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Article

Hop extracts and their utilizations: perspectives based on the last 10 years of research

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Hops (*Humulus lupulus* L.) is of notorious importance in the brewing industry, providing bitterness, aroma and acting as a preservative. Besides beer, innovative technologies are being developed on hop extracts, and this review aims to highlight their potential applications in the food, veterinary, and pharmaceutical industries. The emphasized topics include extraction methodologies, bioactive compounds, and motivations for use. Publications from the last 10 years that have claiming promising results, of extracts with verified composition or elucidated extraction methodology, in the development of new products such as: functional foods, food preservative, microbiota control in livestock, drug active ingredient or technological adjuvant were selected. This review collected relevant articles and subjected them to bibliometric mapping of the most common terms found in the title or abstract, in order to understand how the themes evolved in the time series and what the most recent trends are. Most of the claimed applications are related to antimicrobial potential, followed by other pharmacological applications.

Keywords: Hop extracts. Xanthohumol. Bioactive compounds. Plant extract. Bibliometric mapping.

INTRODUCTION

The species *Humulus lupulus* L., is a dioecious vine whose flowers, more precisely named cones or strobili, from the female plant, commonly called hops, are used in the brewing process. Its use in beer dates back at least a millennium and has been widely used in popular culture as a medicinal herb (Biendl, Pinzl, 2009). In recent decades, the dedication of science to studying the biological effects that certain plants or their extracts would have according to popular medicine has increased, resulting in numerous publications on methods of extracting and isolating phytocomplexes and their biological effects (Knez Hrnčič *et al*., 2019).

The interest of the industry, whether brewing or others, is in the inflorescences of the female hop plant. These cones have, in their central axis, a large number of trichome-like glands that store a variety of secondary metabolites, with quite different properties. These substances together are called lupulin. The most relevant compounds regarding their various applications are resins, essential oils and polyphenols. The compounds of greatest industrial interest for brewing are soft resins and essential oils, but polyphenols also have an influence on certain attributes of the final product (Dresel, Dunkel, Hofmann, 2015; Hieronymus, 2012).

Fractions of interest from hop compounds and their extraction methods

Resins are divided into two large groups, soft resins and hard resins. The groups have different physicochemical and chemical properties. Soft resins make up about 10-25% of the hops total weight, and are defined by hexane solubility. These comprise substances known as alpha-acids and beta-acids. In the brewing industry, when the wort is boiled, the alpha-acids are isomerized into iso-alpha-acids, compounds primarily responsible for beer bitterness and bacteriostatic properties. They also influence beer foam stability and shelf life. Among the biological effects of soft resins already described in the

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literature are promotion of gastric secretion, sedation, apoptosis inducer, anti-inflammatory, antimicrobial, livestock growth promoter, among others (Ano *et al.*, 2019; Flythe *et al.*, 2017b; Fukuda *et al.*, 2020a; Knez Hrnčič *et al.*, 2019)

The hard resins, insoluble in hexane, represent 3-5% of the hops total weight and have a more solid appearance than the soft resins which are a viscous fluid under standard temperature and pressure conditions. Despite their lower concentration, hard resins have a proportionally smaller impact on beer production compared to soft resins. However, there is evidence suggesting that even at low concentrations, they can positively affect foam formation, sensory aspects, and shelf life (Dresel, Dunkel, Hofmann, 2015). Among the biological effects of hard resins already described are anti-inflammatory, angiogenesis inhibition, metastasis inhibitor, sedative, antimicrobial, among others (Knez Hrnčič *et al.*, 2019)

The volatile oils in hops are responsible for much of the aroma of beer and are made up of terpenes and their oxidation derivatives, aliphatic hydrocarbons, and sulfur-derived compounds. More than 500 compounds of this class have already been identified in hops, and their concentration is around 0.5 to 3% of the weight. Among the most notable biological effects of those compounds are inhibition of tumor necrosis factor-α, inhibition of genotoxicity, insect repellent, among others (Bedini *et al.*, 2015; Lee *et al.*, 2015; Mitić-Ćulafić *et al.*, 2016) and the utilization of some molecules of essential oil as a synergistic enhancer of paclitaxel in antitumoral purposes (Zhang *et al.*, 2015).

Finally, the class of polyphenols comprises quite diverse compounds, many with antioxidant activity or biological effects such as catechins, flavonols, phenolic acids, stilbenes and prenylated flavonoids. Some polyphenols such as xanthohumol are also considered hard resins in some studies (Hofta, Dostalek, Basarova, 2004). The main biological effects are high antioxidant potential, anti-inflammatory, inhibition of tumor growth and formation, prevention, or treatment of cardiovascular diseases, among others (Liu *et al.*, 2015; Samuels, Shashidharamurthy, Rayalam, 2018; Yui, Kiyofuji, Osada, 2014).

In the brewing industry hops are rarely used in their unprocessed form, being more common in the forms of pellets and extracts. Pelletized hops oxidize 3 to 5 times slower than the raw cone, utilization 10 to 15% more, are more homogeneous and easier to pack and transport (Hieronymus, 2012).

Hop extracts are even more stable products and their use in breweries is a reality, from smaller ones to large industries. The extracts can achieve greater standardization than hops in natura, mitigating reproducibility problems caused by harvest variations. There are several types of extracts, some containing a higher fraction of resins, used to impart bitterness to beer, some with a higher fraction of volatile oils, used to add aroma, and some that have both fractions.

Breweries also use advanced extracts such as Iso-Extracts which have isomerized alpha acids and can be used for post-fermentation bittering and Tetra-Extracts which undergo a redox reaction to prevent light-mediated oxidation known to cause an off-flavor known as “light struck” (Huvaere *et al.*, 2005).

The composition of the extracts is derived from the extraction techniques and solvents employed. The main methods have supercritical CO2, liquid CO2 and organic compounds as a solvent. Extractions using CO2 as solvent have been preferred both for the yield and for the harmlessness of the residue, in line with the trend of the food industry. About 90% of all extracts sold today are produced from supercritical CO2 (Knez Hrnčič *et al.*, 2019).

METHODS

The temporal cut-off for this review was from 2012 to 2022. The search “hop extract” was used in the Web of Science, Google Scholar and PubMed search engines. All articles selected had the following criteria:

- Method in which the extract was made and/or detailed composition.
- Defined purpose of application with assays against negative and/or positive controls.

