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Research article

Chemical Analysis and Bioactivities of *Gaultheria procumbens* L. Essential Oil: Herbicidal, Antioxidant and Antibacterial Activities

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Abstract

Keywords

essential oil;
EC-EO;
Gaultheria procumbens;
bioactivity;
herbicidal activity;
antibacterial activity;
antioxidant activity

The chemical composition of wintergreen (*Gaultheria procumbens* L.) essential oil (WEO) was identified by gas chromatography-mass spectrometry (GC-MS) and evaluated for its biological activities. Methyl salicylate (100%) was the main compound in the wintergreen essential oil. The herbicidal efficacy of the WEO was confirmed by germination inhibition, radical and shoot length reduction, and phytotoxicity assessment with *Echinochloa crus–galli* and *Amaranthus tricolor* seedlings. The WEO significantly reduced the germination and seedling growth of both weeds. It showed highly significant inhibition of seed germination (93.37%) and seedling growth of *A. tricolor* at the highest dose (6 µL/petri dish) assayed. The WEO was formulated as an emulsifiable concentrate (EC-EO) for post-emergence application, and was applied at a range of concentrations from 10 to 80 mL/L. Both treated plant leaves appeared wilted and slightly discolored within 1 day of application (DAA). The visible weed control efficacy was most remarkable at 80 mL/L treatment, which was a level that ultimately killed the seedlings of both species at 3 DAA, suggesting promising herbicidal potential for the WEO. The WEO showed weak scavenging activity with a high IC_{50} value (IC_{50} >2×10^4 and >5×10^4 ppm) for DPPH scavenging and metal chelating assays, respectively. The WEO showed moderate antibacterial activity, and its zones of inhibition against bacterial test strains were 7.90±0.8 mm and 23.9±0.9 mm for *Escherichia coli* TISTR 780 and *Staphylococcus aureus* TISTR 1466, respectively. Therefore, the results suggest the possibility of using WEO as an active ingredient to produce natural herbicides.

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1. Introduction

In recent years, increased environmental and human health concerns and resistance to synthetic pesticides in many pest species have increased the demand for natural products. This is even more highlighted in a global scale to avoid the entrance of hazardous chemicals and pesticides into the food chain [1, 2]. Among the natural plant products, essential oils (EOs) have been extensively reported that they are used in organic farming systems [3]. Because EOs are volatile and can evaporate quickly at a particular vapor pressure [4], they do not persist in soil and do not leach into groundwater [3, 5]. These secondary metabolites from the mevalonic and shiquimic pathways have a range of biological activities, including their antioxidant, antimicrobial, antiviral, insect repellent and pest control functions [6-8]. Among commercial EOs, *Gaultheria procumbens* L. (wintergreen) essential oil (WEO) is well-known and mainly used in the aromatherapy and perfume industries. The essential oil of wintergreen is usually obtained from the *Gaultheria*, and most commonly from the two wintergreen species *G. procumbens* and *G. fragrantissima* [9] because these species yield a high concentration of methyl salicylate, which is an aromatic ester structurally related to aspirin. It is found at high concentration in WEO, and is an active ingredient that efficiently relieves pain caused by muscle fever [10]. Methyl salicylate represents more than 99% of the composition of most wintergreen species oils after distillation [9, 11, 12]. Wintergreen oil is significant in a number of applications but the secondary metabolite ratio in the oil shows instability due to climatic, geographic, seasonal, and genetic factors. Oil-in-water (o/w) emulsion formulations are water-based systems, so they are not only environmentally friendly; they are also less toxic to the consumer and safer to transport [13]. In an emulsion, droplet agglomeration can lead to phase separation, which decreases emulsion stability and reduces bio-efficacy. About 95% of pesticides seem to have a common effect, which is associated with the production and accumulation of free radicals [2]. Reactive oxygen species (ROS) are generated by normal metabolic processes and can be formed through different mechanisms [9]. Over-production of ROS can damage biomolecules, interfere with protein oxidation and lipid peroxidation, and result in cellular apoptosis [14]. Furthermore, excess ROS levels can lead to the development of cell and tissue injuries [15].

