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Original article

Evaluation of the anti-mycobacterial activity and composition of *Carlina acaulis* L. root extracts

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Abstract

Nontuberculous mycobacteria (NTM) have recently emerged as important bacterial pathogens of animals and humans. Of particular concern is the high level of antimicrobial resistance displayed by these organisms, which complicates treatment and potential successful outcomes. Here, we evaluated the potential of *Carlina acaulis* L. as a source of novel anti-mycobacterial agents. Our goal was to measure the activity of aqueous, ethanol, and chloroform *C. acaulis* root extracts against 99 NTM strains. GC-MS spectroscopy analyses were performed to deliver qualitative and quantitative data on the composition of *C. acaulis* extract. In our study, we have shown for the first time the activity of *C. acaulis* extracts against NTM. The highest activity was exhibited by the chloroform extract, which inhibited the growth of more than 90% of the strains at the dose of 100 µg/mL (MIC_{90} = 100 µg/mL). The results of the GC-MS analysis of the *C. acaulis* chloroform extract contributed to the identification of 37 compounds, with carlina oxide as the most representative compound (69.52%) followed by 3,4-dihydro-2H-phenanthren--1-one (6.54%) and stigmast-5-en-3-ol (4.14%). Our results indicate that *C. acaulis* chloroform and ethanol extracts have potential for treatment of NTM infections and that this plant contains anti-mycobacterial compounds.

Key words: *Carlina acaulis*, mycobacterium, NTM, minimum inhibitory concentration

Introduction

The *Mycobacterium* genus includes mycobacteria that cause tuberculosis (MTB), mycobacteria that cause leprosy, and non-tuberculous mycobacteria (NTM), i.e. a widely diverse group of species ranging from saprophytes to pathogens of humans and animals. The rate of growth has also been a classifying factor for the *Mycobacterium* spp., dividing them into rapid growers (RGM) forming colonies within 3–7 days and slow growers (SGM) for which colony formation lasts longer than 7 days (Wee et al. 2017). Currently, the number of NTM infection cases almost outweighs that of MTB in developed countries where NTM infection has been growing due to population aging, immunodeficiency, comorbidity with chronic diseases, and immunosup-

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pressive therapy (Pereira et al. 2020). NTM infections are associated with a multiplicity of diseases, including skin infections, which are mostly aquatic in origin (Balagué et al. 2015). NTM infections are difficult to treat and require a long time before remission (Balagué et al. 2015). Natural products are potentially valuable as a source of medicine due to their natural origin and low toxicity with lesser side effects (Mickymaray et al. 2020).

Carlina acaulis L., also known as carline thistle, stemless carline thistle, dwarf carline thistle or silver thistle, is a monocarpic perennial from the *Asteraceae* family occurring in South and Central Europe. Carlina root has been used in medicine since ancient times. It was mainly used for treating skin diseases; other commonly mentioned uses were based on its anthelmintic and diuretic effects and application in treatment of dental and gastrointestinal (emeticum, laxative) diseases (Strzemski et al. 2019). Additionally, there are ethno-pharmacological reports on the specific use of *C. acaulis* against leishmaniasis and leprosy (Strzemski et al. 2019). Comparatively little research has been performed to verify the ethno-pharmacologically claimed antimicrobial effects of *C. acaulis*, and even fewer publications have dealt with the phytochemistry of this species. However, in accordance with the traditional medicinal uses of *C. acaulis*, some researchers have demonstrated *in vitro* its antimicrobial properties against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Bacillus subtilis* and *Candida albicans* (Đorđević et al. 1998, Đorđević et al. 2007, Stojanović-Radić et al. 2012, Rosato et al. 2021). It has been also shown that the bacterial cell membrane is primary target site of *C. acaulis* essential oil (Stojanović-Radić et al. 2012). Taking into consideration the high-level drug resistance displayed by NTM, the toxicity of anti-mycobacterial pharmacological agents, and the potential of natural medicines, we conducted this study to analyze the biochemical properties of *C. acaulis* and estimate the efficacy of its extracts against NTM. To this end, we applied GC-MS spectrometry analyses of the *C. acaulis* chloroform extracts to determine the composition of the most active extract and to identify their major constituents.

