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Research Article

Ethanol-Methanol Interchangeability in Berry Extracts: Enhancing Safety and Cost-Effectiveness for Antimicrobial Research

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Abstract

The primary focus of this manuscript is to compare ethanol and methanol as solvents, excluding detailed antibacterial results that have been previously published. By concentrating on the solvent comparison, the viability of ethanol as a substitute for methanol is emphasized without diminishing its distinct contribution. The antibacterial potential of northern berry extracts against common foodborne pathogens was explored, alongside the influence of solvent choice on extract efficacy. Statistical analyses were performed to compare bacterial counts from extracts prepared using ethanol and methanol, using one-way ANOVA with p-values > 0.05 indicating non-significance.

Berry extracts were prepared with ethanol and methanol, then tested against *Staphylococcus aureus*, and *Listeria monocytogenes*. Statistical analysis showed no significant differences in antibacterial activity between solvent types. Ethanol proves an effective substitute for methanol, offering economic and safety advantages due to its lower toxicity and cost-effectiveness. This research provides insights into solvent selection for antibacterial extracts, enhancing experimental practices with broader implications for antimicrobial studies

Keywords: solvent interchangeability, berry extracts, antibacterial activity, ethanol, methanol, foodborne pathogens

Introduction

Interest in the utilisation of natural sources rich in bioactive compounds, such as phenolics and flavonoids, has witnessed substantial growth due to their diverse beneficial biological properties, health-enhancing attributes, and potential therapeutic effects [1, 2, 3]. Berries, characterised by their red, blue, or purple hues, have emerged as noteworthy reservoirs of phenolic compounds, flavonoid anthocyanins, and organic acids, rendering them significant contributors to human health [3, 4, 5]. With botanical names such as *Aronia melanocarpa*, *Ribes nigrum* L., *Vaccinium* species, and *Rubus* genus members, various berries encompass bilberry, blackcurrant, blueberry, cranberry, lingonberry, raspberry, and blackberry [6, 7, 4]. The health-promoting attributes of flavonoids and anthocyanins skirted an array of effects, including anti-inflammatory, anti-allergic, anti-carcinogenic, antihypertensive, and antimicrobial properties [1, 8]. Additionally, these compounds, functioning as natural colourants, possess antioxidant, free-radical-scavenging, and metal-chelating capabilities, which offer potential alternatives to synthetic pigments, antioxidants, and chelators [1, 8, 9]. Extensive research has investigated the antimicrobial efficacy of berries against both human and foodborne pathogens, positioning them as potential natural antimicrobial agents and food preservatives [10, 11, 4, 2, 8,12]. *Berry plants: origin, history, use, and research* Berries, with their vibrant spectrum of colours and diverse origins, have garnered attention for centuries. From Aronia, native to North America, to blackcurrant, originating in Europe and Asia Pacific, each berry variety boasts a unique history and utility. Blackcurrant, revered for its cold-curing properties, is a mainstay in the production of juices, jellies, and preserves. Likewise, blueberries, hailing from the *Vaccinium* genus, have a global presence and offer both nutritional and commercial value. With historical uses ranging from cold remedies to fragrance components, berries have been a source of human well-being for generations [13].

Among these berries, *Aronia melanocarpa*, *Ribes nigrum* L., *Vaccinium* species, and *Rubus* genus members have shown promise in combating bacterial pathogens. Aronia, long utilised by Native Americans for treating colds, has evolved into a staple for juice and jam production. Blackcurrant, valued for its role in alleviating respiratory infections, is now integral to various culinary delights. Blueberries, renowned for their antioxidant potential, are processed into a multitude of products, while raspberries and blackberries are cherished for their nutritional and medicinal attributes [14].

While the scientific inquiry has unveiled the antimicrobial potential of these berries, a key aspect of our research delves into the extraction process itself. By scrutinising the use of ethanol and methanol as solvent bases, we aim to ascertain their interchangeability for extracting berry bioactive compounds. Our study elucidates whether the choice of solvent, an integral part of the extraction methodology, influences the antibacterial potency of the extracts. Within the realm of prior investigations centered on the antibacterial attributes of berries [12]. this paper emphasises a unique facet: the interchangeability of ethanol and methanol as extraction solvents. By illuminating this pivotal aspect, advancement towards a more economical and safer methodology for preparing berry extracts for antimicrobial assessments is contributed. Through this inquiry, an existing research void is

addressed, ultimately showcasing the viability of ethanol as a practical alternative to methanol in the preparation of berry extracts for antimicrobial analyses. Focus and Contribution This manuscript's sole focus is comparing ethanol and methanol as solvents, excluding previous antibacterial and downregulation data from the main thesis. By isolating solvent comparison, we underscore ethanol's feasibility as a methanol alternative without overshadowing its unique contribution. This study addresses a pivotal aspect of antimicrobial research: solvent choice for berry extract preparation and its impact. Our research fills a gap by elucidating the feasibility of using ethanol as a safer and cost-effective alternative to methanol in extracting antimicrobial compounds from berries.