The aims of the present work were to identify the components of commercial wintergreen oil, and to evaluate its pre- and post-herbicidal activities on barnyard grass (*Echinochloa crus–galli*) and amaranth (*Amaranthus tricolor*), and its antioxidant activity. Furthermore, the antibacterial activity of WEO was also performed using the disc diffusion method against *Escherichia coli* TISTR 780 and *Staphylococcus aureus* TISTR 1466. The chemical composition and various medicinal biological activities of WEO have been previously researched. However, to the best of our knowledge, this is the first research project concerned with formulating commercial WEO and evaluating its bioactivity for agricultural applications.

2. Materials and Methods

2.1 Essential oil and analysis of its chemical composition

Commercial grade essential oil from the leaves of the wintergreen plant (*Gaultheria procumbens* L.) was purchased from Chemipan Corporation Co., Ltd. (Bangkok, Thailand) and used in all experiments. The essential oil composition was analyzed by gas chromatography-mass spectrometry (GC–MS) using an Agilent 6890 N gas chromatograph with an Agilent 5973 mass detector equipped with an HP-5 silica capillary column (30 m × 0.25 mm ID, 0.25 µm film thickness). The oven temperature was programmed to rise from 40ºC for 3 min to 100ºC at 10ºC/min, then to further increase at 5ºC/min to 260ºC, and finally to remain isothermal at 260ºC for 5 min. The carrier gas

was helium with a 1 mL/min flow rate. MS analysis was carried out over a detection range of 30-500 amu. Typically, 0.2 µL of the sample was injected, and split mode injection (ratio 50: 1) was employed. The injector and detector temperatures were maintained at 250ºC and 270ºC, respectively. Individual compound identification was performed by comparison with the Kovat's retention indices (KI) calculated using a reference to a homologous series of n - alkanes. Percentage compositions were determined based on GC peak area and retention time as calculated by a Shimadzu CR6A data processor.

2.2 Preparation and characterization of emulsifiable concentrate formulation

The emulsifiable concentrate formulation of the essential oil (EC-EO) was done using the method described by Laosinwattana *et al*. [16] with an emulsifying agent system, Tween 80 as surfactant, and N-Dimethylformamide as co-surfactant. The formulation was prepared by mixing Tween 80 (30% w/v) and N-Dimethylformamide (20% w/v) with a magnetic stirrer at 1500 rpm for 10 min. Then, wintergreen oil (50% w/v) was added to the mixture, which was stirred continuously for 10 min. Various amounts of distilled water were added to adjust the WEO (active ingredient; AI) concentration, and the resulting solutions were constantly stirred for 10 min. The formulations were kept at room temperature (25±2ºC) for 3 h to observe their emulsion stability. After 3 h, the formulations were evaluated for particle size and polydispersity index (PI) by a dynamic light scattering (DLS) technique using a Nanoplus 3 (Micromeritics, Japan). Each measurement was computed in five replications using the program NanoPlus version 5.10/3.00. Each formulation was stored at room temperature and evaluated for post-emergence and antioxidant activity in later experiments.

2.3 Herbicidal activity: Pre-emergence

The seeds of *Echinochloa crus-galli* (barnyard grass) and *Amaranthus tricolor* (amaranth) were selected as representatives of mono- and dicotyledonous weeds, respectively. The *E. crus-galli* seeds were collected from paddy fields in Ladkrabang, Thailand, dried in the shade at room temperature for 3 months, and then incubated in a hot-air oven at 60ºC for 2 days to break their dormancy. The *A. Tricolor* seeds were purchased from Thai Seed and Agriculture Co. Ltd Bangkok, Thailand. The pre-emergent herbicidal activity assay of the essential oil was conducted under laboratory conditions. Petri dishes (9 cm diameter) were lined with germination paper that was moistened with 5 mL of distilled water, and 20 healthy seeds of the tested plants were placed in separate petri dishes. To test the inhibitory effects, various volumes of the pure essential oil (0, 3 and 6 µL) were loaded onto pieces of filter paper attached to the inner sides of the upper petri dishes, which were then sealed with parafilm® (in order to ensure that the volatile oil did not evaporate out). Treatment with distilled water (ddH_2O) was used as an experimental control. The experiment was conducted following a completely randomized design (CRD) replicated five times for each treatment. The petri dishes were maintained in a growth chamber (LAC-1075-N, Longyue, Shanghai) at 27±2ºC, 12/12 h light/dark, and around 80% relative humidity. After 7 days of incubation, the germination of seeds, radicals, and shoot length (cm) of weeds were measured. Then, the inhibition percentages were calculated according to the equation described by Laosinwattana *et al.* [17] and Chotsaeng *et al*. [18] with slight modifications as stated below:

$$\text{Inhibition (\%)} = 1 - \frac{\text{Length of WEO treatment (cm)}}{\text{Length of control treatment}} \times 100 \quad (1)$$

