International Journal of Secondary Metabolite
2023, Vol. 10, No. 3, 385–393

https://doi.org/10.21448/ijsm.1232606

Published at https://dergipark.org.tr/en/pub/ijsm

Research Article

Antimicrobial evaluation of the Patchouli (*Pogostemon cablin* Benth.) leaf essential oil combination with standard antimicrobial compounds

Nazlı Şenay Beşirik [1], **Gamze Göger** [2,*]

[1]Trakya University, Institute of Science, Department of Biotechnology and Genetic, Edirne, Türkiye
[2]Afyonkarahisar Health Sciences University, Department of Pharmacognosy, Faculty of Pharmacy, Afyonkarahisar, Türkiye

Abstract: *Pogostemon cablin* Benth's leaves, which belong to the Lamiaceae family, are used to make patchouli essential oil (PEO). Sesquiterpenes are the main compounds in essential oil. Antibacterial, antifungal, antiviral, antiemetic, and anti-inflammatory activities are just a few of the many pharmacological actions that are well-known. In this investigation, cefuroxime (CEF), moxifloxacin (MOX), clarithromycin (CLA), fluconazole (FLU), and terbinafine (TER) were combined with patchouli (*Pogostemon cablin* leaf essential oil). Gram-positive *Staphylococcus aureus* ATCC 6538, Gram-negative *Escherichia coli* ATCC 8739, and yeasts *Candida albicans* ATCC 10231, and *C. tropicalis* ATCC 750 were tested against all combinations. Drug interaction was given as fractional inhibitory concentrations (FIC) after combinations of the essential oils were tested for their minimum inhibitory concentrations (MIC).

The checkerboard method was used to assess antimicrobial interactions, Fluconazole, terbinafine, cefuroxime, and clarithromycin combined with essential oil demonstrated an "additive effect" against *Candida* strains together with *E. coli* and *S. aureus*.

ARTICLE HISTORY

Received: Jan. 17, 2023
Revised: May 25, 2023
Accepted: May 27, 2023

KEYWORDS

Pogostemon cablin,
Essential oil,
Checkerboard method,
Antibacterial,
Antifungal

1. INTRODUCTION

In modern medicine, antimicrobial substances especially antibiotics have been used for the protection of global health for the last eighty years (Laxminarayan *et al.*, 2016). With the discovery and development of antibiotics over time, it has been seen that infections can be controlled and prevented. However, the overuse and misuse of antibiotics used against microorganisms have led to the formation of antimicrobial resistance in microorganisms (Kapoor *et al.*, 2017). Antibiotic resistance, in addition to being a multi-sectoral problem that destroys decades of progress in medicine, food safety, and public health, causes very high rates of death worldwide, regardless of the level of development of countries (Laxminarayan *et al.*, 2016). Due to the increase in antimicrobial-resistant microorganisms and the inability to produce new antimicrobials easily, alternative strategies must be found to combat infections caused by drug-resistant organisms (Ahmad *et al.*, 2021; Langeveld *et al.*, 2014).

*CONTACT: Gamze Göger gamze.goger@afsu.edu.tr Afyonkarahisar Health Sciences University, Department of Pharmacognosy, Faculty of Pharmacy, Afyonkarahisar, Türkiye

e-ISSN: 2148-6905 / **© IJSM 2023**

Synergistic, additive, indifferent, and antagonistic effects can be produced when combining essential oils and antimicrobials. When the combined antimicrobial activity of the two compounds is larger than the combined antimicrobial activity of the two separately administered compounds, this is known as a synergistic effect (Göger *et al*., 2018). The synergic effect is thought to be caused by four different theoretical mechanisms, including blockage of a specific metabolic pathway, inhibition of enzymes that break down antimicrobials, interactions of some antimicrobials with the cell wall, and/or membrane interactions that result in an increase in the number of antimicrobial compounds taken up by the cell (Hyldgaard *et al*., 2012).