Studies with Tetra-extracts were also considered, due to the particularity that they are subjected to a well-known molecular modification. Articles that only presented composition with application suggestion but did not present trials were excluded. Results of antimicrobials trials that present half maximal inhibitory concentration (IC50) above 100µg/ml were not considered (Cos *et al.*, 2006)

Bibliometric Mapping

For a better visualization of how knowledge evolves according to a focal theme, bibliometric mapping is an information science resource that helps in the representation of clusters through data analysis (Börner, Chen, Boyack, 2003). The maps were included in this review as a tool for synthesizing the cited articles, in order to help understand how the themes developed over the years and the strength of the relationship between the terms, expressed by the distance between them as well as the links between terms.

The maps were generated using the VOSViewer software (version 1.6.18, Leiden, Netherlands), and aimed to represent the most frequent terms in the cited articles, clustering them by area. The software is suitable for generating maps with substantial amounts of terms, allowing the reader to quickly see the details of the terms and in which years they were more frequent (van Eck and Waltman, 2010). The setup used to generate the maps was the co-occurrence of terms present in the title and abstract of the files, from a .RIS text file. Terms with at least two occurrences were considered and approximately 80% of the most relevant terms were selected, manually excluding units (e.g.: mg, mg/L, etc.) and words with little scientific relevance (e.g.: day, week, subject, etc.). The rest of the parameters have been kept as default.

DISCUSSION

Emerging Technologies

Of all the properties that hop compounds present, antimicrobial activity is the one that provided the greatest number of new product development projects, among the articles evaluated in this review. The reason for the greater number of research around this property is the fact that antimicrobials are a product of cross-sectoral interest, with applications ranging from drugs to preservatives. On a lesser scale of importance are antioxidant properties and other pharmacological applications, detailed in Figure 1.

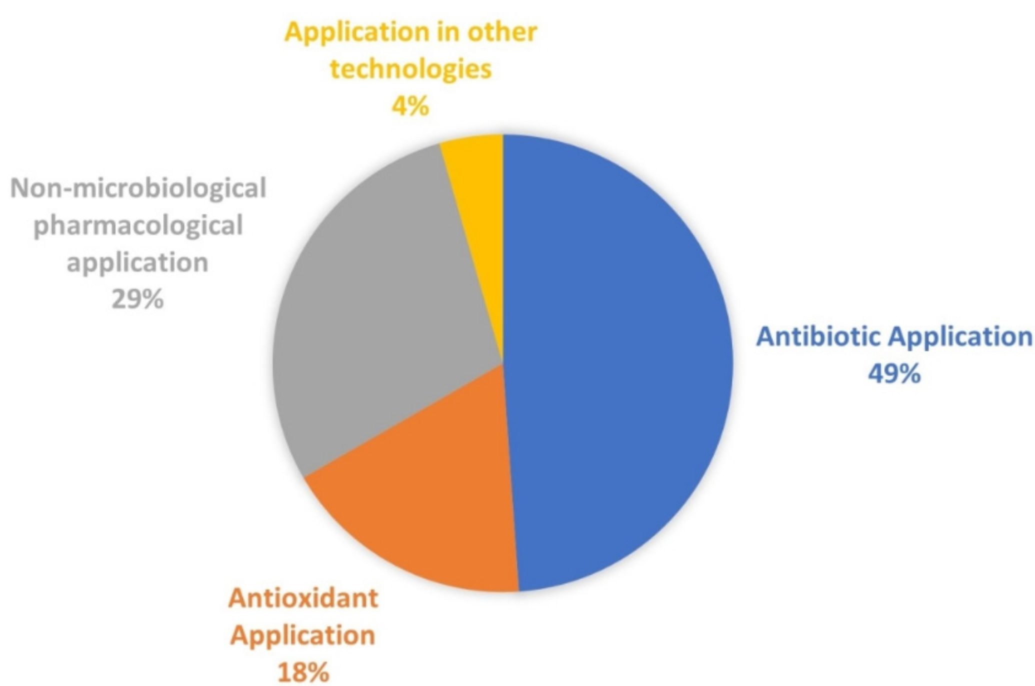

FIGURE 1 - Proportion of articles considered in this review by type of hop extract application.

Food

Emerging technologies with food utilization based on hop extracts can be divided into two broad classes: food adjuvant, acting to improve a certain characteristic of the final product, and food preservative, with the more specific aim of protecting food from microbial contamination.

Among the publications that showed potential for use as a food additive, antioxidant and possibly functional properties stand out, summarized in Table I.

Extracts based on water and milk at 90-95°C have been shown to be effective in increasing the strength and shape-holding ability of bread dough, and may be effective in improving the quality of flours with a weak gluten structure (Sokolova, 2021). Increases in polyphenols, antioxidant activity and total free amino acids were reported in bread and bread with a heterofermentative process (popularly called sourdough or "wild" fermentation) (Nionelli *et al.*, 2018). Similarly, an increase in antioxidant activity and total polyphenols was observed in bread with rice bran (Irakli *et al.*, 2019). Still in dough products, both hops infusion and hops in natura had the effect of reducing lipid and protein oxidation in lamb patties, in addition to increasing color stability (Villalobos-Delgado *et al.*, 2015).

The demand from the food industry for methods of microbiological protection is increasing. Emergence and prevalence of antibiotic-resistant microbiological contaminants are already considered an element of consideration in risk characterization, due to the greater severity of infections (FAO and WHO, 2021). Hop-derived products have attracted attention because, in addition to having antimicrobial properties, their use in beer is an indication of safety and harmlessness.

Although the bacteriostatic properties of hops have been described for a long time, research and technologies are only emerging that use this characteristic for purposes other than beer production. Some works also point to broader effects on other classes of microorganisms, different from what was thought about being restricted to gram-positive bacteria, but also antifungals. Significant inhibition of the proliferation of some molds was observed in breads (Nionelli *et al.*, 2018). Another work also found activity against Penicillium fungi, and several bacteria with a lower minimum inhibitory concentration than conventional antibiotics, but coming from hop seed extract, a less conventional approach, since the effects were not attributed to bitter acids or xanthohumol, but catechin and epicatechin (Alonso-Esteban *et al.*, 2019), and in this work the extract had 13.7 ± 0.5 mg/g of catechin and 3.9 ± 0.2 mg/g of epicatechin, amounts on the same scale of magnitude of extract obtained from hop flowers with microwave-assisted extraction (Carbone *et al.*, 2020).

The use of extracts with liquid or supercritical CO2 started to be used in breweries and they prioritize obtaining alpha-acids. The residue of the extractions was studied to verify if the compounds that are not extracted have applications. Weak antibacterial and antifungal activities, such as some anti staphylococcal and anti-fusarium activity, have been reported, which could be a starting point for research on synthetic derivatives since the extraction waste can be an economically viable raw material. However, the MIC80 values of the best compound, a molecular modified derivative of xanthohumol, for Staphylococcus in all cases were higher than that of ampicillin. The MIC50 for Fusarium was, for all extracts and isolated compounds, higher than that of amphotericin B (Bartmańska *et al.*, 2018). Therefore, some well-established antibiotics presented better activities than the hop extracts and derivatives. Table I presents all the hop extracts applications discussed.

