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Research Article

Clove and Peppermint Essential Oils Effect on Pathogenic Gut Micro-Biota in Chronic Hepatic Disease Patients

Mohamed T. Shaaban, Gehan M. Fahmy , Esraa H. Saad, and Hussein S. Salama*

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Abstract
Multidrug-resistant bacteria infections in cirrhotic patients are currently increasing and associated with greater morbidity and mortality. The aim of this study was to decrease complications and bacterial infections that probably occurred after a patient undergoes liver transplantation surgery in Egypt using natural edible nontoxic peppermint and clove essential oils. All patients undergo clinical, laboratory, and microbiological testing. The recorded results showed that administered minimum inhibitory concentration (MIC) of clove and peppermint essential oils significantly reduced the total colony forming unit (CFU) count of multidrug-resistant pathogenic bacteria isolated from patients with chronic hepatic disease. The gas chromatography-mass spectrometry (GC-MS) analysis of clove and peppermint oil revealed the presence of active constituents with antibacterial activities as indicated in previous reviews.

Keywords: liver-cirrhosis, multidrug-resistant bacteria, clove oil, peppermint oil

1. INTRODUCTION

Cirrhosis refers to scarring of liver tissue caused by long-term damage which prevents the liver from functioning properly. Cirrhosis is considered as the end-stage of liver disease because it occurs after other stages of liver injury. Thus, preventing any bacterial infection in cirrhosis liver patients is very important due to the weak immunity of these patients [1]. Multidrug-resistant bacteria complications increased rapidly in recent years [2], especially in patients with decompensated cirrhosis and with acute-on-chronic liver failure in Europe causing dangerous problem for these patients [1][3]. Looking forward to an alternative natural antibacterial agent such as essential oils (EOs) rather than using antibiotics is the latest tren during the last decades [4]-[6].

Recently, more than 3000 EOs have been extracted, mostly from families including Lamiaceae, Rutaceae, Myrtaceae, Zingiberaceae, and Asteraceae. Among them, more than 300 EOs are commercialized in the fragrance and food markets with anticipated growth reaching more than $15 billion by 2025 [7]. Recently, EOs and their derivatives have gained attention due to their good tolerability and effectiveness in the prevention and treatment of diseases, including cancer and metabolic syndrome, in both animal and human studies [8]. Clove (*Syzygium aromaticum*, Myrtaceae) is an aromatic plant widely cultivated in tropical and subtropical countries. Clove's EO is rich in volatile compounds and antioxidants such as eugenol, β-caryophyllene, and α-humulene. It has considerable interest due to its wide application in the perfume, cosmetic, health, medical, flavoring, and food industries. Eugenol is the major compound accounting for at least 50% which is approved to have antibacterial activity [9][10]. On the other hand, peppermint (*Mentha piperita*, Lamiaceae) is native to Europe and the Middle East, but it is cultivated in different parts of the world. In folk medicine, EOs and extracts of peppermint have been widely used to treat inflammation of the oral mucosa, and cold. The EOs of peppermint have been reported to have antioxidant, anti-inflammatory, antibacterial, and antifungal activities [11]-[13]. Therefore, this study aimed to use edible nontoxic clove and peppermint essential oils to prevent the colonization of pathogenic gut microbiota in patients with chronic hepatic disease.

2. MATERIALS AND METHODS

2.1. Sample Collection, Isolation and Counting of

Table 1. MIC values of clove oil against *E. coli* and *K. pneumoniae.*

Bacteria /inhibition percentage	Concentration (mL/mL)								
	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.02	0.01
E. coli	51%	45%	37%	22%	11%	11%	0%	0%	0%
K. pneumoniae	48%	39%	27%	9%	9%	0%	0%	0%	0%

Table 2. MIC of peppermint oil against *E. coli* and *K. pneumoniae*.

Bacteria /inhibition percentage	Concentration (mL/mL)								
	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.02	0.01
E. coli	47%	31%	10%	9%	0%	0%	0%	0%	0%
K. pneumoniae	33%	12%	11%	0%	0%	0%	0%	0%	0%

Bacterial Colonies

Fecal samples were collected from ten patients before and after treatment by clove and peppermint EOs according to the hospital policy on days 2, 7, and 14. The samples were weighed and homogenized. Collected samples were serially diluted and plated on MacConkey agar medium for analysis of Gram-negative bacteria then incubated overnight at 37 °C. After isolation, Chrom agar medium was used for counting the antibiotic resistant bacterial colonies using colony forming unit (CFU). Then, purified bacterial isolates were identified using VITEK 2 [14].

