

Antibacterial activity of northern Ontario medicinal plant extracts

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Hassan, H. M., Jiang, Z.-H., Asmussen, C., McDonald, E. and Qin, W. 2014. **Antibacterial activity of northern Ontario medicinal plant extracts.** *Can. J. Plant Sci.* **94**: 417–424. In the present study, the antibacterial activity (*in vitro*) of the leaf and/or flower crude extracts of *Anaphalis margaritacea* (L.) Benth & Hook.f., *Grindelia squarrosa* (Pursh) Dunal, *Apocynum androsaemifolium* L., *Arctostaphylos uva-ursi* (L.) Spreng, *Cornus canadensis* L. and *Xanthium strumarium* L. medicinal plants was analyzed through the hole-plate diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays against *Escherichia coli*, *Aeromonas caviae*, *Paenibacillus alvei*, *Micrococcus luteus*, *Mycobacterium avium* subsp. *avium* and *Bacillus cereus* bacteria. In addition, the time-kill dynamic processes of these extracts against *A. caviae*, *B. cereus*, *P. alvei*, and *M. luteus* bacteria were also tested. The leaf and flower extracts of *Anap. margaritacea* and *G. squarrosa* possess significant antibacterial activity against all the bacteria tested, with inhibition of *A. caviae*, *P. alvei* and *M. luteus* within 1–12 h of incubation at MBC. Particularly, at higher concentrations of the *Anap. margaritacea* flower crude extract (2–3 × MBC), inhibition of *A. caviae*, *B. cereus*, *P. alvei*, and *M. luteus* bacteria is achieved between 0.5 and 4 h of incubation. In addition, these extracts exhibit high inhibition diameters (majority > 18 mm) and low MIC and/or MBCs (majority ≤ 1.25 mg mL⁻¹). In contrast, the leaf extracts of *Arct. uva-ursi*, *X. strumarium*, *Apoc. androsaemifolium* and *C. canadensis* plants demonstrate moderate to low activity. These results indicate that extracts from *Anap. margaritacea* and *G. squarrosa* could be a source of novel antimicrobial scaffolds, compounds or pharmacophores with implications in the pharmaceutical industry and medicine.

Key words: Antibacterial, phytochemicals, northern Ontario, medicinal plants, Gram positive, Gram negative

Hassan, H. M., Jiang, Z.-H., Asmussen, C., McDonald, E. et Qin, W. 2014. **Activité antibactérienne des herbes médicinales du nord de l'Ontario.** *Can. J. Plant Sci.* **94**: 417–424. La présente étude porte sur le pouvoir antibactérien (*in vitro*) des extraits bruts de feuilles ou de fleurs de *Anaphalis margaritacea* (L.) Benth & Hook.f., *Grindelia squarrosa* (Pursh) Dunal, *Apocynum androsaemifolium* L., *Arctostaphylos uva-ursi* (L.) Spreng, *Cornus canadensis* L. et *Xanthium strumarium* L. L'activité antibiotique de ces herbes médicinales a été analysée par diffusion dans la gélose, concentration minimale inhibitrice (CMI) et concentration minimale bactéricide (CMB) avec *Escherichia coli*, *Aeromonas caviae*, *Paenibacillus alvei*, *Micrococcus luteus*, *Mycobacterium avium* ssp. *avium* et *Bacillus cereus*. Les auteurs ont également testé la dynamique temporelle des processus bactéricides avec *A. caviae*, *B. cereus*, *P. alvei* et *M. luteus*. Les extraits foliaires et floraux d'*Anap. margaritacea* et de *G. squarrosa* possèdent un pouvoir antibactérien significatif contre toutes les bactéries testées, l'inhibition de *A. caviae*, *P. alvei* et *M. luteus* survenant après une à 12 h d'incubation à la CMB. Aux concentrations plus élevées (2–3 × CMB), l'extrait brut des fleurs d'*Anap. margaritacea*, en particulier, inhibe la croissance de *A. caviae*, *B. cereus*, *P. alvei* et *M. luteus* après ½ à 4 h d'incubation. Ces extraits créent aussi des plages d'inhibition d'un grand diamètre (> 18 mm pour la plupart) et des plages plus petites à la CMI ou à la CMB (≤ 1,25 mg mL⁻¹ pour la plupart). À titre de comparaison, les extraits foliaires de *Arct. uva-ursi*, *X. strumarium*, *Apoc. androsaemifolium* et *C. canadensis* n'ont qu'une activité allant de faible à modérée. Ces résultats indiquent que les extraits de *Anap. margaritacea* et de *G. squarrosa* pourraient constituer une nouvelle source d'échafaudages, de composés ou de pharmacophores antimicrobiens dont on pourrait se servir dans l'industrie pharmaceutique ou en médecine.