Materials and Methods

Strains and growth conditions

The study involved 99 NTM strains originally isolated from diseased ornamental fish between January 2015 and December 2016 in the Department of Biology and Fish Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Poland. The atypical mycobacteria studied were as follows: *M. abscessus* (n=1), *M. chelonae* (n=16), *M. fortuitum* (n=10), *M. gordonae* (n=15), *M. marinum* (n=33), *M. mucogenicum* (n=1), *M. neoaurum* (n=2), *M. peregrinum* (n=12), *M. salmoniphilum* (n=1), *M. saopaulense* (n=1), *M. senegalense* (n=4), *M. septicum* (n=2), and *M. szulgai* (n=1). The mycobacterial strains were identified on the basis of molecular characters as described previously (Puk and Guz 2020). *M. fortuitum* ATTC 6841, *M. peregrinum* ATTC 700686, *M. marinum* ATCC 927, and *M. gordonae* ATCC 14470 were used as reference strains.

Preparation of plant extracts

Dried *C. acaulis* roots were purchased from the herb wholesale company NANGA (Przemysław Figura, Złotów, Poland). Extractions were performed with chloroform, ethanol 96%, or water using the Dionex ASE 150 apparatus (Thermo Fisher Scientific Inc., Waltham, MA USA). The following conditions were set: extraction temperature of 80°C, static time of 5 min, flush volume of 60%, gas purging time of 300 s, 4 static cycles. The obtained extract volumes were reduced using a rotary evaporator Hei-VAP Precision (Heidolph, Germany). Next, the aqueous extract was subjected to lyophilization (Labconco, FreeZone 2.5, Kansas, MO, USA), whereas the ethanol and chloroform extracts were evaporated to dryness under nitrogen at 25°C using an SE 400 Dionex solvent evaporator (Thermo Fisher Scientific, Waltham, MA, USA). After drying, the extracts were stored at a temperature of -20°C.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations (MICs) of *C. acaulis* root extracts were determined by the resazurin microtiter assay (REMA) as previously reported (Choi 2017) with minor modifications. Briefly, NTM log-phase cultures were adjusted with additional sterile water equal to a 0.5 McFarland turbidity standard (approximately 10^7 colony forming units (CFU) per mL), and 50 µL of bacterial suspension were transferred to 10 ml of Middlebrook 7H9 supplemented with OADC. Bacterial suspensions were inoculated into a 96-well microtiter plate containing final concentrations of *C. acaulis* root extract of 6.25-800 µg/mL. All wells (including controls) contained 1.0% DMSO, and the final volumes were 200 µL. Controls consisting of 7H9 medium alone, 7H9 medium + drug/extract, or 7H9 medium + bacterial culture were included. An established anti-mycobacterial drug (amikacin) was tested as a further control. The plates were covered with

Table 1. Minimum inhibitory concentrations (MIC) (μg/mL) of *C. acaulis* chloroform and ethanol extracts against mycobacteria isolated from ornamental fish and reference strains.

	Strain	No. of strains	Chloroform			Ethanol			Amikacin
			Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	MIC_{90}
RGM	*M. abscessus*	1	100	100	100	400	400	400	2
	M. chelonae	16	50-200	100	200	200-800	400	800	32
	M. fortuitum	10	12.5-100	100	100	200-800	400	800	2
	M. mucogenicum	1	100	100	100	800	800	800	1
	M. neoaurum	2	100	100	100	800	800	800	1
	M. peregrinum	12	12.5-100	50	100	200-800	400	400	1
	M. saopaulense	1	50	50	50	800	800	800	1
	M. septicum	2	50-100	50	100	400	400	400	1
	M. salmoniphilum	1	100	100	100	400	400	400	2
	M. senegalense	4	50	50	50	200-400	200	400	8
SGM	*M. gordonae*	15	12.5-50	50	50	50-200	100	200	2
	M. marinum	33	12.5-50	25	25	25-100	50	50	2
	M. szulgai	1	25	25	25	100	100	100	2
Total	RGM	50	12.5-200	100	100	200-800	400	800	16
	SGM	49	12.5-50	25	50	25-200	50	100	2
	RGM + SGM	99	12.5-200	50	100	25-800	200	800	8
RS	*M. fortuitum*	1	100	100	100	400	400	400	2
	M. peregrinum	1	100	100	100	400	400	400	1
	M. gordonae	1	25	25	25	100	100	100	2
	M. marinum	1	25	25	25	50	50	50	2