Material And Methods

Bacterial strain and culture conditions the bacterial strains employed in this study are outlined in Table 1. The cultivation conditions varied based on the strains. *S. aureus* was cultured on Mueller-Hinton agar (MHA) or Tryptone soya agar (TSA) and in the same broth with agitation (150–180 rpm). *L. monocytogenes* was cultured on Brain heart infusion agar (BHIA) or in the same broth with agitation. All strains were grown aerobically at 37°C and maintained as frozen stock strains at -80°C. Prior to experiments, strains were streaked on agar and incubated overnight. Cultures from a single colony were subculture in medium broth, incubated overnight, and used as the inoculum for experiments.

Strain	Agar	Broth	Source
Gram-positive bacteria			
Listeria monocytogenes EGD-e (serovar1/2a)	BHI	BHI	(*15*)
Staphylococcus aureus strain Newman (NCTC 8178)	MH and TSA	MH	(*16*)

Table 1. List of the bacterial strain and culture conditions

Plant material

Dried powder forms of Aronia (*Aronia melanocarpa*), blackcurrant (*Ribes nigrum* L.), and blueberry (*Ericaceae Vaccinium*) were obtained from Berrifine, Denmark.

Chemicals

A list of chemicals used in the study is provided in Table 2.

Material name	Abbreviation	Trade ID	Company
Brain heart infusion	BHI	CM1135	Oxoid
Casein hydrolysate (ACID)			Oxoid
Ethyl alcohol 70%	Ethanol 70%	UN.NR 1170	Kemetyl
Ethyl alcohol 99.9%	Ethanol 100%	UN.NR 1987	Kemetyl
Meat extract		1,03979	Merck
Methyl alcohol	Methanol	M 3641	Sigma- Aldrich, Inc.
Methyl alcohol	Methanol	M 322415	Sigma- Aldrich, Inc.
Mueller-Hinton agar	MH A	CM0337	Oxoid
Mueller-Hinton broth	MH B	CM0405	Oxoid
Phosphate buffered saline, pH 7.4,	PBS		
Sodium carbonate anhydrous			Merck
Sodium Hydroxide Pellets		402	Baker analyzed
Starch, soluble		S9765	Sigma- Aldrich, Inc.
Tryptone soya agar	TSA	CM0131	Oxoid
Tryptone soya broth	TSB	CM0129	Oxoid
Tween 80		CN.103170	MP Biomedicals

Table 2. List of materials used in this study

Extraction and preparation

In evaluating the antimicrobial properties of berry extracts, the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined. Berry powders were extracted using either 50% aqueous methanol or ethanol (100 mg×ml-1) [17,18]. with complete extraction ensured through 24-hour shaking at 40°C [19]. After filtration and removal of residual compounds, extracts were sterilised using a 0.45 µm filter (Syringe Filter Q-Max 25 mm 0.45 µm CA membrane sterile, Frisenette ApS). Subsequently, extracts were neutralised with 1M sodium hydroxide to achieve pH 7, followed by evaporation to dryness at 40°C. The dried extracts were then reconstituted in phosphate-buffered saline (PBS) and stored at 4°C.

Antimicrobial testing and food ingredient effects

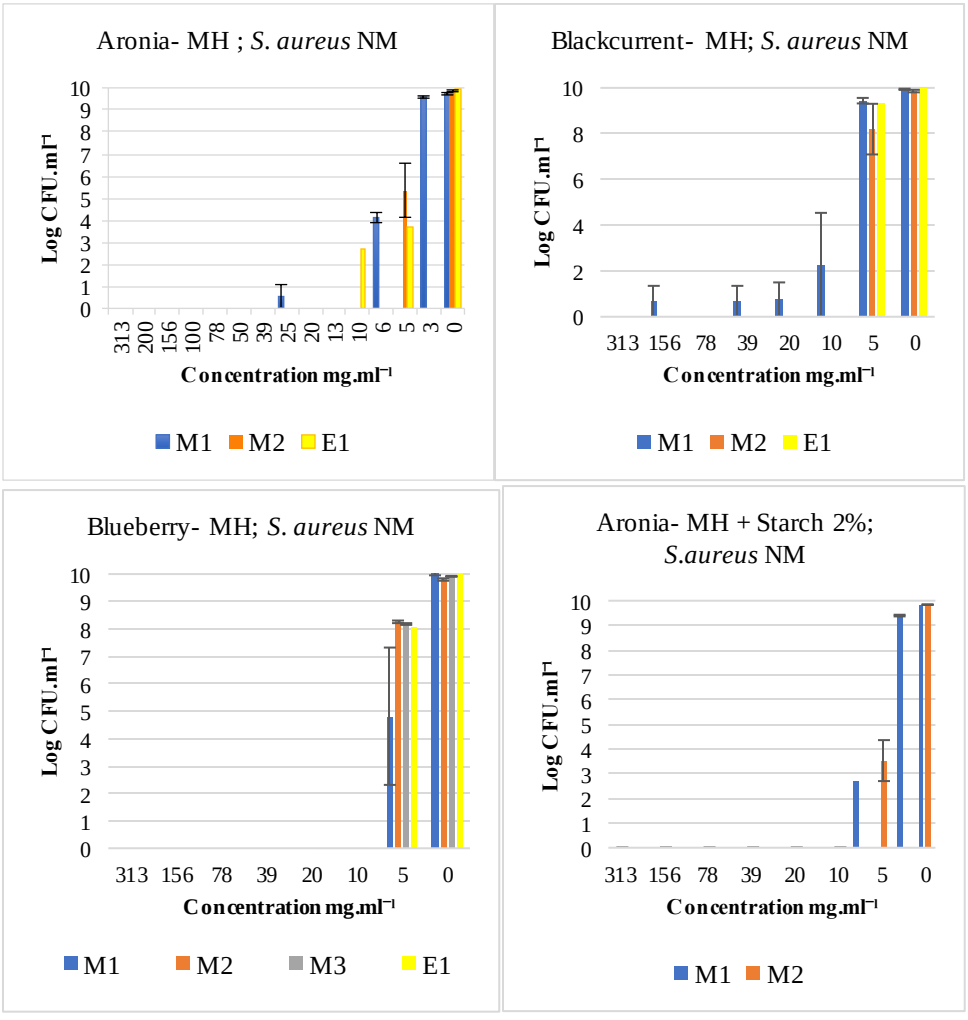
Antimicrobial experiments were determined using a 2-fold micro-dilution method in broth, with concentrations ranging up to 313 mg/ml of medium. Initial extracts were placed in the first row of a microtiter plate, with subsequent rows undergoing 2-fold dilutions. Overnight cultures of microorganisms were normalised, ten-fold diluted, and inoculated into the plates. Microplates were incubated at 37°Cover 24 hours, followed by a number of colonies forming unit (CFU) indications. To determine CFUs, the simultaneous drop plate method was employed. Microtitre plate contents were homogenised and transferred to new plates. Dilutions were prepared, spotted onto agar plates, and incubated for CFU formation. To assess the potential consistency of the results in various conditions, experiments were conducted in the presence of various food ingredients. Ingredients included meat extract, acid-hydrolyzed casein, sunflower oil, starch, and UHT milk. *Statistical analysis* A one-way ANOVA was performed to analyse the bacterial counts among various batches of extracts using methanol and ethanol as solvents. Non-significant results were indicated by p-values > 0.05.