Mots clés: Antibactérien, phytochimie, nord de l'Ontario, herbes médicinales, Gram positif, Gram négatif

Infectious diseases pose a considerable threat to public health due to the evolution of multiple-drug resistant strains of pathogenic bacteria (Ahmad et al. 1998). In addition to the escalating costs of intervention strategies, the current situation has expedited the re-emergence of previously benign diseases and a substan-

tial spread of opportunistic and chronic infections in developing countries (Ako-Nai et al. 2003). This is particularly alarming due to a decline in commercially accessible antibiotics, which may be attributed to the

Abbreviations: CFU, colony forming units; LB, liquid broth; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; NCE, new chemical entity; PBS, phosphate buffer saline; ZOI, zone of inhibition

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retraction of natural product screening programs from the advent of alternate drug discovery venues: genomics, in silico drug design and target-based high throughput screening of compound libraries (Lam 2007; Butler 2008; Harvey 2008; Taylor 2013). However, despite the exploitation of these regimes, the inception of new chemical entities (NCEs) to treat infections are steadily declining (Newman et al. 2003). Subsequently, there is a growing interest in medicinal plants as sources of antibacterial phytopharmaceuticals.

Northern Ontario is renowned for investment in forestry, and many industries resort to forestry resources for economic stability and diversification (Duinker et al. 1991). In our initial inquiry of the northern Ontario region, we identified an absence of ethnopharmacological research despite the abundance of plants utilized in traditional medicinal practices. Hence, we accumulated a list of 48 northern Ontario medicinal plants that exhibit anti-inflammatory, antibacterial, antiviral, anticancer and antidiarrheal activity, among others (Hassan et al. 2012). From this list, we identified Pearly Everlasting (*Anaphalis margaritacea* (L.) Benth & Hook.f., *Asteraceae*), Gumweed (*Grindelia squarrosa* (Pursh) Dunal, *Asteraceae*), Dogbane (*Apocynum androsaemifolium* L., *Apocynaceae*), Bearberry (*Arctostaphylos uva-ursi* (L.) Spreng, *Ericaceae*), Bunchberry (*Cornus canadensis* L., *Cornaceae*) and Cocklebur (*Xanthium strumarium* L., *Asteraceae*) medicinal plants based on their uses in the treatment of burns, cuts, inflammation, infections, diarrhea and mouth conditions in indigenous communities. In accordance with the North American First Nations ethnobotanical medicinal plants trend (Jones et al. 2000), plants utilized in aboriginal communities in the treatment of these illnesses may be a source of antibacterial phytochemicals. In this study, the antibacterial bioactivity of the leaf and/or flower extracts of these plants was evaluated through the hole-plate diffusion, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and time-kill assays against *Escherichia coli*, *Aeromonas caviae*, *Paenibacillus alvei*, *Micrococcus luteus*, *Mycobacterium avium* subsp. *avium* and *Bacillus cereus* bacteria. The antibacterial activity of *Anap. margaritacea* and *Apoc. androsaemifolium* has never been studied; also, the activity of *G. squarrosa* (Jacobs et al. 2010) and *C. canadensis* (McCutcheon et al. 1992) plants is crudely reported in literature. Although, the activity of *Arct. uva-ursi* (Vučić et al. 2013) and *X. strumarium* (Kim and Shin 1997) has previously been reported, geographical variation in the northern Ontario region may be present. In addition, we report on the first time-kill analysis of these extracts against *A. caviae*, *P. alvei*, *M. luteus* and *B. cereus* bacteria. The study of antibacterial activity (in vitro) of northern Ontario medicinal plants may provide scientific justification for their use in indigenous communities, and broaden the scope of existing drug discovery programs.

MATERIALS AND METHODS

Chemicals

Resazurin was purchased from Sigma-Aldrich (Canada). Sodium chloride, potassium chloride, sodium bisphosphate, potassium phosphate monobasic, methanol, yeast extract, phytone and peptone were purchased from Fisher Scientific (Canada).

Plant Material

The leaf and/or flower samples of *Anaphalis margaritacea* (C.E.G.51), *Grindelia squarrosa* (A.E.A.56), *Apocynum androsaemifolium* (W.H.70), *Arctostaphylos uva-ursi* (J.A.B.55), *Cornus canadensis* (P.B.71) and *Xanthium strumarium* (O.F.R.I.131) plants were collected in Thunder Bay region, Ontario, between 2011 Jul. 22 and 2011 Aug. 10. The specimens were identified by Erika North, herbarium curator and contract lecturer at Lakehead University, Thunder Bay, Ontario. All voucher specimens were deposited in a -80°C freezer at the Department of Biology lab (CB3037), Lakehead University.