TABLE I - Potential applications of hop extracts in food

Application	Technology	Effect
Bread	Extraction in water or milk at 90-95°C	Increases strength and quality of flours low in gluten. (SOKOLOVA, 2021)
Bread and heterofermentative bread (sourdough)	Aqueous extract (boil, 1h)	Increase in polyphenol content, antioxidant activity and total amino acids.
		Change in the taste and color of the crust (NIONELLI *et al.*, 2018)
Bread with rice bran and heterofermentative bread (sourdough)	Aqueous extract (water boiled for 6 hours then room temperature)	Increased antioxidant activity and total polyphenols in conjunction with rice bran. (IRAKLI *et al.*, 2019)
Lamb patty	Extraction in 2g/50ml boiling H2O and dispersion in natura (hop powder) unfiltered.	Increased antioxidant activity; reduction of lipid and protein oxidation; increased color stability during storage (VILLALOBOS-DELGADO *et al.*, 2015)
Bread and heterofermentative bread (sourdough)	Aqueous extract (boil, 1h)	Significant inhibition of: *A. parasiticus*, *P. carneum*, *P. polonicum*, *P. paneum*, *P. chermesinum*, *A. niger*, and *P. roqueforti*. (NIONELLI *et al.*, 2018)
Food/breads	Solid-liquid extraction of hop seeds in 80:20 methanol:water	Polyphenols with inhibition of *B. cereus, L. monocytogenes, E. faecalis, E. coli, Salmonella typhimurium and* fungus *Penicillium* (ALONSO-ESTEBAN *et al.*, 2019)
Chicken	Aqueous extract (boil 2g/L 30min)	Significant reduction ($p < 0.05$) in lactic bacteria count and *Brochothrix thermosphacta* without taste change (NIETO *et al.*, 2020)
Food	Ethyl acetate, ethanol or methanol extract from supercritical CO2 extraction residues	Moderate inhibition of *Fusarium* and *Staphylococcus* (BARTMAŃSKA *et al.*, 2018).
Food and crops	Extraction of essential oils by Clevenger apparatus	Activity against *Sitophilus granarius* (PAVENTI *et al.*, 2020).
Food and crops	Xanthohumol extract	Activity against pests such as the *Varroa destructor* mite (DEGRANDI-HOFFMAN *et al.*, 2012) and various insects (AYDIN *et al.*, 2017)
Package	Chitosan silica film with 0.3% beta acids	Improvement in markers of oxidative and microbiological stability (TIAN *et al.*, 2021).

The time series map of most common food technology terms is shown in Figure 2. The most cited term in articles referring to food is "hop extract" being the most frequent citations between 2020 and 2022 and related to baking. The crosslinks with "sourdough" reflect a recent interest to the foods with plant ingredients or with health claims, popularly called "natural". The term "hop infusion" also appears, however from 2016 to 2018. As the infusion is still an extract with simpler technology, it is an initial approach, which was further developed later. The most distant grouping has "antifungal activity" as the most relevant term, not addressed in more recent articles.

Most of the findings on antifungal activities had shown to be related with xanthohumol, which can be seen in the links between these terms and the "xanthohumol", so it can explain why it did not receive more extensive research in the following years.

The evolution of research themes began with the antimicrobial activities that are the most elucidated properties related to hops. In a novel way, the most current themes wanted to use the extracts as a technological adjuvant for baking. It is important to highlight that hops have strong bitter taste, so one challenge in using hops in food is not only finding its use as a conservative but adjusting the concentration that have the effect without much impact on taste.

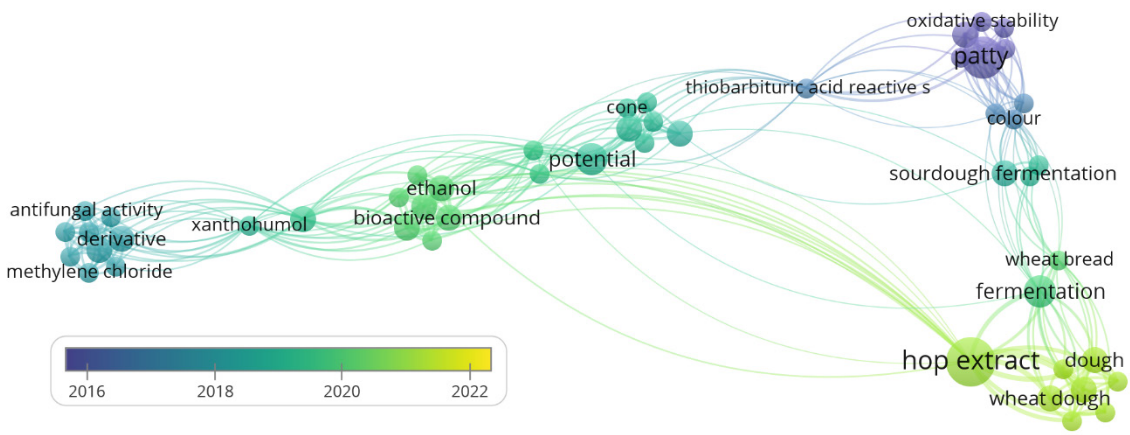

FIGURE 2 - Bibliometric mapping of the most common terms of application of hop extracts in food technology.

Zootechnics

Many diseases that prevail in society originate or occur concomitantly with livestock activity. Because of this, today some farms use protocols for the use of antibiotics to prevent certain pathogens from circulating in the herd. However, the use of many of these compounds is questioned due to the impact of their residues and antibiotic resistance. Despite the historical reduction in the cost of cattle, medical costs from infections caused by resistant microorganisms are not considered in this account (van Boeckel *et al.*, 2015), and safer alternatives such as some natural products are of interest to be developed. The potential use of hop compounds as growth promoters was considered, with the hypothesis that they are substitutes for ionophore antibiotics (Flythe, *et al.*, 2017).

Some emerging products studied are food additives in animal feed and hop derivatives have already been used. The research referring to these products can be divided between those that work directly with the herds, called here *in vivo* studies. The studies that use digestive models with organs will be called ex-vivo and the rest *in vitro*.

