Antioxidant capacity and combinatorial antimicrobial effects of Nardostachys jatamansi essential oil with conventional antibiotics against some drug resistant bacteria

Majeed, Aasiya; Guleria, Sanjay; Sharma, Neha; Salaria, Khalid Hussain; Aiman, Faiqa; Singh, Bikarma; Gupta, Vijai Kumar

Published in:
Current Research in Biotechnology

DOI:
10.1016/j.crbiot.2022.100118

Print publication: 01/01/2023

Document Version
Publisher's PDF, also known as Version of record

Current Research in Biotechnology 5 (2023) 100118

Contents lists available at ScienceDirect

Current Research in Biotechnology

journal homepage: www.elsevier.com/locate/crbiot

Antioxidant capacity and combinatorial antimicrobial effects of *Nardostachys jatamansi* essential oil with conventional antibiotics against some drug resistant bacteria

Aasiya Majeed [a], Sanjay Guleria [a,*], Neha Sharma [a], Khalid Hussain Salaria [a], Faiqa Aiman [a], Bikarma Singh [b], Vijai Kumar Gupta [c]

[a] Natural Product-cum-Nano Lab, Division of Biochemistry, Faculty of Basic Sciences,Sher-e- Kashmir University of Agricultural Sciences and Technology of Jammu, Main Campus Chatha, Jammu- 180009, Jammu and Kashmir, India
[b] Botanic Garden Division, CSIR-National Botanical Research Institute, Lucknow 226001, Uttar Pradesh, India
[c] Biorefining and Advanced Materials Research Center, SRUC, Kings Building, West Mains Road, Edinburgh EH9 3JG, UK

ARTICLE INFO

Keywords:
Nardostachys jatamansi
Antibiotics
Synergistic antimicrobial activity
Antioxidant activity

ABSTRACT

The antibacterial and antioxidant properties of essential oils (EOs) have long been recognized. The present study was conducted to investigate the antioxidant capability of *Nardostachys jatamansi* essential oil and to see if it has a synergistic antimicrobial effect with antibiotics against two Gram negative (*Klebsiella pneumoniae and Escherichia coli*) and three Gram positive (*Bacillus subtilis, Micrococcus luteus* and *Staphylococcus aureus*) bacterial strains. Guaia-6,9-diene (11.96 %), calarene (10.44 %), jatamansone (8.11 %), α-gurjunene (7.42 %), valencene (6.46 %), α-maaliene (5.24 %), sprojatamol (5.06 %), and caratol (5.06 %) were found to be the primary components of *N. jatamansi* EO. According to antioxidant studies, *N. jatamansi* EO has moderate DPPH radical scavenging activity, reducing power, and ferric reducing antioxidant power. Similarly, *N. jatamansi* EO also showed significant antibacterial activity, with inhibition zones, MIC, and MBC values ranging from 10.5 ± 0.5 to 14.0 ± 0.4 mm, 1.5 to 3.1 mg/mL, and 1.8 to 3.5 mg/mL respectively. The results of *N. jatamansi* EO interactions with conventional antibiotics revealed that amoxicillin, erythromycin, chloramphenicol, and ampicillin MICs were reduced by 5 to 10 fold, 4 to 9.09 fold, 4 to 10.5 fold, and 4 to 8.0 fold, respectively. The findings of this study are noteworthy because no previous reports of *N. jatamansi* EO's synergistic interaction with conventional antibiotics have been published, and therefore may constitute an important strategy for addressing problem of drug resistant bacteria.

1. Introduction

The dynamic evolution and ubiquitous occurrence of multi-drug resistant bacterial strains is presenting a global challenge to disease management (Lambert, 2000). Antimicrobial resistance is most commonly caused by uncontrolled antibiotic use, self-medication, inability to follow an antibiotic course, acquired infections, inadequate biomedical waste management, and antibiotic use in veterinary clinics (Srivastava et al., 2014). Safe natural ingredients, such as herbal products, are increasingly in demand to replace synthetic preservatives in food and maintain antibacterial effectiveness (Fowler, 2006). Essential oils and plant extracts are natural sources of physiologically active substances (Celiktas et al., 2007). Essential oils are complex mixtures of substances that represent the most regal part of the plant, appearing as tiny droplets in the petals of flowers, the skin of fruits, the resin and bark of trees, and the roots of herbs and aromatic plants (Carson and Hammer, 2011). These extracts are generally volatile elements that are soluble in alcohol and oil but not in water. Each essential oil can contain over 100 different chemical components, including alcohols, aldehydes, ketones, esters, phenols, sesquiterpenes, and terpenes. Each of these classes is distinguished by a homologous group of substances that differ solely in elemental composition, structural

Abbreviations: GC/MS, Gas chromatography/mass spectrometry; DPPH, 2, 2-diphenyl-1-picrylhydrazyl; EO, Essential oil; MIC, Minimum inhibitory concentration; MBC, Minimum bactericidal concentration.

* Corresponding author.
E-mail address: guleria71@gmail.com (S. Guleria).

https://doi.org/10.1016/j.crbiot.2022.100118
Received 2 June 2022; Revised 12 November 2022; Accepted 19 November 2022

(isomers), or spatial arrangement (enantiomers). Various studies have shown that essential oils have antibacterial properties (Lu et al., 2014; Thielmann et al., 2019). The capacity of EOs to enhance bacterial cell permeability, coagulate cytoplasm, decrease intracellular ATP pool, and cause cytoplasmic and membrane protein molecules to malfunction is responsible for their antibacterial effect (Nazzaro et al., 2013; Prasch and Bucar, 2015). The use of EOs in combination with conventional antibiotics has recently piqued scientific interest (Sharma et al., 2020). In addition, EOs in combination with antibiotics may have various antibacterial mechanisms of action, and so may play an essential role in discovering novel ways to combat bacterial drug resistance (Yap et al., 2013). As a result, combinatorial antibiotic therapy incorporating EOs and conventional antibiotics could be a viable alternative in which the combined antibacterial activity exceeds the antimicrobial activity of the individual components (Yahiaoui et al., 2017).