RGM – rapid growing mycobacteria; SGM – slow growing mycobacteria; RS – reference strains.

breathable paper and plastic lids, placed in plastic bags, and incubated at 37°C for 3-6 days for RGM and 7 days for SGM. Plates with *M. marinum*, *M. chelonae*, and *M. salmoniphilum* were incubated at 30°C. After this time, 20 μL of a 0.02% (w/v) resazurin solution was added to each well and incubated for 24 h. A change in the color from blue to pink indicated bacterial growth. The MIC was defined as the lowest concentration of the drug/extract that prevented visible color change. MIC_{50} and MIC_{90} values were defined as drug concentrations that inhibited 50% and 90% of the isolates, respectively.

GC/MS analyses

The gas chromatography/mass spectrometry (GC/MS) analyses were repeated three times for each sample using a Thermo TRACE GC Ultra gas chromatograph (Thermo Fisher Scientific Inc., Waltham, MA, USA). The gas chromatograph was equipped with a fused silica capillary column DB-5MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm) (Agilent Technologies, Santa Clara, USA) and coupled with a Thermo ITQ 1100 ion trap mass spectrometer detector (Thermo Fisher Scientific Inc., Waltham, MA, USA). The injector and interface were operated at 250 and 265°C, respectively. The flow rate of the mobile phase (carrier gas: helium) was set to 1 mL/min. The temperature program (oven temperature) was set at 50°C for 1 min and then raised to 200°C at 5°C/min and to 265°C at 10°C/min. The injection volume was 1 μL; the split ratio was 20:1. The ionization mass spectroscopic analysis was done at 70 eV. The mass spectrum was recorded for the mass range of 30-625 m/z for 63.52 minutes. The percentage composition was computed from the GC peak areas without the use of correction factors. Qualitative analyses of the extract constituents were based on comparison of their mass spectra with those from NIST/EPA/NIH Mass Spectral Library 2.0. (https://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:nistlibs).

Data analysis

Data collected through the laboratory analyses were entered and analyzed in Microsoft Excel 2016.