Extraction

The plant samples were dried in the dark over a 24–48 h period at 30°C . The dried plant samples (2 g) were ground, milled through no. 40 mesh and extracted at room temperature with 95% ethanol (2×50 mL, 24 h each). All extracts were filtered through Whatman No. 1 filter paper, evaporated to dryness through rotary evaporator at 34°C under vacuum and further dried under high vacuum overnight. The dried extracts were stored in parafilm glass vials at -80°C until required for analysis.

Test Microorganisms

The microorganisms utilized in this study were representative of Gram negative, Gram positive, aerobic and facultative anaerobic categories. They comprised of *Bacillus cereus*, *Escherichia coli*, *Micrococcus luteus*, *Mycobacterium avium* subsp. *avium*, *Paenibacillus alvei* and *Aeromonas caviae*. The isolates were streak plated on liquid broth (LB) agar and maintained at 4°C in the dark. Stock freezer cultures were kept at -80°C .

Hole-plate Diffusion Assay

The procedure was followed according to Daud et al. (2005) with modifications. In this assay, $100 \mu\text{L}$ of 2×10^6 CFU mL^{-1} bacterial suspensions in $1 \times$ phosphate buffer saline (PBS) were homogeneously seeded into 25 mL of LB agar and poured into 100-mm (diameter) petri dishes. Then, 6 mm wells were aseptically bored into agar and $40 \mu\text{L}$ of 10 mg mL^{-1} methanolic extracts were aseptically pipetted into the wells. The plates were kept at 4°C for 3 h, and then incubated at 32°C for 18–24 h in the dark. Chloramphenicol ($100 \mu\text{g mL}^{-1}$) and ampicillin ($100 \mu\text{g mL}^{-1}$) were used as positive controls, and methanol was used as the negative control. All results were recorded as the mean of triplicate

measurements and the zones of inhibition were determined as the diameter of inhibition zones around the 6-mm wells.

MIC and MBC Assay

Minimum inhibitory concentration (MIC) represents the lowest concentration of extract treatment that inhibits the visible growth of microorganisms, whereas, the minimum bactericidal concentration (MBC) is the lowest extract concentration that completely exterminates the bacterial population. The MIC assay was performed according to Sarker et al. (2007) with modifications. The MIC assay utilized is compatible with the CLSI antimicrobial susceptibility-testing standards, as described in the M7-A7 document (Wikler 2006). The dried extracts were re-suspended in 99.85% methanol to a concentration of 20 mg mL⁻¹. Then, 50 µL of 20 mg mL⁻¹ extract was aseptically added to 230 µL of LB broth in the first well of a sterile 96-well microplate. To each subsequent well, 180 µL of LB broth was added. Twofold serial dilutions, in 100-µL aliquots, were performed using a multichannel pipette to achieve a concentration gradient of 5.00–0.04 mg mL⁻¹. Prior to each subsequent dilution, precaution was taken to maximize the homogeneity of the solution. Then, 20 µL of 2 × 10⁶ CFU mL⁻¹ bacterial suspensions in 1 × PBS buffer was inoculated in their respective wells (to a final volume of 200 µL), the plate was wrapped with parafilm and placed in a plastic bag with mildly wet paper towel to prevent dehydration of the bacteria. The plates were incubated at 32°C for 24 h in the dark. After incubation, 10 µL of 0.04% resazurin in 1 × PBS buffer was added and the plate was further incubated in the dark at 32°C for 90–180 min. Micro-plates were viewed under short-wavelength UV lamp for fluorescence and color change. The lowest concentration in which color changed from blue to pink, and displayed fluorescence, was taken as MIC. To determine the MBC of extracts, a 15 µL aliquot was appropriated from the wells without visible growth and plated on LB agar (Karaman et al. 2003). Wells exhibiting complete annihilation of bacteria after incubation of the LB agar plates at 32°C for 18–24 h in the dark were taken as MBC. Chloramphenicol and ampicillin (dissolved in 99.85% methanol) were used as positive controls, and 99.85% methanol as negative control in parallel experiments. The tests were performed in duplicate.

Time-kill Analysis

Time-kill curves represent an analysis of the bacterial population dynamics upon plant extract treatments over a certain time interval. Time-kill analysis was performed according to Imazato et al. (1999). In this assay, bacterial suspensions prepared in 1 × PBS buffer were added to 2 mL of LB broth to a final concentration of 2 × 10⁶ CFU mL⁻¹. The LB broth solution was prepared with a defined concentration of the plant extracts and incubated at 32°C with shaking at 200

rpm. After 30 min, 1 h, 2 h, 4 h, 8 h, 12 h and 24 h of incubation, a 40-µL aliquot was acquired, serially diluted (10¹, 10², 10³) and plated on LB agar plates. The plates were incubated at 32°C for 18–24 h and the number of viable microorganisms was determined by counting the colonies. Each assay included a growth control with 99.85% methanol treatment in the absence of the plant extracts. The test was performed in triplicate.