Essential oils exhibit a wide range of activities against pathogenic microorganisms. According to research that has been published, various combinations of essential oils and antibacterial compounds are efficient against microorganisms (Hemaiswarya & Doble, 2009; Van Vuuren *et al*., 2009; Göger *et al.*, 2018; Sharma *et al.*, 2020; Zhong *et al.*, 2021).

Pogostemon cablin L., a plant belonging to the Lamiaceae family, is used mostly in traditional medicine in Southeast Asia (Dechayont *et al.*, 2017). The word "cablin" is derived from the term "cabalam," which is also the local name for the patchouli plant in the Philippines and these are synonymous (Swamy & Sinniah, 2015). *P. cablin* has often been used in traditional medicine to promote digestion, restore function, and lower fever and chronic weariness (Dechayont *et al.*, 2017; Picheansoonthon & Jerawong, 2001).

At least 140 biologically active chemicals, including terpenoids and flavonoids, are present in the significant medicinal plant *P. cablin* (Tang *et al.*, 2019). There are several pharmacological qualities, including antibacterial, anti-inflammatory, antiplatelet, antioxidant, antithrombotic, aphrodisiac, analgesic, antimutagenic, antiemetic, antidepressant, fibrinolytic, and cytotoxic activity (Ribeiro *et al.*, 2018; Swamy & Sinniah, 2015).

Patchouli (*Pogostemon cablin* L.) leaf essential oil (PCLO), which is derived from the leaves of *P. cablin*, has tremendous significance due to the structure of its twenty-four distinct sesquiterpenes (Donelian *et al.*, 2009; Silva-Filho *et al.*, 2016). Scientists have looked at patchouli essential oil's therapeutic qualities, including its cytotoxic, antioxidant, anti-inflammatory, antibacterial, and antidepressant effects (Aisyah *et al.*, 2021; Jain *et al.*, 2021; Paulus *et al.*, 2020).

For this study, we combined *P. cablin* leaf essential oil with antimicrobial compounds such as, cefuroxime, moxifloxacin, clarithromycin, fluconazole, and terbinafine against strains of Gram-positive *Staphylococcus aureus* ATCC 6538, Gram-negative *Escherichia coli* ATCC 8739, and yeasts *Candida albicans* ATCC 10231, and *C. tropicalis* ATCC 750. To the author's knowledge, this is the first study to investigate the effects of combining essential oil of *Pogostemon cablin* with antimicrobial compounds.

2. MATERIAL and METHODS

2.1. Essential Oil

An amber glass bottle of PCLO was purchased from Bade Natural Ltd. in Istanbul, Turkey. The essential oil was kept in a 4°C refrigerator. Analyses of the essential oil supplied by the provider using Gas Chromatography (GC).

2.2. Materials

As conventional antimicrobial drugs, Sanovel Pharmaceutical Company (Istanbul, Turkey) provided the following: cefuroxime (CEF), moxifloxacin (MOX), clarithromycin (CLA), fluconazole (FLU), and terbinafine (TER). To determine antimicrobial activity and checkerboard microdilution assays, RPMI-1640 medium with L-glutamine and Mueller Hinton

Broth (MHB, Sigma) were used. RPMI medium was buffered to a pH of 7 with 3-[N-morpholino]-propane sulfonic acid (MOPS).

2.3. Antimicrobial Activity Studies

2.3.1. *Bacterial and Yeast strains*

The standard strains were *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Candida* albicans ATCC 10231, and *C. tropicalis* ATCC 750 from the American Type Culture Collection (ATCC).

2.3.2. *Determination of minimum inhibitory concentration* (MIC)

Susceptibility studies for PCLO were performed using microdilution methods adapted from CLSI guidelines (CLSI, 2002; CLSI, 2006). The essential oil was dilutedtwo-fold initially, with a final concentration range of (40-2560 µg/mL), for standard antibacterial drugs (0.125- 64 µg/mL), and antifungal drugs (0.25-128 µg/mL).

