Artículo original

Chemical profiles and efficacy of essential oils obtained from three spices against *Helicobacter pylori*

Perfiles químicos y eficacia de los aceites esenciales obtenidos a partir de tres especias contra *Helicobacter pylori*

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ABSTRACT

Introduction: the medicinal herbs constitute one of the most preferred alternative solutions as antimicrobial agents due to their availability, biodegradability, fewer side effects. Unfortunately, rare are the studies focused on the efficacy of plant extracts against *Helicobacter pylori* to either confirm or refute their effectiveness.

Objective: determinate the chemical profiles and evaluate the antibacterial activity against *Helicobacter pylori* of the essential oils obtained from *Eugenia caryophyllata, Foeniculum vulgare* and *Carum carvi*.

Methods: the essential oils obtained from flower buds of *Eugenia caryophyllata* and seeds of *Foeniculum vulgare* and *Carum carvi* were extracted by hydrodistillation. The antibacterial potency against local clinical isolate of *Helicobacter pylori* was tested using agar disc diffusion and minimum inhibitory concentration techniques and the chemical composition was determined via gas chromatography-mass spectrometry.

Results: the phytochemical analysis identified *trans*-anethole (43.01%), estragole (27.04%) and fenchone (06.63%) as main compounds in *F. vulgare* oil. Carvone and α -pinene were defined as major components of *C. carvi* oil with 63.92% and 8.43% respectively, whereas the abundant constituents of *E. caryophyllata* were eugenol

(65.22%), eugenyl acetate (18.77%) and *trans*-caryophyllene (9.92%). The strongest anti *H. pylori* activity was exhibited by *F. vulgare* oil reaching similar action of Clarithromycin ($p < 0.05$) used as positive control.

Conclusion: this is the first report showing the potency of essential oils from spices against *H. pylori*, the results indicated that these resources can constitute potential natural treatment.

Keywords: carvone; *trans*-anethol; eugenol; medicinal herbs; gastric cancer.

RESUMEN

Introducción: las hierbas medicinales constituyen una de las soluciones alternativas preferidas para desempeñar el papel de agentes antimicrobianos debido a su disponibilidad, biodegradabilidad y pocos efectos secundarios. Desafortunadamente, son raros los estudios centrados en la eficacia de los extractos de las plantas contra *Helicobacter pylori* que confirman o refutan su eficacia.

Objetivo: evaluar la actividad antibacteriana contra *Helicobacter pylori* de los aceites esenciales de *Eugenia caryophyllata*, *Foeniculum vulgare* y *Carum carvi*.

Métodos: los aceites esenciales obtenidos de los brotes de la flor del *E. caryophyllata*y, de semillas del *F. vulgare* y del *C. carvi* fueron extraídos por el *hydrodistillation*. La potencia antibacteriana contra el aislante clínico local *Helicobacter pylori* fue probada usando la difusión del disco del agar. Las técnicas de concentración inhibitoria mínima y la composición química fueron determinadas vía cromatografía del gas, espectrometría de masas.

Resultados: los análisis fitoquímicas de los aceites esenciales identificado *trans*-anethole (43,01 %), estragole (27,04 %) y fenchone (6,63 %) como compuestos mayoritarios en *F. vulgare*. El aceite extraído del *C. carvi* reveló la presencia del carvone y α -pinene con los compuestos abundantes a 63,92 % y 8,43 % respectivamente, mientras que los componentes principales para *E. caryophyllata* eran el eugenol (65,22 %), eugenyl acetate (18,77 %) y *trans*-caryophyllene (9,92 %). De los aceites esenciales obtenidos, el de *F. vulgare* presentó mayor actividad anti-*H. pylori*, con la acción similar al del Clarithromycin ($p < 0,05$) usada como control positivo.

Conclusión: los aceites esenciales de *Eugenia caryophyllata*, *Foeniculum vulgare* y *Carum carvi* pueden constituir un potencial tratamiento natural contra *H. pylori*.

Palabras clave: carvone; *trans*-anethol; eugenol; hierbas medicinales; cáncer gástrico.

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Introduction

Infectious diseases caused by microbial pathogens affect millions of people around the world without frontiers with variation in the effects depending on their pathogeneicity and development level of countries/regions affected. Indeed, the majority of infections occur in developing nations where it can attain 100% of population.[1]

Helicobacter pylori, a ubiquitous gastric microbe, infects more than half of the world's population and can accompany human for decades since the early childhood.[2] Infection with *H. pylori* is a major risk factor to arouse peptic ulcer disease and increases the probability to express gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma by three to six folds.[3] In relation to the high risk of gastric cancer of this bacterium, the International Agency for Research on Cancer[4] has categorized *H. pylori* infections as type I of carcinogen.