Statistical Analysis

The results from hole-plate diffusion and time-kill assays are represented as mean ± standard deviation (SD) of three replicate measurements. The MIC and MBC assays were run in duplicate, and the values represent measurements from two independent experiments. PRISM V was utilized for the generation of graphs.

RESULTS

The antibacterial activity of the leaf and/or flower extracts of medicinal plants was determined through the hole-plate diffusion, MIC, MBC and time-kill assays. In the hole-plate diffusion assay, all eight extracts exhibit antibacterial activity with zone of inhibition diameters (ZOI) in the range of 9.3–35.3 mm, representing a variation of approximately fourfold (Table 1). The leaf and/or flower extracts from *Anap. margaritacea* and *G. squarrosa* plants display high inhibition diameters (majority >16 mm) towards *A. caviae*, *B. cereus*, *M. luteus*, *M. avium* subsp. *avium* and *P. alvei* bacterium. These extracts also exhibit relatively low MIC and MBC's (Table 2). In contrast, the extract of *X. strumarium* leaf displays the highest inhibition diameters against the bacteria tested (ZOI > 25.0 mm); however, it performs inadequately in the MIC and MBC assays. The extracts from *Arct. uva-ursi*, *Apoc. androsaemifolium* and *C. canadensis* display moderate activity in the agar diffusion and micro-dilution studies. Overall, only the strains from *A. caviae*, *P. alvei*, *M. luteus* and *B. cereus* display susceptibility to the plant extracts in the hole-plate diffusion, MIC and MBC assays. Hence, the time-kill dynamics of plant extracts against these bacteria were evaluated.

In the time-kill assays, most plant extracts inhibit the growth of *A. caviae*, *P. alvei* and *M. luteus* bacteria within 12 h of incubation at MBC (Fig. 1). However, the leaf extract of *C. canadensis* induces a bacteriostatic effect against *A. caviae* despite its high inhibitory concentration (2.50 mg mL⁻¹). In addition, none of the extracts proved lethal to *B. cereus* at their respective MBCs. Instead, an inhibitory effect is observed in 1–2 h of incubation; then, the colony count either returns to the initial starting point (*Apoc. androsaemifolium* leaf, *X. strumarium* leaf, *G. squarrosa* leaf and flower) or declines to 10^{5.2}–10^{5.6} CFU mL⁻¹ (*Anap. margaritacea* flower and *C. canadensis* leaf) between 4 and 8 h after contact.

Table 1. Hole-plate diffusion assay of the northern Ontario medicinal plants

Medicinal plants	Plant part	Zone of inhibition (mm)					
		Gram negative		Gram positive			
		<i>E. coli</i> ^z	<i>A. caviae</i> ^y	<i>B. cereus</i> ^x	<i>M. luteus</i> ^w	<i>M. avium</i> ^v	<i>P. alvei</i> ^u
<i>Anap. margaritacea</i>	Leaf	–	–	22.7±3.1	19.3±1.2	18.6±1.2	–
	Flower	–	22.0±1.2	21.3±3.1	19.3±1.2	22.7±1.2	23.0±1.2
<i>Arct. uva-ursi</i>	Leaf	16.6±1.2	30.0±0.0	16.7±2.3	17.3±1.2	18.0±2.0	15.0±0.0
<i>Apoc. androsaemifolium</i>	Leaf	15.3±1.2	–	9.3±1.2	–	16.0±2.0	–
<i>C. canadensis</i>	Leaf	17.3±1.2	26.7±1.2	11.3±1.2	14.7±3.1	11.3±1.2	12.0±0.0
<i>G. squarrosa</i>	Leaf	–	18.0±0.0	15.0±1.0	19.3±1.2	13.3±1.2	15.3±0.6
	Flower	–	21.3±1.2	19.3±2.3	23.3±1.2	15.0±1.0	16.0±0.0
<i>X. strumarium</i>	Leaf	–	–	26.7±1.2	35.3±1.2	30.0±1.2	25.0±1.2
Chloramphenicol (100 µg mL ⁻¹)		32.0±0.0	–	42.0±0.0	52.0±0.0	50.0±0.0	50.0±0.0
Ampicillin (100 µg mL ⁻¹)		30.0±0.0	–	22.0±0.0	44.0±0.0	36.0±0.0	36.0±0.0
99.85% methanol (40 µL ⁻¹)		–	–	–	–	–	–

Note: all results are expressed as mean ±SD of three replicate measurements.