For the preparation of cell suspensions in MHB for bacterial strains and RPMI medium for yeasts, fresh overnight cultures of the tested microorganisms were employed to achieve 10^6 colony forming units (CFU)/mL and 1-2x10^3 cells/mL, respectively. The experiments were conducted in a 96-well plates, and inoculated microplates were incubated at 37°C for 24 hours for bacteria and 48 hours for yeast, respectively. Adding 15 µL of 0.01% resazurin allowed for the observation of microbial growth. MICs were established as the lowest concentrations of resazurin that did not result in bacterial or fungal growth or a color change.

2.3.4. *Combination of EO and standard antimicrobial compounds*

The antimicrobial interactions of the antibiotics (CEF, MOX, and CLA) and antifungals (TER, FLU) with PCLO were studied using the checkerboard method. The 10-to-7-well design was used on 96-well plate. The same solvents (media) used in the MIC test were used to generate seven serial dilutions of PCLO and ten dilutions of antimicrobial drugs (CEF, MOX, CLA, TER, and FLU).

In the first row A of a 96-well plate, 200 µL of PCLO were placed. Then the essential oil was serially diluted to the other six rows in horizontal direction. Similarly, 200 µL aliquots of each antimicrobial drug (CEF, MOX, CLA, TER, FLU) were added in a vertical (column) orientation, and serially diluted. Similarly, 200 µL of antimicrobial drugs CEF, MOX, CLA, TER, and FLU were introduced and serially diluted in vertical column orientation. Thus, various concentrations of essential oil and antimicrobial drugs were formed in the plate. The positive growth controls were used free of antimicrobial drugs in the wells. To evaluate sterility, negative growth controls were also established. Microorganisms were individually pipetted into all wells except for negative controls, with a McFarland value of 0.5 for each microorganism, 50 µL for bacteria, and 100 µL for Candida, respectively. Bacteria were incubated for 24 hours and candida for 48 hours at 37°C. Following incubation, 15 µL of 0.01% resazurin was added to each well and incubated for 2 hours at 37°C. The color of the medium changes from blue to pink as it grows.

The following formula (Van Vuuren *et al.*, 2009) was used to calculate the interaction of combinations based on the sum of the fractional inhibitory concentration (FIC):

$$\text{FIC of essential oil} = \frac{\text{MIC of essential oil in combination with antimicrobial drugs}}{\text{MIC of essential oil alone}}$$

$$\text{FIC of antimicrobial drug} = \frac{\text{MIC of antimicrobial in combination with essential oil}}{\text{MIC of antimicrobial drug alone}}$$

$$\text{FICI} = \text{FIC of essential oil} + \text{FIC of antimicrobial drug}$$

The impacts were classed as follows:

Table 1. The effects and ranges of combinations.

	Ranges	Effect
FICIs	≤0.5	synergistic
	0.5≤1	additive
	1–4	indifferent
	≥4	antagonistic

3. RESULTS

3.1. Specifications of *Pogostemon cablin* L. Leaf Essential Oil

The supplier at Bade Natural provided the analysis certificate for PCLO. Specifications for *Patchouli* essential oil was given in Table 2. The major components of PCLO were identified as patchouli alcohol (30.0–60.0%), guaiene (10.0–30.0%), and caryophyllene (1.0–5.0%) (GC) by using Gas Chromatography.

Table 2. Specifications for PCLO from Bade naturel.

Compounds	%
Patchouli alcohol	30.0 - 60.0%
α-guaiene	(10.0 - 30.0%)
β-caryophyllene	(1.0 - 5.0 %)
The density (at 20°C)	0.943-0.983 g/mL
Refractive index (at 20°C)	1.502-1.514

3.2. Minimum Inhibitory Concentrations (MIC)

The minimum inhibitory concentration values PCLO and standards were found for each microorganism and represented in Table 3. In this study, the MIC of PCLO in the range of >2560-640 µg/mL against bacteria and yeasts. The PCLO was found to be more effective against *Staphylococcus aureus* ATCC 6538 at MIC= 640 µg/mL than *Escherichia coli* ATCC 8739 at MIC= 2560 µg/mL. The MIC values were found at 1280-2560 µg/mL for *C. tropicalis* ATCC 750 and *C. albicans* ATCC 10231 strains, respectively.