2.4 Herbicidal activity: Post-emergence

The effects of foliar-sprayed WEO on *E. crus-galli* and *A. tricolor* were studied in post-emergent herbicidal experiments. Plastic pots (15 cm high and 15 cm wide) that contained soil (soil: sand: manure ratio of 3: 1: 1) were used. In each pot, the weed seeds (15 seeds in each pot) were sown at 1–cm depth. The pots were kept in greenhouses under natural light conditions. The plants in the pots were irrigated daily with tap water. Fourteen days after sowing (DAS), equally sized healthy emergent plants were selected, and thinning to 5 plants per pot was performed. The EC-EO formulations were prepared with 10, 20, 40 and 80 mL/L of WEO (AI) as described above. Foliar spraying with EC-WEO and distilled water (control) was done onto the randomized pots, 20 DAS. Moreover, 30% w/v of Tween 80 and 20% w/v of N-Dimethylformamide were also tested to check for potential interference with the WEO activity. The sprays were applied using handheld sprayers at a rate of 1000 L/ha. Five replicates per treatment were maintained in a completely randomized design (CRD). After treatment, plant visual damage due to phytotoxicity, weed control efficacy was estimated as percentage growth reduction compared to the untreated control at 0, 1, 3, 5, 7,14 and 21 DAA, with 0% indicating no injury tissue and 100% representing complete destruction. The Brazilian Society of Weed Science published a detailed symptom assessment report, which is shown in Table 1 below.

Table 1. Description of phytotoxicity assessments for percentage growth reduction estimation

Growth Reduction (%)	Description
0 – 20	No injury or no damaging effect
20 – 40	Slight damage and/or reduction of growth but rapid recovery
40 – 60	Moderate injury and/or reduction of growth and slow recovery
60 – 80	Severe injury and/or reduction of growth and no recovery
80 – 100	Destruction of weeds and/or only a few living weeds

Source: The Brazilian Society of Weed Science [19]

2.5 Determination of *in vitro* antioxidant activity of essential oil

There are numerous methods for the determination of antioxidant activity. In this study, a DPPH bioassay and metal chelating assay were applied. As described above, the EC-WEO formulation was prepared with different concentrations for the antioxidant determination.

2.5.1 DPPH radical scavenging

DPPH radical scavenging activity was measured using the method of Ebrahimzadeh *et al.* [20]. In brief, a 2 mL volume of each WEO concentration (500, 1×10^3, 2×10^3, 1×10^4, and 2×10^4 ppm) was mixed with 2 mL of 100 µM DPPH prepared in ethanol. The reaction mixture was vortexed thoroughly and kept in the dark at room temperature for 30 min. The absorbance was read at 517 nm. The radical scavenging activity was calculated as a percentage of DPPH discoloration utilizing the equation:

$$\text{Radical scavenging activity (\%)} = \frac{A_C - A_S}{A_C} \times 100 \quad (2)$$

The EC-WEO concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition % against oil concentration; A_C is the absorbance of the solution containing all reagents except WEO, and A_S is the absorbance of WEO treatment. Ascorbic acid (vitamin C) and butylated hydroxytoluene (BHT) were the standards.

2.5.2 Metal chelating

The chelating ability of WEO for ferrous ions (Fe^{2+}) was quantified following a modified version of the method of Jamuna *et al.* [21]. Briefly, 1 mL of each concentration (1×10^4, 2×10^4, 3×10^4, 4×10^4, and 5×10^4 ppm) of each WEO was individually mixed with 50 µL of 2 mM $FeSO_4\cdot7H_2O$. The reaction was initiated by adding 100 µL of 5 mM ferrous solution into the mixture. The mixture was shaken and maintained at room temperature for 10 min before the absorbance at 562 nm was determined. The inhibition of Fe^{2+} complex formation was calculated using the same equation (2) as was used for the DPPH assay. The results were expressed as IC_{50} values.