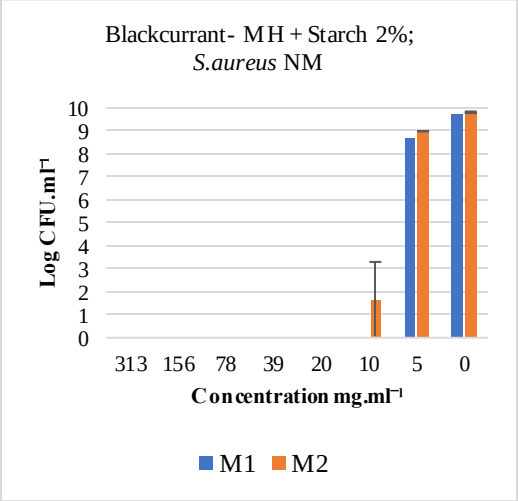
Results and Discussion

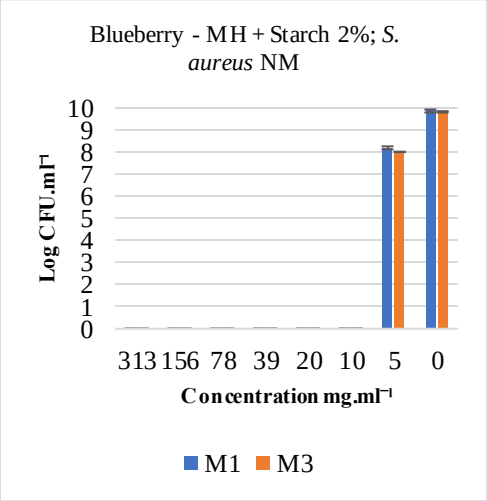
The comprehensive antibacterial properties of the studied berries have already been thoroughly detailed in Attarianshandiz (2022). To solely spotlight solvent interchangeability, specific antibacterial details are omitted here [12]. The primary objective of this manuscript is to demonstrate the viability of ethanol as an alternative solvent to methanol for berry extract preparation. This work not only enhances experimental practices but also underscores the applicability of ethanol in various experiments, promoting cost-effectiveness and safety. Impact of primary solvents (methanol or ethanol) on berry extract activity