Table 3. Minimum inhibitory concentrations (µg/mL).

Compounds	*E.coli* ATCC 8739	*S.aureus* ATCC 6538	*C.albicans* ATCC 10231	*C.tropicalis* ATCC 750
PCLO	>2560	640	2560	1280
CEF	64	64	-	-
MOX	0.25	0.25	-	-
CLA	64	0.25	-	-
TER	-	-	32	16
FLU	-	-	2	4

PCLO: *Pogostemon cablin* L. leaf essential oil- CEF: Cefuroxime, MOX: Moxifloxacin, CLA: Clarithromycin, TER: Terbinafine, FLU: Fluconazole

3.3. Combination of Essential Oil and Standard Antimicrobial Compounds

The checkerboard method was used to determine the antimicrobial interaction of antibiotics (CEF, MOX, and CLA) with PCLO. In the present study, the antimicrobial activity of *Patchouli* essential oil was tested on *S. aureus* and *E. coli* strains. While an additive effect was obtained with cefuroxime and clarithromycin, an indifferent effect was observed with moxifloxacin. According to the results of the combination, essential oil with cefuroxime and clarithromycin showed an additive effect (FICI=1.0) against *S.aureus* ATCC 6538. The essential oil in combination with moxifloxacin was defined as having an indifferent effect (FICI=1.25 for the same strain) (in Table 4).

The combination of essential oil with cefuroxime and clarithromycin showed additive effects (FICI=1.0), and (FICI=0.75), respectively, against *E. coli*. The combination of essential oil with moxifloxacin had an indifferent effect (FICI=1.25) against the same strain (in Table 5). The combination of the essential oil with fluconazole had an additive effect for both *Candida* strains, while the combination with terbinafine had an indifferent effect for *C.albicans* (in Table 6) and an additive effect for *C.tropicalis* (in Table 7). Briefly, our test organisms demonstrated neither synergistic nor antagonisticeffects.

Table 4. Combination of essential oil with antibiotics against *S. Aureus.*

Combinations	PCLO			Standard Comp.			FICI	Result
	Alone	Comb.	[1]FIC	Alone	Comb.	[2]FIC		
PCLO+ CEF	640	320	0.5	4	2	0.5	1	Additive
PCLO+ MOX	640	160	0.25	0.031	0.031	1	1.25	Indifferent
PCLO+ CLA	640	320	0.5	0.5	0.25	0.5	1	Additive

PCLO: *Pogostemon cablin* L. leaf essential oil - CEF: Cefuroxime, MOX: Moxifloxacin, CLA: Clarithromycin

Table 5. Combination of essential oil with antibiotics against *E. coli.*

Combinations	PCLO			Standard Comp.			FICI	Result
	Alone	Comb.	[1]FIC	Alone	Comb.	[2]FIC		
PCLO + CEF	20480	10240	0.5	32	16	0.5	1	Additive
PCLO + MOX	10240	2560	0.25	0.031	0.031	1	1.25	Indifferent
PCLO + CLA	10240	5120	0.5	256	64	0.25	0.75	Additive

PCLO: *Pogostemon cablin* L. leaf essential oil- CEF: Cefuroxime, MOX: Moxifloxacin, CLA: Clarithromycin

Table 6. Combination of essential oil with antifungals against *C. albicans.*

Combinations	PCLO			Standard Comp.			FICI	Result
	Alone	Comb.	[1]FIC	Alone	Comb.	[2]FIC		
PCLO + TER	2560	640	0.25	8	8	1	1.25	Indifferent
PCLO + FLU	2560	40	0.0157	1	0.5	0.5	0.5157	Additive

PCLO: *Pogostemon cablin* L. leaf essential oil- TER: Terbinafine, FLU: Fluconazole