2.2. Minimum Inhibitory Concentration (MIC) of Essential Oils

The minimum inhibitory concentration (MIC) of clove and peppermint oils was evaluated *in vitro* by using the broth micro-dilution technique in 96-well plates. Different dilutions of oils were put in the 96-well plate wells in the culture of 100 µL suspension of the isolated pathogenic bacteria in Muller Hinton broth media with a concentration of 10^6 CFU/ mL. The results were recorded using ELISA [15].

2.3. Treatment by Essential Oils

Regarding to the MIC results, clove and peppermint EOs were administered to patients for two weeks. After treatment, the samples were collected and CFU were estimated again on Chrom agar medium.

2.4. GC-MS Analysis of Essential Oils

The most prominent active constituents in clove and peppermint essential oils were analyzed using gas chromatography-mass spectrometry (GC-MS) spectroscopy (Shimadzu GCMS-QP2010, Kyoto, Japan). All the mass spectra were recorded applying the following conditions: (equipment current) filament emission current, 60 mA; ionization voltage, 70 eV; ion source, 200 °C. Diluted samples (1% v/v) were injected with split mode (split ratio 1: 15) [16].

2.5. Statistics

All experiments were done in triplicates. Statistic calculations were done using the SPSS software. The data represented as average values ± standard deviation (S.D.) and the significance between treated and non-treated groups were considered only when the p-value ≤ 0.05 between the compared treatments.

3. RESULTS AND DISCUSSIONS

The different bacterial isolates were isolated from patients who suffered from liver diseases varied from patients finally prepared for liver transplantation surgery suffered from ascites to end-stage liver disease, liver cirrhosis and HCV positive which caused liver failure. MacConkey agar medium was used for isolation and identification. The results of identification using VITEK 2

indicated the most prominent isolates were *Escherichia coli* and *Klebsiella pneumonia* [1][3]. Table 1 showed that the MIC of clove oil against *E. coli* was 0.04 mL/mL with an inhibition percentage of 11%. While the MIC of clove oil against *K. pneumoniae* was 0.05 mL/mL with an inhibition percentage of 9 %. In Table 2, the MIC of mint oil against *E. coli* was 0.06 mL/mL with an inhibition percentage of 9%. The MIC of mint oil against *K. pneumoniae* was 0.07 mL/mL with an inhibition percentage of 11%.

After that, the CFU of multidrug resistant bacteria were estimated on Chrom agar medium from ten patients before and after treatments with MICs of clove and peppermint EOs. Antibiotic resistance is a phenomenon increasing rapidly during last decades and it has become a major cause of global mortality in patients with chronic liver disease [3]. As shown in Table 3, the bacterial colony count after the patients treated with clove oil significantly reduced as compared with the bacterial colony count before treatment. There were statistically significant differences between the mean readings of the research samples before and after using clove oil as the p-value lower than 0.05, where Z value was 4.67. These results indicate that using clove oil was effective to significantly reduce colony forming unit of pathogenic bacteria in patients suffered from chronic hepatic disease**.** Moreover, Ginting *et al*. reported the efficiency of clove oil against *K. pneumoniae* [17].

Table 4 illustrated the reduction of bacterial colony count after treatment with mint oil when compared with bacterial count before treatment. There were statistically significant differences between the mean readings of the research samples before and after mint oil treatment as the p-value < 0.05, where Z value was 3.65. These results indicate that using peppermint oil for treatment of patients with chronic hepatic disease was effective to significantly reduce pathogenic bacteria but not as clove oil. Moreover, Nazzaro *et al*. and Upadhyay *et al.* reported the efficiency of peppermint oil against *E. coli* and *K. pneumoniae* [12][18][19].

Finally, the analysis using GC-MS spectroscopy were done to find out the bioactive compounds. The

Table 3. The bacterial colony forming unit of samples collected from liver transplantation patients before and after treatment with clove oil (the data were represented as mean value ± SD).

Patient	Before treatment with clove oil (CFU)	After treatment with clove oil (CFU)
1	$6.63\times10^{7}\pm3.10\times10^{6}$	$8.50\times10^{6}\pm1.01\times10^{6}$
2	$1.76\times10^{7}\pm3.51\times10^{6}$	$9.73\times10^{5}\pm3.41\times10^{5}$
3	$8.56\times10^{7}\pm1.01\times10^{7}$	$1.04\times10^{7}\pm1.40\times10^{6}$
4	$8.63\times10^{7}\pm6.10\times10^{6}$	$1.90\times10^{6}\pm8.19\times10^{5}$
5	$8.90\times10^{7}\pm1.73\times10^{7}$	$1.90\times10^{6}\pm5.56\times10^{5}$

Table 4. The bacterial count of samples collected from liver transplantation patients before and after treatment with peppermint oil (the data were represented as mean value ± SD).