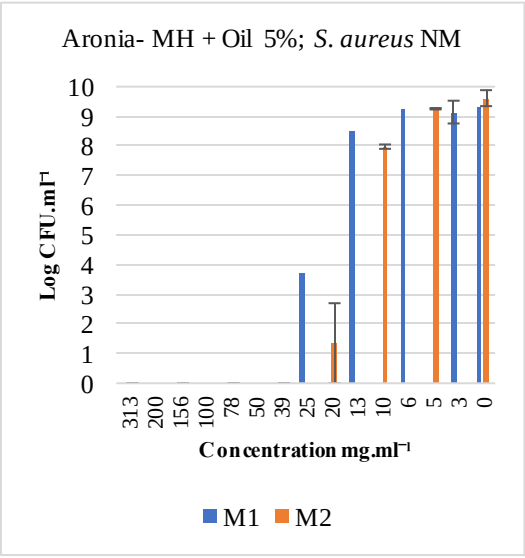
Figures 1 concentration is expressed as mg berry per ml media. Control is BHI broth with initial inocula of ~5×10^6 CFU.ml^{-1}. M: Methanol based extracts. E: ethanol-based extracts. 1 to 4 represents different batches of extracts. and 2 depict the investigation into different extract batches and primary solvents (methanol and ethanol) on *S. aureus* NM and *L. monocytogenes*. Ethanol's consideration as a solvent stem from methanol's toxicity and incompatibility with food. Statistical analysis indicates comparable results between extract batches and solvents ($P > 0.05$), asserting ethanol's interchangeability with methanol. Antimicrobial activity of neutralised berry extracts' antimicrobial efficacy against *S. aureus* NM, *L. monocytogenes*, *E. coli* 0157, and *Salmonella* Typhimurium is demonstrated in my previous paper [12]. The finding aligns with Puupponen-Pimiä et al. (2001), highlighting Nordic berries' antimicrobial potential against various bacteria, including pathogens [8]. Concurrent with Lacombe et al. (2010) and Siddiqi et al. (2011), who fractionated fruit extracts into soluble and neutralised phenolic and anthocyanin fractions, our findings validate berries' antimicrobial potential [12,20,21].Nohynek et al. (2006) further corroborate the outer membrane disruption phenomenon caused by phenolic extracts from cloudberry and raspberry in *Salmonella* strains [*4*]. All in all, in alignment with other studies using berry extract in standard media cultures, solvent selection (ethanol and methanol) proves interchangeable.

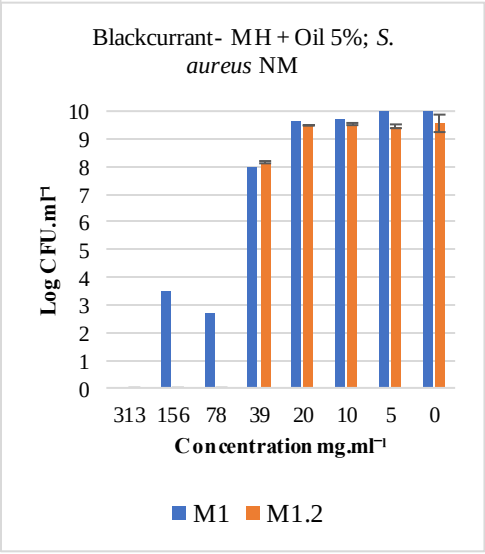
Antimicrobial activity within varying food compositions: The study highlights nuanced outcomes of different berry extracts in various food compositions [12]. It also affirms ethanol's viability for extracting these compounds. Our research encompasses multiple aspects, uncovering insights into berry extract antibacterial properties and solvent selection. Despite the variation caused by supplementing standard media with various food ingredients, no significant variations were observed between the results concerning the solvent as another variable in each individual experiment (see Figure. 1 and Figure. 2).

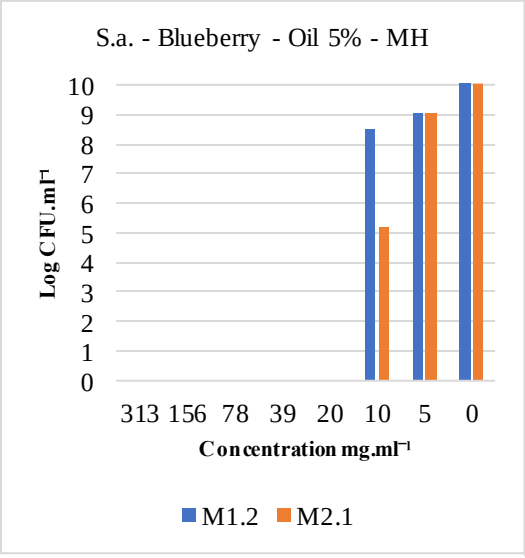


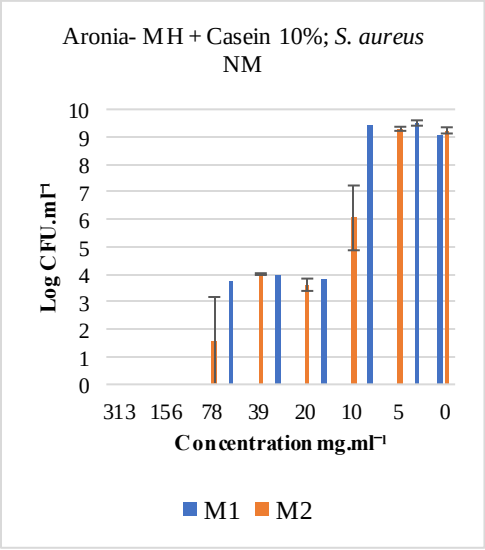

Blackcurrant- MH + Starch 2%; S.aureus NM
Log CFU.ml⁻¹
0 1 2 3 4 5 6 7 8 9 10
313 156 78 39 20 10 5 0
Concentration mg.ml⁻¹
M1 M2


Blueberry - MH + Starch 2%; S. aureus NM
Log CFU.ml⁻¹
0 1 2 3 4 5 6 7 8 9 10
313 156 78 39 20 10 5 0
Concentration mg.ml⁻¹
M1 M3


