beverages

MDPI

Brief Report

The Antibacterial Effect of *Humulus lupulus* (Hops) against *Mycobacterium bovis* BCG: A Promising Alternative in the Fight against Bovine Tuberculosis?

James Blaxland [1,*], Richard Thomas [1] and Leslie Baillie [2]

[1] ZERO2FIVE Food Industry Centre, School of Sport and Health Sciences, Llandaff Campus, Cardiff Metropolitan University, Cardiff CF5 2YB, UK; rwthomas@cardiffmet.ac.uk
[2] School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Redwood Building, King Edward VII venue, Cardiff CF10 3NB, UK; bailliel@cardiff.ac.uk
* Correspondence: jablaxland@cardiffmet.ac.uk

Abstract: The female flowers of the *Humulus lupulus* plant or Hops have been used extensively within the brewing industry for their aroma and bitterness properties. It was also found that beer that contained hops was less likely to spoil, thus revealing the antimicrobial potential of these plants. One species of bacteria, *Mycobacterium* spp., is of particular interest as it is the causative agent of both human and animal forms of tuberculosis (TB). In this study an aqueous extraction process was employed to analyse the antibacterial properties of 50 hop extracts (45 individual variants); against *M. bovis* BCG. Using an agar well diffusion assay we found that all hops exhibited a level of inhibitory activity which ranged from 1.2 mm (+/− 0.08 mm) in the case of hop variant; Target, to 15.7 mm (+/− 0.45 mm) in the case of hop variant Citra. The Citra variant had a minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 16% *v*/*v*. This is the first study to analyse a wide range of hops for their antimicrobial potential against *M. bovis* BCG and recommends that further research focuses on other *Mycobacteria* spp., the potential for antimicrobial synergy and the antibacterial effect of individual components.

Keywords: *Humulus lupulus*; hops; mycobacteria; *M. bovis* BCG; natural products; antibiotic resistance

Citation: Blaxland, J.; Thomas, R.; Baillie, L. The Antibacterial Effect of *Humulus lupulus* (Hops) against *Mycobacterium bovis* BCG: A Promising Alternative in the Fight against Bovine Tuberculosis? *Beverages* **2022**, *8*, 43. https://doi.org/10.3390/beverages8030043

Academic Editor: Dimitris P. Makris

Received: 30 June 2022
Accepted: 21 July 2022
Published: 25 July 2022

1. Introduction

Plants and their derivatives represent a diverse resource from which discovering new, efficient and safe antibiotics is a priority. Research is currently focusing on novel sources and early pharmacopeias' to discover plants or extracts which were once considered effective but perhaps fell out of favour with the introduction of antibiotics. The plant *Humulus lupulus*, known as the hop, is a potential source of novel antimicrobial activity [1].

The hop plant itself has been employed in the brewing industry due to its positive effect on flavouring and aroma, its perhaps less known for its antibacterial properties against a range of clinical and food related microorganisms such as *Listeria monocytogenes* [2]. The antibacterial effect can be attributed in part to the presence of humulone (alpha acid); lupulone (beta acid) and the polyphenol xanthohumol [2]. The addition of hops in the beer brewing process has been observed to prevent beer spoilage when compared to its 'unhopped' counterpart [3]. Current research has focused on developing the commercial potential of hops such as: The use of specific hop extracts to improve the aroma and flavour of beer [4], The acceleration of lautering [5], reduction of methane emissions [6] and as a food preservative [2].

Given the emergence of multidrug resistant strains of *Mycobacterium* [7–10]. Several studies have investigated the role of the hop plant as a treatment or preventative agent against *Mycobacterium tuberculosis* infection [11–13]. Serkani and colleagues observed that drug resistant strains of *M. tuberculosis* were inhibited by exposure to hop extracts

highlighting the potential for hops or their extracts to be exploited for their antimicrobial potential, particularly against mycobacterium spp. [12].

A related organism to *M. tuberculosis* is *M. bovis* which is responsible for the animal form of the disease; Bovine tuberculosis (bTB). Current legislation prevents the use of antimicrobials (without written consent of the Welsh Government (Wales) [14] and The secretary of State (England) [15] in cattle either as a treatment or preventative measure, the only option is for infected animals to be culled (TB in Wales 2010) [16]. Further to this, current European legislation has been updated to reduce the use of antibiotics in animals as growth promotors (Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC). The legislation which came into force in January 2022 prevents the routine administering of antibiotics in groups of animals, bans the preventive use of antimicrobials via medicated feed [17].

In Wales, UK, between March 2020 and March 2021, 10,258 cattle were slaughtered for TB control which was a 11% decrease on the previous 12 months when 11,559 cattle were slaughtered, bovine infection with this pathogen is estimated to cost the UK economy over £100 million per year [16].

M. bovis, similar to *M. tuberculosis*, is also classed as a Category III pathogen and replicates at a similar rate making it an impractical bacterium to use in high through put screening assays. In contrast, the vaccine strain of *M. bovis*, *Mycobacterium bovis* Bacillus Calmette- Guérin [BCG) rarely causes disease in healthy individuals and replicates in the laboratory at a similar rate to *M. smegmatis* [18]. In vitro studies have shown that BCG has a similar level of antibacterial sensitivity to anti-tuberculosis compounds making it a suitable model organism with which to screen for antibacterial activity [19,20].

In this study the inhibitory effect of fifty aqueous hop extracts against *Mycobacterium bovis* BCG was investigated; Hops were purchased as either pelleted varieties or as whole (unmacerated) hops, the alpha acid content was determined by the supplier. A previously optimized aqueous extraction procedure was undertaken to increase bioactive component extraction and agar-based methods were used to determine overall inhibitory effects and minimum inhibitory [MIC) and bactericidal concentrations (MBC) were identified.

2. Materials and Methods

All materials were purchased from Fisher scientific, UK unless otherwise stated.