^z*Escherichia coli*.

^y*Aeromonas caviae*.

^x*Bacillus cereus*.

^w*Micrococcus luteus*.

^v*Mycobacterium avium* Subsp. *avium*.

^u*Paenibacillus alvei*.

The zone of inhibition (ZOI) is defined as the inhibition diameter around the 6-mm holes bored in the agar.

–, denotes an absence of ZOI.

In this study, the *Anap. margaritacea* flower displays the most promising antibacterial activity. This extract eradicates *P. alvei*, *M. luteus* and *A. caviae* bacteria within 2–8 h of contact at a concentration of 0.08 mg mL⁻¹ (MIC), 1.25 mg mL⁻¹ (MBC) and 1.25 mg mL⁻¹ (½ MBC), respectively (Fig. 2). At 2 × MBC, complete extermination of these bacteria is observed within 1–4 h of incubation. In contrast, the growth of *B. cereus* is inhibited only at 3 × MBC (2.50 mg mL⁻¹). Between the MIC–2 × MBC (0.31–1.25 mg mL⁻¹) range, the colony count of *B. cereus* varies between 10³ and 10⁶ CFU mL⁻¹ in the 24-h incubation period.

DISCUSSION

The spread of multiple drug resistant bacteria, elevated production costs of the synthetic NCEs, and the subsequent decrease in antibiotic drug discovery has compelled pharmaceutical industries to reconsider medicinal plants as alternative venues for the discovery of potent antibacterial drugs (Nimri et al. 1999; Sucher 2013). In the present study, the antibacterial activity of the crude extracts of *Anap. margaritacea*, *G. squarrosa*, *Apoc. androsaemifolium*, *Arct. uva-ursi*, *C. canadensis* and *X. strumarium* medicinal plants was evaluated against a wide range of microorganisms on the basis of hole plate diffusion, MIC, MBC, and time-kill assays.

The inhibition diameters, MICs and MBCs of *X. strumarium* and *Arct. uva-ursi* crude extracts were comparable to known literature values (Mehta et al. 1983; Jahodár et al. 1985; Jawad et al. 1988; Anjoo and Ajay 2010; Vučić et al. 2013), with differences attributable to alterations in growth conditions, bacterial

strains studied and/or seasonal variations in the secondary metabolites. Different parts of the plants exhibit varying activity, which could be attributed to their respective components. The inhibitory activity of *Arct. uva-ursi* may be associated with arbutin (Jahodár et al. 1985), the main component in the extract (Vučić et al. 2013), which has been reported to exhibit antibacterial activity (Kruszewska et al. 2004). *X. strumarium* belongs to the *Asteraceae* family, which is a rich source of sesquiterpenoid phytochemicals with documented antibacterial activity (Wu et al. 2006). The *Xanthium* genus in particular is renowned for its use in alternative medicine for the treatment of infectious diseases (Anjoo and Ajay 2010). Its antibacterial activity has been attributed to the presence of xanthanolide sesquiterpenoids, xanthol and xanthanin (Sato et al. 1997).

To our knowledge, the antibacterial activity of *Anap. margaritacea* and *Apoc. androsaemifolium* has not been reported before. Although, the inhibition diameters of *G. squarrosa* and *C. canadensis* leaf extracts were comparable to those reported in existing literature (McChesney and Adams 1985; McCutcheon et al. 1992; Borchardt et al. 2008; Jacobs et al. 2010), we are the first to report their MICs, MBCs and time-kill dynamics. The leaf extracts of *C. canadensis* and *Apoc. androsaemifolium* display moderate antibacterial activity, with inhibitory effects in 1–12 h of incubation at a concentration range of 1.25–5.00 mg mL⁻¹. Previous studies have isolated iridoid glycosides from the aerial parts of *C. canadensis* (Stermitz and Krull 1998) and glycosides, triterpenoid saponin and apocynins from *Apoc. androsaemifolium* (Moore 1909; Yamatova and

Table 2. MIC and MBC values for the crude extracts of northern Ontario medicinal plants