Aronia- MH + Oil 5%; S. aureus NM
Log CFU.ml⁻¹
0 1 2 3 4 5 6 7 8 9 10
313 200 156 100 78 50 39 25 20 13 10 6 5 3 0
Concentration mg.ml⁻¹
M1 M2


Blackcurrant- MH + Oil 5%; S. aureus NM
Log CFU.ml⁻¹
0 1 2 3 4 5 6 7 8 9 10
313 156 78 39 20 10 5 0
Concentration mg.ml⁻¹
M1 M1.2


S.a. - Blueberry - Oil 5% - MH
Log CFU.ml⁻¹
0 1 2 3 4 5 6 7 8 9 10
313 156 78 39 20 10 5 0
Concentration mg.ml⁻¹
M1.2 M2.1


Aronia- MH + Casein 10%; S. aureus NM
Log CFU.ml⁻¹
0 1 2 3 4 5 6 7 8 9 10
313 156 78 39 20 10 5 0
Concentration mg.ml⁻¹
M1 M2

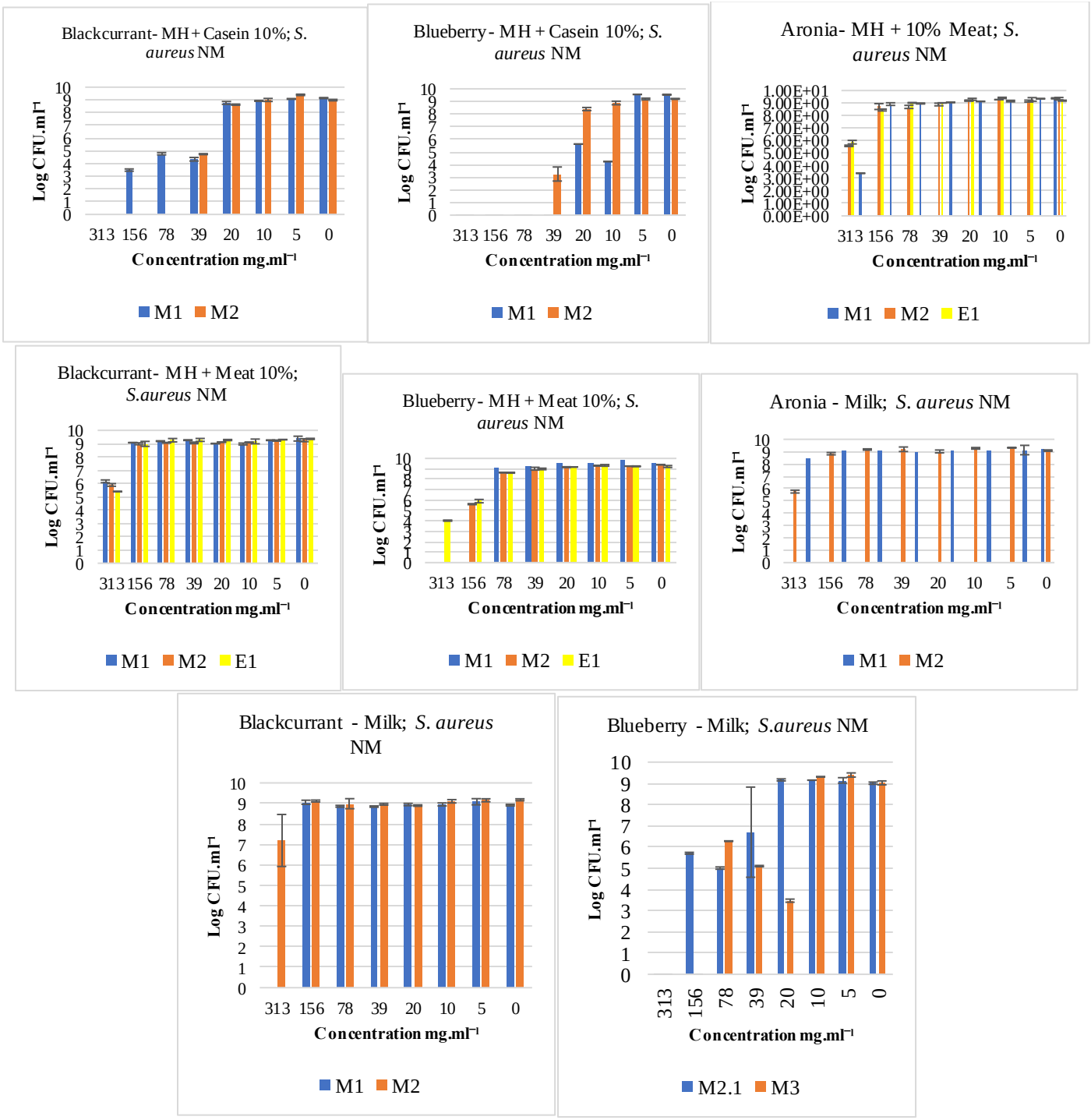
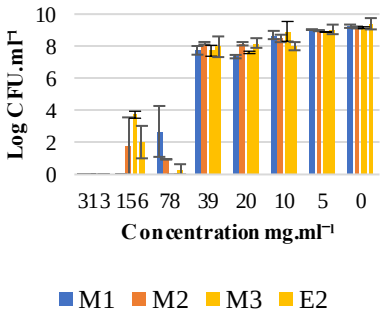
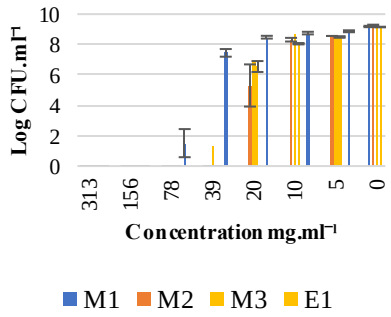