Table 7. Combination of essential oil with antifungals against *C.tropicalis.*

Combinations	PCLO			Standard Comp.			FICI	Result
	Alone	Comb.	[1]FIC	Alone	Comb.	[2]FIC		
PCLO + TER	2560	1280	0.5	32	4	0.125	0.625	Additive
PCLO + FLU	2560	1280	0.5	1	0.031	0.031	0.531	Additive

PCLO: *Pogostemon cablin* L. leaf essential oil- TER: Terbinafine, FLU: Fluconazole

4. DISCUSSION and CONCLUSION

According to published research, the chemical composition of patchouli oil from Samia Aromatherapy was revealed to contain patchoulol (25.21%), δ-guaieno (11.49%), α-gurjunene (11.26%), seicheleno (9.61%), α-guaieno (9.56%), benzyl alcohol (6.73%), vidreno (3.12%), aromadendrene (2.81%), α-cedrol (2.63%), and β-patchouleno (1.57%). The density value was found to be 1009 mg/mL (Murbach Teles Andrade *et al.*, 2014).

The Chinese Pharmacopoeia defines the essential oil of patchouli, which is derived from the leaves of *Pogostemon cablin* (Lamiaceae) (Pharmacopoeia, 2015). The characteristics of patchouli essential oil include its color, scent, specific gravity (between 0.95 and 0.98), specific rotation (43°-66°), and refractive index (1.503 and 1.513). Patchoulol should have a minimum content of 26.0%. (Pharmacopoeia, 2015; van Beek & Joulain, 2018).

In the previously published study, the patchouli essential oil was compared by three different detectors on five different GC systems for the analysis of patchoulol using internal standardization and found absolute percentages at 31.0, 30.6, 30.9, 30.7, and 30.9% (van Beek & Joulain, 2018). Patchouli essential oil often displayed a variety in its chemical composition, but these findings are consistent with the literature.

Yang and colleagues reported that the antimicrobial activity of *Patchouli* essential oil MIC values found between 1.0 to 6.5 mg/mL for *E. coli* and *S. typhi*, respectively (Yang *et al.*, 2013). However, Das and colleagues reported MIC between the range 250 to 1000 µg/mL in their study against nine bacterial strains (Das *et al.*, 2013). Andrade *et al.* reported *the* antimicrobial activity of *P. patchouli* essential oil against *S. aureus*, *E. coli*, and *P. aeruginosa*. The minimum inhibitory concentration was defined as MIC_{50} and MIC_{90} values. The results of essential oil were found MIC_{50}=0.25, MIC_{90}=0.25 for *Staphylococcus aureus*, ($MIC_{50}\geq30.27$ and $MIC_{90}\geq30.27$) for *E. coli*, and ($MIC_{50}>30.27$, $MIC_{90}\geq30.27$) *P. aeruginosa*, respectively (Murbach Teles Andrade *et al.*, 2014).

In another study, Singh et al (2019) reported that the antimicrobial activity of *Patchouli* essential oil was tested against clinically important 80 bacterial isolates. The results of the study Gram-positive bacteria were significantly more sensitive to *Patchouli* essential oil than Gram - negative bacteria. These findings are in agreement with our study.

Kocevski et al (2013), the antifungal activity of *Allium tuberosum*, *Cinnamomum cassia*, and *Pogostemon cablin* essential oils were investigated on *Aspergillus flavus* and *Aspergillus oryzae* According to these data, it was reported that *A. tuberosum* and *C. cassia* essential oils inhibited *A. flavus* 32758 and 34408, and *A. oryzae* with a MIC 250 ppm, while patchouli essential oil was found to be > 1500 ppm. The essential oils showed an inhibitory effect on colony growth at 100, 175, and 250 ppm for *A. tuberosum*, and was8 found to be 25, 50, and 75 ppm for *C. cassia*. Also, the essential oil *P. cablin* showed inhibitory an effect at 100, 250, and 500 ppm (Kocevski *et al.*, 2013).