Medicinal plant	Plant part		Extract concentration (mg mL ⁻¹)					
			Gram negative			Gram positive		
			<i>E. coli</i> ^z	<i>A. caviae</i> ^y	<i>B. cereus</i> ^x	<i>M. luteus</i> ^w	<i>M. avium</i> ^v	<i>P. alvei</i> ^u
<i>Anap. margaritacea</i>	Leaf	MIC	> 5	2.50	0.63	1.25	> 5	0.16
		MBC	> 5	2.50	1.25	1.25	> 5	0.63
	Flower	MIC	> 5	2.50	0.31	0.31	5.00	0.08
		MBC	> 5	2.50	0.63	1.25	> 5	0.16
<i>Apoc. androsaemifolium</i>	Leaf	MIC	> 5	> 5	1.25	1.25	2.50	1.25
		MBC	> 5	> 5	2.50	1.25	> 5	1.25
<i>Arct. uva-ursi</i>	Leaf	MIC	> 5	5.00	> 5	2.50	> 5	1.25
		MBC	> 5	5.00	> 5	2.50	> 5	1.25
<i>C. canadensis</i>	Leaf	MIC	> 5	1.25	1.25	1.25	2.50	1.25
		MBC	> 5	2.50	2.50	1.25	> 5	1.25
<i>G. squarrosa</i>	Leaf	MIC	> 5	5.00	0.63	1.25	> 5	0.31
		MBC	> 5	5.00	1.25	1.25	> 5	0.63
	Flower	MIC	> 5	5.00	0.63	2.50	> 5	1.25
		MBC	> 5	5.00	0.63	2.50	> 5	1.25
<i>X. strumarium</i>	Leaf	MIC	> 5	> 5	> 5	> 5	> 5	2.50
		MBC	> 5	> 5	> 5	> 5	> 5	2.50
Chloramphenicol		MIC	0.05	0.05	0.05	0.05	0.05	0.05
		MBC	0.05	0.05	0.05	0.05	0.05	0.05
Ampicillin		MIC	0.05	0.05	0.05	0.05	0.05	0.05
		MBC	0.05	0.05	0.05	0.05	0.05	0.05
99.85% methanol			–	–	–	–	–	–

Note: The MIC and MBCs of plant extracts represent measurements from two independent experiments.

^z*Escherichia coli*.

^y*Aeromonas caviae*.

^x*Bacillus cereus*.

^w*Micrococcus luteus*.

^v*Mycobacterium avium* Subsp. *avium*.

^u*Paenibacillus alvei*.

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; > 5 denotes that the MIC or MBC value observed is beyond the 5.00 mg mL⁻¹ range tested.

–, denotes a lack of inhibition.

Abubakirov 1965; Murzagaliev et al. 1977); however, their association with antibacterial activity is currently unknown.

The leaf and flower extracts of *G. squarrosa* and *Anap. margaritacea* display the highest antibacterial activity in this study. The flower extract of *Anap. margaritacea* inhibits *P. alvei* within 2 h of incubation at 80 µg mL⁻¹ (MIC). Subsequently, at higher concentrations of the extract (1–3 × MBC), inhibition of *P. alvei* is observed within 2 h or less. Findings in this study illustrate the high potency of this extract, especially against the Gram-positive bacteria. In addition, the extract of *G. squarrosa* flowers displays inhibition diameters in excess of 19.3 mm and the extermination of *A. caviae*, *M. luteus* and *P. alvei* bacteria within 1–4 h at MBC. Previous studies have isolated hydroxylactones and flavonoids from *Anap. margaritacea* and polyphenolics and diterpene resin acids from *G. squarrosa* (Ahmed et al. 2004; Hoffmann et al. 1993; Khattab 1998; Nowak and Rychlinska 2012); however, their correlation with antibacterial activity is open to future investigations. Overall, our data reveal that there is no uniform response between the different species of bacteria in terms of susceptibility

to the crude extract of medicinal plants (Tables 1 and 2; Figs. 1 and 2). These types of differences may be explained by variation in cell wall structures and/or the presence of transmittable inheritance genes on plasmids. In addition, due to the differences in the speed of growth of microorganisms and components in the crude extracts of medicinal plants, time-kill dynamics against each bacterium presents unique inhibitory trends.

In the antimicrobial query, bactericidal influences on the Gram-positive bacteria are more pronounced than on the Gram-negative bacteria. This finding correlates well with previous studies (Vlietinck et al. 1995; Olano et al. 1996; Kudi et al. 1999) indicating that Gram negative bacteria are more resistant. This innate resistance may be associated with differences in cell wall structure, with the outer membrane of Gram negative bacteria providing a protective barrier against antibiotics, detergents and environmental substances (Palombo and Semple 2001). In addition, none of the shortlisted extracts proved lethal to *B. cereus* at their respective MBC values; instead, a bacteriostatic effect was observed. Similar findings have been reported in a prior study (Viljoen et al. 2005), suggesting that *B. cereus* may