The ideal tendency to avoid *H. pylori* pathologies is to eradicate this rogue pathogen. In fact, defeat in the eradication route (patients with peptic ulcer disease) increase the ulcer recurrence at more than 55% compared with 10% for success cases.[5] However, successful *H. pylori* elimination may be not an optimal clinical achievement without suitable eradication regimen even with complex therapeutic treatment.[6]

Currently, the triple therapy involving two antibiotics metronidazole and clarithromycin accompanied with inhibition of acid secretion is the conventional clinical treatment in the fight against *H. pylori*.[7] However, the major obstacle of this heavy treatment is related to the antibiotic resistance.[8] This great hindrance is generally related to various factors such as half-life and instability of antibiotics in stomach acidity[6] and limited antibiotic action on mucosal surface without effect on "*invasive H. Pylori*"[3] that can colonize intercellular spaces and the lamina propria.[9,10] Thus, some researchers have argued the effectiveness of nature to control *H. pylori* infections.[7,8,9,10,11]

The medicinal herbs constitute one of the most preferred alternative solutions as antimicrobial agents for the modern communities due to their availability, biodegradability, fewer side effects and less toxicity. Unfortunately, rare are the studies

focused on the effect of plant extracts against *H. pylori* to either confirm or refute their effectiveness. To the best of our knowledge, no previous scientific reports have been published on antibacterial effects of spices on *H. pylori*.

Therefore, the aim of this study is to determine the chemical profiles of essential oils of three commercial and widely consumed spices *Eugenia caryophyllata*, *Foeniculum vulgare* and *Carum carvi* and to test their possible action against clinical isolate of *H. pylori*.

Methods

Plant material and essential oil extraction

The flower budsofclove (*Eugenia caryophyllata*), fennel seeds (*Foeniculum vulgare*) and caraway seeds (*Carum carvi*) were purchased at a local market of Chlef (North-West of Algeria). 100 g of each spice were separately subjected to hydrodistillation using a Clevenger-type apparatus for 3 h. The oils were dried over anhydrous sodium sulphate and then stored in dark glass vials at +4 °C until analysis and bioassay.

GC-MS analysis

The gas chromatography-mass spectrometry (GC-MS) analysis was performed on Hewlett-Packard 6890 series GC systems (Agilent Technologies) coupled to a quadruple mass spectrometer (model HP 5973) equipped with an HP5 MS capillary column (5 % phenyl methylsiloxane, 30 m×0.25 mm, 0.25 μm film thickness). Analytical conditions were: injector temperature 250 °C, oven temperature: isothermal 60 °C, 8 minutes, to 250 °C at 2 °C/minute then, isothermal, 30 minutes; carrier gas helium at 0.5 mL/minute; split 1/20; ionization voltage, 70 eV; scan range, 35-500 uma.

Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of n-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra (MS) built up from pure substances and components of known oils and MS literature data.[12]

Isolation and growth conditions of bacterial strain

The *Helicobacter pylori* strain was an endoscopic specimen received systematically at *Enterobacteriaceae* service's in Pasteur Institute of Algiers for diagnostic and antimicrobial susceptibility. Isolation was performed on Columbia agar supplemented with 10% horse blood and Helicobacter selective supplement (SR147E, Oxoid, England). Under microaerophilic atmosphere (5% O_2, 10% CO_2 and 85% N_2) in an anaerobic jar using BD GasPak EZ Pouch Systems at 37 °C for 2-14 days. *H. pylori* isolate was stored in brain heart infusion broth (BHIB) supplemented with 20% glycerol at -80 °C until tests.

Antibacterial bioassay

Antibacterial activity of essential oils was tested using disk diffusion method following Semeniuc *et al*.[13] protocol. In bref, *H. pylori* suspension prepared at 10^8 CFU/mL, was inoculted on Mueller Hinton agar supplemented with 10% (v/v) horse blood (MHA10%HB) plates by using cotton swabs. Sterile filter paper disks (Whatman paper no.3; Ø: 6 mm) were impregnated with 40µl of the essential oiland placed in the centre of agar surface. Disks without saturation were used as negative control, whereas the Clarithromycin (1µg/ml) was used as positive control. Each plate received one disc and all tests were carried out in triplicate. The plates were incubated for 48 H at 37 °C under microaerophilic conditions (5% O_2, 10% CO_2 and 85% N_2) in an anaerobic jar using BD GasPak EZ Pouch Systems. Zones of inhibition appearing around disks were measured and recorded in mm.

Determination of minimum inhibitory concentration (MIC)

The MIC assay of essential oils was tested using the modified method of Medjkane *et al*.[7] Briefly, aliquots of essential oils were dissolved separately in liquid MHA10%HB supplemented with 5% (v/v) Tween 80 at 45 °C in test tubes (final volume: 15 mL) to obtain different concentrations (1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000 and 1/5000 (v / v)). Rapidly vortexed, the emulsions were cooled in Petri dishes (Ø: 90 mm), then inoculated with the bacterial suspension (10^6 CFU/ml) with a STEERS apparatus. In parallel, the negative control (essential oil free) was performed. All Plates were incubated in microaerophilic environment for 48 hours. The MIC was determined as the lowest concentration that completely inhibited the bacterial growth.