Patient	Before treatment with peppermint oil (CFU)	After treatment with peppermint oil (CFU)
6	$1.50\times10^{7}\pm1.00\times10^{7}$	$2.56\times10^{6}\pm1.52\times10^{5}$
7	$1.63\times10^{7}\pm2.08\times10^{6}$	$1.20\times10^{6}\pm2.00\times10^{5}$
8	$7.67\times10^{7}\pm1.53\times10^{7}$	$1.57\times10^{7}\pm1.15\times10^{6}$
9	$1.37\times10^{8}\pm1.53\times10^{7}$	$1.77\times10^{7}\pm1.53\times10^{6}$
10	$1.53\times10^{8}\pm2.08\times10^{7}$	$1.70\times10^{6}\pm1.73\times10^{5}$

Table 5. GC-MS analysis of clove oil.

Peak	Retention Time (min)	Area%	Name	Base m/z
1	14.386	15.91	5-Methyl-2-(1-methylethyl)-cyclohexanol	71.05
2	19.808	46.55	2-Methoxy-3-(2-propenyl)phenol	164.10
3	21.496	17.36	Caryophyllene	41.00
4	22.405	1.84	Humulene	93.05
5	24.274	3.80	2-Methoxy-4-(2-propenyl) acetate	164.10
6	25.724	1.41	Caryophyllene oxide	40.95
7	35.609	3.16	Palmitic Acid	117.05
8	43.169	2.61	Decyl Undec-10-ynoate	67.00
9	53.377	8.81	Eugenol	149.05

Table 6. GC-MS analysis of peppermint essential oil.

Peak	Retention Time (min)	Area%	Name	Base m/z
1	7.075	1.86	α-Pinene	93.10
2	8.325	2.55	β-Pinene	93.05
3	9.923	3.52	(S)-1-Methyl-4-(1-methylethenyl)-cyclohexene	68.05
4	13.790	12.27	5-Methyl-2-(1-methylethyl)-cyclohexanone	41.00
5	14.111	7.57	5-Methyl-2-(1-methylethyl)-cyclohexanone	41.00
6	14.427	60.02	1-Methyl-4-(1-methylethyl)-cyclohexanol	41.00
7	17.958	3.23	Menthyl acetate	43.00
8	42.045	2.24	2-Methyloctacosane	57.10
9	43.670	2.03	Tetracosane	57.05
10	52.034	4.69	Hexatriacontane	57.05

results in Tables 5 and 6 revealed that, in peppermint oil the bioactive compounds were α-pinene, β-pinene, menthyl acetate, 2-methyloctacosane, tetracosane and hexatriacontane. Also, Nascimento *et al.* and Hawrył *et al*. [13][20] found β-pinene and menthyl acetate as the active constituents in mint EO. On the other hand, the compounds revealed in clove EO were caryophyllene, humulene, 2-methoxy-4-(2-propenyl) acetate, and eugenol. This is in consonance with the work of Marya *et al*. and Agatonovic-Kustrin *et al*. [21][22] who reported the same active constituents in clove EO.

4. CONCLUSIONS

This research reveals that the edible nontoxic clove and peppermint EOs revealed a good effect in reduction of total colony forming unit counts of pathogenic bacteria isolated from patients with chronic hepatic disease. Therefore, we recommend using clove and peppermint oil with patients suffering from chronic liver diseases to prevent any secondary infection by pathogenic bacteria. Further studies are needed to estimate antioxidant, anti-inflammatory, and cytotoxocityeffects on patients with chronic hepatic disease.

AUTHOR INFORMATION

Corresponding Author

Hussein S. Salama — Botany and Microbiology Department, Menoufia University, Menoufia-6131567 (Egypt);
Email: husseinsalama@science.menofia.edu.eg
orcid.org/0000-0002-8803-5675

Authors

Mohamed T. Shaaban — Botany and Microbiology Department, Menoufia

University, Menoufia-6131567 (Egypt);
orcid.org/0000-0003-1808-9771
Gehan M. Fahmy — Clinical Microbiology Department, Ain Shams University, Cairo-11566 (Egypt);
orcid.org/0009-0000-6204-6600
Esraa H. Saad — Botany and Microbiology Department, Menoufia University, Menoufia-6131567 (Egypt);
orcid.org/0009-0003-4205-4023

Author Contributions

Conceptualization, M.T. S., G. M. F. and E. H. S.; Methodology, E. H. S.; Software, H. S. S.; Validation, E. H. S.; Formal analysis, H. S. S.; Investigation, M. T. S. and G. M. F.; Supervision, M. T. S. and G. M. F.; Data curation, H. S. S.; Writing original draft, E. H. S.; Writing- Review & Editing, M. T. S., G. M. F. and H. S. S.

Conflicts of Interest

The authors declare no conflict of interest.

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