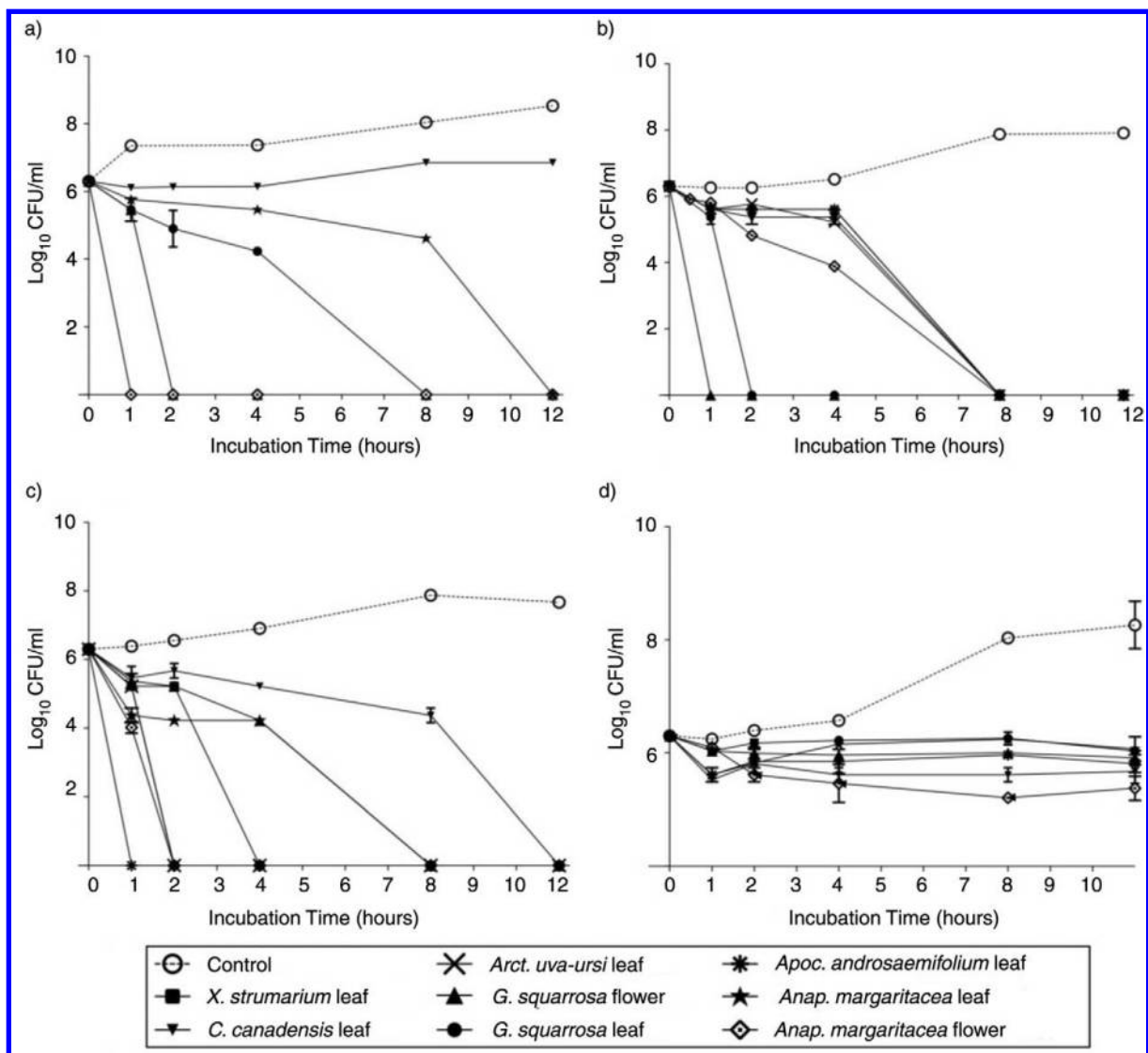


Fig. 1. Time-kill plot of the northern Ontario medicinal plants against *A. caviae* (a), *M. luteus* (b), *P. alvei* (c) and *B. cereus* (d) bacteria. All extracts were analyzed at their respective MBC concentration. The control treatment did not contain any plant extract. The results represent mean \pm SD from three replicate measurements.

develop resistance to antibiotics when grown in favorable conditions.

The increasing resistance to currently accessible antibacterial agents and the cost of drug discovery have led researchers to investigate medicinal plants for the presence of antibacterial entities. In this study, we have identified several medicinal plant candidates that could be the target of future disease treatment and drug exploration strategies. The leaf and flower extracts of *Anap. margaritacea* and *G. squarrosa* display high inhibition diameters, low MIC and MBC values and eradicate *A. caviae*, *P. alvei* and *M. luteus* bacteria within 1–12 h of incubation at MBC. In particular, the flower extract of *Anap. margaritacea* annihilates *P. alvei* within 2 h of incubation at 80 $\mu\text{g mL}^{-1}$ (MIC).