2.6 Determination of *in vitro* antibacterial activity of essential oil

Bacterial strains, *Staphylococcus aureus* TISTR 1466 (Gram-positive) and *Escherichia coli* TISTR 780 (Gram-negative), were obtained from the School of Food Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The bacterial culture comprised nutrient broth (NB; HiMedia, India) containing 1 g/L of Tween 80 to aid oil diffusion through the growth medium. Tween 80 was solubilized in NB, filter-sterilized (0.45 µm, MS®, USA), and sterilized in an autoclave at 121ºC and 1 atm for 15 min. For activation of the tested strains, a loopful of each of *S. aureus* and *E. coli* were transferred to cultured medium. Each culture was incubated at 30°C overnight. Each overnight culture was used as inoculum in the experiments. Bioassay for the antimicrobial activity of WEO was carried out by the paper-disc diffusion method. A 100 µL aliquot of each bacterial suspension (10^8 CFU/mL) was uniformly inoculated using a sterile L-glass rod onto the sterilized nutrient agar (NA) plates. After the surfaces of the NA plates were left to dry, sterile filter paper discs with diameters of 6 mm (Whatman, England) were placed over these plates. The sterile discs were impregnated with 20 µL of pure WEO (100%). Amoxicillin (50 µg/mL) and sterile distilled water was used as positive and negative controls. Finally, the plates were kept at 4ºC for 2 h to allow dispersal, and then incubated at 37±2ºC for 24 h. The diameters of inhibition zones (mm) around the paper discs were measured for bacteria and antibiotics, and data were recorded on the backs of the inverted plates.

2.7 Statistical analysis

All data were analyzed using analysis of variant (ANOVA); the means were compared using Tukey's multiple range tests, considering a significance level of $p<0.05$. The experiment was arranged in a completely randomized design with five replications.

3. Results and Discussion

3.1 Chemical composition

The color of the essential oil extracted from the *G. procumbens* leaves (Chemipan, Thailand) was light yellow. According to the results of the GC-MS analysis, only one component representing 100% of the total commercial wintergreen essential oil composition was found. It had a retention

time (RT) of 10.09 min (Figure 1), and was identified as being methyl salicylate. Others also found that methyl salicylate was the main compound in oil extracted from the *Gaultheria* species [22, 23]. According to the Ibáñez and Blázquez [24] analysis, wintergreen essential oil (*G. procumbens*) had two aromatic compounds (99.83±0.01%); methyl salicylic (99.63±0.02%) and ethyl salicylic (0.18%), four monoterpene hydrocarbons (0.04±0.01%), and three oxygenated monoterpenes (0.07±0.01%). Moreover, numerous plants have been reported to be natural sources of essential oils that are high in salicylates. Examples are the essential oils from *Cimicifuga racemose* rhizome, *Filipendula ulmaria* and *Spiraea ulmaria* flower, *Salix* sp. bark and some *Viola* sp. [25]. However, our detection differed in the proportion of methyl salicylate. These variations in composition are the result of differences in climatic, geographic, and seasonal conditions [26].

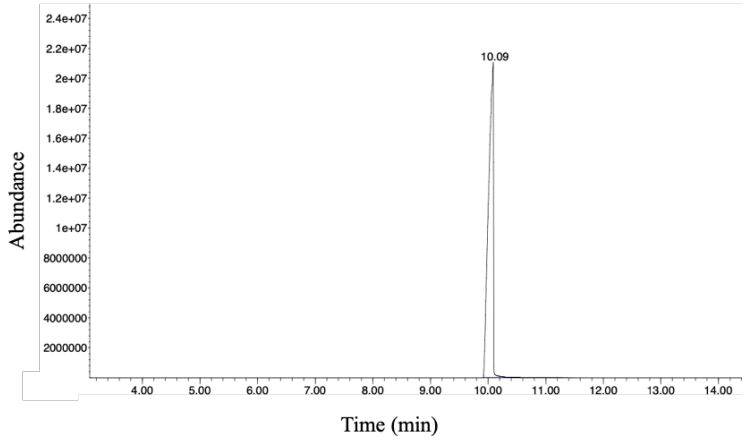

Figure 1. GC-MS total ion count chromatograms for the commercial essential oil of *G. procumbens* leaves

3.2 Characterization of the EC-EO formulation

The WEO was formulated as an EC-EO. The formulation characterization was investigated with analysis of droplet size and PI, 3 h after preparation. When diluted in water, the formulations gave a stable milky emulsion and were stable for 24 h without creaming and oil phase separation. The DLS technique was used to determine the mean particle size and the particle size distribution of the emulsion. The average droplet size was 457.8 nm, with a symmetrical distribution (data not shown). The EC-EO produced average PI values of 0.352; the obtained PI values were less than 0.5, indicating a droplet size distribution that was homogenous and suitable for agriculture [27].

