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Antimicrobial potential of pyroligneous extracts – a systematic review and technological prospecting

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ABSTRACT

Pyroligneous extract is applied in diverse areas as an antioxidant, an antimicrobial, and an anti-inflammatory agent. The discovery of new cost-effective antimicrobial agents of natural origin remains a challenge for the scientific community. This study aimed to conduct a systematic review and a technological forecasting of the existent evidence regarding the use of pyroligneous extract as a potential antimicrobial agent. Studies were identified through an investigation of various electronic databases: PubMed, SciFinder, Web of Science, Scopus, Scielo, Google scholar, and ProQuest. Patents were searched through INPI, Google patents, Espacenet, Patents online, USPTO, and WIPO. The literature on antimicrobial activity of pyroligneous extract are limited given the short duration of studies and variability in study design, use of pyroligneous preparations, and reports on results. However, evidence suggests the potential of pyroligneous extract as a natural antimicrobial agent. The most studied activity was the role of PE as a food preservative. However, pyroligneous extracts are also effective against pathogenic bacteria in the oral microflora and treatment of candidal infections. Further research is needed using standardized preparations of pyroligneous extracts to determine their long-term effectiveness and ability as antimicrobial agents.

Introduction

Pyroligneous extract (PE), also called pyroligneous acid, liquid smoke, or wood vinegar, is a crude condensate produced from the distillation of smoke generated in wood carbonization. This extract is a complex mixture of compounds derived from the chemical breakdown of wood components through the condensation of vapors and gases generated during the pyrolysis of a limited amount of oxygen.[1] PE is a complex and highly oxygenated aqueous liquid fraction; it results from

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the thermochemical breakdown or pyrolysis of plant biomass components, such as cellulose, hemicellulose, and lignin.[2,3]

PE finds application in diverse areas, acting as an antioxidant, antimicrobial, and anti-inflammatory agent. PE also acts as a source of valuable chemicals and imparts a smoky flavor and antimicrobial protection in food.[3] PE has been reported to possess extreme antifungal activity against several plant pathogenic fungi[4] and a termiticidal activity.[5] Several researchers have reported the antibacterial activity of PE against several pathogenic bacteria, including plant pathogens.[6] Recently, the medicinal use of PE has been studied intensively in the field of oriental medical science, where some natural resources have been used for investigating biological activities.[7] However, the antimicrobial potential of PE against human and animal pathogenic microorganisms has not been elucidated. Therefore, the present work is carried out to evaluate the antimicrobial potential of PE against human and animal pathogenic microorganisms and organize its present status. PE, a by-product of charcoal-making and often considered as waste, was selected because of its availability in Rio Grande do Sul, Brazil.

Systematic reviews are considered the gold standard for evidence and used to evaluate the benefits and harms of healthcare interventions.[8] Such reviews are much more likely to yield valid conclusions.[9] The levels of evidence of the studies are ranked according to the degree of confidence, which is related to the methodological quality. Thus, systematic review of the literature occupies the top of the pyramid, followed by randomized clinical trials, cohort studies, case-control, case series, case reports, and lastly, expert opinion and research in animals or *in vitro*.[10] This study aimed to search the various electronic databases for articles and online systems of patents and included assays of PE antimicrobial potential for humans and animals.

Material and methods

Eligibility criteria: The inclusion criteria comprised articles and patents that investigated the antimicrobial activity of PE against pathogenic microorganisms of humans and animals.

Information sources and search

This systematic review was conducted according to the guidelines of the Cochrane Handbook for Systematic Reviews of Interventions,[11] following the four-phase flow diagram of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement.[12] This report is based on the PRISMA Statement. The following databases were screened: MedLine (PubMed), SciFinder, Web of Science, Scopus, Scielo, Google scholar, and ProQuest. For patents, the sources searched comprised the following: INPI, Google patents, Espacenet, Patents online, USPTO, and WIPO.

Table 1 – Search strategy.