Considering studies in live animals, the addition of β-acids extracted from hops to chicken feed showed a positive change in metabolites relevant to meat quality, related to oxidative stability, when at a concentration of 30 mg/kg, although higher concentrations have had not very promising results (Zawadzki *et al.*, 2018), similar to a study carried out with a sample of 1440 chickens that showed weight gain and a higher feed conversion rate at the concentrations of 30 mg/kg and 60 mg/kg, although higher concentrations resulted in a decrease in weight (Bortoluzzi *et al.*, 2014). Also with this sample size of 1440 chickens, this same research group obtained significant results in the substitution of zinc bacitracin by β-acids as an antimicrobial (Bortoluzzi *et al.*, 2015). A product made with hops, wheat germ and chicory showed a therapeutic effect in a study with 1400 chickens in reducing the severity of intestinal damage caused by Clostridium perfringens, in addition to promoting weight gain during the infection (Vecchi *et al*., 2021). Another *in vivo* study with β-acids was carried out, evaluating the impact of adding the extract to the diet of 200 piglets weaned at different concentrations for 35 days, comparing with a negative control group that did not receive the addition of the extract and another control group that received a widely used antimicrobial, colistin. Diets with β-acids showed linear improvements ($P<0.05$) in live weight, weight gain, feed efficiency, and apparent digestibility of ether extract in the diet. Additionally, they demonstrated reduced fat, increased protein, and less lipid oxidation of the meat (Sbardella *et al*., 2018, 2016)

Methicillin-resistant Staphylococcus aureus is currently a concern in wound infections, and β-acids applied to wounds as well as *in vitro* analysis showed significant therapeutic effect against these infections in swine (Sleha *et al*., 2021)

In both *in vivo* and *in vitro* analysis, another hop extract, this time from the isolated compound xanthohumol, showed therapeutic effects in pigs against the Porcine Reproductive and Respiratory Syndrome virus (Liu *et al*., 2019a), corroborated by transcriptome scanning that revealed antiviral activity against the same virus in addition to oxidative stress reduction (Liu *et al*., 2019b).

Also *in vitro* studies or using digestive models were performed. Due to a preliminary study in an artificial rumen model which concluded that different varieties of hops in the diet increased energy efficiency and obtained a reduction in methane and total gas emissions (Narvaez *et al*., 2013b) sequence was given to understand which compounds would be responsible for this effect. A similar result was observed with alpha and β-acid extracts replacing monensin and also synergy with monensin and monensin with saponins, also altering the intestinal flora in *in vitro* analysis (Narvaez, Wang, McAllister, 2013a). Urinary excretion of excess ammonia produced by the rumen microbiota tends to be inversely proportional to the animal's weight gain, and hops not used in the industry, stored for 5 years, were still effective in reducing ammonia production by Clostridium sticklandii (Flythe *et al*., 2017a) and may be further subjected to extraction to obtain a standardized final product.

One consequence of the anti-microbial effects of hop compounds, which is being explored recently, is the ability to reduce gas emissions that contribute to the greenhouse effect, since livestock activity corresponds to 41% of the production of this gas whose effect is about 25 times higher than CO2 (USEPA, 2006). An *in vitro* analysis evaluated the emission of methane by Methanobrevibacter ruminantium, the main archaeal producer of gas in ruminants, and both treatments with aqueous extract of hops and commercial extracts of tetrahydro-iso-alpha-acids or beta-acids showed a reduction in methane emission (Blaxland, Watkins, Baillie, 2021). Table II presents all applications in zootechnics researched.

TABLE II - Potential applications of hop extracts in zootechnics

Application	Technology	Effect
Chicken	Addition of extract with 30 mg of beta acids / kg of food	Reduction of radical formation in the meat and increase of oxidative stability in general (Zawadzki *et al*., 2018)
Chicken	Addition of 30-60mg of beta acids in microencapsulated form per kg of food.	Effect similar to zinc bacitracin as a performance enhancer by increasing the feed conversion ratio (Bortoluzzi *et al*., 2015)
Swine	Addition of up to 360mg of microencapsulated beta acids per kg of food	Feed efficiency gain similar to colistin (Sbardella *et al*., 2016) increase in protein content, decrease in fat content and increase in fat oxidative stability (Sbardella *et al*., 2018)
Chicken	Additive containing extracted and modified hops.	Gut microbiota modulation, reduction of injury caused by *C. perfringens* and growth promoter similar to bacitricin (Vecchi *et al*., 2021)

TABLE II - Potential applications of hop extracts in zootechnics

Application	Technology	Effect
Swine	Pure beta acids obtained by extract purification with supercritical CO2	Activity against methicillin-resistant S. aureus in skin infections *in vivo* (Sleha *et al*., 2021) and *in vitro* (Bogdanova *et al*., 2018a)
Swine	Purified xanthohumol in intramuscular application 25mg/kg every 3 days.	Therapeutic effect in piglets infected with porcine reproductive and respiratory syndrome virus (Liu *et al*., 2019b)
Ruminant	Methanolic extract with alpha and beta acids (49 and 38mg/g, respectively)	Rumen microbiota modulation and reduction in methane emission (Narvaez, Wang, Mcallister, 2013)
Ruminant	Beta acid extract by supercritical CO2 or tetra-hydro-iso-alpha-acids	Reduction of methane emission from *Methanobrevibacter ruminantium* comparable to metronidazole (Blaxland, Watkins, Baillie, 2021)

The bibliometric mapping of the applications in zootechnics of hop extracts are shown in Figure 3. The two terms with the highest occurrence are “chicken” and “performance”, more frequent in the years 2018 and 2019.