3.3 Herbicidal activity: Laboratory and foliar spray pot bioassays

Two species of weeds, *E. crus-gilli* and *A. tricolor,* were tested to evaluate the herbicidal activity of WEO as a pre-emergence herbicide, and the petri dish assay results were considered. According to the analysis of the variance table, the percentage of germination, shoot length and root length of tested weeds were affected by the WEO, with depicted significant differences ($p \leq 0.05$) among treatments (Figure 2). In *A. tricolor* treatments, the mean germination at 7 DAA was 76.25 and 6.25% at 3 and 6 µL/dish concentrations, respectively. While for *E. crus-gilli* treatments, the germination percentage at both concentrations was higher than 80% (or 20% inhibition), demonstrating low effectiveness in controlling the weed (Figure 2A). Application of WEO at a high

concentration level (6 µL/dish) significantly reduced germination compared to the lower concentration (3 µL/dish) level. The data showed that WEO with 6 µL/dish highly suppressed *A. tricolor* seed germination, exhibiting a mean germination of 6.25% (or 93.75% inhibition). In a similar pattern, WEO and various concentrations also considerably influenced the shoot and root length of the target weeds, as can be seen in Figures 2B and 2C. The mean data showed a strong inhibitory effect (100% inhibition) on the seedling growth of *A. tricolor*, and treatment at 6 µL/dish had a more significant impact than 3 µL. Considering the pre-emergence evaluation, 6 µL presented a herbicidal activity of 100% for *A. tricolor*. The differences in the effect of WEO on the two types of weeds can be associated with the amount and types of essential oil, which were selective and interacted with the seed biological structure differently; *E. crus-gilli* belongs to the monocotyledonous plants while *A. tricolor* belongs to the group of dicot plants. The effect of WEO against the tested weeds was similar to that observed in previous publications that showed *E. crus-gilli* seed possessed stronger resistance against plant extracts and EOs [28]. In earlier research in 2010 [29], the allelopathic effect of *Zanthoxylum limonella* extract on Chinese amaranth and barnyard grass was investigated, and the research revealed that at a concentration of 2500 µM, xanthoxyline completely inhibited seed germination and growth of Chinese amaranth, while this compound showed a significantly inhibitory effect on seed germination of barnyard grass by 43.59%. The results showed that germination of the dicotyledonous plant was inhibited at a low concentration. Furthermore, in 2018, Chotsaeng *et al.* [18] studied the effects of various aldehydes on both weed species. The investigation revealed that the herbicidal effect of EOs was concentration dependent and species specific. In a similar study, Souri and Bakhtiarizade [30] showed that plant essential oils could have bio-stimulation effects on mineral uptake in a hydroponic culture of tomato seedlings.

The herbicidal potential of EC-EO applied as a foliar spray in the post-emergence was studied in a pot experiment at 20 DAS in both weed species under natural environmental conditions. Using the abovementioned method, the EC-EO was prepared with different concentrations (0, 10, 20, 40 and 80 mL/L of WEO). Figure 3 shows the weed control efficacy of the WEO with increasing time after application (DAA). The plants showed significant injury within 1 DAA in all treatments, ranging from 31 to 83% and 20 to 94% weed control efficacy in *E. crus-gilli* and *A. tricolor*, respectively. However, 3 days after applying 10 and 20 mL/L of AI, the plants rapidly recovered and became healthy (Figures 3A, 3B), while the 80 mL/L of AI ultimately killed both seedling species at 3 DAA (Figures 3C, 3D). The result indicates that WEO with an 80 mL/L active ingredient was highly effective against the tested weeds. The surfactants were also assessed for their phytotoxicity against the tested plants. The result shows the mixture of 30% w/v Tween 80 and 20% w/v N-Dimethylformamide caused slight wilt on both weeds with 10% weed control efficacy. These observations revealed that the EC-EO induces severe plant injuries upon contact. This work also considered other damage in the post-emergence; slight discoloration and small spots on the surface of the leaves were observed in most cases. The injuries found in the foliar spray pot experiment were similar to those seen in other studies [31]. Consequently, the findings obtained in our research are well corroborated by reports in the scientific literature, which show the potential herbicidal activity of the essential oil. Because the tested oil was 100% methyl salicylate, the observed result which was the phytotoxic activity against seed germination and growth of the tested weeds, was probably attributable to the effects of methyl salicylate. Previous researchers studied and developed a pesticide for fungi and bacteria using a methyl salicylate-like compound, pelargonic acid (Beloukha®, Katoun®) as the active ingredient [32]. It is a compound obtained from rapeseed essential oil. In terms of phytotoxicity, methyl salicylate was observed to control *Portulaca oleracea* with germination inhibition percentage of 44.19%, and root and shoot elongation inhibitions of 47.75% and 52.34% respectively, at 1 µL/mL [33]. Methyl salicylic is involved in plant defense and can act as a critical airborne signal that activates disease resistance and the