Search strategy
Pubmed
#1 (“wood vinegar” OR “pyroligneous acid” OR “pyroligneous extract” OR “pyroligneous” OR “liquid smoke”)
AND
#2 (“Anti-Infective Agents” [MESH] “Antimicrobial activity” OR “Antibacterial activity” OR “Antifungal activity” OR “Anti-Infective Agents” OR “Agents, Anti-Infective” OR “Anti Infective Agents” OR “Antiinfective Agents” OR “Agents, Antiinfective” OR “Microbicides” OR “Antimicrobial Agents” OR “Agents, Antimicrobial” OR “Anti-Microbial Agents” OR “Agents, Anti-Microbial” OR “Anti Microbial Agents”)
Web of science
#1 Topic: (“wood vinegar”) OR Topic: (“pyroligneous acid”) OR Topic: (“pyroligneous extract”) OR Topic: (“pyroligneous”) OR Topic: (“liquid smoke”)
AND
#2 Topic: (“antimicrobial activity”) OR Topic: (“Anti-Infective Agents”)
Scopus
#1 (“wood vinegar”) OR (“pyroligneous acid”) OR (“pyroligneous extract”) OR (“pyroligneous”)
AND
#2 (“antimicrobial activity”) OR (“Anti-Infective Agents”)
SciFinder
#1 (“antimicrobial activity of Wood Vinegar”) OR #2 (“antimicrobial activity of pyroligneous acid”) OR #3 (“antimicrobial activity of pyroligneous extract”)
Scielo
#1 (“wood vinegar”) OR (“pyroligneous acid”) OR (“pyroligneous extract”) OR (“pyroligneous”) OR (“liquid smoke”)
AND
#2 (“antimicrobial activity”) OR (“Anti-Infective Agents”)
Google Scholar and ProQuest
#1 (wood vinegar) OR (pyroligneous acid) OR (pyroligneous extract) OR (pyroligneous) OR (liquid smoke)
AND
#2 (antimicrobial activity)
Patents databases: INPI, Google patents, Espacenet, Patents on line, USPTO and WIPO.
#1 (wood vinegar) OR (pyroligneous acid) OR (pyroligneous extract) OR (pyroligneous) OR (liquid smoke)
AND
#2 (antimicrobial activity)

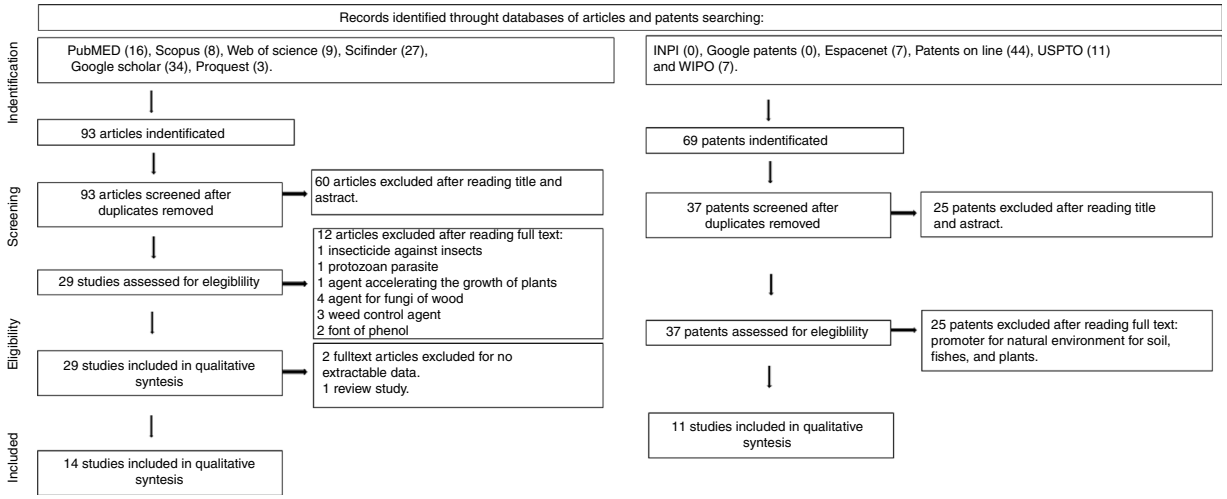

Fig. 1 – Flow chart.

The search strategy is described in Table 1, and the focused question is as follows: What is the antimicrobial potential of PE against human and animal pathogenic microorganisms?

Study selection and data collection

Study characteristics, demographic information, enrollment criteria, microorganisms tested, antimicrobial assay types, duration, results, control and groups, and sample size (Tables 2 and 3) were extracted independently for two reviewers (J.L.S.S. and V.B.S.G.). Missing information was sought from authors and/or inventors. The full text papers and patents were assessed independently and in duplicate by the reviewers. Any disagreement on the eligibility of studies included was resolved through discussion and consensus, and in case of disagreement, a third reviewer (R.G.L.) decided whether the article should be included. All titles and abstracts of articles and patents initially found were analyzed and selected in accordance to the eligibility criteria. No restrictions were considered regarding the language and year of publication. The reference lists of studies included were hand-searched for additional articles. Full copies of all potentially relevant studies were identified. Studies that met the inclusion criteria or for which insufficient data were available in the title and abstract to make a decision were selected for full analysis. Authors of the studies were contacted in case of missing data (*e.g.*, data provided in graphs); these studies were only included if the authors provided the missing information. Data extraction was conducted by consensus between the two researchers who conducted the collection.

Assessment of risk of bias

The risk of bias for all the included studies was assessed based on The Cochrane Collaboration's tool,[11] and the methodological quality was adapted from another systematic review of antimicrobial monomers used in dental materials.[13] The parameters used for the evaluation of methodology assays were discussed by the researchers involved, and judgment was carried out by group discussion. Assessment of risk of bias was conducted using Review Manager 5.3 software.