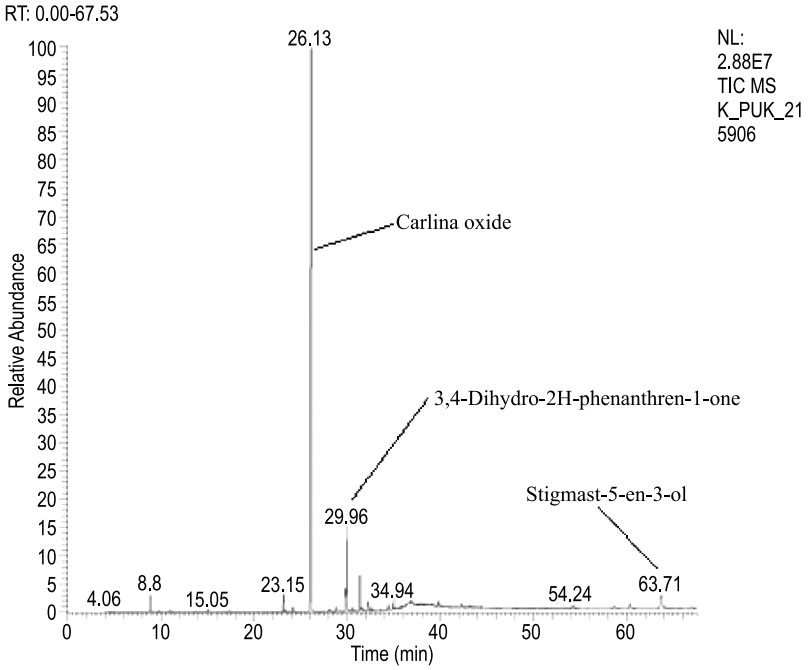

Fig. 1. Gas chromatography mass spectrometry chromatogram profile representing the highest intensity of carlina oxide (retention time: 26.13).

Results

The results revealed that the bioactivity of the aqueous extract obtained from the roots of *C. acaulis* was non-inhibitory (data not shown), whereas the chloroform and ethanol extracts inhibited the tested NTM strains (Table 1). The *M. marinum* strains (n=33) were the most susceptible to both the ethanol and chloroform extracts. In contrast, the *M. chelonae* strains (n=16) were the most resistant to the extracts, compared to the other strains. The SGM species (n=49) were more susceptible to both ethanol and chloroform extracts than the RGM species (n=50) (Table 1). The comparative MIC values (MIC range, MIC_{50}, MIC_{90}) of *C. acaulis* root ethanol and chloroform extracts against 99 strains of NTM and reference strains are presented in Table 1. The reference strain of *M. marinum* ATCC 927 was the most susceptible strain to both ethanol and chloroform extracts (Table 1). The MIC results of both the ethanol and chloroform extracts showed lower anti-mycobacterial activities against all strains in comparison to amikacin (Table 1).

The chemical constituents of the chloroform extract obtained from roots of *C. acaulis,* determined by the GC-MS analyses are reported in Table 2. A total of 37 compounds were identified in the chloroform extract of *C. acaulis.* Carlina oxide was the main component, accounting for 69.52% of the total peak areas (Fig. 1). Among the minor components, 3,4-dihydro-2H-phenanthren-1-one (6.54%) was the most representative compound followed by stigmast-5-en-3-ol (4.14%) (Table 2).

Discussion

Mycobacteria infecting fish include zoonotic pathogens that can cause both localized and disseminated infections in man. The population at risk includes people who are exposed to aquatic environments, mainly workers in the fishing industry and aquarists (Puk and Guz 2020). Given the importance of the drug resistance as well as the increasing incidence of NTM infections, novel therapeutic strategies against NTM are needed. A broad range of plant-derived constituents from various classes have been evaluated for their *in vitro* anti-mycobacterial activity. Such compounds include alkaloids (Gibbons et al. 2003), flavonoids (Sutthivaiyakit et al. 2009, Castellar et al. 2011, Mickymaray et al. 2020), terpenoids (Cantrell et al. 2001, Newton et al. 2002), quinonoids (Dey et al. 2014), and steroids (Gutierrez-Lugo et al. 2005). Numerous studies have also shown the efficacy of plant extracts against mycobacteria (Gupta et al. 2018, Hernández-García et al. 2019, Martini et al. 2020). Current research proves that *C. acaulis* contains many compounds of valuable biological properties, and it seems reasonable to reintroduce it to phytotherapy (Strzemski et al. 2019). It has been also shown that *C. acaulis* essential oil and extracts have high antimicrobial activities against the number of bacterial human pathogens. The most prominent activity was the one exhibited against *S. aureus* followed by the activity against *P. vulgaris* and *P. aeruginosa* (Stojanović-Radić et al. 2012). However, clearly evident is the lack of studies demonstrating the efficacy of *C. acaulis* extracts against mycobacteria,

Table 2. Compounds identified in the chloroform extract of *Carlina acaulis* by gas chromatography – mass spectrometry.