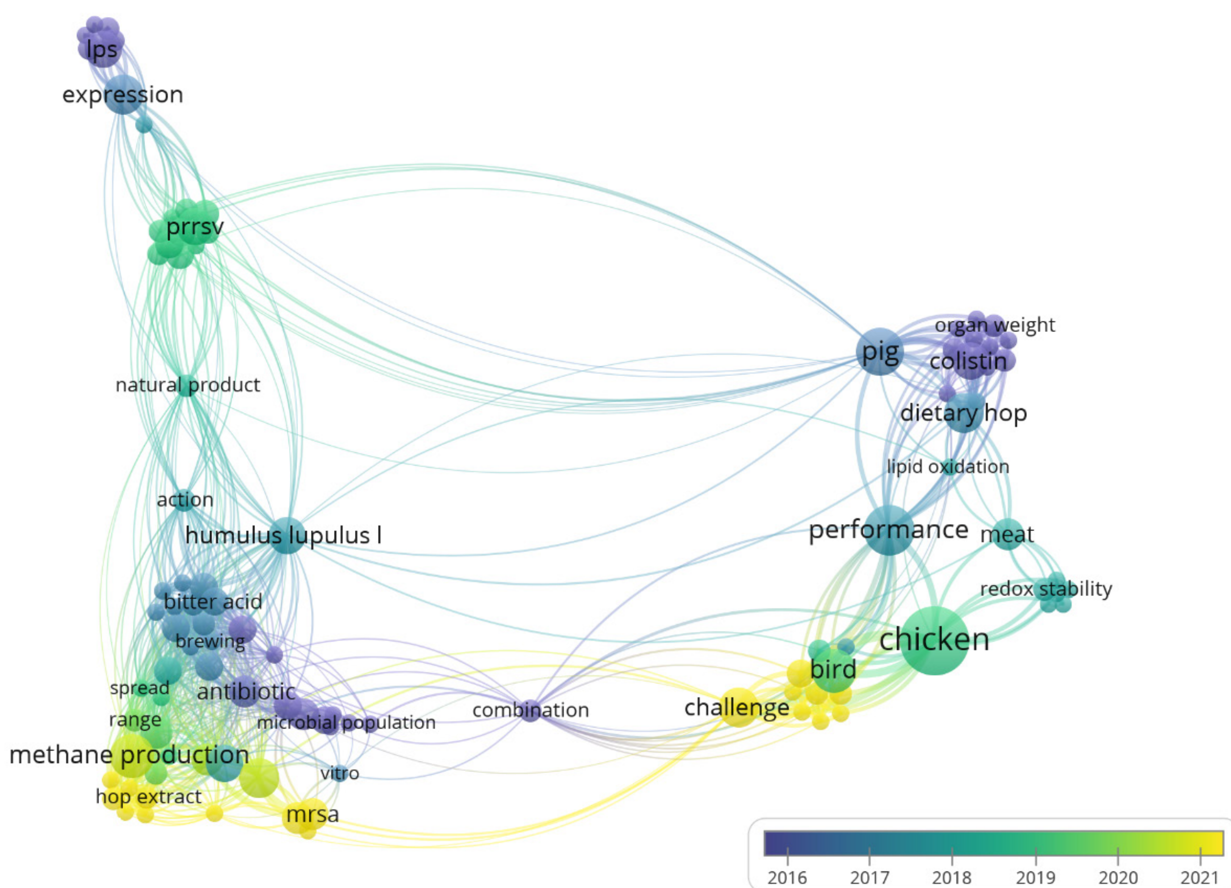

FIGURE 3 - Bibliometric mapping of the most common terms of application of hop extracts in zootechnics.

The map shows two main clusters, one more related with the intent of use as a performance enhancer related to lipid metabolism and feed conversion ratio; the second also focuses on applications such as antibiotics but is more concerned with the issue of methane production. The activity on methane producing archaea received more attention in the last years, and the other effects as an antibiotic needs more research, since the claims can be contradicted by the historic lack of evidence of activity against gram-negatives bacteria, which are a large part of the infectious agents in cattle and chicken.

The most recent term of greatest relevance is "methane production". Most publications deal with the use of hop compounds to optimize livestock activity, whether fighting infectious agents ("mrsa", "c perfringen"), improving yield or with environmental concerns, as demonstrated by "methane production", in line with more modern concerns about the impact of livestock on climate change.

Pharmacology

Due to antimicrobial activities, applications of hop extracts in pharmaceuticals have a long research history. However, in the last two decades, several other distinct biological effects have been found. In the case of resins, anti-inflammatory activities, gastric secretion promoters, sedative, apoptosis inducer, tumor inhibition and angiogenesis inhibition were found. In polyphenols anti-inflammatory, sedative, protection against genotoxicity, decreased mutated DNA replication, apoptosis inducer, angiogenesis inhibitor, metastasis inhibitor, anti-atherosclerotic, estrogenic, chemo-preventive, inhibition of nuclear factor kappa B activation, leukemic cell growth inhibition, anti-endometriotic, among other effects of polyphenols that are found in other sources such as resveratrol and quercetin (Hrnčič *et al.*, 2019).

Isolated xanthohumol is one of the most studied hop compounds in the last decade. The results obtained from isolated extracts had antimicrobial, antiviral, anti-angiogenic effects among others. Among the *in vivo* studies conducted with extracts composed of xanthohumol, many used rodents to elucidate effects related to lipid metabolism. Mice fed a high-fat diet were supplemented with xanthohumol at concentrations of 0, 30, and 60 mg/kg/day for 12 days. A dose-dependent reduction in weight gain was observed, along with decreases in plasma concentrations of low-density lipoprotein, insulin, and interleukin 6 (Miranda *et al.*, 2016). Similarly, it was observed not only a reduction in weight gain in mice fed the same type of diet, but also a decrease in liver weight gain, a relative decrease in plasma triacylglycerols and an increase in fat excretion in the feces (Yui, Kiyofuji, Osada, 2014).

Still related to dyslipidemias, transgenic mice were fed an atherogenic diet with 1% w/w of cholesterol and in one of the groups, xanthohumol at 0.05% p.p. was also added, keeping it for 18 days. Although food consumption was similar in all groups, a reduction in cholesterol accumulation in atherosclerotic regions was observed, inhibiting Cholesterol Ester Transfer Protein (Hirata *et al.*, 2012). Regarding age-related chemoprotective effects, xanthohumol contributed to modulate inflammation and promote liver protective effect in orally supplemented mice (Fernández-García *et al.*, 2019).

The anti-obesity mechanisms of xanthohumol were also associated with the AMPK pathway, and at concentrations of 6.25 μM and 25 μM it inhibited adipogenesis and induced lipolysis (Samuels, Shashidharamurthy, Rayalam, 2018).

With regard to effects related to diabetes, mice fed xanthohumol at a concentration of 10 mg/L and 8-prenylnaringenin (another hop flavonoid) at the same concentration showed, in addition to a reduction in fat accumulation, an increase in insulin sensitivity, glucose tolerance and improvement in lipid profile compared to control (Costa *et al.*, 2017), corroborating with the *in vitro* studies that elucidated that the probable mechanism is the reversible and non-competitive inhibition of α-glucosidase (Liu *et al.*, 2014)

In another approach, an attempt was made to make an extract that simulated brewery residue. This aqueous extract, using 100 g/L of water at 60°C, was prepared with hops heated for 120 h at 60°C and chemically treated to obtain the oxidized compounds, such as humulinones and iso-humulinones, analogously to the reactions that occur in the boiling of beer wort (Taniguchi *et al.*, 2015). An important consideration is that the heat treatment in the production of beer wort occurs in a different condition than that used in the publication, less time, and more temperature, around 95-100°C for 60-90 min, depending on the type of beer and altitude from brewery location. This extract was tested in adipose tissue cell culture and secretion of the gastrointestinal hormone cholecystokinin was observed, activating sympathetic nerves related to appetite inhibition, elucidating the mechanism that supports the hypothesis of prevention of metabolic diseases by these hop compounds (Yamazaki *et al*., 2019). The anti-obesity mechanism of oxidized or matured compounds seems to be different from the mechanism observed in iso-alpha acids, which is related to inhibition of intestinal absorption and hepatic lipolysis (Morimoto-Kobayashi *et al*., 2015). Finally, a clinical study in 200 humans, double-blind, placebo-controlled, evaluated aqueous extract of oxidized compounds in fat loss in people with BMI between 25 and 30 kg/m² with positive results in fat reduction without changing habits. No adverse effects were observed (Morimoto-Kobayashi *et al*., 2016).