Results

Results of searches

After database screening [Pubmed (16), Scopus (8), SciFinder (27), Web of Science (9), Scielo (0), Google scholar (34), and ProQuest (3)] and removal of duplicates, 89 studies were identified. After title and abstract screening, 29 studies remained, and this number was reduced to 14 after careful examination of the full texts. The last electronic search was conducted on December 4th, 2017. Fig. 1 shows a flowchart summarizing the selection process of articles and patents.

Of the 89 articles initially recovered from all databases, 75 articles were excluded because they were not related to the antimicrobial activity of PE and failed to satisfy the selection criteria. A total of 60 articles were excluded after reading the title and abstract, and 12 were excluded after screening the full text because they tested properties other than antimicrobial pathology for humans or animals. One study tested an intestinal protozoan parasite that causes diarrhea in both humans and domestic animals[14]; one article used an agent accelerating the growth of plants and the development of roots.[15] PE has been studied as a repellent and insecticide against insect pests to crops,[5] as an antimicrobial agent for wood fungi, and as a weed control agent.[4,6,16–19] Two studies used the PE as phenol source and tested the antibacterial activity of separate components.[20,21] In addition, two articles were excluded as the researchers featured no access to full text versions,[22,23] and one article was a review study.[24]

In the patent databases, INPI (0), Google patents (0), Espacenet (7), patents on line (44), USPTO (11), and WIPO (7), the search strategy initially retrieved 69 patents. After removal of duplicates, this number was reduced to 37. A total of 25 patents were excluded after reading the titles and abstracts (Fig. 1) as they were not related to PE. Of the remaining 12

patents, 1 patent was excluded because it reported a promoter of environmental properties in soil, plants, and fish.[25] Twelve patents were included in the analysis.

Study characteristics

Table 2 describes the microorganisms tested, the source of PE, concentrations, methodologies used and the application area for each study from articles, and patents selected in the search. Table 3 shows the data of the patents included in this review.

All articles were published between 1998 and 2014. Most studies showed different cellulose sources to burn and generate different types of PE for its test as an antimicrobial agent. Several sources were commercial extracts,[1,7,26–33] and others were experimental extracts.[19,34,35] One study tested microbial strains isolated from dogs and cats.[26] One article analyzed four strains of pathogenic *Candida albicans*, which were isolated from patients suffering from urinary tract infection (two strains), vaginitis, and onychomycosis (one each),[7] and one tested another *C. albicans* strain.[30] One study tested PE as an agent to prevent viral epidemics in agricultural and human environments.[27] Various studies reported PE as a food preservative.[28,30,31,33,36,37] Only one *in vivo* study used *Salmonella*-infected Balb/c mouse model.[35] All *in vitro* studies reported the bacterial or antifungal activity of PE. The methods used for the tests included disk diffusion and minimum inhibitory concentration. The growth profile of the bacteria was examined via time-kill and viral inactivation assays.[27]

Regarding patent documents, the data showed 11 patents deposited from 1981 to 2009. Antibacterial, antifungal, and preservative proprieties of PE were found in these patents, which claimed PE incorporation into additives for the treatment of animal feedstuffs,[38] fiber-reinforced cellulosic food casing,[39] compositions for food preservation,[40–42] carbon fiber,[43] cosmetic composition,[44] biodeodorizing agent,[45] antimicrobial compositions,[46] pharmaceutical composition for symptoms of atopic dermatitis,[47] and compositions for oral microbes.[48]

Discussion

From the literature reviewed, most researchers have reported promising results for PE as an antimicrobial agent. The most studied activity for PE was for food preservative, with seven articles[28–33] and five patents presenting related results.[38,39,41,42,48] The PE was effective for the following microorganisms important for the food industry: *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella muenster*, *Salmonella seftenburg*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Lactobacillus plantarum*, *Listeria innocua*, *Listeria monocytogenes*, *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Saccharomyces cerevisiae*, and *Aspergillus niger*. Table 2 shows all the strains tested in each study. PE was used as antimicrobial in food preservation and has demonstrated abilities to reduce or inhibit pathogenic and spoilage organisms. Comparing results of studies presented difficulty given their various differences. These studies used different methodologies for the antimicrobial activity and for preparation of PE. Some studies provided no information on the preparations. Most commercial extracts were tested for use as food preservative; one was for viral epidemics, two for antibacterial activity, and another for antifungal infections.