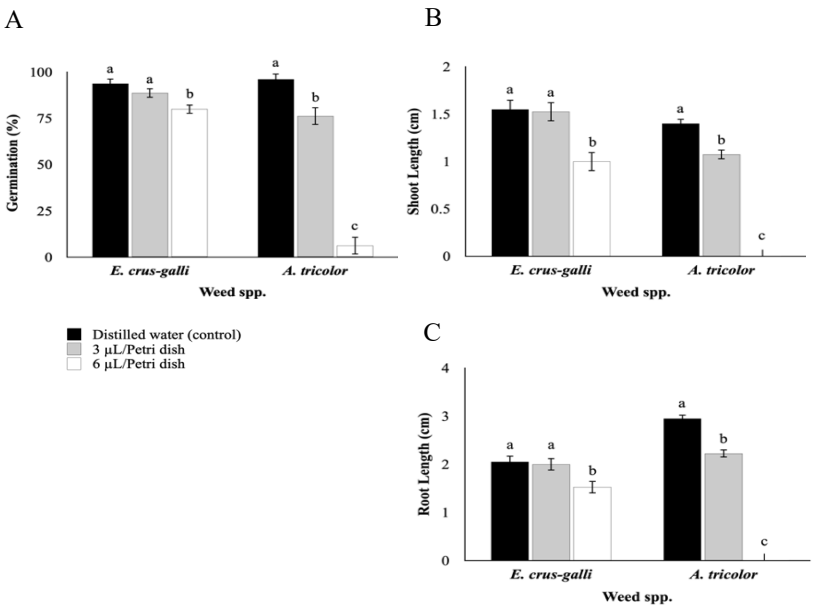

Figure 2. Effect of WEO on germination (A) and seedling growth (B, C) of *E. crus-galli* and *A. tricolor*. The different letters show significant differences ($p < 0.05$) in each weed separately.

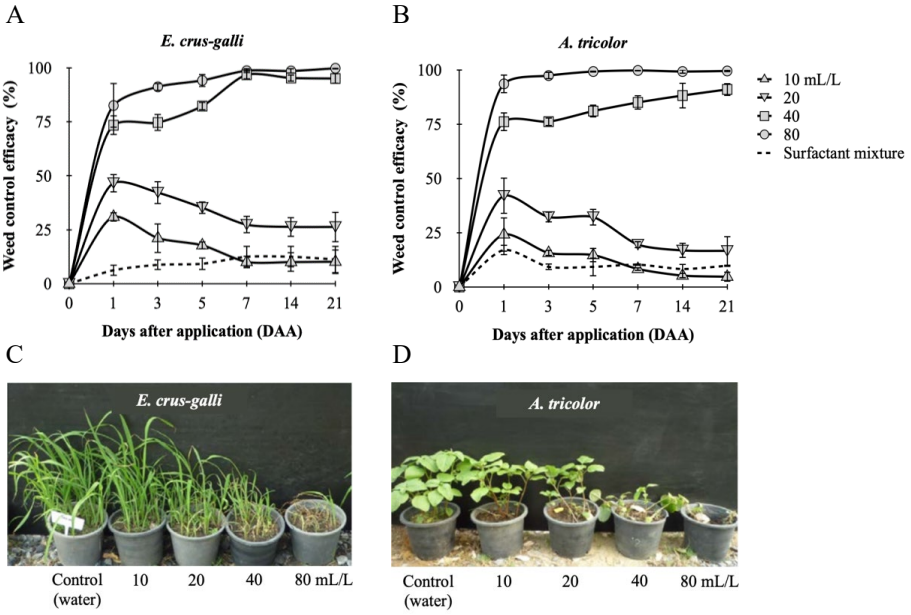

Figure 3. Effects of foliar sprayed WEO at different concentrations on *E. crus-galli* (A) and *A. tricolor* (B) at 1, 3, 5, 7, 14, and 21 DAA. The pictured plants were at 3 DAA (C and D).

expression of defense-related genes in neighboring healthy plants [24]. On the other hand, these observations may have been the result of the high concentrations of methyl salicylate that came into direct contact with leaf surfaces causing phytotoxic effects on the tested weeds.