Reduced alpha acids, in an extract in which the tetrahydro-iso-alpha acids were with different chemical substitutions, were administered to mice with obesity and diabetes for 8 weeks, in conjunction with a high-fat diet. Mice submitted to the treatment had a reduction in weight gain and normalized insulin sensitivity markers, in addition to an increase in the plasma concentration of anti-inflammatory cytokines and a reduction in the concentration of pro-inflammatory cytokines (Everard *et al*., 2012). As the extract is heterogeneous, future research should be carried out to verify whether the effect is due to specific active principles or to the set of molecules.

Aqueous extract of hops was added to a high-fat diet for rats at concentrations of 2 and 5% for 20 weeks. In relation to the control, there was a reduction in adipose tissue gain, adipocyte diameter and a decrease in glucose tolerance in different fractions of the analyzed extracts, indicating obesity prevention (Sumiyoshi, Kimura, 2013).

Regarding inflammation, some mice with induced senescence were treated with xanthohumol at 5mg/kg/day for 30 days. Treated mice had fewer pro-inflammatory molecules in their brains compared to the control group, preventing the aging effects of nervous tissue. (Rancán *et al*., 2017). Neuroprotective effect was also observed in rats with induced cerebral ischemic attack, reducing infarct size in groups treated with 0.2 and 0.4 mg/kg in intraperitoneal injections (Yen *et al*., 2012). Xanthohumol isolated in an *in vitro* study was also able to reduce oxidative stress, endoplasmic reticulum stress and chemoprotective effects related to the development of Alzheimer's disease (Huang *et al*., 2018).

Some cancer-related experiments were also performed. Xanthohumol showed cytotoxicity in breast cancer cells and inhibited their proliferation more than other chalcone (Kim, Lee, Moon, 2013). By inhibiting nuclear factor kappa B activation, xanthohumol also inhibited angiogenesis in human cell pancreatic cancer induced in rats fed 10 mg/kg/week (Saito *et al*., 2018).

The set of anti-inflammatories, anti-obesity, cancer-preventive and immunological effects may be an interesting approach in the prophylaxis of nutrition-related non-communicable diseases (Iniguez, Zhu, 2021).

Antiviral effects of xanthohumol have also been studied *in vivo*. Tree shrews infected with hepatitis-c virus and submitted to intraperitoneal application of xanthohumol showed less liver damage and reduced steatosis and fibrosis (Yang *et al*., 2013) supported by *in vitro* study that demonstrated inhibition of virus replication (Lou *et al*., 2014). Similarly, there was inhibition of the main protein of the coronavirus, related to replication, in a test in monkey kidney cells infected by the virus (Lin *et al*., 2021).

The increase in the occurrence of infections acquired in the hospital environment, originating from bacteria resistant to antibiotics, makes the search for new efficient compounds necessary. One of the most common genera in hospital-acquired diarrhea is Clostridium, with Clostridium difficile currently being an emerging infectious agent of great concern. The MIC and MIB for this bacterium were evaluated separately for alpha acids, beta acids and xanthohumol, and for xanthohumol the MIC order of magnitude was similar to that found in metronidazole (Cermak *et al*., 2017).

Gram-positive bacteria such as S. aureus, S. epidermidis and Cutibacterium acnes are mainly responsible for the occurrence of infectious skin diseases such as those that cause acne, and these microorganisms are often resistant to antibiotics and easily form biofilms. Strains of S. aureus (five), S. epidermis (three) and C. acnes (one) had their inhibition and biofilm formation analyzed in the presence of hydroalcoholic extract of hops. The extract showed a MIC lower or equivalent to ciprofloxacin in 6 of the 9 strains investigated and in relation to the reduction in the biofilm the hops extract was able to cause a significantly greater reduction in 5 of the 9 strains (Di Lodovico *et al.*, 2020). Although not a broad-spectrum result, it is notable that some antibiotic-resistant strains are sensitive to the extract. The authors also associated the results not only with alpha acids and with beta acids, but also demonstrated their possible correlation with phenolic compounds such as resveratrol, gallic acid and rutin. Similar activity has already been reported in relation to infection with Leishmania mexicana, Trypanosoma brucei and methicillin-resistant S. aureus, but attributed to xanthohumol, lupulone and desmethylxanthohumol (Bocquet *et al.*, 2019).

Extracts with isomerized alpha acids have been studied for their activities on the nervous system. Mice fed a high-fat diet and given daily iso-alpha-acids had less neuronal loss and hippocampus atrophy, indicating less cognitive impairment than controls, indicating protection against obesity-induced dementia (Ayabe *et al.*, 2018). These results converge with a study also carried out in rodents that observed a reduction in inflammation in the hippocampus and an improvement in brain activity in an Alzheimer's model in rats with short-term ingestion of iso-alpha-acids (Ano *et al.*, 2019). In addition to the isomerized acids, oxidation derivatives were administered for 12 weeks in a double-blind clinical study to assess their impact on the cognition of adults aged 45-64 years. The group that received the extract with oxidized bitter acids showed improvement in verbal fluency and less mental fatigue than the group that received the placebo. (Fukuda *et al.*, 2020b).

Extract with about 30% iso-alpha-acids had a positive effect in attenuating hepatic steatosis induced by alcohol consumption, possibly due to the mechanism of induced nitric oxide synthetase, although the authors relativize that the effect may also be due to other compounds in the extract or synergies between molecules (Hege *et al.*, 2018).

Antioxidant properties were also studied in the pharmacological approach, using *in vitro* human plasma as a model. Extraction was performed sequentially with methanol and hexane, and both fractions were tested independently to verify the antioxidant potential according to their respective compositions. The fraction of the hexane layer, which was composed of both alpha and beta acids, had greater free radical scavenging activity than the methanolic layer, which was enriched in alpha acids and xanthohumol. However, both fractions had only moderate activity, which is to be expected from phenolic compounds. Similarly, the extract with a mixture of alpha and beta acids inhibited lipid peroxidation more than the extract with alpha acids and xanthohumol, indicating that alpha and beta acids have an important contribution to antioxidant activity and possibly greater than xanthohumol (Kontek *et al.*, 2021). Table III presents the potential applications of hop extracts in pharmacology.

