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Research Article

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In vitro antibacterial efficacy of the commercial wintergreen (*Gaultheria procumbens* L.) essential oil against some Gram-positive and Gram-negative strains

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Article Details:
Received: 2023-04-28
Accepted: 2023-05-23
Available online: 2023-05-31

DOI: https://doi.org/10.15414/ainhlq.2023.0013

The aim of the current study was *in vitro* antimicrobial profiling of commercial wintergreen essential oil derived from leaves of *Gaultheria procumbens* (Natural essential oil – Wintergreen oil Bamer®) against Gram-positive strains such as *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®51299™) (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®29212™), *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC®29213™, *Staphylococcus aureus* NCTC12493™, and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula ATCC®27853™, *Escherichia coli* (Migula) Castellani and Chalmers ATCC®25922™, and *Escherichia coli* (Migula) Castellani and Chalmers ATCC®35218™. The testing of the antibacterial activity of wintergreen EO was carried out *in vitro* by the Kirby-Bauer disc diffusion technique. This study demonstrated that commercial wintergreen essential oil derived from leaves of *Gaultheria procumbens* (Natural essential oil – Wintergreen oil Bamer®) possesses significant antimicrobial activity against Gram-positive bacteria, such as *Enterococcus faecalis* strains. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* strains were resistant to commercial wintergreen essential oil derived from leaves of *Gaultheria procumbens*. This study showed that commercial wintergreen essential oil derived from leaves of *Gaultheria procumbens* could be a potential preparation as a source of natural antibacterial properties. Future pharmacological studies and development in other areas are thus warranted.

Keywords: *Gaultheria procumbens*, antibacterial activity, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* strains

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Introduction

Many publications prove the antibacterial properties of essential oils from medicinal plants (Vergnes et al., 2014). *Gaultheria procumbens* L. (American wintergreen, Ericaceae) is an aromatic, evergreen shrub native to north-eastern North America and cultivated worldwide in regions of temperate climate as an ornamental and medicinal plant (Kiran and Prakash, 2015; Michel et al., 2019; Lawson et al., 2021). Many studies have revealed that the extracts and compounds derived from *Gaultheria* plants exhibit a wide spectrum of pharmacological activities *in vitro* and *in vivo*, covering anti-inflammatory, analgesic, anti-oxidative, and antibacterial properties (Liu et al., 2013; Luo et al., 2018). It is known that *Gaultheria* plants accumulate a wide variety of polyphenols (Middleton, 1992), including methyl salicylate as the main component of the essential oil (Magiera et al., 2019), as well as nonvolatile compounds such as salicylate glycosides, procyanidins, and flavonoids (Liu et al., 2013; Magiera et al., 2019; Michel et al., 2020). Traditionally, plants rich in polyphenols, especially salicylates, have been used worldwide in the form of extracts, tinctures, infusions, and decoctions to treat a number of inflammatory diseases that are cross-related with oxidative stress (Michel et al., 2022).

In traditional medicine, the aerial parts (stems and leaves) of *G. procumbens* and other *Gaultheria* species, as well as methyl salicylate-rich essential oils distilled from the plants, are used (both externally and internally) in the treatment of disorders connected with inflammation, pain, and infection, including rheumatoid arthritis, influenza, the common cold, tracheitis, pharyngitis, pleurisy, fever, prostatitis, swelling and muscular pain, and some skin and periodontal problems (Olszewska et al., 2021). Essential oil from *G. procumbens* is mainly composed of methylsalicylate (MeSA) (>96%), a compound that can be metabolized in plant tissues to salicylic acid, a phytohormone inducing plant immunity against microbial pathogens (Mullen et al., 2014; Olszewska et al., 2021).

In the current study, *in vitro* antimicrobial profiling of commercial wintergreen essential oil derived from leaves of *Gaultheria procumbens* (Natural essential oil – Wintergreen oil Bamer®) was performed, exhibiting inhibitory activity against Gram-positive and Gram-negative strains.

Material and methodology

Wintergreen essential oil

The wintergreen EO was provided by Polish essential oil manufacturers (Bamer®, Włocławek, Poland). The investigated sample did not contain additives or solvents and was confirmed to be natural by the manufacturers. Product description: Natural essential oil – Wintergreen oil Bamer®. The highest quality, pure, natural essential oil, is obtained from fresh leaves of the wintergreen (*Gaultheria procumbens* Leaf Oil). Laboratory tested.