No	RT	Compound name	MF	MW	PeakArea* %
1	8.88	Benzaldehyde	C7H6O	106	1.36
2	9.52	1,1,3,3,5,5,7,7,9,9-Decamethylpentasiloxane	C10H30O4Si5	355	0.07
3	9.77	1-methylenebenzocyclobutane	C9H8	116	0.09
4	10.98	Benzenemethanol	C7H8O	108	0.24
5	13.70	Phenylacetone	C9H10O	134	0.07
6	14.20	Methyl octadeca-9,12,15-trienoate	C19H32O2	292	0.04
7	15.03	Benzoic acid	C7H6O2	122	0.44
8	17.32	Phenylacetic acid	C8H8O2	136	0.25
9	18.26	Thymol	C10H14O	150	0.04
10	21.21	Vanillin	C8H8O3	152	0.03
11	23.15	Curcumene	C15H22	202	1.12
12	23.33	Alpha-Farnesene	C15H24	204	0.1
13	23.44	Zingiberene	C15H24	204	0.09
14	23.77	Beta-Caryophyllene	C15H24	204	0.08
15	24.16	Beta-Funebrene	C15H24	204	0.31
16	26.13	Carlina oxide	C13H10O	182	69.52
17	28.05	Boronal	C14H22O	206	0.13
18	28.16	Methyl 2,5-octadecadiynoate	C19H30O2	290	0.19
19	29.83	2-Phenylphenol	C12H10O	170	1.84
20	29.96	3,4-Dihydro-2H-phenanthren-1-one	C14H12O	196	6.54
21	30.32	(E)-2-Benzylidenecyclohexanone	C13H14O	186	0.15
22	30.56	Aromadendrene oxide-(1)	C15H24O	220	0.24
23	31.37	Benzo[h]quinolin-4(1H)-one, 2,3-dihydro-	C13H11NO	197	2.90
24	31.58	3H-Phenoxazin-3-one	C12H7NO2	197	0.32
25	32.31	5-ethoxy-2-phenyloxazole-2-carbonitrile	C12H10N2O2	214	0.83
26	32.51	Methyl 14-methylpentadecanoate	C17H34O2	270	0.08
27	32.66	2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-4a-phenyl-, (R)-	C16H18O	226	0.10
28	34.43	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	C20H38O2	311	0.24
29	34.94	6,9-octadecadienoic acid, methyl ester	C19H34O2	294	0.42
30	36.89	[(10Z)-4,8,9,13-Tetraacetyloxy-12-hydroxy-3,6,6,10,14-pentamethyl--2-tricyclo[10.3.0.05,7] pentadec-10-enyl] acetate	C30H44O11	580	3.81
31	39.79	6-Amino-3-methyl-4-(2,4,5-trimethoxyphenyl)-1,4-dihydropyrano [2,3-c]pyrazole-5-carbonitrile	C17H18N4O4	342	0.39
32	42.28	2-Methoxy-3,4,5-tris(3-methylbut-2-enyl)-6-[(6E)-2,7,11-trimethyl--5,6,8-tris(3-methylbut-2-enyl)dodeca-2,6,10-trien-5-yl]phenol	C52H80O2	737	0.41
33	43.16	[(10Z)-4,8,9,13-Tetraacetyloxy-12-hydroxy-3,6,6,10,14-pentamethyl--2-tricyclo[10.3.0.05,7]pentadec-10-enyl] acetate	C30H44O11	580	0.21
34	54.24	5,10-dihexyl-5,10-diihydroindolo[3,2-b]indole-2,7-dicarbaldehyde	C28H34N2O2	430	0.62
35	58.70	64807-01-8	C27H36O10	520	0.75
36	60.38	Stigmasta-5,22-dien-3-ol	C29H48O	412	1.26
37	63.71	Stigmast-5-en-3-ol	C29H50O	414	4.14

RT – retention time, MF – molecular formula, MW – molecular weight, * – means of three replicates.