Microorganisms: *Mycobacterium bovis* BCG (Bacillus Calmette-Guerin) NCTC 5692 was employed in this study. The strain was purchased from the Health Protection Agency, Culture Collections, UK. The culture was stored in 10% glycerol on Microbank™ Cryo-protective beads (Pro-Lab Diagnostics Ltd., Birkenhead, UK) at −80 °C until required.

General laboratory media: All laboratory media and reagents were prepared as per manufacturer's instructions and were sterilised at 121 °C for 15 min at 15 PSI. All analyses were performed in triplicate on at least three occasions. Middlebrook 7H9 Broth and 7H10 Agar (Sigma, UK) were used for the propagation of *M. bovis* BCG NCTC 5692.

Hops: Hops were purchased from the Malt Miller Ltd All hops were delivered at room temperature in vacuum packaging and were protected from light exposure. Once opened, hop material was stored at 3 °C in the dark until extraction, typically this was within 24 h. In Table 1 the hop variants employed in the study along with their level of processing (whole hop cones or pelleted cones) and the stated alpha acid content (% *w*/*w*) is detailed.

Aqueous Hop Extraction Method: Upon delivery, hop material was stored at 3 °C within an airtight container. Prior to extraction, 5 g of hop material was mechanically macerated and passed through a filter with pore size of <1 mm and suspended, with mixing at 30 RPM, in 200 mL of sterile deionized water and heated at 100 °C for 60 min. This method was previously optimized by the authors and is detailed in [13].

Table 1. The hop variants employed in this study with stated alpha acid content (as determined by supplier). Hop material was supplied either as whole hops or pelleted hops under vacuum and stored at 3 °C until extraction.

Hop Name/Type	Pellet or Whole Hop	Stated Alpha Acid Content (% *w*/*w*)
Amarillo	Whole	6.9
Amarillo	Pellet	6.9
Apollo	Whole	19.5
Aurora	Whole	7.8
Boadicea	Whole	6
Bobek	Whole	3.9
Brambling Cross	Whole	6
Cascade	Whole	5.5
Cascade	Pellet	5.5
Centennial	Whole	8.6
Centennial	Pellet	8.6
Challenger	Whole	7.6
Chinnok	Whole	12.7
Chinook	Pellet	11
Citra	Whole	15
Citra	Pellet	16.5
Colombus	Whole	16.2
Delta	Whole	6.5
First Gold	Whole	7.9
Fuggles	Whole	4.9
Fuggles	Pellet	4.9
Galaxy	Whole	15.1
Galena	Whole	13
Glacier	Whole	6.7
Goldings	Whole	5.8
Green Bullet	Pellet	13.7
Hallertauer	Whole	3.2
Liberty	Whole	4.5
Magnum	Whole	14.5
Motueka	Pellet	6.7
Nelson Sauvin	Whole	12.8
New Zealand Rakau	Whole	10.8
Northdown	Whole	8
Northern Brewer	Whole	8
NZ Pacifica	Whole	6.1
Pacific Gem	Whole	17
Pacific Jade	Whole	15.1
Perle	Whole	9.6
Progress	Whole	5.5
Savinjski Goldings	Whole	20.75
Simcoe	Whole	12.2
Sonnet	Whole	4.1
Sorachi Ace	Whole	14.9
Summer	Pellet	6
Summit	Whole	14.3
Syrian Goldings	Whole	2.75
Target	Whole	12.5
Tettnang	Whole	4.5
Warrier	Whole	18.2
Willamette	Pellet	4.5

The hop solutions were allowed to cool prior to coarse filtration using a 1 mm gauze. The resultant solution was termed the hop extract.

Bacterial standardisation: A fresh streak plate agar culture of *M. bovis* BCG NCTC 5692 was prepared and incubated at 37 °C for 72 h and a subculture of a single colony was transferred to the appropriate broth for the same duration. The culture was centrifuged at 3000 RPM for 15 min at 4 °C. The supernatant was removed via aspiration and the resultant bacterial pellet resuspended in sterile phosphate buffered saline (PBS). An optical density- aerobic colony count was performed at 600 nm (Jenway 7300 spectrophotometer). In all tests the concentration of test microorganisms was adjusted to approximately 1×10^6 CFU/mL^{-1} using PBS.

Agar Well Diffusion Assay: Middlebrook 7H10 were prepared as previously described and allowed to cool to 40 °C. The agar was then gently inverted, and 20 mL aliquots were decanted into 45 × 90 mm petri dishes which were then allowed to cool and set within a laminar flow cabinet.

Plates were then inoculated with 150 µL of a bacterial suspension which had been standardised to approximately 1×10^6 CFU/mL^{-1} using a spectrophotometer, an even spread was made across the plate using a sterile flat sided spreader, plates were then incubated statically at 37 °C for 3 h. A previously sterilised 6 mm borer was then used to create 4 equally distanced wells per plate [13].

For each hop extract or test bacterium, newly sterilised equipment was used. In each well 80 µL of the aqueous hop extract was pipetted; in addition to this, on each plate 80 µL of H_2O was also pipetted into a well as a negative control and 80 µL of oleic acid (Merck Life Science UK Ltd, Gillingham, UK) at a concentration of 8 µg mL^{-1} in 50% DMSO as a positive control. Plates were transferred to a static 37 °C incubator (Thermo Fisher Scientific, Newport, UK) for 72 h. Inhibition of bacterial growth was observed by a clear zone of inhibition around the test well. The size of the zone of inhibition was determined by measuring two diameters at right angles across the zone and subtracting the diameter of the well itself (6 mm). Each assay was performed on at least 3 separate occasions in triplicate on each bacterial species with each hop extract [13].

