guidelines of the U.S. National Institutes of Health and with the approval of the TransPharm Institutional Animal Care and Use Committee. Eight female BALB/c (Harlan) mice weighing 17 to 19 g were obtained. Each mouse was housed with 1 partner in a static cage with corn-cob bedding and had free access to food and water. Table I outlines experimental procedures and time points.

Mice were randomly assigned to 1 of 4 treatment groups: (1) no treatment, (2) 78 mM of topical PCA, (3) 39 mM of topical PCA, and (4) 19.5 mM of topical PCA (n = 2 mice per group). On the day before treatment, mice were anesthetized with a 5% isoflurane induction chamber and a 2.0 × 2.0-cm region on the dorsal area was cleared of hair and delineated as the infection site. *C. acnes* was selected for testing as it is responsible for the majority of shoulder SSIs[20,21], and testing against this organism may allow for some assessment of the skin-penetration properties of test reagents[24].

Cultures of *C. acnes* (American Type Culture Collection 6919) were grown for 96 hours at 37°C, in an anaerobic atmosphere on trypticase soy (TS) agar plates supplemented

TABLE I Animal Challenge, Treatment, and Harvesting Schedule*

Group	No.	Intradermal *C. acnes*	Treatment	Treatment Schedule†	Skin Harvest†
1	2	6.0 log CFUs	Untreated	NA	96 hr
2	2	6.0 log CFUs	Topical PCA, 78 mM	2, 24, 48, 72 hr	96 hr
3	2	6.0 log CFUs	Topical PCA, 39 mM	2, 24, 48, 72 hr	96 hr
4	2	6.0 log CFUs	Topical PCA, 19.5 mM	2, 24, 48, 72 hr	96 hr

*NA = not applicable, CFU = colony-forming unit, and PCA = protocatechuic acid. †Time defined relative to infectious challenge at 0 hour.

TABLE II Antimicrobial Efficacy of Plant Secondary Metabolites Against Skin and Wound Pathogens*

Treatment (100 mM)	*S. aureus*	*C. acnes*	MRSA	*P. aeruginosa*
PCA	I/CZ/8 mm	I/CZ/22 mm	I/CZ/9 mm	I/CZ/10 mm
Delphinidin	I/CZ/10 mm	I/NZ	I/CZ/7 mm	NI/NZ
Pelargonidin	I/CZ/3 mm	I/CZ/1 mm	I/CZ/4 mm	NI/NZ
Cyanidin Cl	I/CZ/1 mm	NI/NZ	I/CZ/3 mm	NI/NZ
28% C-3-G	I/NZ	I/CZ/23 mm	I/CZ/4 mm	I/CZ/2 mm
2,4,6-THBA	I/CZ/13 mm	NI/NZ	I/CZ/14 mm	I/CZ/3 mm
Vanillic acid	I/CZ/1 mm	NT	I/CZ/1 mm	I/NZ
Hippuric acid	NI/NZ	NT	I/CZ/1 mm	NI/NZ

*PCA = protocatechuic acid, I = inhibition of growth under the sample, CZ = clear zone of inhibition surrounding the sample and zone width in millimeters, NZ = no zone of inhibition surrounding the sample, NI = no inhibition of growth under the sample, Cl = chloride, C-3-G = cyanidine-3-glucoside, 2,4,6-THBA = 2,4,6-trihydroxybenzoic acid, and NT = not tested.

with 5% sheep blood. The culture was aseptically swabbed and transferred to tubes of TS broth and allowed to grow for 72 hours. Cultures were diluted to provide an infectious inoculum of 6.0 log colony-forming units (CFUs) per 50 μL in phosphate-buffered saline (PBS) solution. On day 0, mice were again anesthetized and 50 μL of the *C. acnes* suspension was administered via intradermal injection[25].