Indian spikenard, or *Nardostachys jatamansi*, is a Valerianaceae perennial herb that grows to a height of 10–60 cm. It can be found at heights of 3000–5000 m above mean sea level in the alpine Himalayas, which span from Punjab to Sikkim and Bhutan. It has antidepressant, anticonvulsant, anti- parkinson's, hepato-protective, antibacterial, and cardio-protective characteristics, among others (Rao et al., 2005; Ahmad et al., 2006; Subashini et al., 2006; Joshi and Parle, 2006, Kumar et al., 2006; Dandagi et al., 2008; Khan et al., 2012; Razack et al., 2015). Furthermore, there are just a few studies on the antioxidant, anti-inflammatory and antibacterial effects of *Nardostachys jatamansi* EO in the literature (Parveen et al., 2011; Singh et al., 2014). There is currently no data available on the antibacterial activity of *N. jatamansi* EO in combination with conventional antibiotics against drug-resistant *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Micrococcus luteus.* As a result, the goal of this study was to investigate the antibacterial and antioxidant effects of *N. jatamansi* EO and to see if it may work in synergy with conventional antibiotics like amoxicillin, erythromycin, chloramphenicol, and ampicillin.

2. Materials and methods

2.1. Experimental materials and chemicals

Natural Biotech Products, Baggi, Himachal Pradesh, India, provided the essential oil of *Nardostachys jatamansi* for this study. The bacterial strains employed in this investigation were *E. coli* MTCC 2127, *K. pneumoniae* MTCC 7172, *M. leuteus* MTCC 4821, *S. aureus* MTCC 7443, and *B. subtilis* MTCC 2389, all purchased from IMTECH, Chandigarh, India. Antibiotics (amoxicillin, erythromycin, chloramphenicol, and ampicillin) were procured from Hi-Media Laboratories, Mumbai, India.

2.2. Determination of chemical composition by GC–MS analysis

Separation and chemical profiling of essential oils is usually carried out by gas chromatography mass spectrometry (GC/MS) technique (Rubiolo et al., 2010). GC/MS generates representative chromatograms of volatile compounds, together with the associated mass spectra for each separated component peak, which are then used for identification of metabolites present in essential oils. The gas chromatography mass spectrometry analysis of *N. jatamansi* EO was performed on a Varian GC (Varian Inc., Palo Alto, California, USA) equipped with HP-5 MS column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies, Santa Clara, California, USA); carrier gas was helium (1.0 mL/min). Working conditions were: the injector temperature was set at 280 °C; initial temperature of column held at 50 °C for 5 min and then steadily increased by 3 °C/min to 300 °C; finally held constant at 280 °C for seven minute. 0.2 μL of *N. jatamansi* EO solution in ethyl acetate was injected into the column. The MS scan was recorded with the following conditions: mass range 40–500 *m/z*; electron impact ionization voltage, 70 eV. Volatile components of *N. jatamansi* EO by comparing their spectra and retention indices with authentic reference compounds (Adams, 2007).

2.2. Screening of antibacterial activity

The antibacterial activity of *N. jatamansi* EO was determined using the disc diffusion method (NCCLS, 1997). The selected test bacterial strains were inoculated in Muller Hinton broth (MHB) and incubated for 24 h at 37 °C. Turbidity of the resulting bacterial suspension of active cultures was measured by taking absorbance at 600 nm using spectrophotometer (Labomed, USA) and the optical density was adjusted equivalent to 0.5 Mcfarland standard by dilution with MHB. Twenty milliliter of sterilized nutrient agar (NA) medium was poured into the petri plates and allowed to solidify. Then 100 μL of bacterial suspension (10^8 cfu/mL) was uniformly spread over the NA plates which were then kept to dry for five minutes. Whatman No. 1 sterile filter paper discs (6 mm diameter) impregnated with 3 μL of essential oil were placed on the media. The plates were left for thirty minutes and then they were incubated at 37 °C for 24 h. After this, inhibition zone was measured using transparent ruler. Discs impregnated with chloramphenicol were used as positive control.

2.3. Determination of antioxidant activity

2.3.1. DPPH radical scavenging assay

The DPPH radical is a stable molecule that is soluble in methanol and distinguished by its deep violet colour with a maximum absorption at 515 nm. By donating this stable radical an electron or hydrogen atom, antioxidants are able to reduce it to 2,2-diphenyl-1-picrylhydrazine (DPPH-H), which has a pale yellow colour and can be easily measured using a spectrophotometer. DPPH radical scavenging activity of *N. jatamansi* EO was determined according to Bozin et al. (2006). Briefly, one milliliter of a 90 μM DPPH methanolic solution was mixed with 1 mL of various dilutions of EO (1 mg/ mL stock solution) and final volume was made to 4 mL with methanol. After 1 h incubation in the dark at 25 °C, the absorbance was recorded as A_{sample} at 517 nm using a UV/VIS spectrophotometer (Labomed, USA). Solution without the test material constituted the blank and the absorbance was recorded as A_{blank}. The free radical scavenging activity was calculated as percent inhibition according to the following equation:

$$\% \text{ inhibition} = 100 \times (A_{blank} - A_{sample})/A_{blank}.$$

Test compounds radical scavenging activity was expressed as IC_{50}, or the amount of test substance required to result in a 50 % reduction in the initial DPPH concentration.

2.3.2. Reducing power assay

Reducing power of *N. jatamansi* EO was determined according to Oyaizu (1986). 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferricyanide were mixed with each test sample, and the mixture was then incubated for 30 min at room temperature. Then, 2.5 mL of 10 % trichloroacetic acid was added, and the mixture was centrifuged at 1036 g for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of 0.1 % ferric chloride. The absorbance was then measured at 700 nm against a blank. From the graph of absorbance against concentration, the test sample concentration that provided 0.5 of absorbance (IC_{50}) was determined.