Determination of the minimum inhibitory and minimum bactericidal concentration (MIC and MBC): Agar was produced at double its standard concentration (Middlebrook 7H10 19 g 450 mL^{-1} diH_2O), autoclaved and allowed to cool to 40 °C. Aqueous hop extracts, sterile distilled water and molten agar where then added to a 24 well plate (Thermo Fisher Scientific, Newport, UK) and were mixed via pipette aspiration to give a final hop extract concentration between 0 and 33% (v/v) as detailed in Table 2.

After cooling, the plate was transferred to a static incubator for 3 h at 37 °C. A bacterial suspension was then standardised to 1×10^6 cfu mL^{-1} and two 20 µL aliquots were pipetted onto the surface of each well. Plates were then incubated for 72 h.

The MIC was determined by visually inspecting each well after incubation, the lowest concentration of hop extract which inhibited growth, was considered the MIC. To determine the minimum bactericidal concentration (MBC) the surface of each well was swabbed with a sterile swab and transferred to 10 mL Middlebrook 7H9 broth [13].

The inoculated media was then incubated with shaking at 150 rpm at 37 °C for 72 h. After incubation, the absorbance of each solution was read at 600 nm (UV -6010, Thermo Fisher Scientific, Newport, UK), using sterile broth as a negative control, the lowest concentration of hops that prevented the growth of bacteria was considered the minimum bactericidal concentration (MBC) determined by a 0-absorbance reading compared to the control at 600 nm.

The method was repeated on at least 3 separate occasions in triplicate for each test bacteria unless otherwise specified.

Table 2. The volumes of double concentrated agar, SDW and aqueous hop extract used to generate a range of hop concentrations between 0 and 33% (v/v) within the agar incorporation assay.

Well Number	SDW Amount (µL)	Agar Amount (µL)	Hop Amount (µL)	% Volume of Hop Extract
A1	0.00	2000	1000	33.00
B1	100	2000	900	30.00
C1	200	2000	800	26.00
D1	300	2000	700	23.00
A2	350	2000	650	21.60
B2	400	2000	600	20.00
C2	450	2000	550	18.30
D2	500	2000	500	16.00
A3	550	2000	450	15.00
B3	600	2000	400	13.00
C3	650	2000	350	11.60
D3	700	2000	300	10.00
A4	750	2000	250	8.00
B4	800	2000	200	6.60
C4	820	2000	180	6.00
D4	840	2000	160	5.30
A5	850	2000	150	5.00
B5	860	2000	140	4.60
C5	880	2000	120	4.00
D5	900	2000	100	3.30
A6	910	2000	90	3.00
B6	920	2000	80	2.66
C6	945	2000	55	0.83
D6	0.00	3000	0	0.00

3. Results

We analysed all hop extracts (n = 50) against M. bovis BCG. The least active hop extract was determined to be Target whole hop (Average zone of inhibition 1.2 mm (+/− 0.08 mm)) whilst the greatest effect was observed to be Citra pellets (15.7 mm (+/− 0.45 mm)). It was interesting to note that all hops exhibited some form of inhibitory effect against the test bacterium (Figure 1).

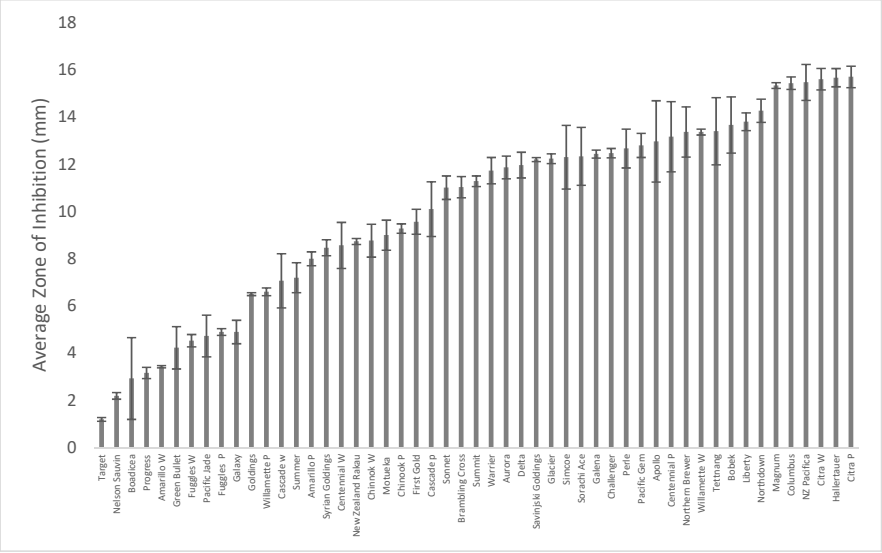

Figure 1. The average zone of inhibition of aqueous hop extracts (n = 50) against *M. bovis* BCG. Experiments are the results of 3 replicates on three occasions +/− standard deviation. (p = Pelleted variant; W = Whole; unstated = whole).

Previous studies [21,22] have suggested that the alpha acid content is linked to antibacterial activity, in Figure 2 the alpha acid content (as determined by the supplier) was plotted against the average zone of inhibition.