Suñen et al. (1998) tested seven commercial preparations of PE used in food industry in Spain against *L. monocytogenes* and other pathogenic microorganisms.[30] In 2001, the same author tested four other commercial preparations of PE used in the Spanish food industry and evaluated their antimicrobial properties at low temperature against *A. hydrophila*, *Y. enterocolitica*, and *L. monocytogenes*. All four extracts effectively eliminated or suppressed the growth of *A. hydrophila* after 21 days.[31] Another study on food preservative examined the effects of selected PEs on the control of *L. monocytogenes* in frankfurters. Treatments with PE reduced and controlled *L. monocytogenes* growth in the most permissive franks for 10 weeks.[32] Milly et al. (2008) used PE fractions applied on ready-to-eat meat products to control the growth of inoculated *L. innocua* M1.[28]

In 2012, Van Loo et al. investigated the antibacterial activity of eight commercial PE samples against *S. enteritidis*, *S. aureus*, and *E. coli*, demonstrating that the commercial smokes inhibited the growth of these foodborne pathogens.[36]

Harada et al. (2013) determined the maximum inhibitory dilutions of bamboo PE against 104 *E. coli*, 112 *Staphylococcus pseudintermedius*, and 58 *P. aeruginosa* strains isolated from dogs and cats. The results indicated that bamboo pyroligneous acid exerts significantly inhibit the growth of representative bacterial pathogens from companion animals, although inhibition differed among species.[26]

Bamboo PE-inactivated picornavirus and encephalomyocarditis virus showed that phenol is the sole germicidal component, and that acetic acid augmented the phenol inactivating activity. These findings suggest that bamboo PE is a potentially useful agent to prevent viral epidemics in agricultural and human environments.[27]

Ibrahim et al. (2013) tested the PE, concentrated PE, and dichloromethane extracts of CPA, namely, DCM A and B, against four pathogenic strains of *C. albicans*. The results exhibited significant inhibition zones. The results also revealed that extract DCM B of CPA showed the most significant potential as an anti-candidal agent.[7] In 2014, the author concluded that *Rhizophora apiculata* PE may also be a broad antimicrobial agent against pathogenic bacteria.[49]

Other authors investigated the effects of temperature on antimicrobial properties of two commercial PE fractions and PE derived from pecan shells, against two common foodborne pathogens, *Listeria* and *Salmonella*. Understanding how storage temperature affects the efficacy of antimicrobials is an important factor that can contribute to reducing high levels and costs of antimicrobials and ultimately improve food safety for consumers.[33]

Three papers reported the development of new experimental PE. They tested different kinds of cellulose as feedstock: walnut tree branches, *Eucommia ulmoides*, olive branch, and rice hull. Wei et al. (2010) prepared and collected PE by pyrolizing walnut tree branches at three temperature ranges: 90 °C–230 °C, 230 °C–370 °C, and 370 °C–450 °C. All the PEs exhibited antibacterial activity. The high level of antibacterial activity of WP3 indicated that pyroligneous acids collected

Table 2 – Demographic data from articles and patents considered in this study.

	Microorganism tested	Pyroligneous extract source	Concentrations	Methods					Application area
				Assays	Sample size (per group)/ repetition of assays	Period	Positive control	Negative control	
[30]	*Bacillus cereus* (CECT 495), *Bacillus subtilis* (CECT 38), *S. aureus* (CECT 239 and 976), *L. monocytogenes* (CECT 932), *L. inocua* (CECT 4030), *Brochothrix thermosphacta* (CECT 847), *L. plantarum* (CECT 220), *L. brevis* (CECT 216), *L. coryniformes* (CECT 982), *L. lactis* ssp. *cremoris* (CECT 697), *L. lactis* ssp. *lactis* (CECT 185), *Leuconostoc carnosum* (CECT 4024), *Carnobacterium divergens* (CECT 4016), *E. coli* (CECT 533, 471, 405), *S. tyhimurium* (CECT 443), *S. enteritidis* (CECT 556), *Y. enterocolitica* (CECT 559), *P. aeruginosa* (CECT 378), *Vibrio vulnificus* (CECT 529) and *Rhodotorula rubra* (CECT 1159). Strains of *C. albicans*, *S. cerevisiae* and *P. aeruginosa* 022 were from their own collection (Spanish National Collection of Type Cultures Valencia, Spain).	Seven commercial smoke condensates.[1]	0.05, 0.1, 0.2 and 0.4% for L1, L4, S2 and S3; 0.2, 0.3, 0.4, 0.6 and 0.8% for L2; 0.5, 1, 2, 4, 8% for L3 and 0.5, 1, 1.5% for S2.	Agar dilution methods	$n = 2$/Twice.	24 and 48 h	TSA and MRS agar without smoke inoculated with the working cultures.	No	As food preservative.
[31]	*L. monocytogenes* (932), *A. hydrophila* (839) and *Y. enterocolitica* (559) (Spanish National Collection of Type Cultures, Valencia, Spain).	Four commercial smoke condensates.[1]	1% for the dried extract, 0.4% for L1, 0.6% for L2 and 4% for L3.	Broth and agar dilution methods	$n = 1$/three times	0, 1, 2, 7, 14 and 21 days	Inoculated TSB without smoke extracts served as positive controls.	Non-inoculated flasks were used for sterility control.	As food preservative.
[29]	*S. muenster, S. seftenburg, S. typhimurium, E. coli* 8677, and *P. putida; L. plantarum* and *L. innocua* M1; *S. cerevisiae* and *A. niger.*	Nine commercial liquid smokes.[1]	Fractions (v/v) were 0.5%, 0.75%, 1.0%, 1.5%, and 2.0% to 10.0%.	Broth or agar dilution methods	$n = 3$/three times	24 h	Petri dishes with no smoke extracts that were inoculated.	No.	As food preservative.
[32]	*L. monocytogenes* Scott A-2 (serotype 4b, clinical isolate), V7-2 (serotype 1/2a, milk isolate), 39-2 (retail frankfurter isolate), and 383-2 (ground beef isolate).	Two commercial liquid smokes.[1]	Dipped for 5, 15, 30, 60, and 90 s with liquid smoke extract.	Direct spiral plating methods	$n = 3$	10 weeks	Frankfurters not dipped.	No.	As food preservative.
[28]	*L. innocua* M1, a strain of *Listeria* resistant to the antibiotics streptomycin and rifampicin.	Four commercial liquid smokes.[1]	2%	Direct spiral plating methods	$n = 15$	2 and 4 weeks	Three samples of each meat product.	No.	As food preservative.