Following *C. acnes* inoculation, topical PCA in sterile water was administered in a dose volume of 0.1 mL at 2, 24, 48, and 72 hours post-challenge. Ninety-six hours following challenge, mice were humanely killed by carbon dioxide overexposure, and skin was aseptically removed from the infection site. Skin samples were placed in homogenization vials with 2.0 mL of PBS solution, weighed, and homogenized using a mini-bead beater. Homogenate was serially diluted and plated anaerobically on TS agar plates for the enumeration of CFUs, transformed to a decimal logarithm. To control for variations in harvested tissue weights, mean values were reported per gram of skin tissue.

Statistical Analysis

All statistical analyses were performed using Stata (version 13.0; StataCorp). Mean (and standard deviation) bacterial burdens per gram of skin tissue were calculated and were compared between untreated mice and mice treated with each dosage of PCA using a 2-tailed t test. A p value of <0.05 was considered significant for all tests.

Results

Antimicrobial Susceptibility Testing

The antimicrobial efficacies of the test compounds against *S. aureus*, *C. acnes*, MRSA, *and P. aeruginosa* are shown in Table II. Saturation with 100 mM of PCA resulted in complete zones of inhibition of at least 8 mm against each of the organisms selected for primary analysis, with the greatest activity shown against *C. acnes* (22 mm of growth inhibition). None of the other compounds tested demonstrated antimicrobial activity against all organisms. The control, amoxicillin, demonstrated growth inhibition against *S. aureus, C. acnes,* and MRSA, but failed to inhibit the growth of *P. aeruginosa* (see Appendix Table 1 for the results of the antimicrobial susceptibility testing against microbes not included in the primary analysis).

Antimicrobial Activity Against C. acnes

None of the mice displayed acute adverse events associated with the treatments, and no treatment group displayed adverse signs beyond those expected for mice that have received a superficial bacterial infection.

Ninety-six hours following infection, the mean bacterial burden for the untreated mice was 6.65 log CFUs per gram of skin. Compared with the untreated group, daily topical application of 78 mM of PCA was associated with a significantly lower *C. acnes* CFU burden in mice skin (mean, 5.51 log CFUs; $p = 0.0295$). Both lower dosages of topical PCA failed to show an effect (Table III).

Discussion

The present study was undertaken to investigate the potential of PCA as a topical antimicrobial for surgical skin antisepsis. PCA demonstrated antimicrobial efficacy in vitro against pathogens prevalent in SSI, and topical PCA demonstrated a dose-dependent reduction of *C. acnes* loads in the skin and deeper

TABLE III *C. acnes* Mouse Skin Loads by Treatment

Treatment	*C. acnes* Counts*	P Value†
Untreated	6.65 ± 0.145	—
PCA, 78 mM	5.51 ± 0.249	0.0295
PCA, 39 mM	7.5 ± 0.725	0.4513
PCA, 19.5 mM	5.86 ± 0.429	0.2235

*The values are given as the mean log CFUs (colony-forming units) per gram of skin tissue and the standard deviation. †Determined using a 2-tailed t test to compare *C. acnes* counts between untreated mice and mice treated with each dosage of PCA.

skin appendages of mice. These findings lay the groundwork for studies exploring the antimicrobial efficacy of topical PCA in human skin and provide a theoretical basis for the application of PCA as a deep, penetrating topical skin antiseptic to combat shoulder SSI.

The in vitro antimicrobial efficacy of plant-derived phytochemicals against skin and wound pathogens has been demonstrated previously. Kuete et al. demonstrated the broad-spectrum activity of PCA in a crude extract of *Ficus ovata* against gram-positive bacteria, gram-negative bacteria, and fungi, including drug-resistant organisms[19]. Liu et al. demonstrated the efficacy of PCA derived from Roselle calyx against the drug-resistant organisms MRSA, *Klebsiella pneumoniae,* and *P. aeruginosa*[26]. Lim et al. demonstrated the antibacterial activity of PCA in *Euphorbia supina* against *C. acnes*[18]. Our findings agree, as saturation with PCA resulted in clear zones of inhibition against *C. acnes* (22 mm), *S. aureus* (8 mm), MRSA (9 mm), and *P. aeruginosa* (10 mm). Through the comparison of the antimicrobial efficacy of PCA and that of related plant secondary metabolites, our results build on previous work by identifying PCA as the likely bioactive antibacterial compound contributing to the activity of these plant-based therapies against tested skin and wound pathogens.