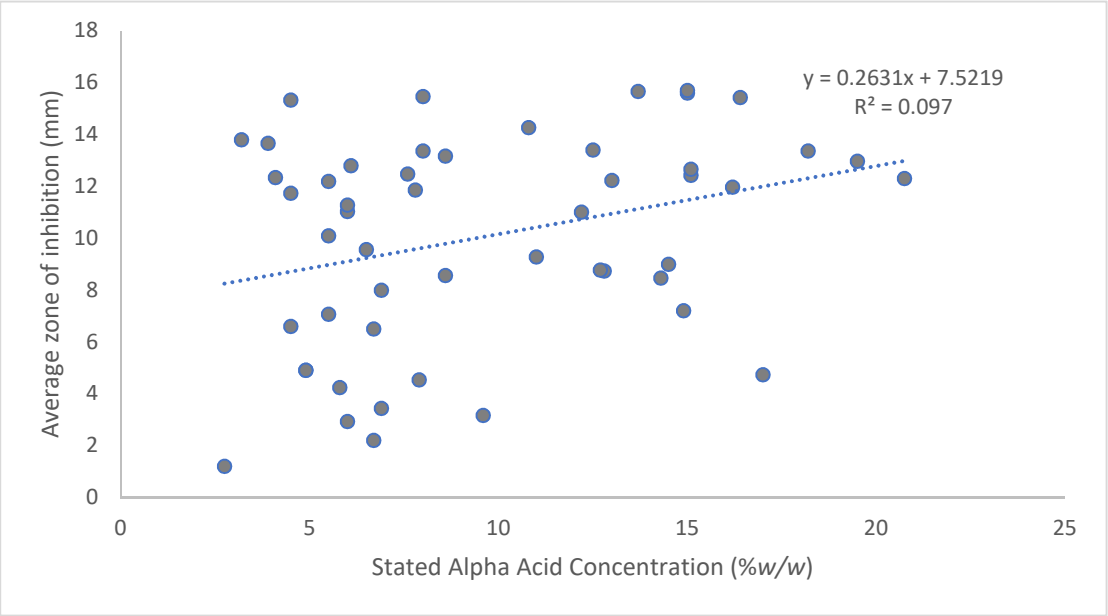

Figure 2. Correlation of the average zone of inhibition against M. bovis BCG and the stated alpha acid content of the hop variants.

Although no direct correlation was observed between the stated alpha acid content and the average zone of inhibition; The MIC and MBC were next investigated as these are generally more sensitive methods for the determination of antimicrobial activity (Table 3).

Table 3. The MIC and MBC (%*v*/*v*) of 14 hop variants against M. bovis BCG as determined by the agar incorporation assay. All other hop variants exhibited a similar profile to 'Pacific Gem' with an MIC and MBC exceeding 33%.

Hop Variant	Average Zone of Inhibition (mm)	MIC (%*v*/*v*)	MBC (%*v*/*v*)
Pacific Gem	12.8	>33%	>33%
Apollo	12.9	26%	>33%
Centennial (Whole)	13.1	26%	>33%
Northern Brewer	13.3	26%	26%
Willamette (Whole)	13.3	26%	26%
Tettnang	13.4	26%	26%
Bobek	13.6	26%	26%
Liberty	13.8	26%	26%
Northdown	14.2	23%	26%
Magnum	15.3	23%	26%
Columbus	15.4	23%	26%
NZ Pacifica	15.4	16%	26%
Citra (Whole)	15.6	16%	26%
Hallertauer	15.6	16%	26%
Citra (Pellet)	15.7	16%	16%

4. Discussion

In this study it was observed that all aqueous hop extracts had an inhibitory effect against the growth of *Mycobacterium bovis* BCG; The MIC and MBC for each hop

was determined and it was found that the Citra hop variant had the greatest level of antimicrobial activity.

The use of hops as an antimicrobial has been well documented, in Table 4 some of the current and pertinent published literature regarding the study of hops, their extracts (including commercial CO_2 extracts) and their individual components such as humulone, lupulone and xanthohumol in vitro against bacteria, viruses, fungi and their potential for biofilm disruption and in rumen applications can be observed.

Table 4. An overview of recent and pertinent antimicrobial, antifungal, antiviral and antibiofilm studies concerning the use of hops and their extracts. The authors found no references to studies that investigated the antimicrobial effect of hops effect against *Mycoacterium bovis*.

Type of Analysis	Reference	Microorganisms Analyzed	Hop Varieties /Components Employed
Antimicrobial (Gram-positive and Gram-negative)	[23]	*S. aureus*	Hop extracts and spent hop extracts
	[2]	*S. epidermidis, L. monocytogenes, S. typhimurium*	Hop extracts—alpha/beta acids, xanthohumol
	[24]	*L. monocytogenes, S. aureus, salmonella enterica, E. coli.*	Purified hop components α-bitter acids (humulones), β-bitter acids (lupulones) and xanthohumol, and a commercial CO_2 hop extract of bitter acids
		Vancomycin and methicillin resistant Gram-positive bacteria	
Antimycobacterial	[13]	*Mycobacterium abscessus*	Whole Hops and Pellets (Inflorescences)
	[11]	*Mycobacterium fortuitum*	Hexane Extracts
	[12,25]	*Mycobacterium tuberculosis*	Alcohol (polar extracts) and Xanthohumol extracts
Antifungal	[26]	*Zymoseptoria tritici*	Hydro-alcoholic crude extracts from leaves, stems, rhizomes, and female cones
	[23]	*Botrytis cinereal, Fusarium oxysporum, F. culmorum, and F. semitectum M. hiemalis* and *P. purpurogenum*	Solvent extracts; hop flavonoids
Antiviral	[27]	BVDV, HIV, FLU-A, FLU-B, Rhino, RSV, YFV, CMV, HBV and HSV-1, HSV-2.	CO_2 extrctas, isomerized kettle extract, xanthohumol enriched extract, *iso*-α-Acids, β-Acids, Hop oil, xanthohumol and *iso*-xanthohumol
	[28]	HIV-1	Xanthohumol
Antibiofilm	[29]	*S. aureus, S. epidermidis* and *C. acnes*	Purified Hop Extracts
	[21]	*S. mutans*	Purified Hop Extracts
	[30]	*S. aureus*	Purified hop extracts containing 51% xanthohumol
Rumen Fermentation and Methane Reduction	[31]	*Fibrobacter succinogenes, Ruminococcus albus*	Artificial Rumen System
	[6]	*Methanobrevibacter ruminantium*	Aqueous (polar hop extracts); in vitro

It was interesting to note from Table 4 that although there are a number of studies which investigated the antimicrobial effect of hops against mycobacteria [11–13,25] and as additives in ruminant feedstuffs [31] or for their potential use in methane reduction [6], there were no studies which investigated the use of hops against *Mycoacterium bovis*.