Table 2 (*Continued*)

	Microorganism tested	Pyroligneous extract source	Concentrations	Methods					Application area
				Assays	Sample size (per group)/ repetition of assays	Period	Positive control	Negative control	
[36]	*S. Enteritidis* (PTA 13A), *E. coli* 0157:H7 (ATCC 43888), *S. aureus* (ATCC 25923 and ATCC 6538), and two methicillin-resistant *S. aureus* (MRSA). *L. monocytogenes* 174 (serotype l/2a), *L. monocytogenes* 163 (serotype 4b), *S. typhimurium* 29, *S. typhimurium* LT2 (ATCC 19585), and *S. aureus* Col (MRSA).	Eight commercial liquid smoke extracts.	96%–0.375%.	Broth microdilution method	$n = 3$	24 h	Control containing PBS solution.	No.	As food preservative.
[27]	*Picornavirus, encephalomyocarditis* virus.	Two commercial liquid smokes.	*Not informed.*	Viral inactivation assay	*Not informed.*	6 h	*Not informed.*	*Not informed.*	As an agent for preventing viral epidemics in agricultural and human environments.
[26]	104 *E. coli*, 112 *S. pseudintermedius* and 58 *P. aeruginosa* strains isolated from dogs and cats.	One commercial liquid smokes.	Serial dilutions of BPA (*i.e.* 1/2, 1/3, 1/4, 1/5, 1/6, 1/7, 1/8, 1/9, 1/10, 1/11, 1/12, 1/13, 1/14, 1/15, 1/16, 1/17, 1/18, 1/19, and 1/20).	Maximum inhibitory dilution/agar method	$n = 1$	18 h	*E. coli*, *E. faecalis*, *P. aeruginosa*, *S. aureus* and *S. pseudintermedius* were used as quality controls.	No.	For antibacterial infections in animals.
[7]	Four strains of pathogenic *C. albicans* which were isolated from patients with suffering from urinary tract infection (two strains), vaginitis and onychomycosis (one each).	Four extract of liquid smokes.	Between 0.39 and 100.00 mg/mL.	Disk diffusion method; broth dilution method; time-kill assay	$n = 3$	24 and 48 h	*Not informed.*	No.	As an antifungal agent especially to treat candidal infections.
[1]	*B. cereus, B. subtilis, B. spizizenni, S. aureus*, MRSA, *S. epidermidis, Streptococcus pyogenes, S. faecalis, Citrobacter freundii, E. coli, Erwinia sp., K. pneumonia, P. mirabilis, P. aeruginosa, Salmonella typhi and Yersinia sp. (Industrial Biotechnology Research Laboratory Culture Collection.*	Four extract of liquid smokes.	Between 0.39 and 100.00 mg/mL.	Disk diffusion method; broth dilution method; time-kill assay	$n = 3$	24 h	Chloramphenicol (Sigma, Germany) at the concentration of 30 μg/mL was used as a positive control.	Commercial antibiotic disk GF A (Whatman, England) with 6.0 mm.	As an antimicrobial agent against pathogenic bacteria.

Table 2 (*Continued*)