This extract also displays MBC values in the range of 0.16–2.50 mg mL^{-1} , which is considerably more potent than its competitors. The leaf extracts of *Apoc. androsaemifolium*, *Arct. uva-ursi* and *C. canadensis* display moderate antibacterial activity with MIC and MBC in the range of 1.25–5.00 mg mL^{-1} , whereas *X. strumarium* displays low antibacterial activity. Overall, the scrutiny of antibacterial activity suggests that the crude extracts of *Anap. margaritacea* and *G. squarrosa* possess compounds with antibacterial properties and can be used as antimicrobial agents in phytomedicine, food preservatives or new drugs for therapeutic purposes. In addition, the observed bioactivities of the plants studied correlate with their medicinal uses in aboriginal communities of the northern Ontario region.

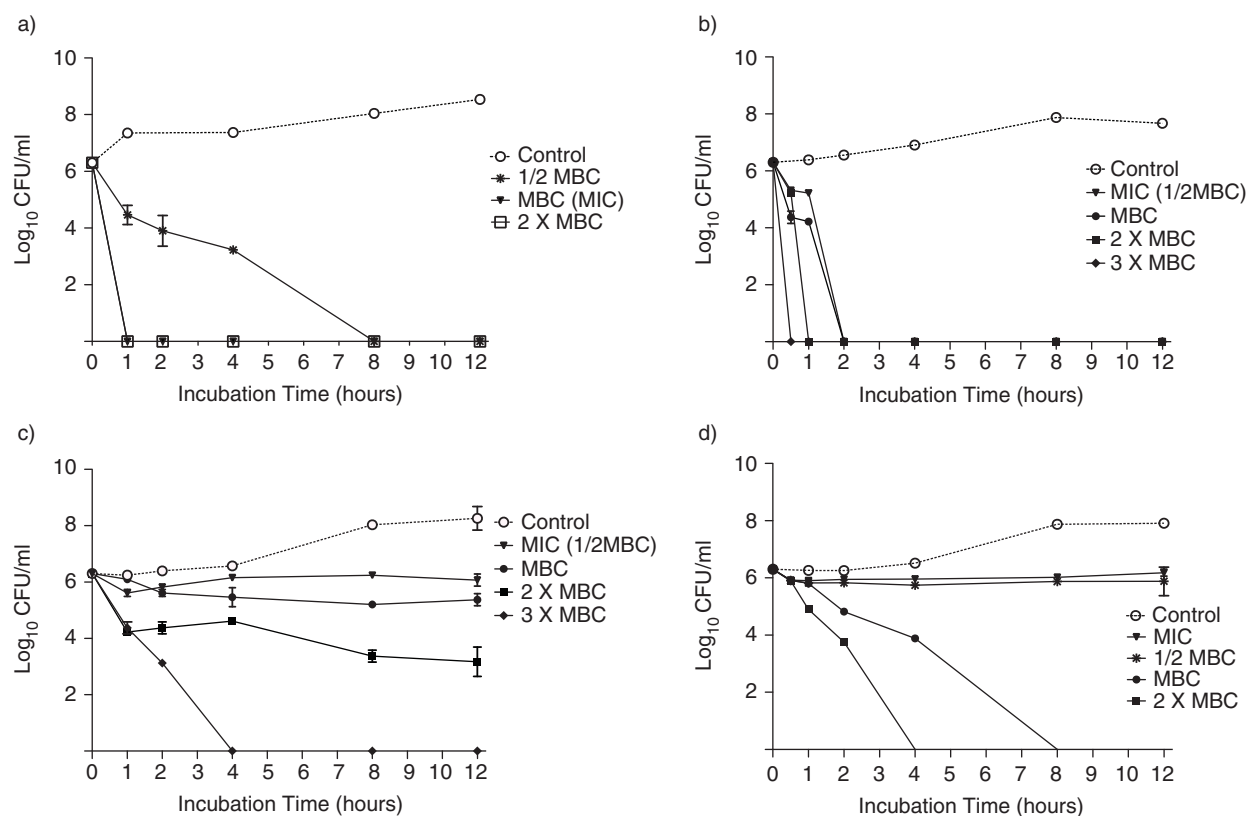


Fig. 2. Time-kill plot of the crude extract of *Anaphalis margaritacea* (L.) Benth & Hook.f. flower against *A. caviae* (a), *P. alvei* (b), *B. cereus* (c) and *M. luteus* (d) bacteria. The extract of *Anap. margaritacea* flower was used at MIC, $\frac{1}{2}$ MBC, $1 \times$ MBC, $2 \times$ MBC, and $3 \times$ MBC depending upon the respective antibacterial concentrations (MIC and MBC) against specific bacterium. The control treatment did not contain any plant extract. The results represent mean \pm SD from three replicate measurements.

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