It is estimated that up to 50.9% of SSIs in the United States are caused by microorganisms resistant to existing prophylactic antibiotics, and this rate is increasing[27]. Compared with infection with antibiotic-susceptible strains, SSI caused by resistant pathogens has consistently been linked to poorer outcomes and increased costs following orthopaedic procedures[28-32]. In the present study, PCA demonstrated activity against all tested drug-resistant organisms, including *P. aeruginosa,* MRSA, Legionella, *K. pneumoniae,* and methicillin-resistant *S. epidermidis* (MRSE). In contrast, *P. aeruginosa, K. pneumoniae,* and MRSE were unaffected by saturation with the control (see xref ref-type="sec" rid="app1"), amoxicillin, suggesting that some bacterial-resistance patterns developed in the presence of classic antimicrobials may not provide the same resistance to this compound. In conjunction with the extremely limited number of reported cases of microbial resistance to plant secondary metabolites[33], these findings provide further support for investigations currently underway exploring PCA, either as monotherapy[19] or in combination with existing antimicrobial agents[34,35], to reduce infection with drug-resistant organisms[26,36]. Taken together, our laboratory investigations suggest that research into the clinical application of PCA as a skin antiseptic agent may yield promise in reducing the health-care impact of SSI.

Despite promising in vitro findings, few studies have explored in vivo applications of the antimicrobial activity of PCA. Yin and Chao demonstrated its potential as a food preservative, as PCA inhibited growth of antibiotic-resistant Campylobacter and aerobic bacteria in a dose-dependent fashion in meat[37]. The present study is the first, to our knowledge, to examine the efficacy of topical PCA at reducing *C. acnes* bacterial loads in living skin, an important therapeutic target for the prevention of SSI caused by this pathogen.

The *C. acnes* rodent injection model employed here was selected to simulate the chronic inflammatory process associated with *C. acnes* colonization in the dermis[25], the layer of the skin where commercial preoperative antiseptic agents have been shown to provide inadequate eradication of *C. acnes*[6]. Our animal study tested the potential of PCA as a deep, penetrating topical agent to reduce dermal *C. acnes* colonization. Untreated mice demonstrated increases in *C. acnes* loads, from 6.0 log CFUs to 6.65 log CFUs, suggesting that *C. acnes* successfully established a steady intradermal colonization in the skin of BALB/c mice following injection. When administered topically, daily application of 78 mM of PCA resulted in an approximately 13.8-fold (or 1.14 $\log_{10}$) reduction in *C. acnes* CFU counts per gram of mouse skin. However, the effect was only observed at the highest dose tested. These results agree with previous findings[13,18], as the in vivo skin-penetration properties and activity of PCA against *C. acnes* were dose-dependent, suggesting that higher concentrations of topical PCA may be required to achieve antimicrobial activity against this organism in living skin.

On the basis of our current findings, the effective dose of topical PCA to reduce dermal *C. acnes* loads was 78 mM, which equates to 12.021 mg of PCA/mL. In a 100-mL volume of solution sufficient for application in human skin surrounding the shoulder joint, this would equate to an approximate dose of 1.2 g of topical PCA. It should be noted that the dose of PCA varies depending on its application and the drug vehicle[10], and the optimal concentration and drug formulation for antimicrobial efficacy in intact human skin remain a topic of additional study. Nonetheless, our data suggest that investigations into topical PCA may yield the potential for reducing *C. acnes* loads in the dermal layer of skin. Given the relative abundance of *C. acnes* in the skin surrounding the shoulder joint and the lack of available skin antiseptic agents proven to reduce infection rates following shoulder surgery[24,38], topical PCA may serve as an attractive alternative or adjunctive agent to existing shoulder skin antisepsis protocols for the prevention of shoulder SSI. As a next step toward the potential clinical translation of these findings, our group performed a pilot study involving the human shoulder in which we explored the application of topical PCA for shoulder skin antisepsis among healthy volunteers[39].