	Microorganism tested	Pyroligneous extract source	Concentrations	Methods					Application area
				Assays	Sample size (per group)/ repetition of assays	Period	Positive control	Negative control	
33	*L. monocytogenes* 2045 (Scott A, serotype 4b, from Dr. Martin Weidemann, Department of Food Science, Cornell University, Ithaca, NY), *L. monocytogenes* 10403S (serotype l/2a, from Dr. Aubrey Mendonca, Department of Food Science and Human Nutrition, Iowa State University, Ames), *L. innocua* ATCC 33090, *L. innocua* Ml ATCC 33091, *S. typhimurium* LT2 ATCC 19585, and *S. heidelberg* ATCC 8326 (American Type Culture Collection, Manassas, VA).	Three commercial liquid smokes from Mesquite, Hickory and pecan shell.	Eight serial dilutions ranged to 48 and 0.375% (v/v).	Broth micro dilution/agar method	$n = 3$	24 h	Controls containing only PBS plus bacteria were included.	No	As food preservative.
19	*S. aureus, E. coli, Bacterium proteus, Bacterium prodigious*, and *Aerobacter aerogenes*.	Walnut tree branches.	Concentrations of 40, 20, 10, 5, 2.5, 1.25 and 0.625 mg/mL.	Disk diffusion and (EC50).	*Not informed.*	*Not informed.*	*Not informed.*	*Not informed.*	As natural germicide.
34	*S. aureus, E. coli, B. prodigious, B. subtilis, A. aerogenes, Pseudomonas sp.* and others non-human pathogenic.	*Eucommia ulmoides* Oliv. Branch.	Concentrations of 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.781 mg/mL.	Disk diffusion method.	$n = 1$/three times	48 h	Sterile water.	No.	As germicide.
35	*Salmonella enterica* serovar typhimurium (ATCC #14028) (S. typhimurium) American Type Tissue Culture Collection (ATCC, Manassas, Va., U.S.A.).	Rice (*Oryza sativa* L.) hull.	Concentrations of 0.1%, 0.5%, and 1.0% (v/v).	Disk diffusion method.	$n = 3$	24 h	*Not informed.*	*Not informed.*	As antimicrobial flavor formulations for application to human foods and animal feeds.
			For *in vitro* assay and (1.0%, v/w) for *in vivo* assay.	*Salmonella*-infected Balb/c mouse model.	Three groups of 10 mice each.	12/12 h for 48 h	PBS-treated control.	Vancomycin (20 mg/mL).	

Table 2 (*Continued*)

	Patents data				
[38]	*Bacillus stearothermophilus* ATCC 7953, *S. typhimurium* ATCC 14028, *C. albicans* ATCC 10231, *Aspergillus flavus* ATCC 9643, *Aspergillus niger* ATCC 9642, *Chaetomium globosum* ATCC 6205, *Penicillium funiculosum* ATCC 11797, *Chaetomium globosum* ATCC 6205, *Gibberella zeae* ATCC 24688, *Trichoderma viride* QM 9123, *Bacillus cereus*, *Enterobacter aerogenes*, *Serratia marcescens*, *Pseudomanas* sp., *Proteus* sp., and *Enterobacter* sp.	Cellulosic fiber materials, mainly hard wood fibers, such as hickory, maple and other hard woods.	*Not informed*.	Disk diffusion method	As antimicrobial formulations for application to animal feedstuffs.
[39]	P.sub.2B *Penicillium* P.sub.2C No easily recognizable conidial state; may belong in Mycelia Steriles P.sub.2D *Penicillium* P.sub.2E *Penicillium* P.sub.4 *Trichoderms* P.sub.5 *Paecilomyces* P.sub.9 *Paecilomyces* P.sub.11A *Penicillium* P.sub.11B *Penicillium* P.sub.12A *Aspergillus* P.sub.12B *Penicillium* S.sub.1 *Fusarium* S.sub.2 *Penicillium* S.sub.3 *Monocillium* S.sub.4 *Penicillium* S.sub.5 *Penicillium* S.sub.6 *Penicillium* R.sub.1 *Penicillium* R.sub.2 *Penicillium* R.sub.3 *Penicillium* V.sub.1 *Penicillium* V.sub.2 *Penicillium Penicillium* (either P.sub.2D or P.sub.12B) *Aspergillus glaucus* (source: T. LaBuza U. of Minnesota) *A. niger* ATCC 1004.	Royal Smoke AA.sup.(a); Royal Smoke A.sup.(a); Royal Smoke B.sup.(a); Royal Smoke 16.sup.(a); Charsol C-12.sup.(b); Charsol C-10.sup.(b); Charsol X-11.sup.(b); Charsol C-6.sup.(b); Charsol C-3.sup.(b); Smokaroma Code – 12.sup.(c); Code – 10.sup.(c); Code – S.sup.(c); Code – 6.sup.(c). (a) Griffith Laboratories, Inc. 12200 South Central Avenue, Alsip, I. (b) Red Arrow Products Co., P.O. Box 507, Manitowoc, WI. (c) Meat Industry Suppliers, Inc. 770 Frontage Road, Northfield, IL.	89 wt.%	Number of viable molds. Antimycotic action.	As a food preservative.
[41]	*L. monocytogenes*	ZESTI SMOKE (Code 10) Hickory Specialties, Inc. of Brentwood, Tenn.	Acetic acid in a concentration of about 6.5–8.0%; carbonyl 1.0–8.0%; 0.1–1.0%; and water 83–92.4% (w/v).	Wieners sprayed.	As food preservative.
[43]	*B. subtilis, S. aureus, S. epidermidis, E. coli, MidoriMinorikin, Serratia, Salmonella*.	Wood vinegar and bamboo vinegar, $T = 500–900\,^{\circ}C$	*Not informed*.	Disk diffusion method.	Hospital textile industry.
[44]	*E. coli*.	Wood vinegar is Quercus (by Caicos) $T = 80–150\,^{\circ}C$	Between 0.5% and 5.0%.	Detection of bacteria in the wood vinegar.	Cosmetic industry.
[45]	*E. coli*, *Salmonella* sp., *S. aureus*, *Vibrio* sp.	*Not informed*.	Between 3.0% and 5.0%.	Tested as to whether the growth inhibition on.	New natural bio deodorant composition.
[42]	*E. coli* 8677, *S. seftenberg, L. innocua* M1, *L. monocytogenes, S. cerevisiae, A. niger* spores.	ZESTI-SMOKE Code 1O and ZESTI-SMOKE Code V. Mastertaste of Crossville, Tennessee.	Between 0.5% and 5.0%.	Minimum inhibitory concentrations.	As a food preservative.
[46]	*E. coli*, *Salmonella* sp., *Bacillus* sp., *Staphylococcus* sp., *Vibrio* sp., *Aspergillus* sp., *Fusarium* sp., *Tricoderma* sp. *and Candida* sp. (*Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019, *Candida glabrata* ATCC 90.03, *C. albicans* ATCC 64550 and ATCC 90028).	*Not informed*.	Between 100 μL to 1600 μL.	Minimum inhibitory concentrations.	As antiseptic is added to the food.
[47]	*Trichophyton rubrum*.	Purified wood vinegar is acetic acid 2–4% by weight, formic acid 0.05 0.15 wt.%, Propionic acid 0.05 0.15 wt.%.	1–5% of weight of the total weight of the medicament.	Halo test.	A medicament of atopic dermatitis containing refined wood vinegar.
[48]	*Streptococcus mutans, Porphrymonas gingivalis, fusobacterium nucleatin* ssp. *polymorphum*.	ZESTI-SMOKE Code 1O and ZESTI-SMOKE Code V. Mastertaste of Crossville, Tennessee.	Between 0.01% and 50.0%.	Minimum inhibitory concentrations.	As oral antimicrobial.
[40]	*L. monocytogenes*.	ZESTI-SMOKE Code 1O and ZESTI-SMOKE Code V. Mastertaste of Crossville, Tennessee.	Between 0.05% and 5.0%.	Halo test.	As a food preservative.

