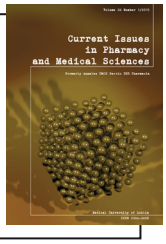
DE GRUYTER OPEN

Current Issues in Pharmacy and Medical Sciences

Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: http://www.curipms.umlub.pl/



Anti-*Helicobacter pylori* activity *in vitro* of chamomile flowers, coneflower herbs, peppermint leaves and thyme herbs – a preliminary report

Anna Malm[1*], Anna Glowniak-Lipa[1], Izabela Korona-Glowniak[1], Tomasz Baj[2]

[1] Department of Pharmaceutical Microbiology with Laboratory for Microbiological Diagnostics, Medical University of Lublin, 1 Chodzki, 20-093 Lublin, Poland

[2] Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, 1 Chodzki, 20-093 Lublin, Poland

ARTICLE INFO

Received 02 March 2015
Accepted 17 March 2015

Keywords:
anti-*Helicobacter pylori* activity,
chamomile flowers,
coneflower herbs,
peppermint leaves,
thyme herbs.

ABSTRACT

Recently, several studies have been undertaken so as to develop more effective therapeutic approaches towards eradicating *Helicobacter pylori*. Among these is phytotherapy. The aim of this study was to investigate the activity *in vitro* of the plant extracts obtained from common herbs cultivated in the Lubelszczyzna region against the reference strain *H. pylori* ATCC 43504. Among these are thyme herbs, chamomile flowers, peppermint leaves and coneflower herbs. Herein, it was found that the MIC values of the assayed extracts were as follows: the extracts from coneflower herbs showed anti-*H. pylori* activity with MIC = 31.3-125 μg/ml; the extracts from chamomile flowers demonstrated MIC = 31.3-62.5 μg/ml; the extracts from peppermint leaves had MIC = 15.6-250 μg/ml; and the extracts from thyme herbs revealed MIC = 15.6-62.5 μg/ml, depending on the solvent used. The most active were the extracts obtained with ethyl acetate or ethanol alcohol absolute 99.8%. These showing MIC within the range of 15.6-62.5 μg/ml, while the lowest activity was observed in case of the extract obtained with 70% aqueous ethanol. This last showing MIC within the range of 62.5-250 μg/ml. The MIC values of essential oil components were 15.6 μg/ml for bisabolol and menthol or 31.3 μg/ml for thymol. The obtained data indicate that the assayed herbs possessed promising anti-*H. pylori* bioactivity.

INTRODUCTION

Helicobacter pylori is one of the most common bacterial species worldwide. This bacterial species has colonized the human stomach, with a high prevalence (> 50%) in much of the world, although the infection rates are dropping in some developed countries, reaching < 25%. *H. pylori* is an important cause of gastritis, peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma, and gastric adenocarcinoma [12]. The effectiveness of standard triple therapy for the eradication of *H. pylori* has decreased recently due to its increasing resistance to antibacterials, mostly clarithromycin, with failure rate of up to 40%. Therefore, further studies are performed so as to develop more effective therapeutic approaches, including alternative therapeutic approaches, *e.g.* phytomedicine [12]. However, the available *in vitro* data on the activity of plant extracts or essential oils against *H. pylori* and data from clinical trials concerning the use of herbal medicines for treating *H. pylori* infection are controversial and not complete [14,16,17]. The aim of this study was to investigate the activity of the plant extracts obtained from common herbs cultivated in the Lubelszczyzna region (chamomile flowers, coneflower herbs, peppermint leaves and thyme herbs) against *H. pylori*.