Figure 1 Comparison of antimicrobial effects of different berries' extraction batch and initial extracting solvents of aronia, blackcurrant and blueberry extracts on *S. aureus* NM in broth supplemented with food constituents. Each bar chart represents the average log CFU.ml⁻¹ of at least two replicates after 24 h of incubation. Error bars show SEM. Relative concentration is expressed as mg berry per ml media. Control is MH broth with initial inocula of ~5×10⁶ CFU.ml⁻¹. M: Methanol based extracts. E: ethanol-based extracts. 1 to 4 represents different batches of extracts.

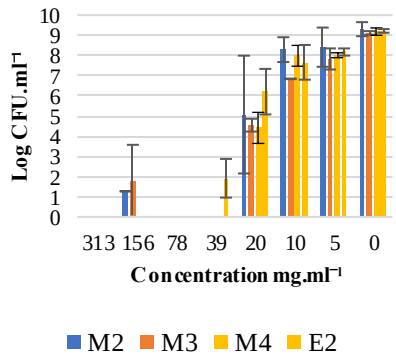
Blackcurrant- BHI; *L. monocytogenes*



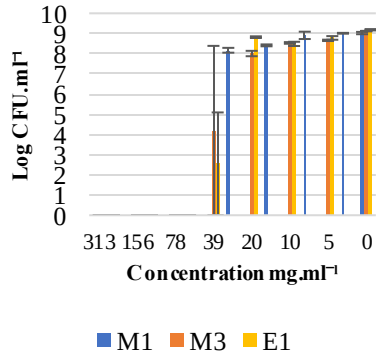
Aronia- BHI; *L. monocytogenes*



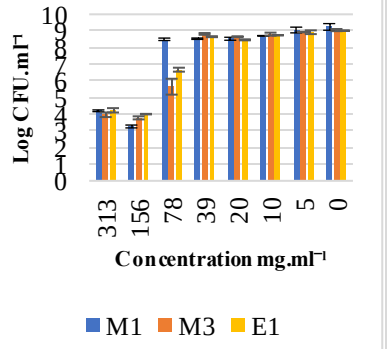
Blueberry- BHI; *L. monocytogenes*



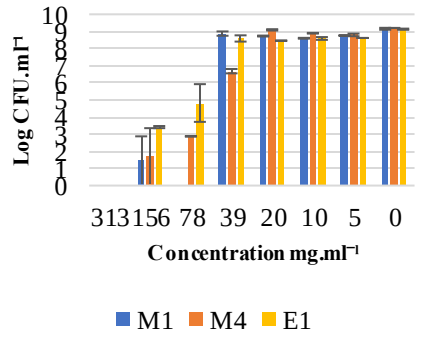
Aronia- BHI + Starch 2%; *L. monocytogenes*



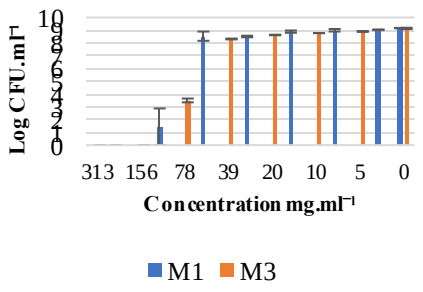
Blackcurrant- BHI + Starch 2%; *L. monocytogenes*



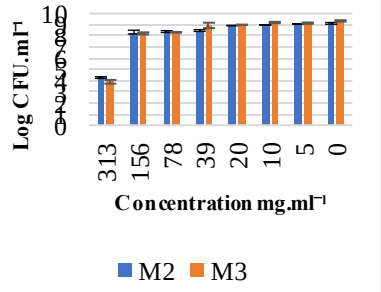
Blueberry- BHI + Starch 2%; *L. monocytogenes*