Table 3 – Patents data, antibacterial compositions and claims related to pyroligneous extracts antimicrobial activity.

Patent	Country	Title	Year	Antibacterial composition	Claimed
US4308293[38]	United States	Antimicrobial treatment and preservation of animal feedstuffs	1981	Pyroligneous acid and pyroligneous acid complexes.	Preservative agents for the treatment of animal feedstuffs.
US4377187[39]	United States	Liquid smoke impregnated fibrous food casing	1983	Liquid smoke.	A fibrous reinforced cellulosic food casing with the impregnated liquid smoke providing antimycotic quality.
US5043174[41]	United States	Meat processing with Listeria monocytogene re-inoculation control stage	1991	Liquid smoke derivative product containing a minimum of carbonyl and phenol.	Compositions for antimicrobial treatment of food products.
JP2000160476 (A)[43]	*Japan*	Production of carbon fiber and carbon fiber produced thereby	2000	Extract of mugwort (*Artemisia princeps*) and one of pyroligneous acid from wood or bamboo.	Carbon fiber that has antimicrobial activity.
KR20030005075 (A)[44]	Korean	Cosmetic composition containing pyroligneous acid solution	2003	The cosmetic composition contains 0.5 to 5.0% by weight of a pyroligneous acid solution, based on the total weight of the composition.	A cosmetic composition containing a pyroligneous acid solution with antimicrobial activity and antioxidant activity for protecting the skin.
KR20030014052 (A)[45]	Korean	Natural biodeodorizing agent composition	2003	The Bacillus strain has a final concentration of 0.5x10 not 7 to 1x10 not 7, based on 3 to 5% pyroligneous solution.	A biodeodorizing agent composition with an excellent antimicrobial activity against putrefactive or pathogenic bacteria and a long-lasting deodorizing effect.
US20050175746 A1[42]	United States	Low flavor anti-microbials derived from smoke flavors	2005	Derivatives of liquid smoke.	Compositions for antimicrobial treatment of food products.
KR20060109757 (A)[50]	Korean	Silver-ionized wood vinegar having enhanced antimicrobial activity and use thereof for improving or preventing disease caused by pathogenic bacteria	2006	The silver-ionized wood vinegar is prepared by ionizing silver in wood vinegar with electrolysis.	Compositions for antimicrobial activity.
KR10200700428 68[47]	Korean	Pharmaceutical composition for ameliorating symptoms of atopic dermatitis without skin irritation Comprising refined nontoxic wood vinegar having no harmful materials	2007	Refined wood vinegar 24 wt.% of acetic acid, 0.05–0.15 wt.% of formic acid, 0.50–0.15 wt.% of propionic acid.	Pharmaceutical composition for ameliorating symptoms of atopic dermatitis and improving antimicrobial activity.
US20070212310 A1[48]	United States	Antimicrobial smoke flavor for oral microflora control	2007	Compositions that include low flavor antimicrobial liquid smoke derivatives.	Compositions and methods for inhibiting the growth of oral microbes in a subject.
US20090011096[40]	United States	Preservatives for food	2009	Combination of N long chain alkyl of di basic amino acid alkyl ester acid salt biocides with liquid smoke compositions.	Compositions for antimicrobial treatment of food products.