TABLE III - Potential applications of hop extracts in pharmacology

Application	Technology	Effect
Obesity and dyslipidemia	Isolated xanthohumol supplemented in food for 12 days at concentrations of 30 and 60mg/kg/day	Decreasing dose-dependent effect of weight gain, plasma LDL and IL-6 concentration in mice (Miranda *et al.*, 2016) Decreased hepatic weight gain, plasma triglycerides and increased fecal excretion in mice (Yui, Kiyofuji, Osada, 2014)
Obesity and dyslipidemia	Isolated xanthohumol supplemented at 0.05% in an atherosclerotic diet	Reduction of cholesterol accumulation in atherosclerotic regions by inhibiting esterified cholesterol transfer protein in mice (Hirata *et al.*, 2012)

TABLE III - Potential applications of hop extracts in pharmacology

Application	Technology	Effect
Obesity and dyslipidemia	Isolated xanthohumol	Inhibitor of adipogenesis and induction of lipolysis partially via AMP-activated protein kinase (Samuels, Shashidharamurthy, Rayalam, 2018).
Diabetes and dyslipidemia	Xanthohumol 10mg/L + 8-prenylnaringenin 10mg/L	Reduced fat accumulation, increased insulin sensitivity, glucose tolerance, and improved lipid profile over control (Costa *et al*., 2017)
Obesity and dyslipidemia	Extract derived from brewer's wort production residue (humolinones and iso-humolinones)	Secretion of the gastrointestinal hormone cholecystokinin in vitro (Yamazaki *et al*., 2019) and fat reduction in a double-blind clinical study (Morimoto-Kobayashi *et al*., 2016)
Obesity and type II diabetes	Tetra-iso-alpha-acids with different chemical substitutions supplemented in the diet	Reduction in weight gain, normalization of insulin sensitivity markers, increase in plasma concentration of anti-inflammatory cytokines and reduction in the concentration of pro-inflammatory cytokines in mice (Everard *et al*., 2012)
Obesity and diabetes	Different fractions of aqueous extract of hops supplemented in the diet at 2 and 5%	Reduction in adipose tissue gain, adipocyte diameter and decreased glucose tolerance (Sumiyoshi, Kimura, 2013).
Alzheimer's disease/ neuroprotection	Isolated xanthohumol supplemented in food	Reduction of pro-inflammatory molecules in nervous tissue (Rancán *et al*., 2017)
Stroke/neuroprotection	Isolated xanthohumol in peritoneal application 0.2 and 0.4 mg/kg	Reduction of infarct size in induced ischemic stroke in treated groups (Yen *et al*., 2012).
Alzheimer's disease/ neuroprotection	Xanthohumol in vitro	Reduction of oxidative stress, endoplasmic reticulum stress and chemoprotective effect against the development of Alzheimer's disease (Huang *et al*., 2018).
Hepatoprotection	Xanthohumol supplemented in diet 1 mg/kg/day	Inflammation modulation and liver protector (Fernández-García *et al*., 2019).
Obesity-induced dementia	Iso-Alpha Acid Extract in diet	Reduction of neuronal loss and hippocampus atrophy (Ayabe *et al*., 2018) Reduced inflammation in the hippocampus and improved brain activity (Ano *et al*., 2019).
Obesity-induced dementia	Extract derived from brewer's wort production residue (humolinones and iso-humolinones).	Improved verbal fluency and less mental fatigue than placebo in double-blind clinical trial (Fukuda *et al*., 2020).
Anticancer	Isolated xanthohumol	Cytotoxicity and inhibition of proliferation in breast cancer cells (Kim; Lee; Moon, 2013). NF-kB inhibitor. Angiogenesis inhibition in pancreatic cancer (Saito *et al*., 2018).
Antiviral	Xanthohumol in peritoneal application	Reduction of liver damage and reduction of steatosis and fibrosis caused by hepatitis C virus (Yang *et al*., 2013)

TABLE III - Potential applications of hop extracts in pharmacology

Application	Technology	Effect
Antiviral	Xanthohumol	Hepatitis C virus replication inhibition (Lou *et al.*, 2014) and protease inhibition related to coronavirus replication (Lin *et al.*, 2021).
Antibiotic	Xanthohumol	MIC and MIB similar to metronidazole in *Clostridium difficile* isolated from patients with nosocomial diarrhea (Cermak *et al.*, 2017)
Antibiotic	Commercial extract of supercritical CO2, lupulone and xanthohumol.	Inhibition and reduction of Methicillin-resistant *Staphylococcus aureus* and Vancomycin-Resistant *Enterococci* biofilm formation (Bogdanova *et al.*, 2018b).
Antibiotic	Hop alcoholic extract	Inhibition and reduction of biofilm formation in gram-positive bacteria related to antibiotic-resistant skin infections (Di Lodovico *et al.*, 2020).
Antibiotic	Hydroalcoholic extract of hop cones, leaves, stems and rhizomes further purified to concentrate xanthohumol, lupulone and deoxyxanthohumol	Antibiotic effect against *Leishmania mexicana*, *Trypanosoma brucei* and methicillin-resistant *S. aureus* (Bocquet *et al.*, 2019).
Antioxidant	Extrato feito por partição hexano e metanol.	Fraction of hexane rich in alpha and beta acids inhibited lipid peroxidation (Kontek *et al.*, 2021).
Control of disease vectors	Essential oil extract	Toxic for mosquitoes and molluscs vectors of diseases (Bedini *et al.*, 2016).

The bibliometric mapping of the most common terms in pharmacology is shown in Figure 4. The substantial number of articles in this area generated a more complex map, with more links between terms, with no cluster that was too isolated. The most frequent term was "xanthohumol" followed by "acid", the latter being related to hop bitter acids. The most recent term of greater relevance is "oxidative stress". Although there are many *in vivo* studies and some clinical trials, the term "oxidative stress" has been inflated due to many *in vitro* tests and its relevance ends up being reduced in fact, since xanthohumol is a chalcone and antioxidant activity is expected from phenolic compounds. This way it needs to be retraced that the bioavailability is of main importance on drugs, and the clinical evidence have to be made until final claims.