3.4 Antioxidant activity

All pesticides seem to have a common effect, which involves the induction of oxidative stress through an accumulation of reactive oxygen species (ROS) [2]. Over-production of ROS can result in plant physiology changes such as cellular apoptosis, damage to biomolecules and cell and tissue injury [15]. To further understand the herbicidal activity of WEO, the antioxidant activity of the oil was determined with DPPH scavenging and metal chelating assays (Figure 4). Weak antioxidant activity means high herbicidal activity. IC_{50} values, displayed in Figure 4, can be defined as the essential oil concentration that causes 50% inhibition. A lower IC_{50} value is associated with higher antioxidant activity. As can be seen, the wintergreen essential oil had weak antioxidant activities with IC_{50} values of $>2\times10^4$ and $>5\times10^4$ ppm for DPPH scavenging and metal chelating assays, respectively, compared to the standard antioxidant vitamin C (3.16 ppm), BHT (25.23 ppm) and EDTA (18.32 ppm). According to Nikolić *et al*. [9], regarding the scavenging activity of the stable free radicle DPPH, *G. procumbens* essential oil exhibited an IC_{50} value of 3.06×10^4 ppm. This result indicated that the WEO showed weak scavenging activity.

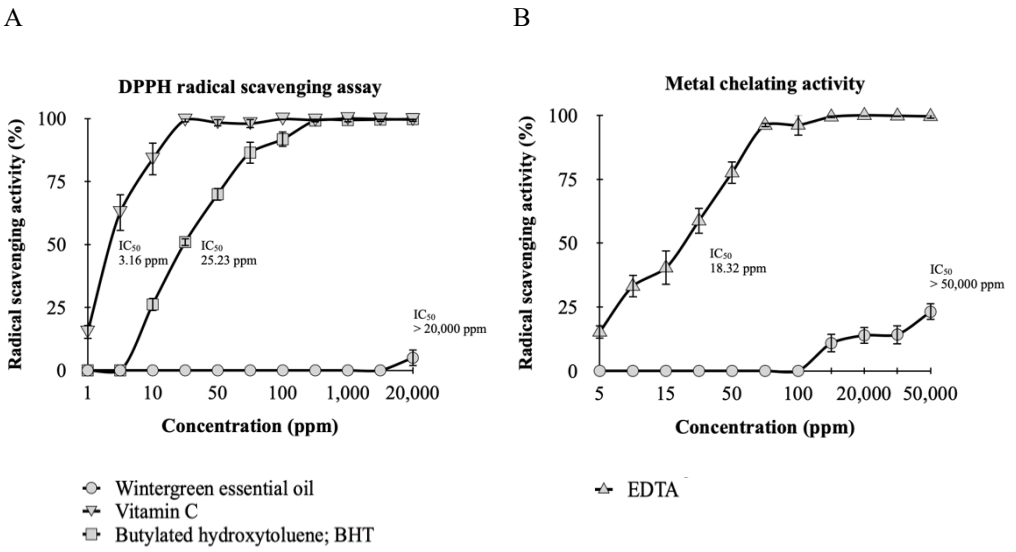

Figure 4. Antioxidant activity of the WEO. Determining DPPH scavenging ability (A) and metal chelating power (B). Vitamin C, BHT, and EDTA were used as positive controls.