About the manufacturer: Bamer® has been offering 100% natural, pure essential oils and fragrance compositions since 1993. Application studies on patients conducted at the Medical Academy confirmed the effectiveness of Bamer® oils in aromatherapy and cosmetics. The products are not tested on animals. Safety assessments, numerous certificates and approvals, compliance with the latest pharmacopoeia Ph.Eur. and IFRA, positive opinion of the National Institute of Hygiene guarantee the highest pharmaceutical and cosmetic quality of oils. Bamer® oils have been submitted to the European Commission via CPNP (Cosmetic Products Notification Portal). Bamer® essential oils are certified by the National Institute of Hygiene, IFRA, and laboratory analyses.

The samples were stored in resalable vials at 5°C in the dark but were allowed to adjust to room temperature before investigation. Geographical origins were excluded as information was mostly not available.

Determination of the antibacterial activity of essential oils by the disk diffusion method

The testing of the antibacterial activity of wintergreen EO was carried out *in vitro* by the Kirby-Bauer disc diffusion technique (Bauer et al., 1966). In the current study, Gram-positive strains such as *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®51299™) (resistant to vancomycin; sensitive to teicoplanin), and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®29212™), *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC®29213™, *Staphylococcus aureus* NCTC12493™, and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula ATCC®27853™, *Escherichia coli* (Migula) Castellani and Chalmers ATCC®25922™, and *Escherichia coli* (Migula) Castellani and Chalmers ATCC®35218™ were used.

The strains were inoculated onto Mueller-Hinton (MH) agar dishes. Sterile filter paper discs impregnated with wintergreen EO were applied over each of the culture dishes. Isolates of bacteria with wintergreen EO were then incubated at 37 °C for 24 h. The Petri dishes were then observed for the zone of inhibition produced by the antibacterial activity of wintergreen EO. A control disc impregnated with 96% ethanol was used in each experiment. At the end of the 24 h period, the inhibition zones formed were measured in millimetres using the vernier. For each strain, eight replicates were assayed (n = 8). The Petri dishes were observed and photographs were taken. The susceptibility of the test organisms to the wintergreen EO was indicated by a clear zone of inhibition around the discs containing the wintergreen EO and the diameter of the clear zone was taken as an indicator of susceptibility. Zone diameters were determined and averaged. The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (S) ≥15 mm, Intermediate (I) = 10–15 mm, and Resistant (R) ≤10 mm (Okoth et al., 2013; Tkachenko et al., 2022).

Statistical analysis

Zone diameters were determined and averaged. Statistical analysis of the data obtained was performed by employing the mean ± standard error of the mean (S.E.M.). All variables were randomized according to the phytochemical activity of the wintergreen EO tested. All statistical calculation was performed on separate data from each strain. The data were analyzed using a one-way analysis of variance (ANOVA) using Statistica v. 13.3 software (TIBCO Software Inc., Krakow, Poland) (Zar, 1999).

Results and discussion

Figures 1 and 2 summarize the results obtained by the mean diameters of the inhibition zone around the growth of Gram-positive strains such as *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®51299™) (resistant to vancomycin; sensitive to teicoplanin), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®29212™), *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC®29213™, *Staphylococcus aureus* NCTC12493™, and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula

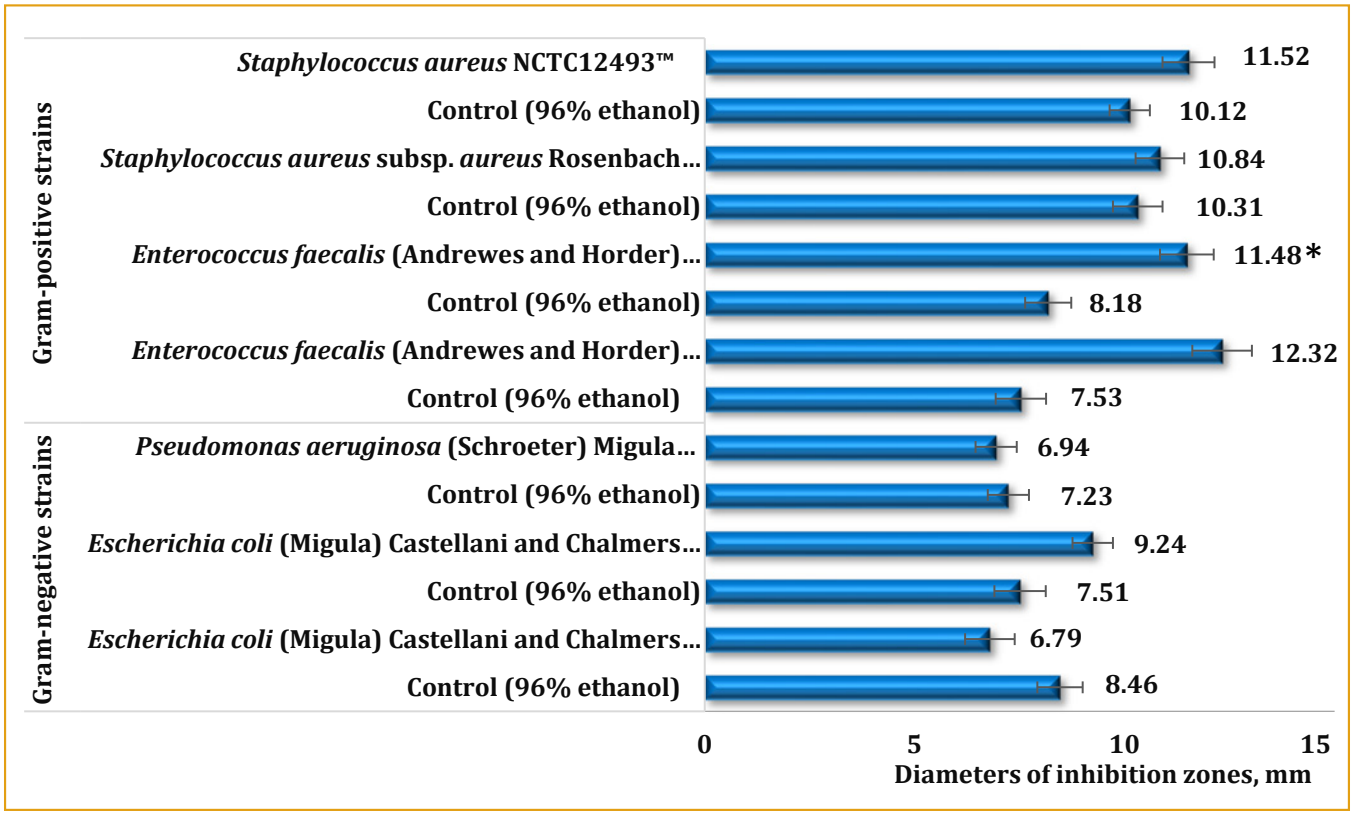

Figure 1 The mean inhibition zone diameters induced by commercial wintergreen essential oil against Gram-positive and Gram-negative strains (M ± m, n = 8)
*– changes are statistically significant compared to the 96% ethanol

ATCC®27853™, *Escherichia coli* (Migula) Castellani and Chalmers ATCC®25922™, and *Escherichia coli* (Migula) Castellani and Chalmers ATCC®35218™ strains induced by wintergreen EO.

After applying wintergreen EO to *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®51299™) strain, we noted a statistically significant increase in the zone of growth inhibition by 40.3% ($p < 0.05$) compared to the control samples (11.48 ±0.64 vs. 8.18 ±0.55 mm). We observed similar trends after *in vitro* application of wintergreen EO against *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®29212™) strain, where we also observed a statistically significant increase in the zone of growth inhibition by 63.6% ($p < 0.05$) against the control samples (12.32 ±0.71 vs. 7.53 ±0.6 mm). *Staphylococcus aureus* strains were resistant to wintergreen EO. After applying wintergreen EO to *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC®29213™ and *Staphylococcus aureus* NCTC12493™ strains, a statistically non-significant increase in the zone of growth inhibition by 5.1% ($p > 0.05$) and by 13.8% ($p > 0.05$) compared to the control samples (10.84 ±0.58 vs. 10.31 ±0.59 mm) and (11.52 ±0.62 vs. 10.12 ±0.48 mm), respectively (Figure 1).

Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula ATCC®27853™, *Escherichia coli*

Figure 2 The diameters of the inhibition zone around the growth of Gram-positive strains such as *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®29212™) (A), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®51299™) (B), *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC®29213™ (C), *Staphylococcus aureus* NCTC12493™ (D), and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula ATCC®27853™ (E), and *Escherichia coli* (Migula) Castellani and Chalmers ATCC®35218™ (F) strains induced by wintergreen EO

(Migula) Castellani and Chalmers ATCC®25922™, and *Escherichia coli* (Migula) Castellani and Chalmers ATCC®35218™ were resistant to wintergreen EO. Adding wintergreen EO to *Escherichia coli* (Migula) Castellani and Chalmers ATCC®25922™ and *Escherichia coli* (Migula) Castellani and Chalmers ATCC®35218™ strains resulted in statistically non-significant changes in the zone of growth inhibition (decrease by 19.7% and increase by 23%, p >0.05) compared to the control samples (6.79 ±0.59 vs. 8.46 ±0.54 mm) and (9.24 ±0.48 vs. 7.51 ±0.61 mm), respectively. Similarly, *Pseudomonas aeruginosa* (Schroeter) Migula ATCC®27853™ strain was also resistant to wintergreen EO. The diameter of the zone of growth inhibition was (6.94 ±0.49 mm) compared to the control (7.23 ±0.49 mm) (Figure 1).

The pharmacological activities of pure compounds and crude extract from the *Gaultheria* genus were mainly focused on anti-inflammatory and analgesic properties (Liu et al., 2013). Some studies were carried out revealing the antibacterial properties of plants belonging to the *Gaultheria* genus. For example, Ma et al. (2001) screened for the anti-bacterial activity of extracts derived from *Gaultheria leucocarpa* var. *yunnanensis* (Franch.) T.Z.Hsu & R.C.Fang. Anti-bacterial tests with extracts derived from water, acetic ester, and n-butanol exhibited that 3 extracts from 22 samples possessed anti-*Staphylococcus aureus* activity, and the extracts from roots and stems showed the same result. Two extracts inhibited the growth of *Escherichia coli* and *Pseudomonas aeruginosa* in a dose-dependent manner. These results suggested that not only essential oil but other ingredients from *G. leucocarpa* var. *yunnanensis* have anti-bacterial activity. Anti-fungal tests of the same extracts didn't indicate remarkable action (Ma et al., 2001).

The chemical constituents and biological activities of essential oil and crude methanol extract of *Artemisia vulgaris* L. and *Gaultheria fragrantissima* Wall. were identified by Pandey et al. (2017). Gas chromatography-mass spectroscopy analysis revealed that leaves of *G. fragrantissima* contained methyl salicylate (95%) and asarone (4.64%). Furthermore, methanol extracts from leaves of *A. vulgaris* and *G. fragrantissima* were found rich in total flavonoids and phenolic content. HPLC analysis revealed the presence of rutin as a major flavonoid compound in the leaves of *G. fragrantissima*. Further, methanol extract of the *A. vulgaris* and *G. fragrantissima* showed the highest antioxidant and antibacterial properties compared to the essential oil (Pandey et al., 2017). The least antibacterial activity was observed with *G. fragrantissima* oil against Gram-positive and negative pathogenic strains. Although the oil of *G. fragrantissima* showed the least activity, the methanol extract revealed comparatively higher antibacterial activities with a zone of inhibition in the range of 11–14 mm (Pandey et al., 2017).

Studies on the antimicrobial activity of essential oil from the leaves of *Gaultheria yunnanensis* (Franch.) Rehder was carried out by Wang et al. (2005). The essential oil from the leaves of *G. yunnanens* presented similar antibacterial effects as methyl salicylate. It has antibacterial activity against *E. coli* and *S. aureus*, but the essential oil is superior to methyl salicylate, and the lowest antimicrobial concentration is 0.3125 and 5%, respectively (Wang et al., 2005). Klūga et al. (2021) have detected the antimicrobial activity of the essential oils on pathogenic microorganisms found in freshwater fish. Essential oil of *Gaultheria procumbens* showed strong antibacterial activity against *Yersinia* spp. and *Vacococcus* spp. (6.25 μL.mL^{-1}) (Klūga et al., 2021). Ojha et al. (2022) carried out a comparative analysis of *Gaultheria fragrantissima* essential oils based on geographical location, distillation time, and varying distillation conditions. Three samples showed notable antibacterial activity against *Staphylococcus epidermidis*, with a minimum inhibitory concentration (MIC) value of 156.3 μg.mL^{-1}. Similarly, one sample showed effectiveness against *Aspergillus niger* (MIC = 78.1 μg.mL^{-1}) (Ojha et al., 2022). Essential oil of *G. procumbens* exhibited the strongest antimicrobial activity against one strain of *S. aureus* with the disc diffusion test (7.33 mm) in the study of Kačániová et al. (2020). A MIC value of 12.50 μL.mL^{-1} was found for *S. aureus*, *S. capitis*, and one strain of *S. haemoliticus*, determined with the broth microdilution method (Kačániová et al., 2020). Higher activity of essential oil of *G. procumbens* against Gram-negative bacteria (*Acinetobacter baumanii*, *Aeromonas sobria*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, and *Serratia marcescens*) in comparison to Gram-positive microorganisms (*Staphylococcus aureus* and *Enterococcus faecalis*) was observed in the study of Hammer et al. (1999). Higher resistance of Gram-positive bacteria against essential oil of *G. procumbens* was shown by Nikolić et al. (2013).