2.3.3. Ferric reducing antioxidant power assay

Method of Benzie and Strain (1996) was used to measure the ferric reducing antioxidant power of *N. jatamansi* EO. FRAP reagent was prepared by mixing 100 mL of 300 mM sodium acetate buffer (pH 3.6), 10 mL of 10 mM TPTZ [2,4,6-tri-(2-pyridyl)-1,3,5-triazine] solution in 40 mM HCl plus 10 mL of 20 mM $FeCl_3$ and 12 mL of distilled water. Test solutions were mixed with 3 mL of FRAP reagent and the absor-

bance was measured at 593 nm against blank after incubating the reaction mixture for 10 min. The FRAP activity was calculated from the calibration curve of ferrous sulphate ($FeSO_4$ $2H_2O$) and expressed as mM Fe^{2+} eq./100 mg of the essential oil.

2.3. Determination of MIC and MBC of N. Jatamansi EO

MIC and MBC of EO were determined according to Wayne, 2003 with minor modifications. Mueller Hinton Broth (MHB) was used in all the tests. *N. jatamansi* EO was dissolved in dimethyl sulfoxide (DMSO) in a 1.0:1.0 (v/v) ratio for stock solution preparation, followed by filtration with a 0.22 μm filter disc before usage. MHB was used to make the final volume to 1 mL. Similarly, MHB was also used to dilute the essential oil in the same way. In sterile microfuge tubes containing 90 μL of MHB, EO dilutions (100 μL) were added. Following that, 10 μL of working bacterial solution (10^6 CFU/mL) was added, resulting in a total volume of 200 μL in the microfuge tubes. Positive control comprised of broth and inoculum but no EO or antibiotic, whereas negative control was devoid of inoculum. After 24 h incubation period at 37 °C, each tube was added 40 μL of 0.4 mg/mL *p*-iodonitrotetrazolium violet (INT) solution and incubated for another 30 min at 37 °C. The development of a pink colour indicated bacterial growth. The MIC was defined as the lowest concentration of essential oil or antibiotic that prevented bacterial growth. After 24 h of incubation, 50 μL of broth was taken from the dilutions with no obvious bacterial growth and sub-cultured on nutrient agar plates, then incubated for another 24 h at 37 °C for MBC determination. MBC was the lowest dose of EO or antibiotic required to kill 99.9 % of inoculated bacteria.

3.4. Interaction studies of N. Jatamansi EO and antibiotics

The broth dilution method was used to assess synergistic interactions between *N. jatamansi* EO and antibiotics (amoxicillin, erythromycin, chloramphenicol, and ampicillin). In microfuge tubes containing 50 μL of antibiotic dilutions and 100 μL of microbial cell suspension, aliquots (50 μL) of *N. jatamansi* EO were introduced and incubated at 37 °C for 24 h. After that, the tubes were incubated for another 30 min at 37 °C with 40 μL of 0.4 mg/mL *p*-iodonitrotetrazolium violet (INT) solution to check for bacterial growth. The emergence of a pink colour indicated bacterial growth. The FIC Index was used to determine the interaction between two drugs (Pei et al., 2009). Didry et al. (1993) method as used to calculate the fractional inhibitory concentration index (FICI) and gain. Fractional inhibitory concentration indices (FICI) were determined as follows:

FIC of A = MIC of A in combination with B/ MIC of A alone.

FIC of B = MIC of B in combination with A/ MIC of B alone.

FICI = FIC of A + FIC of B.

Where A and B are two different essential oils/antibiotics. FICI ≤ 0.5, total synergism;

0.5 < FICI ≤ 0.75, partial synergism; 0.75 < FICI ≤ 2, no effect; FICI > 2, antagonism.

3. Results and discussion

3.1. Composition of Nardostachys jatamansi EO

Essential oils are widely used in the food and cosmetics industry and also in the medical and pharmaceutical fields for various purposes. The semi-volatile and volatile components that make up essential oils can be easily separated, identified, and quantified by gas chromatography and mass spectrometry making them excellent tools for essential oil analysis. Gas chromatography and mass spectrometry are often used in combination and is commonly referred to as GC/MS analysis. Each type of essential oil has a unique GC/MS qualitative fingerprint that is compared to literature (Hu et al., 2006). Therefore, GC/MS has become a part of the routine testing for essential oils and commonly used for detecting adulterations. The qualitative and quantitative profile of *Nardostachys jatamansi* EO determined by gas chromatography mass spectrometry is depicted in Table 1. Nineteen compounds were determined in *N. jatamansi* essential oil with guaia-6,9-diene (11.96 %), calarene (10.44 %), jatamansone (8.11 %), α-gurjunene (7.42 %), valencene (6.46 %), α-maaliene (5.24 %), spirojatamol (5.06 %) and caratol (5.06 %) as dominant compounds. Previous studies have also reported chemical composition of *N. jatamansi* EO from different geographical locations. Chouhan et al. (2017) reported that *N. jatamansi* EO from north and south facing slopes of Tungnath, Uttrakhand, India contained patchouli alcohol (40 to 52 %) as major component. In addition, caryophyllene oxide, cubeb-11-ene, α-patchoulene, seychellene, pogostol and carotol were also present in appreciable amount. Similarly, Singh et al. (2018) demonstrated that EO extracted from air dried rhizomes of *N. jatamansi* from Haridwar, Uttrakhand, India was dominated by calarene (20.4 %), vardiflorene (12.3 %), α-panasinsen (9.7 %), α-santalene (4.6 %), resibufogenin (8.4 %) and epiglobulol (1.9 %). One report on *N. jatamansi* EO from India showed jatamansone (36.7 %); α-cadinol (22.7 %) as the major constituents (Naquvi et al., 2013), while another reported β-gurjunene (20.6 %); maaliol (8.2 %); patchouli alcohol (5.9 %) and 9-aristolan-12-ol (5.8 %) (Vaze, 2003). α-pinene (6.00 – 8.53 %), 2- β-pinene (0.51 – 19.26 %), α – terpinolene (1.32 – 2.73 %) and myrtenyl acetate (0.54 – 8.89 %) were found as predominant compounds in *N. jatamansi* EO from five different regions of Nepal (Sharma et al., 2016). Similarly, ledene oxide [II] (13.021 %), and sesquiterpine patchouli alcohol (9.582 %) were reported as major components of the *N. jatamansi* EO from Pakistan (Parveen et al., 2011). These data showed that several intrinsic and extrinsic factors such as plant age, collection time, geographical location, and genetic factors affect composition of essential oil (Hajdari et al., 2016; Nafis et al., 2019).