To the best of our knowledge this is the first study to investigate the inhibitory effect of such a wide range of hop extracts against *Mycobacterium bovis*. *M. bovis* can cause tuberculosis in cattle (bovine tuberculosis (bTB)). bTB is an endemic, zoonotic disease commonly seen within cattle. In the UK, bTB is especially prevalent in Wales and the South West of England. Between December 2020 and December 2021 there were 665 new herd incidents (positive bTB cases) in Wales an increase of 8.5% compared to December 2019–2020 [16]. As previously mentioned, the requirement to cull infected animals produces a large economic burden for farmers in these circumstances, an alternative control method includes the use of culling potential animals such as badgers which are purported to be the main transmission route between cattle is often met with public controversy [32]. Therefore,

a potential preventative approach using natural products such as hops may be a promising alternative to animal slaughter.

We firstly investigated the antimicrobial potential of each hop variant using an agar well diffusion assay. Although not specific, this method is a useful screening tool for large scale antimicrobial detection [33]. It was interesting to note that in all hop extracts we observed some level of inhibitory activity which ranged between 'Target' at 1.2 mm (+/− 0.08 mm) to Citra pellets at 15.7 mm (+)/− 0.45 mm). It was also observed that in all cases the pelleted version of the variant had a greater observed antimicrobial effect than its whole hop counterpart. This could be due to the reduced particle size of the hop material produced as a result of the pelleting process leading to increased extraction of bioactive components due to increased surface area; It could also be attributed to the compressed nature of the pellet reducing the potential for component oxidation or degradation due to oxygen exposure and indeed a larger amount of hop material being required to manufacture a single hop pellet [34,35].

Whilst we acknowledge that the agar well diffusion assay may not be as sensitive as the broth microdilution method, it has been shown to be a useful tool in the determination of overall antimicrobial activity [33]. The limitations of the method can be attributed to differences in bacterial concentration, agar volume and sample addition, to ensure comparability these were kept constant in our analysis. Due to the turbidity of the hop extracts utilized in the study we did not employ a spectrophotometric method for the determination of the minimum inhibitory and bactericidal concentrations. Instead, we relied upon an agar-based method where the agar and hop extract were homogenized to form a known concentration. Further studies would be improved by increased filtration of the hop extracts to remove large organic matter prior to analysis as demonstrated by Gregory and colleagues [21].

We found that in the majority of our extracts the minimum inhibitory and bactericidal concentrations exceeded our limit of detection (33% v/v). However, in correlation with the zone of inhibition results from 13 variants we observed an MIC range between 16% v/v (Citra Pellets) and 26% v/v (Apollo whole hops). The MBC ranged between 16% v/v (Citra Pellets) and 26% v/v (Northern Brewer). The pelleted version of the Citra variant exhibited a higher zone of inhibition and lower MIC/MBC compared to the whole hop variant. This could be attributed to the pelleting process which macerates hop material and increases surface area for extraction [34,35].

The antimicrobial activity of hops has previously been linked to the hop alpha acid humulone, beta acid lupulone and polyphenol xanthohumol [22,25]. Gerhauser (2005) demonstrated the antibacterial activity of xanthohumol against the Gram-positive microorganisms *Staphylococcus aureus* and *Streptococcus mutans* along with antiviral and antiparasitic activity [36].

In our study, there was no observed link between the stated alpha acid content and the antimicrobial activity against Mycobacterium bovis BCG. It may be the case however that the extraction method which is known to lead to isomerisation of such compounds may have a detrimental effect on these components [37] although isohumulone and xanthohumol can be considered important hop bioactive compounds [22,25,28].

Future research could focus on the characterization of the antibacterial components of the hop extracts utilized in this study. Prencipe and colleagues (2014) developed a high-performance liquid chromatography method for the bioactive compounds within hops in combination with a range of extraction techniques to improve secondary metabolite identification which may improve identification of future antimicrobial components in similar studies [38]. It may be the case that the antimicrobial effect is not due to the presence of a single component found within the hop extract but is perhaps a synergistic combination of different components. We also acknowledge that at this stage we have not assessed the effect of these particular extracts at the tested concentrations for their cell toxicity. Ensuring the safety of such extracts for potential therapeutic use would be pertinent in future studies and would further point towards the basis of antimicrobial activity [39].

In this study we observed that the Citra hop variant had the greatest amount of antimicrobial activity against *M. bovis*. The Citra hop variant also known as 'HBC 394′ was patented by inventors Eugene Probasco and Jason Perrault as a product of a control breeding program by Hop Breeding Co LLC. The variety is a cross between female parent '8801-02′ ('Hallertauer mittlefrueh' × '853–144M') (not patented) and male parent '8801-01M ('Hallertauer mittlefrueh' × '853–144M') (not patented). In general, the Citra variety produces approximately 11–13% Alpha Acids, 3.4–4.5% Beta acids and 22–24% Cohumulone [40]. The alpha acid concentration is slightly lower than that reported by our supplier. It has been previously reported that weather conditions during growing can affect the alpha acid content [41] along with harvesting following flowering [42]; In tea it has been that catechin contents is affected by environmental effects such as growing altitude [43]. Therefore, future similar studies could standardize the growth conditions and undertake alpha acid, beta acid and xanthohumol concentration analysis prior to testing.