* **Corresponding author**
e-mail: anna.malm@umlub.pl
tel./fax: +48 81 448-71-00, +48 81 448-71-02

MATERIAL AND METHODS

Extraction procedure. Plant material for the study were: chamomile flowers (*Matricaria chamomilla* L., *Asteraceae*), coneflower herbs (*Echinacea purpurea* (L.) Moench., *Asteraceae*), peppermint leaves (*Mentha piperita* L., *Lamiaceae*) and thyme herbs (*Thymus vulgaris* L., *Lamiaceae*); this material was purchased from the herbal Trading Company KRAUTEX, Lopiennik Gorny, Poland. The dry

 DOI: 10.1515/cipms-2015-0038

material was first milled and sieved. The dry plants' medicinal components were then extracted by way of different solvents: ethanol alcohol absolute 99,8% (POCh, Gliwice, Poland), ethanol 96% (pure-basic, POCh Gliwice, Poland), 70% aqueous ethanol or ethyl acetate (ACS pure p.o., POCh Gliwice, Poland), at room temperature (about 25°C), by using the maceration method, for 48 h, in a 1:10 material/solvent ratio. After extraction, the samples were filtered using standard filter paper. The solvents were subsequently evaporated by way of employing the rotary evaporator (IKA RV Basic, Germany) under reduced temperature below 50°C. The amorphous solid crude extracts were then tested for microbial properties. In the case of that treated by way of 70% aqueous ethanol solvent, samples were evaporated under reduced pressure, and the obtained residue was frozen in a vertical low temperature freezer (GLF, Germany) at -50°C and then lyophilized (Christ, Alpha 2-4 LD Plus, Germany).

Antibacterial activity assay *in vitro*. The extracts obtained from coneflower herbs, chamomile flowers, peppermint leaves and thyme herbs were screened for activity towards *H. pylori* ATCC 43504 (the reference strain being obtained from the American Type Culture Collection), by way of the micro-dilution broth method, using Mueller-Hinton broth supplemented with lysed horse blood, allowing for the determination of the minimal inhibitory concentration (MIC) of the tested extracts. Serial two-fold dilutions were made in order to obtain final concentrations of the tested extracts, which ranged from 0.98 to 1000 μg/mL. The sterile 96-well polystyrene microtitrate plates (Nunc, Denmark) were prepared by dispensing 200 μl of appropriate dilution of the tested extract in broth medium per well. The inocula were prepared with fresh microbial cultures in sterile 0.85% NaCl to match the turbidity of 1.0 McFarland standard, and 2 μl were added to the wells to obtain a final density of 3.0×10^6 CFU (colony forming units)/ml. After incubation at 35°C for 72 h under microaerophilic conditions (5% O_2, 15% CO_2 and 80% N_2), the MICs were assessed visually as the lowest concentration of the extracts showing complete growth inhibition of the reference strain. A positive control (containing inoculum without the tested extracts) and a negative control (containing the tested extracts without inoculum) were included on each microplate. Bisabolol alpha, menthol, and thymol (P.P.H. "Standard", Poland) were used as the reference substances. Minimal bactericidal concentration (MBC) was determined by subculturing 100 μl of the microbial culture from each well that showed thorough growth inhibition, from the last positive and from the growth control, onto the plates of Mueller-Hinton agar supplemented with horse blood. The plates were incubated at 35° for 72 h under microaerophilic conditions, and the MBC was defined as the lowest concentration of the extracts without growth of microorganisms. The MBC/MIC ratios were calculated to determine the bactericidal or bacteriostatic effect of the assayed extracts. Of note, in our work, antibacterial agents are usually regarded as bactericidal if MBC/MIC is no more than four times the MIC [6]. Each experiment was repeated in triplicate. Representative data are presented.

RESULTS

The activity of the extracts from coneflower herbs, chamomile flowers, peppermint leaves and thyme herbs towards *H. pylori* ATCC 43504 was determined *in vitro* by way of employing the micro-dilution broth method, allowing for the determination of MIC. The extracts were obtained using solvents of different polarity. The obtained data are shown in Table 1. As seen in the table, the MIC values of the assayed plant extracts ranged from 15.6-250 μg/ml, depending on the plant material and solvent. The extracts from chamomile flowers showed activity against the reference strain of *H. pylori* with MIC = 31.3-62.5 μg/ml, while the extracts from coneflower herbs showed MIC = 31.3-125 μg/ml, the extracts from peppermint leaves had MIC = 15.6-250 μg/ml,and the extracts from thyme herbs revealed MIC = 15.6-62.5 μg/ml. The most active were the plant extracts obtained either with ethyl acetate or ethanol (99.8%), both showing MIC within the range of 15.6-62.5 μg/ml, while the lowest activity was observed in case of the extract obtained by way of ethanol (70%) – this showing MIC within the range of 62.5-250 μg/ml. Furthermore, the MIC values of essential oil components were 15.6 μg/ml for bisabolol and menthol or 31.3 μg/ml for thymol.