3.2. Antioxidant activity of N. Jatamansi EO

Findings of antioxidant assays are displayed in Table 2. *N. jatamansi* EO had moderate DPPH radical scavenging activity (IC_{50} of 0.95 ± 0.008 mg/mL), reducing power (IC_{50} of 1.70 ± 0.082 mg/mL), and ferric reducing antioxidant power (154 ± 6.13 mM Fe^{2+} eq./100 mg) according to the results. Previous studies on the antioxidant activity

Table 1
Chemical composition of volatile oil from *Nardostachys jatamansi* roots.

RT	Compound	Relative concentration (%)
7.74	β- Patchoulene	4.83
8.34	Calarene	10.44
8.49	Guaia-6,9-diene	11.96
8.60	α-Gurjunene	7.42
8.67	Seychellene	4.83
8.74	γ-Vetivenene	1.78
8.80	Kessane	4.68
9.03	*epi*-Bicyclosesquiphellandrene	3.11
9.15	Valencene	6.46
9.22	2-Methylene-5-(1-methylvinyl)-8-methyl-bicyclo [5.3.0]decane	3.88
9.44	Dehydroaromadendrene	3.12
9.51	α-Maaliene	5.24
9.98	4,5,9,10-dehydroisolongifolene	2.41
10.29	Spirojatamol	5.06
10.56	Carotol	5.06
11.45	Jatamansone	8.11
12.53	Methyl zizanoate	1.64
12.79	Octahydro-4,7-methano-1H-indenol	4.44
13.28	Vetiselinenol	1.74
Total (%)		96.21

Table 2
Antioxidant activities of *Nardostachys jatamansi* essential oil.

Component	DPPH radical scavenging activity IC_{50} (mg/mL)	Reducing power IC_{50} (mg/mL)	Ferric reducing antioxidant power (mM Fe^{2+} eq./ 100 mg)
Nardostachys jatamansi essential oil	0.95 ± 0.008	1.70 ± 0.082	154 ± 6.13
BHT	0.02 ± 0.002	0.16 ± 0.004	–
BHA	0.015 ± 0.001	0.12 ± 0.01	–

of *N. jatamansi* EO has been limited. Antioxidant capacity of *N. jatamansi* root EO utilizing DPPH radical scavenging assay was reported in one such study from Pakistan (Parveen et al., 2011). Similarly, Chaudhary et al. (2015) found that extract and fractions of *N. jatamansi* had antioxidant and anticancer effects in breast carcinoma. In the same way, the ethanol fraction from *N. jatamansi* rhizomes has been found to have significant antioxidant capacity, with an IC_{50} value of 58.39 μg/mL (Mishra et al., 2014). The antioxidant potential of our *N. jatamansi* EO may be largely due to presence of calarene, seychellene and maaliene in a higher amount. Furthermore, the presence of an allyl structure in these terpenes, which has a lower bond-breaking energy of C—H bonds than alkylic C—H or vinylic C—H bonds, and has been reported to provide them higher scavenging capacities. As a result, the C—H bond of methyl in allyl is easily broken, resulting in the loss of hydrogen atoms for DPPH free radical neutralization (Wu et al., 2020; Wojtunik et al., 2014).

3.3. Antibacterial activity of EO

The results of the antibacterial activity of the *N. jatamansi* EO and conventional antibiotics against two Gram negative (*Klebsiella pneumoniae and Escherichia coli*) and three Gram positive (*Bacillus subtilis, Micrococcus luteus and Staphylococcus aureus*) bacterial strains are depicted in Table 3. It is evident from the above results that *N. jatamansi* EO showed antibacterial activity against all tested strains with inhibition zone diameter (IZD) ranging from 10.5 ± 0.5 mm to 14.0 ± 0.4 mm. EO's MIC and MBC values against all of the investigated bacterial strains ranged from 1500 to 3100 μg/mL and 1800 to 3500 μg/mL, respectively.

Intriguingly, *E. coli*, an antibiotic-resistant Gram-negative bacteria, was most sensitive to *N. jatamansi* EO of all the bacterial strains tested (MIC = 1500 μg/mL; MBC = 1800 g/mL), whereas, *S. aureus*, an antibiotic-resistant Gram-positive bacteria, was least sensitive (MIC = 3100 g/mL; MBC = 3500 g/mL). *N. jatamansi* EO concentrations 0.1 %, 0.5 %, and 1 % v/v were reported to be effective against *S. aureus and E. coli* with inhibition zone of about 12.8 mm and 12.4 mm respectively, which were comparable with chloramphenicol at concentration of 0.1 mg/mL (Singh et al., 2018).

Thielmann et al. (2019) found that commercial *N. jatamansi* EO had a significant inhibitory effect against *S. aureus* (MIC of 50 μg/mL), but was ineffective against *E. coli.* The diffrences in antibacterial activity of *N. jatamansi* EO observed in several studies may be due to quantitative and qualitative differences in chemical composition of *N. jatamansi* EO from different geographical regions and altitudes (Nafis et al., 2019).

It has been suggested that the antibacterial activity of EOs is mostly due to their terpene content. Increased permeability, which leads to breakdown of the plasma membrane and cytoplasmic leakage, is the principal antibacterial action mechanism observed in EOs (Álvarez-Martínez et al., 2021). Furthermore, the molecular mechanisms of action of different terpenes are influenced by their chemical structure. Terpenes containing phenolic OH groups, for example, can breach the bacterial plasma membrane and disturb membrane potential and homeostasis by binding monovalent cations like K^+ and transporting them out of the bacterial cell, disrupting membrane potential and homeostasis (Yang et al., 2015).