Given the potential bioactive properties of hops against *Mycobacteria* spp. Future research could investigate the ability of hop extracts to inhibit the growth of other mycobacterial species such as *Mycobacterium tuberculosis* or *Mycobacterium abscessus*. Additionally, previous studies (Table 4) have explored the use of hops and their extracts as a potential feed addition for cattle to decrease methane production and found that hops and their extracts have an in vitro inhibitory effect on *Methanobrevibacter ruminantium* the main methane emitting microorganisms within ruminants [6]. Similar studies such as those undertaken by Narvaez and colleagues (2011) have investigated the use of hop additions in ruminal fermentation and concluded that the addition of hop extracts may offer a means of decreasing ruminal methane emission without compromising the fermentability of feed [31]. The caveat being that these studies have been completed in vitro and thus more work needs to be undertaken to understand potential in vivo effects. Given the ability of hops to inhibit *Mycobacterium bovis* and the potential to also reduce methane production the addition of certain hop components within cattle feed is an area that we believe justifies more research.

Hops and other plants such as tea [44], seaweed [45] and dandelions [46] offer potential solutions to the growing threat of antimicrobial resistance [47].

These plants offer the potential to be explored for antimicrobial synergistic activity; Natarajan and colleagues (2008) investigated the use of hop derived compounds such as lupulone and xanthohumol in combination with a range of antibiotics against both Gram-positive and Gram-negative microorganisms using agar-based methods [48]. A review by Fahle and colleagues (2022) highlighted the potential for hop components to be used with thirteen antibiotics and found effectiveness against both Gram-positive and Gram-negative microorganisms [49].

Within our own research we found that the majority of studies related to in vitro effects of hop components. Whilst this is promising, future studies could investigate the cellular effect of hop components and the antimicrobial effect in vivo.

5. Conclusions

This study characterized the antimicrobial effect of 50 different hop variants against *Mycoabcteroum bovis* BCG. All hop variants demonstrated inhibition against the microorganism, with the Citra hop variant having the lowest MIC and MBC. Previous studies have also investigated the potential for hops to reduce methane production in ruminants; the ability to both have a protective effect against bovine tuberculosis whilst reducing methane production is an area we recommend for future research. Clinically, future studies could include other *Mycobacteria* spp. along with the potential to include synergistic activity with antimicrobials as well as determining the toxicity of potential bioactive components.

Author Contributions: Conceptualization, J.B. and L.B.; methodology, J.B. and L.B.; investigation, J.B.; writing—original draft preparation, J.B. and R.T.; writing—review and editing, J.B., R.T. and L.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data available from authors upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hieronymus, S. *For the Love of Hops: The Practical Guide to Aroma, Bitterness, and the Culture of Hops*; Brewing elements series; Brewers Publisher, bp: Boulder, CO, USA, 2012; ISBN 978-1-938469-01-5.
2. Kramer, B.; Thielmann, J.; Hickisch, A.; Muranyi, P.; Wunderlich, J.; Hauser, C. Antimicrobial Activity of Hop Extracts against Foodborne Pathogens for Meat Applications. *J. Appl. Microbiol.* **2015**, *118*, 648–657. [CrossRef] [PubMed]
3. Filmer, R. *Hops and Hop Picking*; History in camera; Shire Publications Ltd.: Oxford, UK, 2011; ISBN 978-0-85263-617-6.
4. Rettberg, N.; Biendl, M.; Garbe, L.-A. Hop Aroma and Hoppy Beer Flavor: Chemical Backgrounds and Analytical Tools—A Review. *J. Am. Soc. Brew. Chem.* **2018**, *76*, 1–20. [CrossRef]
5. Karabín, M.; Hanko, V.; Nešpor, J.; Jelínek, L.; Dostálek, P. Hop Tannin Extract: A Promising Tool for Acceleration of Lautering. *J. Inst. Brew.* **2018**, *124*, 374–380. [CrossRef]
6. Blaxland, J.A.; Watkins, A.J.; Baillie, L.W.J. The Ability of Hop Extracts to Reduce the Methane Production of Methanobrevibacter Ruminantium. *Archaea* **2021**, *2021*, 5510063. [CrossRef] [PubMed]