Table 1. The antimicrobial activity of the tested plant extracts towards *Helicobacter pylori* ATCC43504

Extract (solvent)	Plant material	MIC (μg/ml)	MBC (μg/ml)	MBC/MIC ratio
Extract no 1 (ethyl acetate)	coneflower herbs	62.5	125	2
	chamomile flowers	31.3	125	4
	peppermint leaves	15.6	125	8
	thyme herbs	15.6	125	8
Extract no 2 (ethanol 99.8%)	coneflower herbs	31.3	62.5	2
	chamomile flowers	62.5	125	2
	peppermint leaves	15.6	125	8
	thyme herbs	31.3	250	8
Extract no 3 (ethanol 96%)	coneflower herbs	62.5	250	4
	chamomile flowers	62.5	250	4
	peppermint leaves	62.5	250	4
	thyme herbs	31.3	250	8
Extract no 4 (ethanol 70%)	coneflower herbs	125	125	1
	chamomile flowers	62.5	250	4
	peppermint leaves	250	250	1
	thyme herbs	62.5	125	2
Essential oil constituents	bisabolol	15.6	31.3	2
	menthol	15.6	62.5	4
	thymol	31.3	62.5	2

As presented in Table 1, the MBC/MIC values of the plant extracts ≤ 4 suggested their bactericidal activity against *H. pylori* ATCC 43504, while the MBC/MIC values of the extracts > 4 demonstrated their bacteriostatic activity. By way of reference, the essential oil components studied, *i.e.* bisabolol, menthol and thymol, showed bactericidal activity against the reference strain of *H. pylori,* with MBC/MIC values ≤ 4.

DISCUSSION

Numerous plant species have been utilized as traditional medicine (phytotherapy) in many parts of the world, for thousands of years. The employed medicinal plants contain a lot of biologically active compounds, *e.g.* essential oils, and may display potential antimicrobial properties [2,3,8,13]. Indeed, phytotherapy has been used for centuries to treat various gastrointestinal tract disorders. The gastroprotective effects of herbs are multidirectional, including anti-*H. pylori*

activity. Herbs with potent anti-*H. pylori* effects were found to belong to several families, including Amaryllidaceae, *Anacardiaceae*, *Apiaceae*, *Apocynaceae*, *Asclepiadoideae*, *Asteraceae*, *Bignoniaceae*, *Clusiaceae*, *Chancapiedra*, *Combretaceae*, *Cyperaceae*, *Euphorbiaceae*, *Fabaceae*, *Geraniaceae*, *Lamiaceae*, *Lauraceae*, *Lythraceae*, *Menispermaceae*, *Myristicaceae*, *Myrtaceae*, *Oleaceae*, *Papaveraceae*, *Plumbaginaceae*, *Poaceae*, *Ranunculaceae*, *Rosaceae* and *Theaceae* [3,8,14].

The anti-*H. pylori* activity of plant extracts, infusions or decoctions, as well as essential oils or other plant compounds is studied *in vitro* usually by way of the disc diffusion method or the micro-dilution method [1,4,5,7,9,11,15]. The latter is recommended by the Clinical and Laboratory Standards Institute (CLSI) in USA and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for determining MIC of antimicrobial substances [18]. As proposed by O`Donnell et al. [10], the bioactivity of the naturally occurring and synthetic substances should be defined as 'moderate' in case of MIC = 126-500 mg/ml, as 'good' in case of MIC = 26-125 μg/ml, and as 'strong' with MIC = 10-25 μg/ml. In this paper, the activity *in vitro* of the plant extracts obtained from species belonging to *Asteracea* (extracts from coneflower herbs and chamomile flower), and to *Lamiaceae* (extracts from peppermint leaf and thyme herbs) towards the reference strain *H. pylori* ATCC 43504 was determined using the micro-dilution broth method. The obtained MIC data indicate that the assayed extracts, especially those obtained with ethyl acetate or ethanol (99.8%), possessed strong or good *in vitro* activity against *H. pylori* ATCC 43504. These results are comparable to that of the representative components of essential oils such as bisabolol, menthol and thymol. Further studies will be performed to confirm the activity of these extracts against clinical *H. pylori* isolates, including those showing drug resistance, and to study a possible mechanism of their anti-*H. pylori* activity.