Statistical analysis

All tests were carried out in triplicate and in identical conditions. The statistical analysis (ANOVA one way) was accomplished using SPSS 16.0 software for Windows (SPSS Inc., Chicago, Illinois, USA). Differences between diameter inhibitions at $p \leq 0.05$ were regarded to be significant.

Results

Yield and composition of essential oils

The hydrodistillation extraction of essential oils from *E. caryophyllata*, *F. vulgare* and *C. carvi* spices yielded 1.3%, 0.6% and 4.2% (w/w) respectively.

The results about the chemical composition are given in Table 1. The components are listed in order of their retention time on the HP-5MS column. The GC-MS identified twenty compounds in the essential oil extracted from seeds of *F. vulgare* (97.36%) with dominance of *trans*-anethole (43.01%), estragole (27.04%) and fenchone (06.63%).

The chromatographical analysis identified twenty five constituents for *C. carvi* oil's that represent 95.25% of the total composition, the carvone and α -pinene were defined as the main components with 63.92% and 8.43% respectively as well as the limonene represented in the oil by 4.81%.

The quantitatively most important constituents in the essential oil isolated from *E. caryophyllata* were eugenol (65.22%), eugenyl acetate (18.77%) and *trans*-caryophyllene (9.92%), these compounds represents 93.91% from the total percentage identified (97.27%).

Table 1 -Chemical composition of essential oils obtained from three commercial spices.

N°	Compound	L.R.I	*Carum carvi* (%)	*Eugenia caryohyllata* (%)	*Foeniculum vulgare* (%)
1	α -thujene	931	-	-	0.12
2	α -pinene	935	8.43	tr	1.36
3	camphene	955	tr	-	0.27
4	sabinene	973	-	-	3.89
5	β -pinene	985	tr	tr	-
6	myrcene	992	-	-	1.36
7	α –phellandrene	1007	tr	-	1.16
8	eucalyptol	1015	1.83	-	-
9	*p*-cymene	1025	-	tr	-
10	limonene	1037	4.81	-	4.68
11	γ-terpinene	1061	0.30	-	2.27
12	fenchone	1089	-	-	06.63

13	linalool	1100	4.26	tr	3.22
14	*trans*-Perillyl alcohol	1116	tr	-	-
15	isocarveol	1129	2.07	-	-
16	limonene oxide	1140	tr	-	-
17	*trans*-verbenol	1145	tr	-	-
18	camphor	1148	-	-	0.23
19	trans-Perillaldehyde	1150	2.02	-	-
20	terpinen-4-ol	1178	-	-	0.31
21	α- terpineol	1194	0.14	-	-
22	verbenone	1204	-	-	1.03
23	*cis*-Dihydrocarvone	1208	1.47	-	-
24	*trans*-Carveol	1217	0.12	-	-
25	dihydrocarveol	1242	0.14	-	-
26	carvone	1244	63.92	tr	-
27	fenchyl acetate	1247	-	-	0.16
28	*trans*-anethole	1285	-	-	43.01
29	anethole	1288	2.49	tr	0.47
30	thymol	1290	-	-	0.15
31	estragole	1297	tr	-	27.04
32	eugenol	1355	-	65.22	-
33	α-copaene	1377	-	0.83	tr
34	β –cubebene	1390	0.3	-	-
35	β –caryophyllene	1421	1.24	tr	tr
36	*trans*-caryophyllene	1425	-	9.92	-
37	α -amorphene	1477	-	tr	-
38	α -humulene	1457	-	1.53	-
39	α -guaiene	1468	tr	-	-
40	β –selinene	1490	0.71	-	-
41	β -cadinene	1503	0.69	-	-
42	eugenyl acetate	1527	-	18.77	-
43	δ-cadinene	1530	-	0.25	-
44	caryophyllene oxide	1573	-	0.54	-
45	apiole	1678	0.31	-	-
46	farnesol II	1696	-	0.21	-
Total		/	95.25	97.27	97.36

LRI: Linear retention index;
tr: trace (≤ 0.1%).
The components are listed in order of their retention indices on HP-5MS column.