at higher temperature feature stronger inhibition effects on bacteria.[19]

PE of *E. ulmoides* olive branch was collected at different temperature ranges: 90 °C–200 °C, 200 °C–340 °C, and 340 °C–520 °C. The results showed that the maximum amount of the PE was collected at the range 200 °C–340 °C and also showed the most anti-pathogenic activities. After the preliminary analysis, phenols were considered the active components of bacteriostatic activity.[34]

A previously characterized rice hull PE was tested for bactericidal activity against *S. typhimurium* using the disk-diffusion method. The *in vivo* antibacterial activity of rice hull smoke extract (1.0%, v/v) was also examined in a *Salmonella*-infected Balb/c mouse model. The combination of rice hull smoke extract and vancomycin acted synergistically against the pathogen. The beneficial results suggest that the rice hull PE possesses the potential to complement wood-derived smokes as antimicrobial flavor formulation for application in human foods and animal feeds.[35]

Technology prospecting for PE as an antimicrobial agent: the patent search

Studies on PE in patents were older than articles. In 1981, PEs incorporating selective additives were used as antifungal and antibacterial preservative agents for the treatment of animal feedstuffs.[38] In 1983, PE was used in a fibrous reinforced cellulosic food casing with PE to provide antimycotic quality in the casing without separating antimycotic agent.[39] In 1991, a PE derivative product was applied to wieners post-peeling and before packaging to inhibit *L. monocytogenes* reinoculation and extend the shelf life of the wieners without adversely affecting their taste and/or edibility.[41] In 2004 and 2008, other patents developed methods and compositions for antimicrobial treatment of food products.[40,42]

The antimicrobial property of PE was used for the development of carbon fiber by soaking carbon fibers in a heat treatment solution mainly comprising PE from wood or bamboo.[43] PE was also used as biodeodorizing agent composition containing a culture solution; the PE solution showed an excellent antimicrobial activity against putrefactive or pathogenic bacteria and a long-lasting deodorizing effect.[45]

Two studies developed antimicrobial products for skin; a cosmetic composition containing a PE solution featuring an antimicrobial activity and antioxidant activity as a main component was obtained and considered suitable for skin protection.[44] A pharmaceutical composition for ameliorating symptoms of atopic dermatitis comprising refined PE was proven to avoid side effects, such as skin irritation and bad smell, by removing harmful materials and toxicity and improving antimicrobial activity.[47]

One patent developed a silver-ionized PE to enhance antimicrobial activity of PE against pathogenic bacteria: *E. coli*, *Salmonella* sp., *Bacillus* sp., *Staphylococcus* sp., *Vibrio* sp., *Aspergillus* sp., *Fusarium* sp., *Tricoderma* sp., and *Candida* sp.[46] Another patent used PE against pathogenic microorganisms of oral cavity and provided compositions and methods to inhibit the growth of oral microbes and promote oral care.[48]

The examination of PE as an antimicrobial agent, the broad spectrum of its properties, with and without enhancing additives, was evaluated against heat-resistant, spore-forming, aerobic bacilli, gram-negative bacillus associated with avian, and human enteritis. Various saprophytic molds (mycelial fungi) are associated with animal feeds, spoilage and, in several instances, human and animal mycotoxicoses. In each instance, our findings indicate that PE effectively and irreversibly reduces natural and/or experimental microbial contaminants associated to animal feedstuffs.[38]

Cellulosic sources of PE

The cellulose sources reported in the studies included in this systematic review comprised woods of hickory, mesquite, apple, pecan, moso bamboo (*Phyllostachys pubescens*), *Rhizophora apiculata*, walnut tree branches, and *E. ulmoides* olive. Branch and rice hull and five studies presented no information about these cellulose sources.

Future prospects for PE

Further *in vivo* studies are required for the development of new products using PE and the investigation of its possible use as an antimicrobial agent against resistant pathogenic microorganisms and development of pharmaceutical medicines. Many pathogenic microorganisms are tested for use as food preservative; this extract demonstrated a remarkable antimicrobial potential but was not identified in *in vivo* studies for humans or clinical assays.

Conclusion

In conclusion, the evidence suggests that PE features an antimicrobial activity against pathogenic microorganisms for humans and animals. Its use is prolonged and safe in food products. However, only one study was conducted on animals, and no clinical case was found.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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