7. Rattan, A.; Kalia, A.; Ahmad, N. Multidrug-Resistant Mycobacterium Tuberculosis: Molecular Perspectives. *Emerg. Infect. Dis.* **1998**, *4*, 195–209. [CrossRef]
8. Müller, B.; Borrell, S.; Rose, G.; Gagneux, S. The Heterogeneous Evolution of Multidrug-Resistant Mycobacterium Tuberculosis. *Trends Genet.* **2013**, *29*, 160–169. [CrossRef]
9. Perdigão, J.; Silva, C.; Maltez, F.; Machado, D.; Miranda, A.; Couto, I.; Rabna, P.; Florez de Sessions, P.; Phelan, J.; Pain, A.; et al. Emergence of Multidrug-Resistant Mycobacterium Tuberculosis of the Beijing Lineage in Portugal and Guinea-Bissau: A Snapshot of Moving Clones by Whole-Genome Sequencing. *Emerg. Microbes Infect.* **2020**, *9*, 1342–1353. [CrossRef]
10. Rosa, P.S.; D'Espindula, H.R.S.; Melo, A.C.L.; Fontes, A.N.B.; Finardi, A.J.; Belone, A.F.F.; Sartori, B.G.C.; Pires, C.A.A.; Soares, C.T.; Marques, F.B.; et al. Emergence and Transmission of Drug-/Multidrug-Resistant Mycobacterium Leprae in a Former Leprosy Colony in the Brazilian Amazon. *Clin. Infect. Dis.* **2020**, *70*, 2054–2061. [CrossRef]
11. Stavri, M.; Schneider, R.; O'Donnell, G.; Lechner, D.; Bucar, F.; Gibbons, S. The Antimycobacterial Components of Hops (Humulus Lupulus) and Their Dereplication. *Phytother. Res. PTR* **2004**, *18*, 774–776. [CrossRef]
12. Serkani, J.E.; Isfahani, B.N.; Safaei, H.G.; Kermanshahi, R.K.; Asghari, G. Evaluation of the Effect of Humulus Lupulus Alcoholic Extract on Rifampin-Sensitive and Resistant Isolates of Mycobacterium Tuberculosis. *Res. Pharm. Sci.* **2012**, *7*, 235–242.
13. Blaxland, J. The Antibacterial Activity of Humulus Lupulus against Mycobacteria. Ph.D. Thesis, Cardiff University, Cardiff, UK, 2015.
14. The Tuberculosis (Wales) (Amendment) Order 2017. Available online: https://www.legislation.gov.uk/wsi/2017/711/contents/made (accessed on 20 July 2022).
15. The Tuberculosis in Animals (England) Order 2021. Available online: https://www.legislation.gov.uk/uksi/2021/1001/article/1/made (accessed on 20 July 2022).
16. Incidence of Tuberculosis (TB) in Cattle in Great Britain: March 2021. Available online: https://gov.wales/incidence-tuberculosis-tb-cattle-great-britain-march-2021 (accessed on 1 February 2022).
17. *EU 2019/6*; Veterinary Medicinal Products and Repealing Directive 2001/82/EC (Text with EEA Relevance). European Parliament and of the Council: Brussels, Belgium, 2018; Volume 004.
18. Altaf, M.; Miller, C.H.; Bellows, D.S.; O'Toole, R. Evaluation of the Mycobacterium Smegmatis and BCG Models for the Discovery of Mycobacterium Tuberculosis Inhibitors. *Tuberc. Edinb. Scotl.* **2010**, *90*, 333–337. [CrossRef] [PubMed]
19. Fitzgerald, S.D.; Schooley, A.M.; Berry, D.E.; Kaneene, J.B. Antimicrobial Susceptibility Testing of Mycobacterium Bovis Isolates from Michigan White-Tailed Deer during the 2009 Hunting Season. *Vet. Med. Int.* **2010**, *2011*, 903683. [CrossRef] [PubMed]
20. Ritz, N.; Tebruegge, M.; Connell, T.G.; Sievers, A.; Robins-Browne, R.; Curtis, N. Susceptibility of Mycobacterium Bovis BCG Vaccine Strains to Antituberculous Antibiotics. *Antimicrob. Agents Chemother.* **2009**, *53*, 316–318. [CrossRef] [PubMed]
21. Gregory, E.R.; Bakhaider, R.F.; Gomez, G.F.; Huang, R.; Moser, E.A.S.; Gregory, R.L. Evaluating Hop Extract Concentrations Found in Commercial Beer to Inhibit Streptococcus Mutans Biofilm Formation. *J. Appl. Microbiol.* **2022**. [CrossRef] [PubMed]
22. Bland, J.S.; Minich, D.; Lerman, R.; Darland, G.; Lamb, J.; Tripp, M.; Grayson, N. Isohumulones from Hops (*Humulus lupulus*) and Their Potential Role in Medical Nutrition Therapy. *PharmaNutrition* **2015**, *3*, 46–52. [CrossRef]
23. Bartmańska, A.; Wałecka-Zacharska, E.; Tronina, T.; Popłoński, J.; Sordon, S.; Brzezowska, E.; Bania, J.; Huszcza, E. Antimicrobial Properties of Spent Hops Extracts, Flavonoids Isolated Therefrom, and Their Derivatives. *Mol. J. Synth. Chem. Nat. Prod. Chem.* **2018**, *23*, 2059. [CrossRef]
24. Bogdanova, K.; Röderova, M.; Kolar, M.; Langova, K.; Dusek, M.; Jost, P.; Kubelkova, K.; Bostik, P.; Olsovska, J. Antibiofilm Activity of Bioactive Hop Compounds Humulone, Lupulone and Xanthohumol toward Susceptible and Resistant Staphylococci. *Res. Microbiol.* **2018**, *169*, 127–134. [CrossRef]