The essential oils from the root, stem, and leaf of *Gaultheria longibracteolata* R.C.Fang were investigated by anti-bacterial assays by Luo et al. (2021). Oil extracts from *G. longibracteolata* root, stem, and leaf, as well as methyl salicylate, were tested on four bacterial species: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*,

E. coli, and *S. aureus*. According to the literature (Jia and Li, 2005), *G. yunanensis* can be used to treat skin infections. However, according to Luo et al. (2021), the oil extracts did not inhibit the growth of *P. aeruginosa*. In contrast, the oil did show dose-dependent inhibitory activity against the other three bacteria species. The 80% dilution of leaf oil extract showed the strongest inhibitory activity against *K. pneumoniae*; the other extracts also showed inhibitory effects against *K. pneumoniae*, which may explain the use of branches and roots in traditional medicine. For *E. coli*, the 80% dilutions of leaf oil, stem oil, and methyl salicylate provided the strongest inhibitory activities. The *G. yunannensis* was also used traditionally for treating skin infections (Jia and Li, 2005). All oil samples showed relatively weak antibacterial activities against *S. aureus* in the study of Luo et al. (2021). Additionally, methyl salicylate is the most abundant bioactive component of *G. longibracteolata* oil in this study. Nevertheless, the result showed that pure methyl salicylate had relatively weaker activity than those of mixed oil at the same dosage, which raised the possibility that other components also contributed to the observed activity (Luo et al., 2021). Neither essential oil nor methanol extract of *G. longibracteolata* showed any antibacterial activity against *P. aeruginosa*. The study on *G. longibracteolata* essential oil showed a similar result on *E. coli* and *S. aureus*, which could be because the oil of the two species shared the same main component, methyl salicylate (Luo et al., 2021).

Gaultherin, 2-[(6-O-beta-D-Xylopyranosyl-beta-D-glucopyranosyl)oxy] benzoic acid methyl ester, a natural salicylate derivative extracted from *Gaultheria yunnanensis*, has been shown to have analgesic and anti-inflammatory effects and lack gastric ulcerogenic effect compared to aspirin in the primary study of Zhang et al. (2007). Earlier, these researchers investigated the mechanism of action of gaultherin, which may rely on its active metabolite, and the mechanism responsible for the non-ulcerogenic property. The results showed that gaultherin (200 $mg.kg^{-1}$) significantly inhibited the abdominal contractions in the acetic acid-induced writhing test in mice. The anti-inflammatory effect of gaultherin was demonstrated in the croton oil-induced ear edema model in mice. The results showed that gaultherin and equimolar dose of aspirin produced comparable inhibitory effects. The study of the metabolism characteristics of gaultherin in mice and rats indicated that gaultherin could be metabolically converted to salicylate, which produced pharmacological effects and provided effective concentrations for an extended period (Zhang et al., 2006). Also, the extract and salicylate-rich fraction obtained from *Gaultheria trichophylla* Royle showed significant analgesic, anti-inflammatory, and antipyretic effects *in vivo*, *in vitro*, and in silico assays that support its use in traditional medicine (Alam et al., 2023).

Conclusions

In the current study, we assessed *in vitro* antimicrobial profiling of commercial wintergreen essential oil derived from leaves of *Gaultheria procumbens* (Natural essential oil – Wintergreen oil Bamer®) against Gram-positive and Gram-negative strains. This study demonstrated that commercial wintergreen essential oil derived from leaves of *Gaultheria procumbens* (Natural essential oil – Wintergreen oil Bamer®) possesses potential antimicrobial properties against Gram-positive bacteria, such as *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®51299™) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®29212™) strains. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* strains were resistant to commercial wintergreen essential oil derived from leaves of *Gaultheria procumbens*. This study showed that commercial wintergreen essential oil could be a potential preparation as a source of natural antibacterial properties. Future pharmacological studies and development in other areas are thus warranted.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Funding

This work was supported by The International Visegrad Fund, and the authors are cordially grateful for this.

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