There were several limitations to the present study. First, amoxicillin is not used as a topical agent in clinical practice. At the initiation of the study, multiple potential applications for PCA were considered, and preliminary antimicrobial susceptibility testing against a broad spectrum of organisms (full susceptibility data in Appendix Table 1) was necessary to identify the potential for PCA as a topical agent for skin disinfection. Ideally, comparisons to current skin-preparation solutions would have allowed for more robust conclusions regarding the potential of topical PCA application for skin disinfection. Another limitation relates to the small sample size in the in vivo model, predisposing our analysis to a type-II error and limiting the precision of our dosage analysis. Furthermore, only 1 microbe was examined in the in vivo model, perhaps limiting the generalizability to other pathogens of interest.

In conclusion, PCA demonstrated laboratory efficacy against pathogens implicated in SSI, including activity against drug-resistant organisms. Furthermore, topical PCA demonstrated a dose-dependent reduction in intradermal *C. acnes* burdens in a mouse model of skin infection. On the basis of our findings, studies

exploring PCA as a topical antimicrobial for human skin antisepsis are warranted.

Appendix

eA Supporting material provided by the authors is posted with the online version of this article as a data supplement at jbjs.org (http://links.lww.com/JBJSOA/A188). ■

Omid Jalali, MD[1]
Molly Best, MD[2]
Alison Wong, MD[2]
Brett Schaeffer, MD[2]
Brendon Bauer, MD[2]
Lanny Johnson, MD

[1]Keck School of Medicine of USC, Los Angeles, California

[2]Loma Linda Department of Orthopedics (M.B.) and Loma Linda University School of Medicine (A.W., B.S., and B.B.), Loma Linda, California

Email address for O. Jalali: omidjalali3737@gmail.com

ORCID iD for O. Jalali: 0000-0003-2511-4621
ORCID iD for M. Best: 0000-0002-5571-4153
ORCID iD for A. Wong: 0000-0002-0398-6480
ORCID iD for B. Schaeffer: 0000-0002-0784-9141
ORCID iD for B. Bauer: 0000-0002-0666-1411
ORCID iD for L. Johnson: 0000-0002-1433-4410

References

1. Whitehouse JD, Friedman ND, Kirkland KB, Richardson WJ, Sexton DJ. The impact of surgical-site infections following orthopedic surgery at a community hospital and a university hospital: adverse quality of life, excess length of stay, and extra cost. Infect Control Hosp Epidemiol. 2002 Apr;23(4):183-9.

2. Itani KM, Wilson SE, Awad SS, Jensen EH, Finn TS, Abramson MA. Ertapenem versus cefotetan prophylaxis in elective colorectal surgery. N Engl J Med. 2006 Dec 21;355(25):2640-51.

3. Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am. 2007 Apr;89(4):780-5.

4. Kurtz SM, Lau E, Schmier J, Ong KL, Zhao K, Parvizi J. Infection burden for hip and knee arthroplasty in the United States. J Arthroplasty. 2008 Oct;23(7):984-91. Epub 2008 Apr 10.

5. Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J. Economic burden of periprosthetic joint infection in the United States. J Arthroplasty. 2012 Sep; 27(8)(Suppl):61-5.e1. Epub 2012 May 2.

6. Lee MJ, Pottinger PS, Butler-Wu S, Bumgarner RE, Russ SM, Matsen FA 3rd. Propionibacterium persists in the skin despite standard surgical preparation. J Bone Joint Surg Am. 2014 Sep 3;96(17):1447-50.

7. Heckmann N, Sivasundaram L, Heidari KS, Weber AE, Mayer EN, Omid R, Vangsness CT Jr, Hatch GF 3rd. Propionibacterium acnes persists despite various skin preparation techniques. Arthroscopy. 2018 Jun;34(6):1786-9. Epub 2018 Mar 24.

8. Heckmann N, Heidari KS, Jalali O, Weber AE, She R, Omid R, Vangsness CT, Rick Hatch GF 3rd. Cutibacterium acnes persists despite topical clindamycin and benzoyl peroxide. J Shoulder Elbow Surg. 2019 Dec;28(12):2279-83. Epub 2019 Aug 27.