3.4. Interaction of N. Jatamansi EO and antibiotics

According to the results obtained from interaction studies of *N. jatamansi* EO with conventional antibiotics (Tables 4, 5, 6 and 7), of the 70 combinations tested between *N. jatamansi* EO and amoxicillin, erythromycin, chloramphenicol and ampicillin 37 presented total synergism (52.85 %), 23 showed partially synergistic effect (32.85 %) and 10 showed no effect (14.30 %). Interaction of *N. jatamansi* EO and erythromycin (Table 4) presented synergistic interaction against *E. coli, M. luteus, S. aureus* and *K. pneumoniae* (FICI values ranging from 0.44 to 0.50), and partial synergy against *B. subtilis* (FICI = 0.62). Similarly, the interaction between *N. jatamansi* EO and ampicillin (Table 5) displayed complete synergism against *E. coli*, *M. luteus* and *S. aureus* (FICI ranging from 0.37 to 0.50) and partial synergy against *B. subtilis* and *K. pneumoniae* (FICI = 0.58). Also, the combination of *N. jatamansi* EO and chloramphenicol (Table 6) showed synergism against *E. coli*, *M. luteus*, *B. subtilis* and *S. aureus* and FICI values ranged from 0.32 to 0.45 and partial synergy against *K. pneumoniae* (FICI = 0.6). Interestingly, the combination of *N. jatamansi* EO and amoxicillin (Table 7, Fig. 1 & Fig. 2) demonstrated excellent synergism against all of the bacteria tested, with FICI values ranging from 0.32 to 0.45. In the presence of *N. jatamansi* EO (1/4 MIC), the gain in MIC was also assessed and expressed as MIC of antibiotics. Antibiotic MICs were lowered by 4 to 10.5 times in the presence of *N. jatamansi* EO against the tested spe-

Table 3
Inhibition zone diameters, MIC and MBC of *Nardostachys jatamansi* essential oil and conventional antibiotics.

Microorganisms	Essential oil			Amoxicillin			Erythromycin			Chloramphenicol			Ampicillin		
	IZD	MIC	MBC	IZD	MIC	MBC	IZD	MIC	MBC	IZD	MIC	MBC	IZD	MIC	MBC
M. luteus	14.0 ± 0.4	2000	2400	14.2 ± 0.2	75	80	14.2 ± 0.5	1.5	1.5	15.3 ± 0.6	2.5	2.5	24.3 ± 0.6	0.8	0.9
E. coli	10.7 ± 0.4	1500	1800	12.2 ± 1.3	75	80	23.5 ± 0.8	1.0	1.8	15.2 ± 0.2	2.0	2.0	12.0 ± 0.4	0.5	0.8
S. aureus	12.5 ± 1.0	3100	3500	13.7 ± 1.0	70	79	24.7 ± 0.6	1.0	1.5	16.0 ± 1.0	1.5	2.0	12.0 ± 0.4	0.4	0.8
B. subtilis	10.5 ± 0.5	1600	2000	19.2 ± 0.6	70	75	22.3 ± 1.8	1.5	1.5	13.5 ± 0.7	2.0	2.5	12.6 ± 0.5	0.5	0.8
K. pneumnae	11.5 ± 0.9	2500	2900	18.3 ± 0.6	75	75	24.5 ± 0.8	1.0	2.0	15.6 ± 1.0	1.5	2.5	17.2 ± 0.6	0.4	1.0

IZD: Inhibition zone diameter (mm).
MIC: Minimum inhibitory concentration (μg/mL).
MBC: Minimum bactericidal concentration (μg/mL).
Concertation of *Nardostachys jatamansi* essential oil: 2.5 mg/disc.
Concentration of antibiotics: 3 μg/disc.

Table 4
Synergistic interaction of *Nardostachys jatamansi* essential oil and erythromycin.

Component	*E. coli*			*M. luteus*			*B. subtilis*			*S. aureus*			*K. pneumoniae*		
	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain
Nardostachys jatamansi (NJEO)	0.33	–	–	0.25	–	–	0.5	–	–	0.25	–	–	0.33	–	–
Erythromyin (Ery)	0.16	0.49*	6.0	0.25	0.50*	4.0	0.12	0.62**	8.33	0.25	0.50*	4.0	0.11	0.44*	9.09

FIC_{NJEO} = MIC of NJEO in combination with Ery/MIC of NJEO alone.
FIC_{ery} = MIC of Ery in combination with NJEO/MIC of Ery alone.
FIC index = FIC_{NJEO} + FIC_{Ery}.
* Synergism.
**Partial synergism.

Table 5
Synergistic interaction of *Nardostachys jatamansi* essential oil and ampicillin.

Component	*E. coli*			*M. luteus*			*B. subtilis*			*S. aureus*			*K. pneumoniae*		
	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain
Nardostachys jatamansi (NJEO)	0.25	–	–	0.25	–	–	0.33	–	–	0.25	–	–	0.33	–	–
Ampicillin (Amp)	0.16	0.41	6.02	0.25	0.50	4.0	0.25	0.58**	4.16	0.12	0.37	8.0	0.25	0.58**	4.0

FIC_{NJEO} = MIC of NJEO in combination with Amp/MIC of NJEO alone.
FIC_{amp} = MIC of Amp in combination with NJEO/MIC of Amp alone.
FIC index = FIC_{NJEO} + FIC_{Amp}.
* Synergism.
**Partial synergism.

Table 6
Synergistic interaction of *Nardostachys jatamansi* essential oil and chloramphenicol.

Component	*E. coli*			*M. luteus*			*B. subtilis*			*S. aureus*			*K. pneumoniae*		
	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain
Nardostachys jatamansi (NJEO)	0.20	–	–	0.16	–	–	0.25	–	–	0.33	–	–	0.5	–	–
Chloramphenicol (Chl)	0.25	0.45*	4.0	0.16	0.32*	6.0	0.12	0.37*	8.0	0.10	0.43*	10.0	0.1	0.6**	10.5

FIC_{NJEO} = MIC of NJEO in combination with Chl/MIC of NJEO alone.
$FIC_{Ch\ 1}$ = MIC of Chl in combination with NJEO/MIC of Chl alone.
FIC index = FIC_{NJEO} + FIC_{Chl}.
* Synergism.
**Partial synergism.

Table 7
Synergistic interaction of *Nardostachys jatamansi* essential oil and amoxicillin.