3.5 Antibacterial activity

Numerous publications have reported on the antibacterial effects of EOs against plant and human pathogens. For example, oils from *Cymbopogan citratus* [34], *Origanum vulgarae* and *Lavandula officinalis* [35] were reported as having properties against *S. aureus* and *E. coli*. Thus, antibacterial activity was measured on two bacterial species: *S. aureus* and *E. coli*. The results were determined

by the paper disc diffusion method with measurement of the size of the inhibition zone (mm). As described in Table 2, the antibacterial effect was determined against *S. aureus* TISTR 1466, and produced a clearing zone of 23.9±0.9 mm, whereas *E. coli* TISRT 780 showed 7.90±0.8 mm. Amoxicillin showed a significantly higher antibacterial activity in comparison to WEO. The present findings revealed that WEO moderately suppressed the growth of *S. aureus* and *E. coli*, respectively (Figure 5). All WEO treatments showed relatively weak antibacterial activity against tested bacteria. The antimicrobial effect of the tested oil was directly linked to its chemical composition. In our study, methyl salicylate was shown to be the main bioactive component in WEO. Previous reports demonstrated the antibacterial activity of pure methyl salicylate was relatively weaker than that of mixed oil at the same dosage [9]. Thus, the observed activity may have been caused or contributed to by other components. Although EO has shown notable antimicrobial activity *in vitro*, higher amounts are required to achieve it. Increased essential oil concentrations may have a toxic effect on non-target organisms [36]. Therefore, the utilization of EOs in agriculture should be considered very carefully. Moreover, EOs have certain limitations, such as low stability and water solubility [37]. Therefore, novel strategies for nano-encapsulation and combination approaches are advised in order to improve the physical properties of the essential oils. Such challenges can provide an exciting platform for this research area.

Table 2. Antimicrobial activity of wintergreen essential oil against pathogenic microorganisms

Bacterial strains	Zone of inhibition (mm)	
	WEO	**50 µg/mL amoxicillin**
Staphylococcus aureus TISTR 1466	23.9±0.9[b]	33.80±0.2[a]
Escherichia coli TISTR 780	7.90±0.8[b]	19.20±0.1[a]

Values are the mean of 5 replications±S.D. Different superscript letters within a row indicate significantly different groups determined by Tukey's multiple range tests ($p < 0.05$).

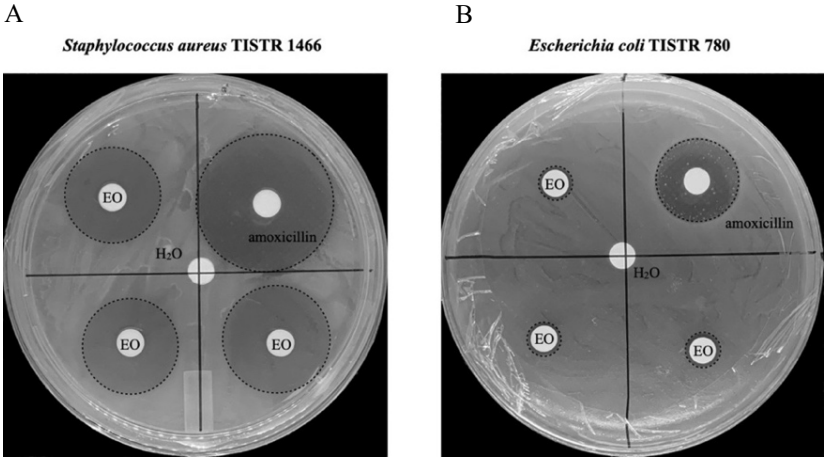

Figure 5. Inhibitory zone of commercial WEO on *S. aureus* TISTR 1466 (A) and *E. coli* TISTR 780 (B) by disc diffusion technique. EO - wintergreen essential oil; amoxicillin 50 µg/mL

4. Conclusions

Wintergreen (*Gaultheria procumbens* L.) essential oil was demonstrated to possess strong herbicidal activity. Pre-emergence, the WEO was shown to ultimately inhibit *A. tricolor* germination and seedling growth, while post-emergence, it can be concluded that it damaged the plants. The WEO eventually killed the seedlings of both species tested at 3 DAA. Wintergreen essential oil could be used as a pre-emergent bioherbicide to control amaranth (*A. tricolor*). In addition, WEO showed powerful post-emergent herbicidal effects against both *A. tricolor* and *E. crus-gilli*. Methyl salicylate effectively controlled weeds in a dose-dependent manner. This aromatic compound from wintergreen oil was assayed for various biological activities and found to have low antioxidant and moderate antibacterial activity. Wintergreen essential oil showed weak scavenging activity suggesting herbicidal potential. Finally, the present findings revealed that WEO moderately suppressed the growth of *S. aureus* (23.9±0.9 mm) and *E. coli* (7.90±0.8 mm). The abovementioned results point to the value of using WEO as an active ingredient for developing natural bioherbicides for organic agricultural systems.

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