When compared to the combined effects of the individual compounds, the essential oils and other components possess particular benefits for antimicrobial activity (Seow *et al.*, 2014). Their mechanism of action has multiple targets on microorganisms, including cell wall destruction, increasing permeability, leakage of the cell content, damaging of the cytoplasmic membrane, membrane protein damage, and coagulation of cytoplasm resulting in metabolic damage and cell death (Betoni *et al.*, 2006; Bhavaniramya *et al.*, 2019).

To the best of our knowledge, this is the first study for a combination of *Pogostemon cablin* L. leaf essential oil with antimicrobial drugs. Various combinations demonstrated mainly additive interactions between Patchouli essential oil and antimicrobial agents. The antagonistic effect did not found with *E. coli*, *S. aureus*, or *Candida* strains. This is probably due to their being generally more sensitive to the effects of essential oil components. The antagonistic effect

was not observed with the strains of *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *C. tropicalis* ATCC 750, and *C. albicans* ATCC 10231. The absence of antagonistic effects in this study suggests that patchouli essential oil has the potential to enhance the antimicrobial activities of other antibacterial drugs for a synergistic antimicrobial impact against various resistant pathogens.

In a study, the antimicrobial activity of patchouli, citronella, and nutmeg essential oils and these oil mixtures were determined against *S. aureus*, *Shigella sp.*, *C. Albicans,* and *Aspergillus niger* by disc diffusion method. The patchouli oil was found to be active against *S. aureus* (z.i=11.36 mm), and *Shigella sp* (z.i= 6.80 mm), respectively. In addition, when compared to citronella and nutmeg oils, patchouli oil was found to be only efficient against *Shigella* sp. Additionally, it was discovered that the patchouli and citronella oil mixtures created a greater inhibitory zone on *S.aureus* than gentamicin. On the other hand, patchouli oil was found to generate an inhibition zone when combined with other oils, however, when applied alone, it had no antifungal impact on *C. albicans* and *A. niger* (Aisyah *et al.*, 2021).

As a result, the current study's findings appear promising and may expand the usage of natural products, demonstrating the potential of this plant essential oil in the treatment of infectious disorders caused by *Staphylococcus aureus*. Future research should be done on the chemical properties of extracts and active ingredients for plants and their antibacterial activities.

Acknowledgments

This work was partially supported by Tübitak Project: 218S812. The authors are grateful to the company Bade Natural for providing the essential oil as well as for the chemical analysis of the sample.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author.

Authorship Contribution Statement

Nazlı Şenay Beşirik: Investigation, and Formal Analysis. **Gamze Göger:** Investigation, Methodology, Resources, Visualization, Software, Formal Analysis, Supervision, and Writing original draft.

Orcid

Nazlı Şenay Beşirik https://orcid.org/0000-0002-8411-8369
Gamze Göger https://orcid.org/0000-0003-2978-5385

REFERENCES

Ahmad, A., Elisha, I.L., van Vuuren, S., & Viljoen, A. (2021). Volatile phenolics: A comprehensive review of the anti-infective properties of an important class of essential oil constituents. *Phytochemistry, 190*, 112864. https://doi.org/10.1016/j.phytochem.2021.112864

Aisyah, Y., Yunita, D., & Amanda, A. (2021). Antimicrobial activity of patchouli (Pogostemon cablin Benth) citronella (Cymbopogon nardus), and nutmeg (Myristica fragrans) essential oil and their mixtures against pathogenic and food spoilage microbes. Paper presented at the IOP Conference Series: Earth and Environmental Science. https://doi.org/10.1088/1755-1315/667/1/012020

Betoni, J.E.C., Mantovani, R.P., Barbosa, L.N., Di Stasi, L.C., & Fernandes Junior, A. (2006). Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus*

diseases. *Memórias do Instituto Oswaldo Cruz, 101*, 387-390. https://doi.org/10.1590/S0074-02762006000400007