CONCLUSION

The results presented in this paper indicate that coneflower herbs, chamomile flowers, peppermint leaves and thyme herbs cultivated in the Lubelszczyna region can be regarded as potential herbal medicines having promising bioactivity anti-*H. pylori,* and having potential for being used in prophylaxis or as adjuvant agents in *H. pylori* infection treatment.

REFERENCES

1. Boyanova L.: Comparative evaluation of the activity of plant infusions against *Helicobacter pylori* strains by three methods. *World J. Microbiol. Biotechnol*., 30, 1633, 2014.
2. Dorman H.J.D, Deans S.G.: Antimicrobial agents from plants: antibacterial activity of plants volatile oils. *J. Appl. Microbiol*., 88, 308, 2000.
3. Edris A.E.: Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. *Phytother. Res*., 21, 308, 2007.
4. Fadda G., Zanetti S.: In vitro activity of essential oil of *Myrtus communis* L. against *Helicobacter pylori. Int. J. Antimicrob. Agents.,* 30, 562, 2007.
5. Falsafi T. et al.: Chemical composition and anti-*Helicobacter pylori* effect of *Satureja bachtiarica* Bunge essential oil. *Phytomedicine*., 15, 173, 2015.
6. French G.L.: Bactericidal agents in the treatment of MRSA infections – the potential role of daptomycin. *J. Antimicrob. Chemother.* 58, 1107, 2006.
7. Hazzit M. et al.: Chemical composition and biological activities of Algerian *Thymus* oils. *Food Chem.,* 116, 714, 2009.
8. Lang G., Buchbauer G.: A review on recent research results (2008-2010) on essential oils as antimicrobial and antifungals. A review. *Flavour Fragr. J*., 27, 13, 2012.
9. Njume C. et al.: Volatile compounds in the stem bark of *Sclerocarya birrea* (*Anacardiaceae*) possess antimicrobial activity against drug-resistant strains of *Helicobacter pylori. Int. J. Antimicrob. Agents*., 38, 319, 2011.
10. O`Donnell F. et al.: A study of the antimicrobial activity of selected synthetic and naturally occurring quinolines. *Int. J. Antimicrob. Agents*., 35, 30, 2010.
11. Ohno T. et al.: Antimicrobial activity of essential oils against *Helicobacter pylori. Helicobacter*, 8, 207, 2003.
12. Papastergiou V., Georgopoulos S.D., Karatapanis S.*: Treatment of Helicobacter pylori* infection: past, present and future. *World J. Gastrointest. Pathophysiol*., 5, 392, 2014.
13. Rios J.L., Recio M.C.: Medicinal plants and antimicrobial activity. *J. Ethnopharmacol.* 100, 80, 2005.
14. Safavi M., Shams-Ardakani M., Foroumadi A.: Medicinal plants in treatment of *Helicobacter pylori* infections. *Pharm. Biol*., 28, 1, 2014.
15. Shikov A.N. et al.: Antibacterial activity of *Chamomilla recutita* oil extract against *Helicobacter pylori. Phytoter. Res*., 22, 252, 2008.
16. Vale F.F., Oleastro M.: Overview of the phytomedicine approaches against *Helicobacter pylori. World J. Gastroenterol*., 20, 5594, 2014.
17. Vitor J.M.B., Vale F.F.: Alternative therapies for *Helicobacter pylori*: Probiotics and phytomedicine. *FEMS Immunol. Med. Microbiol*., 63, 153, 2011.
18. Wiegand I., Hilpert K., Hancock R.E.W.: Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat. Protoc*. 3, 163, 2008.