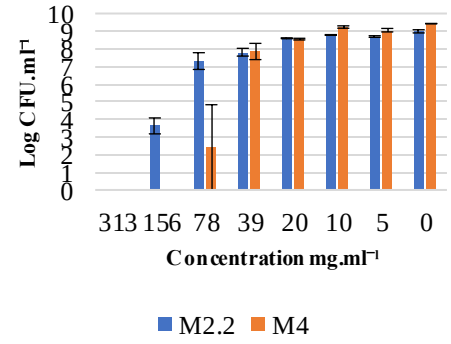
Aronia- Oil 5%; *L. monocytogenes*

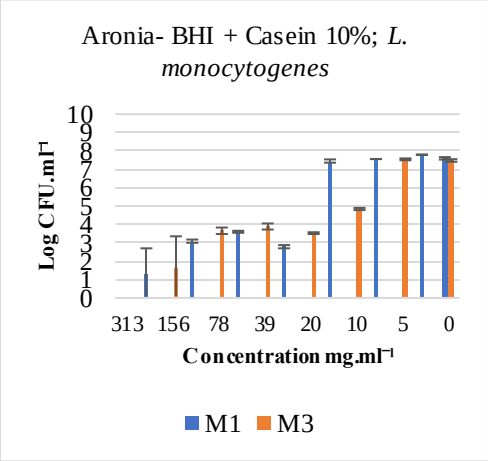


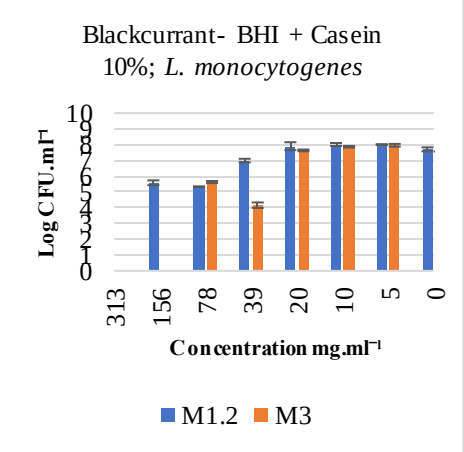
Blackcurrant- BHI + Oil 5%; *L. monocytogenes*

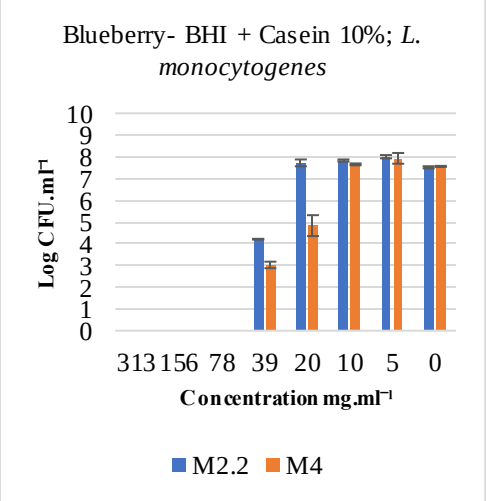


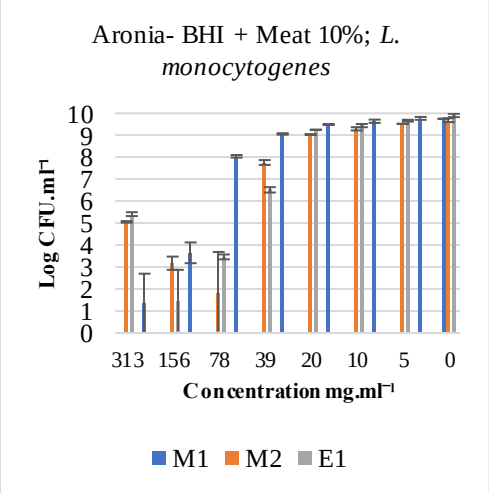
Blueberry- BHI + Oil 5%; *L. monocytogenes*

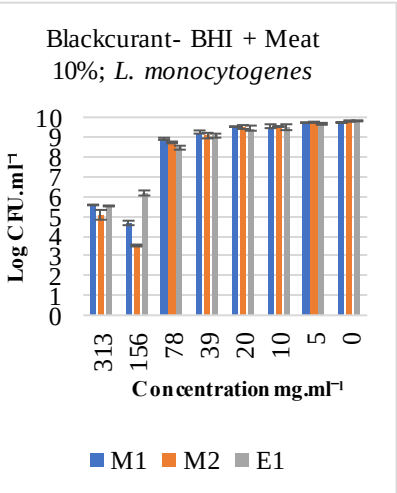



Aronia- BHI + Casein 10%; L. monocytogenes
Log CFU.ml⁻¹
Concentration mg.ml⁻¹
313 156 78 39 20 10 5 0
M1 M3


Blackcurrant- BHI + Casein 10%; L. monocytogenes
Log CFU.ml⁻¹
Concentration mg.ml⁻¹
313 156 78 39 20 10 5 0
M1.2 M3


Blueberry- BHI + Casein 10%; L. monocytogenes
Log CFU.ml⁻¹
Concentration mg.ml⁻¹
313 156 78 39 20 10 5 0
M2.2 M4


Aronia- BHI + Meat 10%; L. monocytogenes
Log CFU.ml⁻¹
Concentration mg.ml⁻¹
313 156 78 39 20 10 5 0
M1 M2 E1


Blackcurant- BHI + Meat 10%; L. monocytogenes
Log CFU.ml⁻¹
Concentration mg.ml⁻¹
313 156 78 39 20 10 5 0
M1 M2 E1