Bhavaniramya, S., Vishnupriya, S., Al-Aboody, M.S., Vijayakumar, R., & Baskaran, D. (2019). Role of essential oils in food safety: Antimicrobial and antioxidant applications. *Grain & Oil Science and Technology, 2*(2), 49-55. https://doi.org/10.1016/j.gaost.2019.03.001

Das, P., Dutta, S., Begum, J., & Anwar, M.N. (2013). Antibacterial and antifungal activity analysis of essential oil of *Pogostemon cablin* (Blanco) Benth. *Bangladesh Journal of Microbiology, 30*(1-2), 7-10. https://doi.org/10.3329/bjm.v30i1-2.28446

Dechayont, B., Ruamdee, P., Poonnaimuang, S., Mokmued, K., & Chunthorng-Orn, J. (2017). Antioxidant and antimicrobial Activities of *Pogostemon cablin* (Blanco) Benth. *Journal of Botany*. https://doi.org/10.1155/2017/8310275

Donelian, A., Carlson, L., Lopes, T., & Machado, R. (2009). Comparison of extraction of patchouli (*Pogostemon cablin*) essential oil with supercritical CO_2 and by steam distillation. *The Journal of Supercritical Fluids, 48*(1), 15-20. https://doi.org/10.1016/j.supflu.2008.09.020

Göger, G., Demirci, B., Ilgın, S., & Demirci, F. (2018). Antimicrobial and toxicity profiles evaluation of the chamomile (*Matricaria recutita* L.) essential oil combination with standard antimicrobial agents. *Industrial Crops and Products, 120*, 279-285. https://doi.org/10.1016/j.indcrop.2018.04.024

Hemaiswarya, S., & Doble, M. (2009). Synergistic interaction of eugenol with antibiotics against Gram-negative bacteria. *Phytomedicine,* 16(11), 997-1005. https://doi.org/10.1016/j.phymed.2009.04.006

Hyldgaard, M., Mygind, T., & Meyer, R.L. (2012). Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Frontiers in Microbiology, 3*, 12. https://doi.org/10.3389/fmicb.2012.00012

Jain, P.L., Patel, S.R., & Desai, M.A. (2021). Patchouli oil: an overview on extraction method, composition and biological activities. *Journal of Essential Oil Research*, 1-11. https://doi.org/10.1080/10412905.2021.1955761

Kapoor, G., Saigal, S., & Elongavan, A. (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. *Journal of Anaesthesiology, Clinical Pharmacology, 33*(3), 300. https://doi.org/10.4103/joacp.JOACP_349_15

Kocevski, D., Du, M., Kan, J., Jing, C., Lacanin, I., & Pavlovic, H. (2013). Antifungal effect of *Allium tuberosum*, *Cinnamomum cassia*, and *Pogostemon cablin* essential oils and their components against population of *Aspergillus* species. *Journal of Food Science, 78*(5), M731-M737. https://doi.org/10.1111/1750-3841.12118

Langeveld, W.T., Veldhuizen, E.J., & Burt, S.A. (2014). Synergy between essential oil components and antibiotics: a review. *Critical Reviews in Microbiology, 40*(1), 76-94. https://doi.org/10.3109/1040841X.2013.763219

Laxminarayan, R., Matsoso, P., Pant, S., Brower, C., Røttingen, J.-A., Klugman, K., & Davies, S. (2016). Access to effective antimicrobials: a worldwide challenge. *The Lancet, 387*(10014), 168-175. https://doi.org/10.1016/S0140-6736(15)00474-2

Murbach Teles Andrade, B.F., Nunes Barbosa, L., da Silva Probst, I., & Fernandes Júnior, A. (2014). Antimicrobial activity of essential oils. *Journal of Essential Oil Research, 26*(1), 34-40. https://doi.org/10.1080/10412905.2013.860409

Paulus, D., Luchesi, L.A., Busso, C., Frata, M.T., & de Oliveira, P.J.B. (2020). Chemical Composition, Antimicrobial and antioxidant activities of essential oils of four species of the Lamiaceae family. *European Journal of Medicinal Plants*, 129-140. https://doi.org/10.9734/ejmp/2020/v31i1030289

Pharmacopoeia, C. (2015). Chinese Pharmacopoeia Commission Chinese Pharmacopoeia, Vol. 1. *Beijing: Chinese Medical Science and Technology Press.*

Picheansoonthon, C., & Jerawong, V. (2001). Thai Pharmaceutical Textbook No. 5 Materia Medica. *Amarin Printing, Bangkok, Thailand*.