Component	*E. coli*			*M. luteus*			*B. subtilis*			*S. aureus*			*K. pneumoniae*		
	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain
Nardostachys jatamansi (NJEO)	0.33	–	–	0.25	–	–	0.33			0.33	–	–	0.33	–	–
Amoxicillin (Amx)	0.12	0.45*	8.0	0.20	0.45*	5.0	0.16	0.49*	6.0	0.10	0.43*	7.0	0.1	0.43*	10

FIC_{NJEO} = MIC of NJEO in combination with Amx/MIC of NJEO alone.
FIC_{amx} = MIC of Amx in combination with NJEO/MIC of Amx alone.
FIC index = FIC_{NJEO} + FIC_{Amx}.
* Synergism.

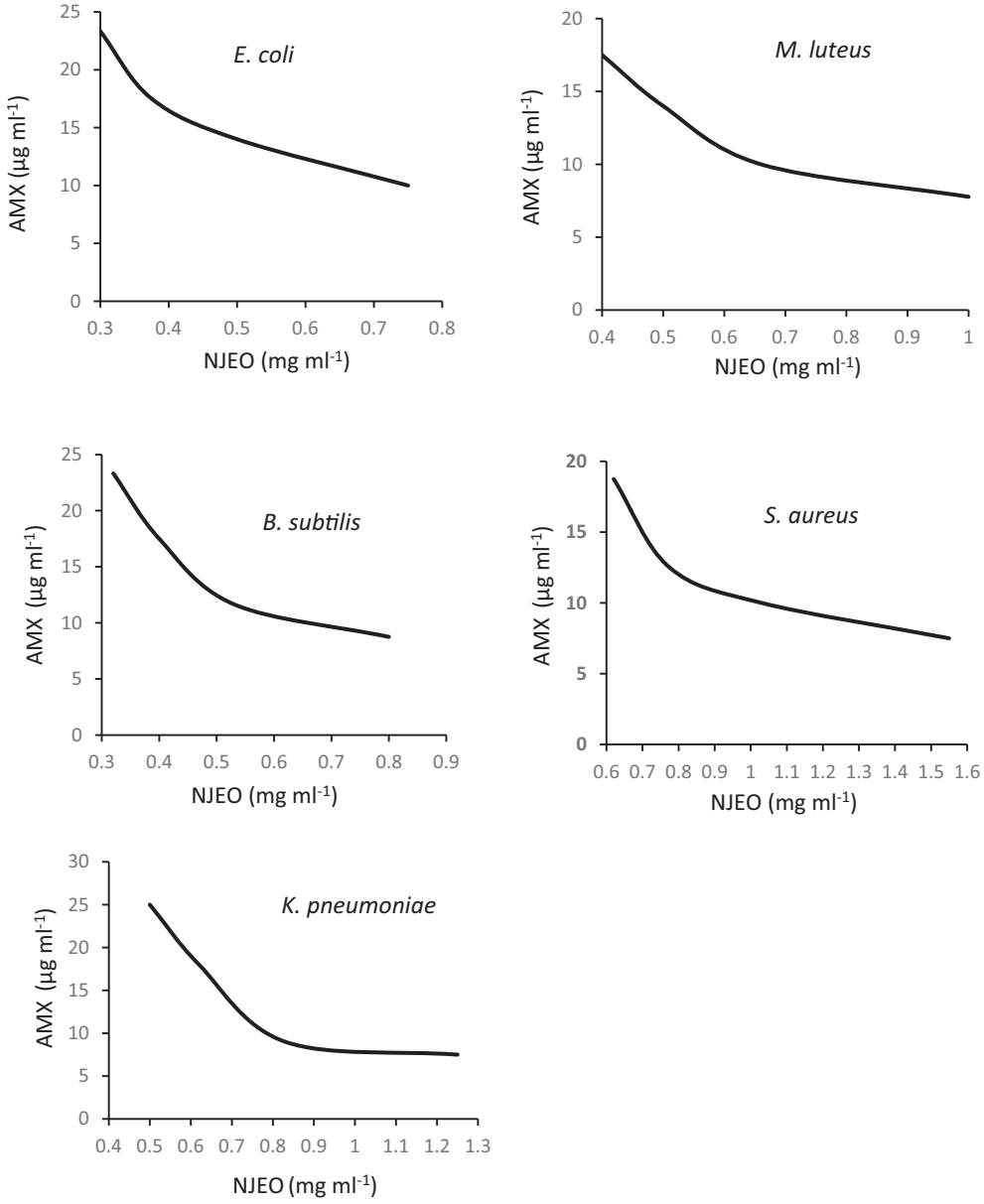

Fig. 1. Synergistic antibacterial activity of *Nardostachys jatamansi* essential oil (NJEO) in combination with amoxicillin (AMX).

cies. With a gain of 10.5 times, *K. pneumoniae* was found to have the highest reduction. *N. jatamansi* EO also increased the Gram-negative bacterium *K. pneumoniae's* susceptibility to the medicines amoxicillin, erythromycin, and chloramphenicol, with gains in MICs of 10, 9.09, and 10.5 fold, respectively. In fact, combinatorial antibiotic therapy with natural products is one of the novel strategy against drug resistant microorganisms. This is the first study on *N. Jatamansi* EO's synergistic action with conventional antibiotics that we are aware of. EOs and antibiotics have been demonstrated to interact synergistically in various studies (Mahadlek et al., 2012; Yap et al., 2013; Aghraz et al., 2018; El Atki et al., 2019; Sharma et al., 2020). EOs from *Cinnamomum zeylanicum*, *Mentha piperita*, *Origanum vulgare*, and *Thymus vulgaris* have been demonstrated to interact synergistically with norfloxacin (Rosato et al., 2020). *Pelargonium endlicherianum* EO in combination with cefepime and gentamicin demonstrated promising synergism against *K. pneumoniae* (Dumlupinar et al., 2020). The most common mechanisms of synergistic antibacterial action are efflux pump inhibition, β-lactamase inhibition, inhibition of shared metabolic pathways, and membrane permeabilization (Aleksic and Knezevic, 2014; Álvarez-Martínez et al., 2021).