and only few publications have dealt with the phytochemistry of this plant. Here we examined for the first time the anti-mycobacterial activities of aqueous, ethanol, and chloroform extracts of *C. acaulis* against 99 strains of NTM. Although the *C. acaulis* root extracts were found to be less active than amikacin, the ethanol and chloroform extracts showed anti-mycobacterial activity. Inactivity of the aqueous extracts confirms the

fact that non-polar (poorly extractable with water) compounds are the main active antimicrobial principles of *C. acaulis* (Stojanović-Radić et al. 2012). Some compounds identified in the investigated chloroform extract of the *C. acaulis* root were reported to have anti-mycobacterial properties earlier. As shown by Gutierrez-Lugo et al. (2005), stigmast-5-ene and stigmasta-5,22-dien steroids have strong activity against *M. tuberculosis*, with MIC values of 1.98 and 1 µg/mL, respectively. Among the minor components identified in the chloroform extract of *C. acaulis*, zingiberene, i.e. a monocyclic sesquiterpene and a predominant constituent of ginger (*Zingiber officinale*) oil was active against *M. tuberculosis* (Priyadarshini and Veeramani 2020). This evidence verifies the anti-mycobacterial effect of the *C. acaulis* root extracts and suggests that its anti-mycobacterial property is most likely attributable to steroid compounds. There are no reports on anti-mycobacterial effects of carlina oxide, i.e. the most abundant compound of *C. acaulis* root extract. However, Wnorowski et al. (2020) have demonstrated that carlina oxide displays toxic effects to cells in culture and to living organisms. Thus, *C. acaulis*-based extracts considered for therapeutic use should be completely deprived of carlina oxide.

Conclusions

In our study, the ethanol and chloroform extracts of *C. acaulis* were active against the tested NTM strains. This could explain the ethnopharmacological uses of *C. acaulis* against skin infections. This study lays the foundation for the development of anti-mycobacterial agents based on *C. acaulis* derivatives.

References

Adams M, Wube AA, Bucar F, Bauer R, Kunert O, Haslinger E (**2005**) Quinolone alkaloids from *Evodia rutaecarpa*: a potent new group of antimycobacterial compounds. Int J Antimicrob Agents 26: 262-264.

Balagué N, Uçkay I, Vostrel P, Hinrikson H, Van Aaken I, Beaulieu JY (**2015**) Non-tuberculous mycobacterial infections of the hand. Chir Main 34: 18-23.

Cantrell CL, Franzblau SG, Fischer NH (**2001**) Antimycobacterial plant terpenoids. Planta Med 67: 685-694.

Castellar A, Coelho TS, Silva PE, Ramos DF, Lourenco MC, Lage CL, Julião LS, Barbosa YG, Leitão SG (**2011**) The activity of flavones and oleanolic acid from *Lippia lacunosa* against susceptible and resistant *Mycobacterium tuberculosis* strains. Rev Bras Farmacogn 21: 835-840.

Choi WH (**2017**) Novel pharmacological activity of artesunate and artemisinin: their potential as anti-tubercular agents. J Clin Med 6: 30.

Dey D, Ray R, Hazra B (**2014**) Antitubercular and antibacterial activity of quinonoid natural products against multi-drug resistant clinical isolates. Phytother Res 28: 1014-1021.

Dordević S, Lukić S, Mraović M, Žižić S (**1998**) Antimicrobial activity of essential oil of *Carlina acaulis* L., *Asteraceae*. Pharmac Pharmacol Lett 8: 126-128.

Dordević S, Petrović S, Dobrić S, Milenković M, Vučićević D, Žižić S, Kukić J (**2007**) Antimicrobial, anti-inflammatory, anti-ulcer and antioxidant activities of *Carlina acanthifolia* root essential oil. J Ethnopharmacol 109: 458-463.

Gibbons S, Fallah F, Wright CW (**2003**) Cryptolepine hydrochloride: a potent antimycobacterial alkaloid derived from *Cryptolepis sanguinolenta*. Phytother Res 17: 434-436.

Gupta VK, Kaushik A, Chauhan DS, Ahirwar RK, Sharma S, Bisht D (**2018**) Anti-mycobacterial Activity of Some Medicinal Plants Used Traditionally by Tribes from Madhya Pradesh, India for Treating Tuberculosis Related Symptoms. J Ethnopharmacol 227: 113-120.