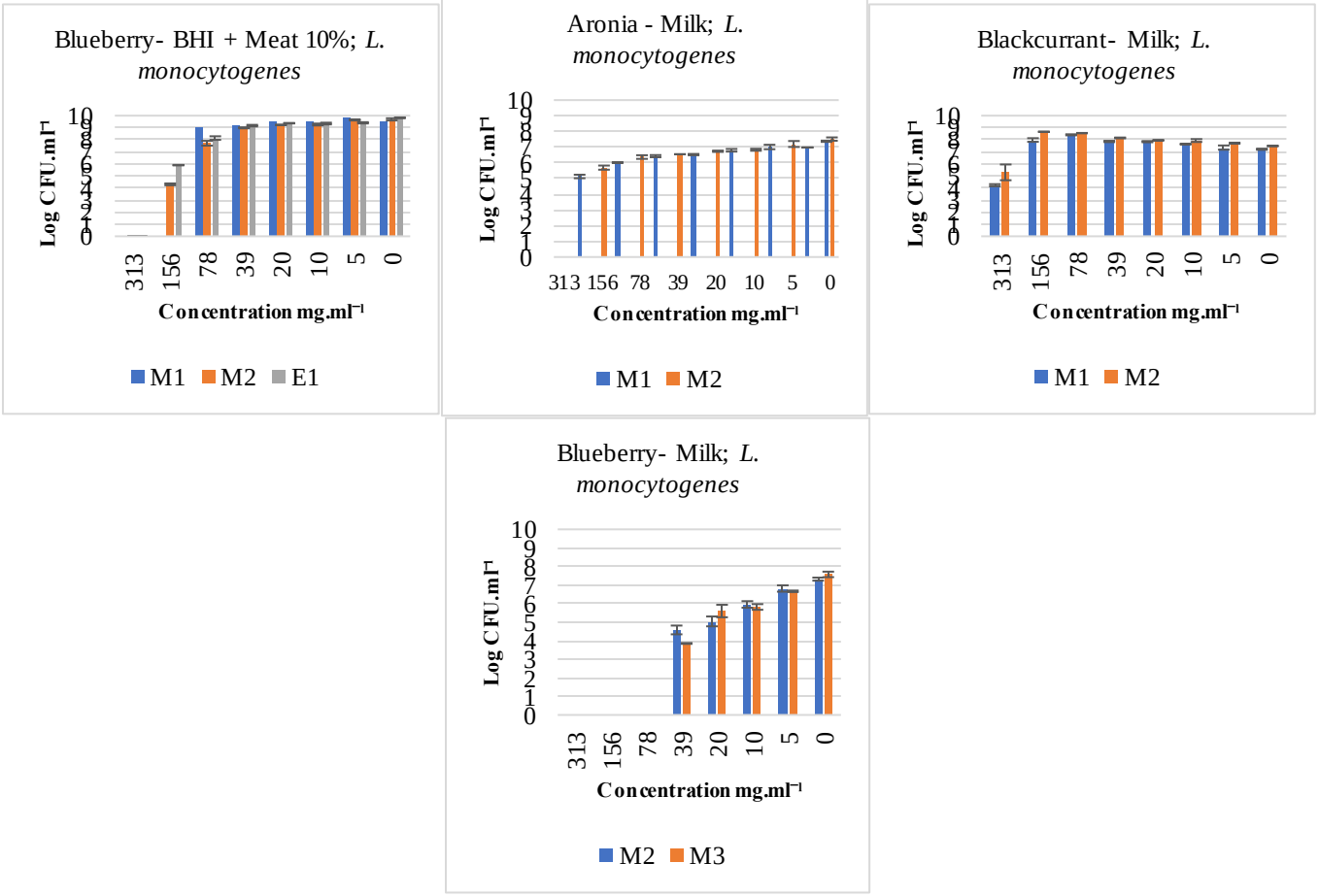


Figure 2: Comparison of antimicrobial effects of different berries' extraction batch and initial extracting solvents of aronia, blackcurrant and blueberry extracts on *L. monocytogenes* in broth supplemented with food constituents. Each bar chart represents the average log CFU.ml⁻¹ of at least two replicates after 24 h of incubation. Error bars show SEM. Relative concentration is expressed as mg berry per ml media. Control is BHI broth with initial inocula of ~5×10⁶ CFU.ml⁻¹. M: Methanol based extracts. E: ethanol-based extracts. 1 to 4 represents different batches of extracts.

Solvent choice impacts Ethanol and methanol as solvent choices for berry extracts yield non-significant differences in antibacterial activity. Ethanol's viability as an adequate substitute offers a safer and moreA economical option, mitigating health and financial concerns associated with methanol.

Conclusions

This manuscript establishes the interchangeability of ethanol and methanol for berry extract preparation, enhancing safety and cost-effectiveness through rigorous analysis. The study also explored the effects of Aronia, blackcurrant, and blueberry extracts on foodborne pathogens, including S. aureus and L. monocytogenes, at neutral pH, even in the presence of food supplements added to the initial media. Antimicrobial Potential: Aronia, blackcurrant, and blueberry extracts demonstrated robust antimicrobial effects against S. aureus NM and L. monocytogenes, even in the presence of food constituents, whether using ethanol or methanol as the solvent. Industrial and Research Value: These insights offer valuable guidance to industries and research groups to leverage berries' potential across applications. This study contributes to the field by focusing on solvent interchangeability, advancing safer and more economical extraction methods.

Acknowledgments

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Conflict of interest

the author (Massoud Attarianshandiz) affirms that there are no conflicts of interest to disclose. Supplementarymaterial Data analysis is provided in the supplementary section. ORCID ID https://orcid.org/0000-0002-2787-7502

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