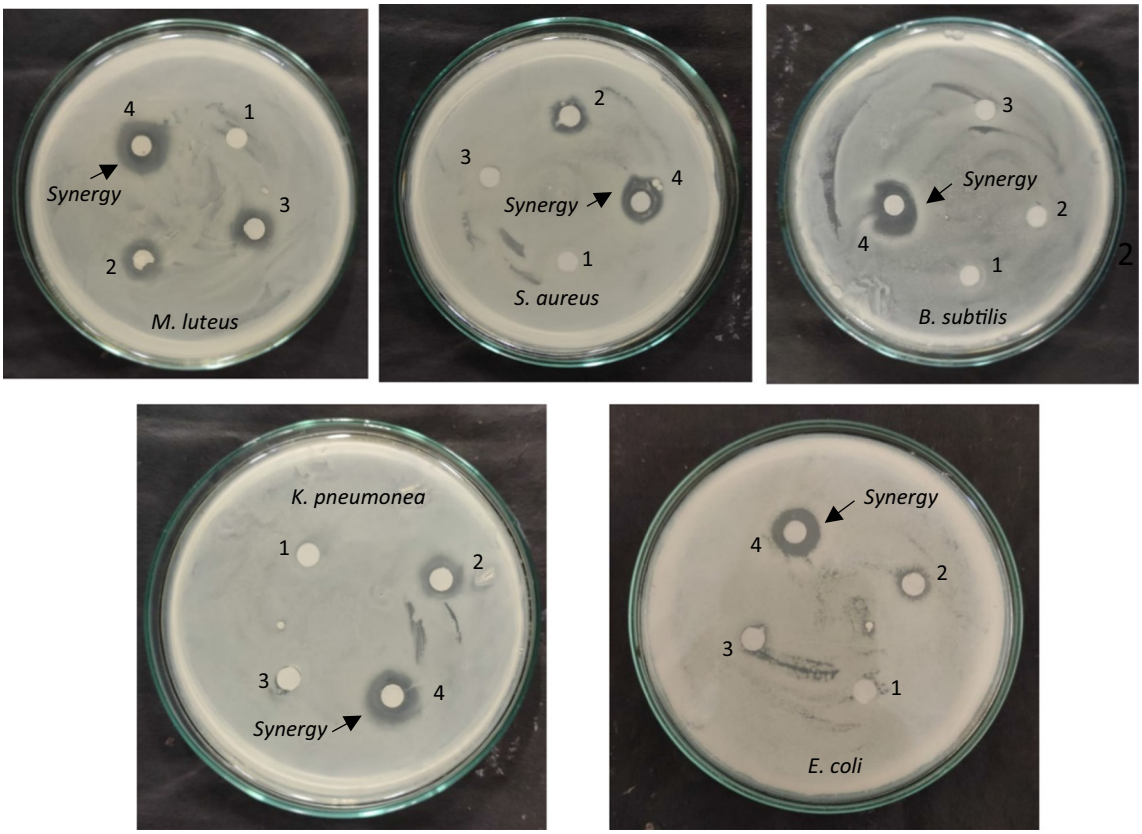

Fig. 2. Antimicrobial effect of NJEO and AMX alone and in combination on five drug resistant microbes. 1. DMSO (3 μL); 2. AMX (0.017 μg/mL); 3. NJEO (187.5 μg/mL); 4. NJEO + AMX (187.5 μg/mL + 0.017 μg/mL). NJEO = *Nardostachys jatamansi* EO; AMX = Amoxicillin.

Conclusion

N. jatamansi EO is characterized by the presence of guaia-6,9-diene, calarene, jatamansone, α-gurjunene, valencene, α-maaliene, sprojatamol, and caratol as major chemicals, according to the findings.The *N. jatamansi* EO also displayed interesting antioxidant and antimicrobial properties. The data showed that *N. jatamansi* EO potentiated the response of antibiotics such as amoxicillin, erythromycin, chloramphenicol and ampicillin against some drug resistant microorganisms. Furthermore, *N. jatamansi* EO lowered the antibiotics' MICs by 4 to 10.5 times against the pathogens tested. On the basis of above findings, it is possible to conclude that combining antibiotics with *N. jatamansi* EO to target drug resistant microbes could result in the development of new antibacterial treatment regimens.

Data availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The study was funded by Indian Council of Agricultural Research (ICAR), New Delhi under development grant.

References

Adams, R.P., 2007. Identification of essential oil components by gas chromatography/mass Spectrometry. Allured Publishing corporation.

Aghraz, A., Benameur, Q., Gervasi, T., Ait Dra, L., Ben-Mahdi, M.H., Larhsini, M., Markouk, M., Cicero, N., 2018. Antibacterial activity of *Cladanthus arabicus* and *Bubonium imbricatum* essential oils alone and in combination with conventional antibiotics against Enterobacteriaceae isolates. Lett. Appl. Microbiol. 67 (2), 175–182. https://doi.org/10.1111/lam.13007.

Ahmad, M., Yousuf, S., Khan, M.B., Hoda, M.N., Ahmad, A.S., Ansari, M.A., Ishrat, T., Agrawal, A.K., Islam, F., 2006. Attenuation by *Nardostachys jatamansi* of 6-hydroxydopamine-induced parkinsonism in rats: behavioral, neurochemical, and immunohistochemical studies. Pharmacol. Biochem. Behav. 83 (1), 150–160. https://doi.org/10.1016/j.pbb.2006.01.005.

Aleksic, V., Knezevic, P., 2014. Antimicrobial and antioxidative activity of extracts and essential oils of *Myrtus communis* L. Microbiol. Res. 169, 240–254. https://doi.org/10.1016/J.MICRES.2013.10.003.

Álvarez-Martínez, F.J., Barrajón-Catalán, E., Herranz-López, M., Micol, V., 2021. Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. Phytomedicine 90, 153626.

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power: The FRAP Assay". Anal. Biochem. 239 (1), 70–76.

Bozin, B., Mimica-Dukic, N., Simin, N., Anackov, G., 2006. Characterization of the volatile composition of essential oil of some lamiaceae species and the antimicrobial and antioxidant activities of the entire oils. J. Agric. Food Chem. 54, 1822–1828. https://doi.org/10.1021/jf051922u.