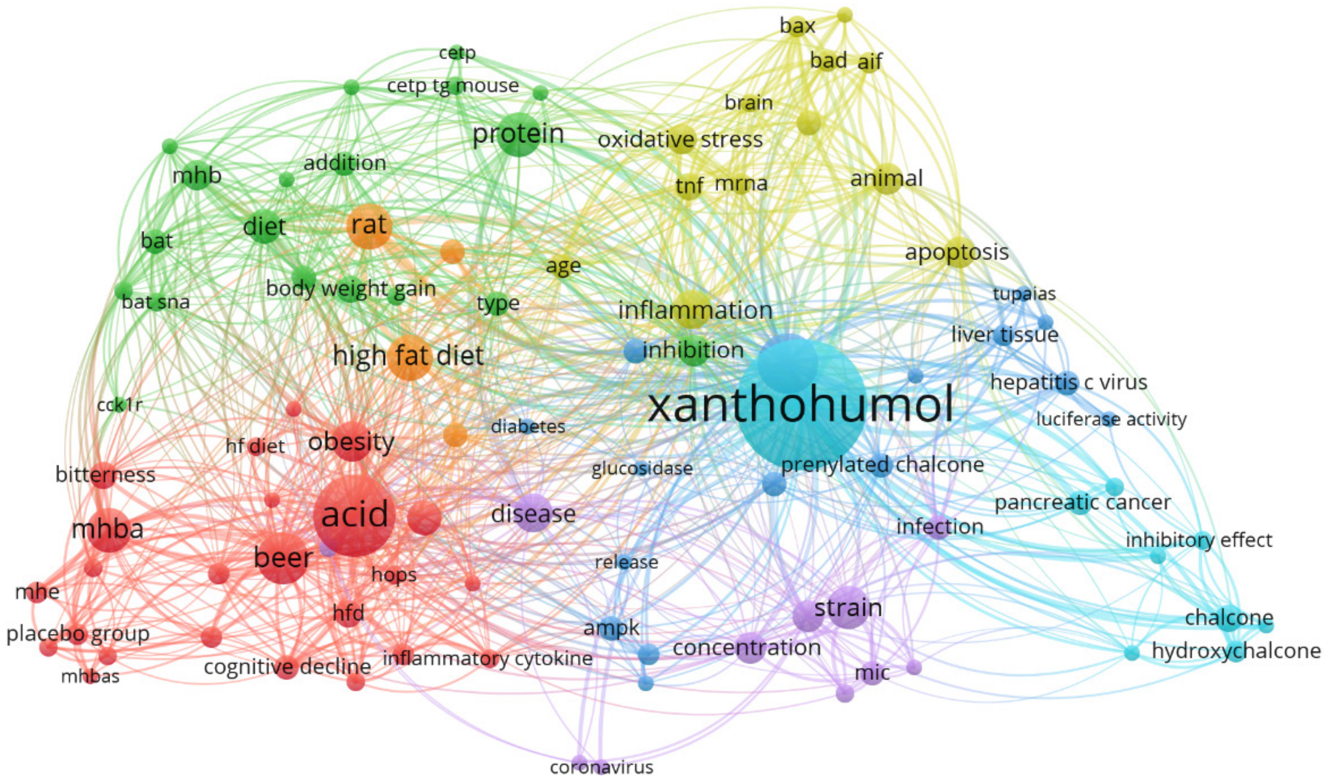

FIGURE 4 - Bibliometric mapping of the most common terms of application of hop extracts in pharmacology.

While the oldest articles in the time series have as main terms "high fat diet", "rat" and "obesity" indicating that the studies carried out between 2014 and 2016 are related to lipid metabolism, the most recent are "oxidative stress", "disease" and "cognitive decline" indicating a trend towards studies of neuroprotection and mechanism of action.

The non-clustered map needs attention, because the general lack of focus on a topic may be an indication that the findings is of limited relevance or that researchers have not yet found the finer line to pursue. Moreover, it is not negligible how xanthohumol drew attention and had connections with almost all sides of the big cluster, as if it were a great gravitational center for research into hop extracts in the pharmaceutical field.

CONCLUSION

This review made a specific cut, highlighting the applications of hop extracts in 3 different areas. The exclusion of articles with hops *in natura* was intended to focus on practical and reproducible applications, since there are at least 105 different hop varieties, with different concentrations of their secondary metabolites, it is inefficient in scientific-technological terms to give the same weight for these results in relation to studies carried out with extracts with the described methodology and, in most cases, with their composition finely analyzed by quantitative methods.

It is noticeable that, in the last 10 years, a lot of research has been done looking for applications of hop compounds. It is clear that antimicrobial properties are transversal to the 3 areas considered here and consist as the main property of hop extracts. This antimicrobial activity meets the global need for diversification of antibiotics, both for human use and for animals that are food raw materials. Some care needs to be taken when antimicrobial and antioxidant activities are reported. In the case of antioxidant activity, it is important to note that this is what would be expected to be found in phenolic compounds, and if not surprising activity, it might be not so relevant. Regarding the antimicrobials tested *in vitro*, caution is required with the method and reported concentrations, as well as the comparison with other antibiotic compounds as a positive control. In all cases, bioavailability must be considered in order not to extrapolate certain findings to an overblown conclusion without performing rigorous clinical trials.

Recent studies on hop extracts as a flour quality enhancer show that there are some possible applications that never were approached. Future studies to determine the activity of isolated compounds or with molecular modifications seeking pharmacological optimization will be necessary for greater assertiveness in applications. Nevertheless, several emerging approaches deserve to be highlighted, such as the possible modulation of lipid metabolism, related to several diseases and with impact in livestock maintenance. It also deserves attention that some research approaches are restricted to the same group or researcher and require reproduction by the international scientific community to confirm the results.

Bibliometric mapping had the role of being a visual resource for researchers to direct their studies, choosing to delve into more recent topics or even closing knowledge gaps left by some past studies. It is notable that, even in 3 areas with their peculiarities, some applications such as antimicrobials and antioxidants are transversal, with studies conducted with extracts with different bioactive compounds from hops. Subjects that have lost relevance over time, even with positive initial results, may represent gaps to be further researched or approaches that presented unsolved difficulties. Researchers should consider these points when dedicating themselves to a certain theme and reflect on whether the fact that the interest in the research has diminished or disappeared, perhaps the active concentrations found are of little relevance or some important methodological problem was the cause.

The hotspots are certainly in the area of microbiology, such as antibiotics, preservatives or intestinal microbiota modulators, and new approaches seeking new technologies related to extracts are interesting, such as the search for nano encapsulated pharmaceutical forms, use in synergy with other drugs or even molecular modifications on certain compounds aiming to obtain a stronger activity.

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Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

AUTHORS' CONTRIBUTIONS

Raul Santiago Rosa was responsible for the idea, literature search, draft, analysis, and bibliometric mapping. Suzana Caetano da Silva Lannes was responsible for critically reviewing the work and suggestions of exclusion criteria.

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