Ribeiro, A.S., Ribeiro, M.S., Bertolucci, S.K., Bittencourt, W.J., Carvalho, A.A., Tostes, W. N., Pinto, J.E. (2018). Colored shade nets induced changes in growth, anatomy and essential oil of *Pogostemon cablin. Anais da Academia Brasileira de Ciencias*, *90*, 1823-1835. https://doi.org/10.1590/0001-3765201820170299

Seow, Y.X., Yeo, C.R., Chung, H.L., & Yuk, H.-G. (2014). Plant essential oils as active antimicrobial agents. *Critical Reviews in Food Science and Nutrition*, *54*(5), 625-644. https://doi.org/10.1080/10408398.2011.599504

Sharma, K., Guleria, S., Razdan, V.K., & Babu, V. (2020). Synergistic antioxidant and antimicrobial activities of essential oils of some selected medicinal plants in combination and with synthetic compounds. *Industrial Crops and Products*, *154*, 112569. https://doi.org/10.1016/j.indcrop.2020.112569

Silva-Filho, S.E., Wiirzler, L.A.M., Cavalcante, H.A.O., Uchida, N.S., de Souza Silva-Comar, F.M., Cardia, G.F.E., Cuman, R.K.N. (2016). Effect of patchouli (*Pogostemon cablin*) essential oil on in vitro and in vivo leukocytes behavior in acute inflammatory response. *Biomedicine & Pharmacotherapy*, *84*, 1697-1704. https://doi.org/10.1016/j.biopha.2016.10.084

Singh, B.R., Sinha, D.K., OR, V.K., Pawde, A.M., De, U.K., & Gupta, V.K. (2019). Comparative antimicrobial activity of *Pogostemon cablin* (patchouli) essential oil (PEO) and conventional antimicrobials against clinically important microbes. *World Journal of Pharmaceutical Sciences*, 47-65.

Swamy, M.K., & Sinniah, U.R. (2015). A comprehensive review on the phytochemical constituents and pharmacological activities of Pogostemon cablin Benth.: an aromatic medicinal plant of industrial importance. *Molecules*, *20*(5), 8521-8547. https://doi.org/10.3390/molecules20058521

Tang, Y., Zhong, L., Wang, X., Zheng, H., & Chen, L. (2019). Molecular identification and expression of sesquiterpene pathway genes responsible for patchoulol biosynthesis and regulation in *Pogostemon cablin. Botanical Studies*, *60*(1), 1-11. https://doi.org/10.1186/s40529-019-0259-9

van Beek, T.A., & Joulain, D. (2018). The essential oil of patchouli, *Pogostemon cablin*: A review. *Flavour and Fragrance Journal*, *33*(1), 6-51. https://doi.org/10.1002/ffj.3418

Van Vuuren, S., Suliman, S., & Viljoen, A. (2009). The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials. *Letters in Applied Microbiology*, *48*(4), 440-446. https://doi.org/10.1111/j.1472-765X.2008.02548.x

Yang, X., Zhang, X., Yang, S.-P., & Liu, W.-Q. (2013). Evaluation of the antibacterial activity of patchouli oil. *Iranian Journal of Pharmaceutical Research 12*(3), 307. https://doi.org/10.22037/ijpr.2013.1320

Zhong, Y., Tang, L., Deng, Q., Jing, L., Zhang, J., Zhang, Y., Huang, B. (2021). Unraveling the novel effect of patchouli alcohol against the antibiotic resistance of *Helicobacter pylori. Frontiers in Microbiology*, *12*, 1169. https://doi.org/10.3389/fmicb.2021.674560