25. Lou, H.; Zhang, F.; Lu, L.; Ding, Y.; Hao, X. Xanthohumol from *Humulus lupulus* L. Potentiates the Killing of Mycobacterium Tuberculosis and Mitigates Liver Toxicity by the Combination of Isoniazid in Mouse Tuberculosis Models. *RSC Adv.* **2020**, *10*, 13223–13231. [CrossRef]
26. Bocquet, L.; Rivière, C.; Dermont, C.; Samaillie, J.; Hilbert, J.-L.; Halama, P.; Siah, A.; Sahpaz, S. Antifungal Activity of Hop Extracts and Compounds against the Wheat Pathogen Zymoseptoria Tritici. *Ind. Crops Prod.* **2018**, *122*, 290–297. [CrossRef]
27. Buckwold, V.E.; Wilson, R.J.H.; Nalca, A.; Beer, B.B.; Voss, T.G.; Turpin, J.A.; Buckheit, R.W.; Wei, J.; Wenzel-Mathers, M.; Walton, E.M.; et al. Antiviral Activity of Hop Constituents against a Series of DNA and RNA Viruses. *Antivir. Res.* **2004**, *61*, 57–62. [CrossRef]
28. Wang, Q.; Ding, Z.-H.; Liu, J.-K.; Zheng, Y.-T. Xanthohumol, a Novel Anti-HIV-1 Agent Purified from Hops Humulus Lupulus. *Antivir. Res.* **2004**, *64*, 189–194. [CrossRef]
29. Di Lodovico, S.; Menghini, L.; Ferrante, C.; Recchia, E.; Castro-Amorim, J.; Gameiro, P.; Cellini, L.; Bessa, L.J. Hop Extract: An Efficacious Antimicrobial and Anti-Biofilm Agent Against Multidrug-Resistant Staphylococci Strains and Cutibacterium Acnes. *Front. Microbiol.* **2020**, *11*, 1852. [CrossRef] [PubMed]
30. Rozalski, M.; Micota, B.; Sadowska, B.; Stochmal, A.; Jedrejek, D.; Wieckowska-Szakiel, M.; Rozalska, B. Antiadherent and Antibiofilm Activity of *Humulus lupulus* L. Derived Products: New Pharmacological Properties. *BioMed Res. Int.* **2013**, *2013*, e101089. [CrossRef] [PubMed]
31. Narvaez, N.; Wang, Y.; Xu, Z.; Alexander, T.; Garden, S.; McAllister, T. Effects of Hop Varieties on Ruminal Fermentation and Bacterial Community in an Artificial Rumen (Rusitec). *J. Sci. Food Agric.* **2013**, *93*, 45–52. [CrossRef]
32. Team, B.T.S. Badger Trust Welcomes End of Selective Badger Cull in Wales and Rethink of BTB Policy. Available online: https://www.badgertrust.org.uk/post/badger-trust-welcomes-end-of-selective-badger-cull-in-wales-and-rethink-of-btb-policy (accessed on 20 July 2022).
33. Holder, I.A.; Boyce, S.T. Agar Well Diffusion Assay Testing of Bacterial Susceptibility to Various Antimicrobials in Concentrations Non-Toxic for Human Cells in Culture. *Burns* **1994**, *20*, 426–429. [CrossRef]
34. Make Hops Pellets for Brewery/Hops Pellet Machine. Available online: https://www.pelletmachines.net/biopellet-plant/hops-pellet-production-line.html (accessed on 1 February 2022).
35. How Are Hop Pellets Made? Available online: https://www.canuckhomebrewsupply.com/blogs/all/how-are-hop-pellets-made (accessed on 1 February 2022).
36. Gerhäuser, C. Broad Spectrum Antiinfective Potential of Xanthohumol from Hop (*Humulus lupulus* L.) in Comparison with Activities of Other Hop Constituents and Xanthohumol Metabolites. *Mol. Nutr. Food Res.* **2005**, *49*, 827–831. [CrossRef] [PubMed]
37. Simpson, W.J.; Smith, A.R.W. Factors Affecting Antibacterial Activity of Hop Compounds and Their Derivatives. *J. Appl. Bacteriol.* **1992**, *72*, 327–334. [CrossRef]
38. Development of a New High-Performance Liquid Chromatography Method with Diode Array and Electrospray Ionization-Mass Spectrometry Detection for the Metabolite Fingerprinting of Bioactive Compounds in Humulus lupulus L.—ScienceDirect. Available online: https://www.sciencedirect.com/science/article/pii/S0021967314007195 (accessed on 20 July 2022).
39. Knez Hrnčič, M.; Španinger, E.; Košir, I.J.; Knez, Ž.; Bren, U. Hop Compounds: Extraction Techniques, Chemical Analyses, Antioxidative, Antimicrobial, and Anticarcinogenic Effects. *Nutrients* **2019**, *11*, 257. [CrossRef]
40. Probasco, E.G.; Perrault, J. Hop Plant Named 'HBC 394' USA. US PP21,289 P3, 14 September 2010.
41. MacKinnon, D.; Pavlovič, V.; Čeh, B.; Naglič, B.; Pavlovič, M. The Impact of Weather Conditions on Alpha-Acid Content in Hop (*Humulus lupulus* L.) Cv. Aurora. *Plant Soil Environ.* **2020**, *66*, 519–525. [CrossRef]
42. Park, K.-Y.; Ree, D.-W.; Min, I.-K. Influence of Days after Flowering and Cultural Environments on Changes of Alpha-Acid Content in Hops (*Humulus lupulus* L.). *Korean J. Crop Sci.* **1987**, *32*, 97–102.
43. Chen, G.-H.; Yang, C.-Y.; Lee, S.-J.; Wu, C.-C.; Tzen, J.T.C. Catechin Content and the Degree of Its Galloylation in Oolong Tea Are Inversely Correlated with Cultivation Altitude. *J. Food Drug Anal.* **2014**, *22*, 303–309. [CrossRef] [PubMed]
44. Vodnar, D.C. Inhibition of Listeria Monocytogenes ATCC 19115 on Ham Steak by Tea Bioactive Compounds Incorporated into Chitosan-Coated Plastic Films. *Chem. Cent. J.* **2012**, *6*, 74. [CrossRef] [PubMed]
45. Pérez, M.J.; Falqué, E.; Domínguez, H. Antimicrobial Action of Compounds from Marine Seaweed. *Mar. Drugs* **2016**, *14*, 52. [CrossRef]
46. Kenny, O.; Brunton, N.P.; Walsh, D.; Hewage, C.M.; McLoughlin, P.; Smyth, T.J. Characterisation of Antimicrobial Extracts from Dandelion Root (*Taraxacum officinale*) Using LC-SPE-NMR. *Phytother. Res. PTR* **2015**, *29*, 526–532. [CrossRef] [PubMed]
47. Murray, C.J.; Ikuta, K.S.; Sharara, F.; Swetschinski, L.; Aguilar, G.R.; Gray, A.; Han, C.; Bisignano, C.; Rao, P.; Wool, E.; et al. Global Burden of Bacterial Antimicrobial Resistance in 2019: A Systematic Analysis. *Lancet* **2022**, *399*, 629–655. [CrossRef]
48. Natarajan, P.; Katta, S.; Andrei, I.; Babu Rao Ambati, V.; Leonida, M.; Haas, G.J. Positive Antibacterial Co-Action between Hop (*Humulus lupulus*) Constituents and Selected Antibiotics. *Phytomedicine* **2008**, *15*, 194–201. [CrossRef]
49. Fahle, A.; Bereswill, S.; Heimesaat, M.M. Antibacterial Effects of Biologically Active Ingredients in Hop Provide Promising Options to Fight Infections by Pathogens Including Multi-Drug Resistant Bacteria. *Eur. J. Microbiol. Immunol.* **2022**, *12*, 22–30. [CrossRef]