9. Spellberg B. The future of antibiotics. Crit Care. 2014 Jun 27;18(3):228. Epub 2014 Jun 27.

10. Kakkar S, Bais S. A review on protocatechuic acid and its pharmacological potential. ISRN Pharmacol. 2014 Mar 26;2014:952943.

11. Khan AK, Rashid R, Fatima N, Mahmood S, Mir S, Khan S, Jabeen N, Murtaza G. Pharmacological activities of protocatechuic acid. Acta Pol Pharm. 2015 Jul-Aug; 72(4):643-50.

12. Semaming Y, Pannengpetch P, Chattipakorn SC, Chattipakorn N. Pharmacological properties of protocatechuic acid and its potential roles as complementary medicine. Evid Based Complement Alternat Med. 2015;593902. Epub 2015 Feb 9.

13. Zillich OV, Schweiggert-Weisz U, Hasenkopf K, Eisner P, Kerscher M. Release and in vitro skin permeation of polyphenols from cosmetic emulsions. Int J Cosmet Sci. 2013 Oct;35(5):491-501. Epub 2013 Jul 17.

14. Sarikaki V, Rallis M, Tanojo H, Panteri I, Dotsikas Y, Loukas L, Papaioannou G, Demetzos C, Weber S, Moini H, Maibach HI, Packer L. In vitro percutaneous absorption of pine bark extract (pycnogenol) in human skin. J Toxicol Cutaneous Ocul Toxicol. 2005;23(3):149-58.

15. Ajiboye TO, Habibu RS, Saidu K, Haliru FZ, Ajiboye HO, Aliyu NO, Ibitoye OB, Uwazie JN, Muritala HF, Bello SA, Yusuf II, Mohammed AO. Involvement of oxidative stress in protocatechuic acid-mediated bacterial lethality. MicrobiologyOpen. 2017 Aug;6(4). Epub 2017 Mar 27.

16. Alves MJ, Ferreira IC, Froufe HJ, Abreu RM, Martins A, Pintado M. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. J Appl Microbiol. 2013 Aug;115(2):346-57. Epub 2013 May 8.

17. Zeouk I, Balouiri M, Bekhti K. Antistaphylococcal activity and phytochemical analysis of crude extracts of five medicinal plants used in the center of Morocco against dermatitis. Int J Microbiol. 2019 Nov 4;2019:1803102.

18. Lim HJ, Jeon YD, Kang SH, Shin MK, Lee KM, Jung SE, Cha JY, Lee HY, Kim BR, Hwang SW, Lee JH, Sugita T, Cho O, Myung H, Jin JS, Lee YM. Inhibitory effects of Euphorbia supina on Propionibacterium acnes-induced skin inflammation in vitro and in vivo. BMC Complement Altern Med. 2018 Sep 27;18(1):263.

19. Kuete V, Nana F, Ngameni B, Mbaveng AT, Keumedjio F, Ngadjui BT. Antimicrobial activity of the crude extract, fractions and compounds from stem bark of Ficus ovata (Moraceae). J Ethnopharmacol. 2009 Jul 30;124(3):556-61. Epub 2009 May 18.

20. Portillo ME, Corvec S, Borens O, Trampuz A. Propionibacterium acnes: an underestimated pathogen in implant-associated infections. BioMed Res Int. 2013; 2013:804391. Epub 2013 Nov 6.

21. Grosso MJ, Frangiamore SJ, Ricchetti ET, Bauer TW, Iannotti JP. Sensitivity of frozen section histology for identifying Propionibacterium acnes infections in revision shoulder arthroplasty. J Bone Joint Surg Am. 2014 Mar 19;96(6):442-7.

22. Biemer JJ. Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method. Ann Clin Lab Sci. 1973 Mar-Apr;3(2):135-40.

23. Thornsberry C. Antimicrobial susceptibility testing of anaerobic bacteria: review and update on the role of the National Committee for Clinical Laboratory Standards. Rev Infect Dis. 1990 Jan-Feb;12(Suppl 2):S218-22.