Gutierrez-Lugo MT, Wang Y, Franzblau SG, Suarez E, Timmermann BN (**2005**) Antitubercular sterols from *Thalia multiflora* Horkel ex Koernicke. Phytother Res 19: 876-880.

Hernández-García E, García A, Garza-González E, Avalos-Alanís FG, Rivas-Galindo VM, Rodríguez-Rodríguez J, Alcantar-Rosales VM, Delgadillo-Puga C, Camacho-Corona MD (**2019**) Chemical composition of *Acacia farnesiana* (L) wild fruits and its activity against *Mycobacterium tuberculosis* and dysentery bacteria. J Ethnopharmacol 230: 74-80.

Martini MC, Zhang T, Williams JT, Abramovitch RB, Weathers PJ, Shell SS (**2020**) *Artemisia annua* and *Artemisia afra* extracts exhibit strong bactericidal activity against *Mycobacterium tuberculosis*. J Ethnopharmacol 262: 113191.

Mickymaray S, Alfaiz FA, Paramasivam A (**2020**) Efficacy and mechanisms of flavonoids against the emerging opportunistic nontuberculous Mycobacteria. Antibiotics (Basel) 9: 450.

Newton SM, Lau C, Gurcha SS, Besra GS, Wright CW (**2002**) The evaluation of forty-three plant species from in vitro antimycobacterial activities; Isolation of active constituents from *Psoralea corylifolia* and *Sanguinaria canadensis*. J Ethnopharmacol 79: 57-67.

Pereira AC, Ramos B, Reis AC, Cunha MV (**2020**) Non-tuberculous mycobacteria: Molecular and physiological bases of virulence and adaptation to ecological niches. Microorganisms 8: 1380.

Priyadarshini N, Veeramani A (**2020**) Antitubercular compounds isolated and characterized in *Tithonia diversifolia* and *Couroupita guianensis*. Int J Mycobacteriol 9: 195-199.

Puk K, Guz L (**2020**) Occurrence of *Mycobacterium* spp. in ornamental fish. Ann Agric Environ Med 27: 535-539.

Rosato A, Barbarossa A, Mustafa AM, Bonacucina G, Perinelli DR, Petrelli R, Maggi F, Spinozzi E (**2021**) Comprehensive evaluation of the antibacterial and antifungal activities of *Carlina acaulis* L. essential oil and its nanoemulsion. Antibiotics (Basel) 10: 1451.

Stojanović-Radić Z, Čomić L, Radulović N, Blagojević P, Mihajilov-Krstev T, Rajković J (**2012**) Commercial Carlinae radix herbal drug: Botanical identity, chemical composition and antimicrobial properties. Pharm Biol 50: 933-940.

Strzemski M, Wójciak-Kosior M, Sowa I, Załuski D, Verpoorte R (**2019**) Historical and traditional medical applications of *Carlina acaulis* L. – a critical ethnopharmacological review. J Ethnopharmacol 239: 111842.

Sutthivaiyakit S, Thongnak O, Lhinhatrakool T, Yodchun O, Srimark R, Dowtaisong P, Chuankamnerdkarn M (**2009**) Cytotoxic and antimycobacterial prenylated flavonoids from the roots of *Eriosema chinense*. J Nat Prod 72: 1092-1096.

Wee WY, Dutta A, Choo SW (**2017**) Comparative genome analyses of mycobacteria give better insights into their evolution. PLoS One 12: e0172831.

Wnorowski A, Wnorowska S, Wojas-Krawczyk K, Grenda A, Staniak M, Michalak A, Woźniak S, Matosiuk D, Biała G, Wójciak M, Sowa I, Krawczyk P, Strzemski M (**2020**) Toxicity of carlina oxide-A natural polyacetylene from the *Carlina acaulis* roots-In vitro and in vivo study. Toxins (Basel) 12: 239.