Antimicrobial activity of essential oils

Antimicrobial screening test using disc diffusion and MIC assays was employed to estimate the potencies of *E. caryophyllata, F. vulgare* and *C. carvi* oils against *H. pylori*. The results are shown in Table 2. As can be seen from this table, all essential oils exhibited anti *H. pylori* activity, the highest potency was exhibited by the *F. vulgare* oil showing 16.83 ± 1.42 mm as inhibition zone diameter with a lowest CMI recorded. This potential is comparable ($p<0.05$) to the activity of Clarithromycin used as positive control prepared at 1µg/mL. The anti-*H. pylori* of essential oils isolated from *E. caryophyllata*

(13.83 ± 1.42 mm) and *C. carvi* (12.66 ± 2.11 mm) was moderate in comparison to the positive control (16.66 ± 0.93 mm), taking into the account the values of MIC test, *C. carvi* (1/500 v/v) exhibited a strong potential than *E. Caryophyllata* (1/1000 v/v) essential oil.

Table 2 -Antibacterial activity of essential oils against *Helicobacter pylori*.

Essential oils	Diameter inhibition (mm)	MIC (v/v)
Foeniculum vulgare	16.83 ± 1.42[a]	1/250
Carum carvi	12.66 ± 2.11[b]	1/500
Eugenia caryohyllata	13.83 ± 1.42[b]	1/1000
Clarithromycin	16.66 ± 0.93[a]	NT

Notes: a-b: Values in the same column sharing different letters are significantly different (P < 0.05).
MIC, Minimum Inhibitory Concentration.
NT: not tested

Discussion

According to Raal *et al.*[14] the yield of essential oil from *C. carvi* seeds from different European countries ranged from 0.6% to 5.4%. Our result is belongs to this interval, whereas the yield obtained from *F. vulgare* seeds is low than the previous extraction by Sellam *et al.*[15] showing 2.8%.

In general, the essential oil yields from medicinal plants is a fluctuate factor that depends on multiple parameters such as, climatic conditions, harvesting date, storage period, origin, variety, cultivar or population.[16]

The compounds *trans*-anethole, estragole and fenchone are reported previously[15,17,18] as main and/or characteristic constituents of *F. vulgare* essential oil. The study realized by Raaletal.[14] on essential oils extracted from twenty samples of caraway commercialized in European countries indicated the dominance of carvone and limonene with amounts ranging between 44.5-95.9% and 1.5-51.3% respectively. In the other hand, carvone and limonene were reported as major constituents for Chinese caraway.[19] The current study is in accordance with all previous investigations.

The most representative components of *E. Caryophyllata* essential oil are frequently reported to be eugenol and *trans*-caryophyllene.[20,21] in another study by Chaieb *et al.*[22] eugenol (88.58%), eugenyl acetate (5.62%) and β-caryophyllene (1.39%) were the major components with 95.59% of the total chemical mixture.

Many previous studies have been reported the powerful antibacterial efficacy of E. *caryophyllata*, *F. vulgare* and *C. carvi* spices oils against human pathogenic bacteria

through *in vitro* tests.[15,22,23,24,25,26,27] To the best of our knowledge, no previous scientific studies have been published on anti *H. pylori* activity of spices oils and our study seems to be the first data on this subject.

The antimicrobial effect of essential oils depends on their chemical compositions. The potency recorded for commercial spices oils in the current study could be attributed to the presence of some major bioactive constituents such as limonene (*F. vulgare* and *C. carvi* oils), α-pinene,[28] *trans*-anethole and fenchone (*F. vulgare* oil).[29] Eugenol found as the most abundant component in *E. Caryophyllata* oil is well known to have antimicrobial activity[22] together carvone (*C. carvi*).[27] However, the possible contribution of minor constituents by synergistic and modulatory functions should not be neglected.[30] These components of essential oils affect generally the cell membrane properties, resulting in the expansion and augmentation of the fluidity of the membrane and enzymatic inhibition.[31]

Medjkane *et al*.[7] evaluated the effect of essential oil isolated from *Pistacia lentiscus* leaves against 48 clinical isolates of *H. pylori*, no isolate was resistant this oil with important inhibition diameter reaching 32±1.00 mm. On the other hand, Ohno *et al*.[32] have been investigated the *in vitro* and *in vivo* antimicrobial activity of thirteen essential oils against *H. pylori*, their findings showed a very strong *in vitro* inhibition by the oils with remarkable bactericidal effect of *Cymbopogon citratus* and *Lippia citriodora* at 0.01%.

The antibacterial efficacy of the widely consumed spices *E. caryophyllata*, *F. vulgare* and *C. carvi* essential oils against *H. pylori* demonstrated a potential action as natural product. However, we think that phytotherapy is not an absolute solution but useful as permanent prevention and should be combined with other therapeutic regimens. Further studies are needed to test the effect of combination of essential oils and their individual constituents on *H. pylori* as well as the *in vivo* investigation.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Authors' contributions

M. Tabti: has carried out the experiments and literature survey with help and supervision of other authors.

A. Merouane: has helped in GC/MS analysis, statistical analysis and prepared the manuscript.

R. Allem: has conceived, designed and supervised the research. All authors have read and approved the final manuscript.