24. Matsen FA 3rd, Butler-Wu S, Carofino BC, Jette JL, Bertelsen A, Bumgarner R. Origin of Propionibacterium in surgical wounds and evidence-based approach for culturing Propionibacterium from surgical sites. J Bone Joint Surg Am. 2013 Dec 4; 95(23):e1811-7.

25. De Young LM, Young JM, Ballaron SJ, Spires DA, Puhvel SM. Intradermal injection of Propionibacterium acnes: a model of inflammation relevant to acne. J Invest Dermatol. 1984 Nov;83(5):394-8.

26. Liu KS, Tsao SM, Yin MC. In vitro antibacterial activity of Roselle calyx and protocatechuic acid. Phytother Res. 2005 Nov;19(11):942-5.

27. Friedman ND, Temkin E, Carmeli Y. The negative impact of antibiotic resistance. Clin Microbiol Infect. 2016 May;22(5):416-22. Epub 2015 Dec 17.

28. Salgado CD, Dash S, Cantey JR, Marculescu CE. Higher risk of failure of methicillin-resistant Staphylococcus aureus prosthetic joint infections. Clin Orthop Relat Res. 2007 Aug;461:48-53.

29. Kilgus DJ, Howe DJ, Strang A. Results of periprosthetic hip and knee infections caused by resistant bacteria. Clin Orthop Relat Res. 2002 Nov;404:116-24.

30. Joshy S, Gogi N, Thomas B, Mahale A, Singh BK. Delayed onset of deep infection after total knee arthroplasty: comparison based on the infecting organism. J Orthop Surg (Hong Kong). 2007 Aug;15(2):154-8.

31. Martínez-Aguilar G, Avalos-Mishaan A, Hulten K, Hammerman W, Mason EO Jr, Kaplan SL. Community-acquired, methicillin-resistant and methicillin-susceptible Staphylococcus aureus musculoskeletal infections in children. Pediatr Infect Dis J. 2004 Aug;23(8):701-6.

32. Inoue S, Moriyama T, Horinouchi Y, Tachibana T, Okada F, Maruo K, Yoshiya S. Comparison of clinical features and outcomes of Staphylococcus aureus vertebral osteomyelitis caused by methicillin-resistant and methicillin-sensitive strains. Springerplus. 2013 Jun 27;2(1):283.

33. Rodrigues T, Reker D, Schneider P, Schneider G. Counting on natural products for drug design. Nat Chem. 2016 Jun;8(6):531-41. Epub 2016 Apr 25.

34. Jayaraman P, Sakharkar MK, Lim CS, Tang TH, Sakharkar KR. Activity and interactions of antibiotic and phytochemical combinations against Pseudomonas aeruginosa in vitro. Int J Biol Sci. 2010 Sep 21;6(6):556-68.

35. Jayaraman P, Sakharkar KR, Sing LC, Chow VT, Sakharkar MK. Insights into antifolate activity of phytochemicals against Pseudomonas aeruginosa. J Drug Target. 2011 Apr;19(3):179-88. Epub 2010 Apr 30.

36. Radulović NS, Blagojević PD, Stojanović-Radić ZZ, Stojanović NM. Antimicrobial plant metabolites: structural diversity and mechanism of action. Curr Med Chem. 2013;20(7):932-52.

37. Yin MC, Chao CY. Anti-Campylobacter, anti-aerobic, and anti-oxidative effects of Roselle calyx extract and protocatechuic acid in ground beef. Int J Food Microbiol. 2008 Sep 30;127(1-2):73-7. Epub 2008 Jun 13.

38. Rao AJ, Chalmers PN, Cvetanovich GL, O'Brien MC, Newgren JM, Cole BJ, Verma NN, Nicholson GP, Romeo AA. Preoperative doxycycline does not reduce Propionibacterium acnes in shoulder arthroplasty. J Bone Joint Surg Am. 2018 Jun 6;100(11):958-64.

39. Jalali O, Best M, Wong A, Schaeffer B, Bauer B, Johnson L. Reduced bacterial burden of the skin surrounding the shoulder joint following topical protocatechuic acid application: results of a pilot study. JBJS Open Access. 2020. [In press].