Carson, C.F., Hammer, K.A., 2011. 'Chemistry and bioactivity of essential oils. In: Thormar, H. (Ed.), Lipids and Essential Oils as Antimicrobial Agents. Wiley, pp. 203–238.

Celiktas, O.Y., Kocabas, E.E.H., Bedir, E., Sukan, F.V., Ozek, T., Baser, K.H.C., 2007. Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. Food Chem. 100 (2), 553–559.

Chaudhary, S., Chandrashekar, K.S., RanganathPai, K.S.R., Setty, M.M., Devkar, R.A., Reddy, N.D., Shoja, M.H., 2015. Evaluation of antioxidant and anticancer activity of extract and fractions of *Nardostachys jatamansi* DC in breast carcinoma. BMC Complement Altern. Med. 15, 50. https://doi.org/10.1186/s12906-015-0563-1.

Chouhan, S., Sharma, K., Guleria, S., 2017. Antimicrobial activity of some essential oils-present status and future perspectives. Medicines (Basel) 4 (3), 58. https://doi.org/10.3390/medicines4030058.

Dandagi, P.M., Patil, M.B., Mastiholimath, V.S., Gadad, A.P., Dhumansure, R.H., 2008. Development and evaluation of hepatoprotective polyherbal formulation containing some indigenous medicinal plants. Indian J. Pharm. Sci. 70 (2), 265–268. https://doi.org/10.4103/0250-474x.41474.

Didry, N., Dubreuil, L., Pinkas, M., 1993. Microbiological properties of protoanemonin isolated from *Ranunculus bulbosus*. Phytother. Res. 7, 21–24. https://doi.org/10.1002/ptr.2650070107.

Dumlupinar, B., Şeker Karatoprak, G., Damar Celik, D., Soyoğul Gürer, Ü., Demirci, B., Gürbüz, B., Rayaman, P., Kurtulus, E.M., 2020. Synergic potential of *Pelargonium endlicherianum* Fenzl. Essential oil and antibiotic combinations against *Klebsiella pneumoniae*. S. Afr. J. Bot. 135, 117–126.

El Atki, Y., Aouam, I., El Kamari, F., Taroq, A., Nayme, K., Timinouni, M., Lyoussi, B., Abdellaoui, A., 2019. Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics. J. Adv. Pharm. Technol. Res. 10 (2), 63–67. https://doi.org/10.4103/japtr.JAPTR_366_18.

Fowler, M.W., 2006. Plants, medicines and man. J. Sci. Food Agric. 86 (12), 1797–1804.

Hajdari, A., Mustafa, B., Nebija, D., Selimi, H., Veselaj, Z., Breznica, P., Quave, C.L., Novak, J., 2016. Essential oil composition of *Pinus peuce* Griseb. needles and twigs from two national parks of Kosovo. Sci. World J., 1–9. https://doi.org/10.1155/2016/5393079.

Hu, L.F., Li, S.P., Cao, H., Liu, J.J., Gao, J.L., Yang, F.Q., Wang, Y.T., 2006. GC-MS fingerprint of *Pogostemon cablin* in China. J. Pharm. Biomed. Anal. 42, 200–206. https://doi.org/10.1016/j.jpba.2005.09.015.

Joshi, H., Parle, M., 2006. *Nardostachys jatamansi* improves learning and memory in mice. J. Med. Food. 9 (1), 113–118. https://doi.org/10.1089/jmf.2006.9.113.

Khan, M.B., Hoda, M.N., Ishrat, T., Ahmad, S., Moshahid Khan, M., Ahmad, A., Yusuf, S., Islam, F., 2012. Neuroprotective efficacy of *Nardostachys jatamansi* and crocetin in conjunction with selenium in cognitive impairment. Neurol. Sci. 33 (5), 1011–1020. https://doi.org/10.1007/s10072-011-0880-1.

Kumar, V.P., Chauhan, N.S., Padh, H., Rajani, M., 2006. Search for antibacterial and antifungal agents from selected Indian medicinal plants. J. Ethnopharmacol. 107 (2), 182–188.

Lambert, R.J.W., 2000. Susceptibility testing: inoculum size dependency of inhibition using the Colworth MIC technique. J. Appl. Microbiol. 89, 275–279. https://doi.org/10.1046/j.1365-2672.2000.01105.

Lu, J., Turnbull, L., Burke, C.M., Liu, M., Carter, D.A., Schlothauer, R.C., Whitchurch, C. B., Harry, E.J., 2014. Manuka-type honeys can eradicate biofilms produced by *Staphylococcus aureus* strains with different biofilm-forming abilities. PeerJ 2, e326.

Mahadlek, J., Charoenteeraboon, J., Phaechamud, T., 2012. Combination effects of the antimicrobial agents and Cinnamon Oil. Adv. Mat. Res. 506, 246–249. https://doi.org/10.4028/www.scientific.net/amr.506.246.

Mishra, A.P., Saklani, S., Milella, L., Tiwari, P., 2014. Formulation and evaluation of herbal antioxidant face cream of *Nardostachys jatamansi* collected from Indian Himalayan region. Asian Pac. J. Trop. Biomed. 4 (2). https://doi.org/10.12980/apjtb.4.2014apjtb-2014-0223. S679–S682.

Nafis, A., Kasrati, A., Jamali, C.A., Mezrioui, N., Setzer, W., Abbad, A., Hassani, L., 2019. Anioxidant activity and evidence for synergism of *Cannabis sativa* (L.) essential oil with antimicrobial standards. Ind. Crops Prod. 137, 396–400. https://doi.org/10.1016/j.indcrop.2019.05.032.

Naquvi, K.J., Ansari, S.H., Ali, M., 2013. Composition of volatile oil of sambulut-teeb (*Nardostachys jatamansi* DC) an endangered species. Anal. Chem. : Indian J. 12, 347–351.

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., De Feo, V., 2013. Effect of essential oils on pathogenic bacteria. Pharmaceuticals (Basel) 6 (12), 1451–1474. https://doi.org/10.3390/ph6121451.

Oyaizu, M., 1986. Studies on products of browning reactions: Antioxidative activities of product of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44, 307–315. https://doi.org/10.5264/eiyogakuzashi.44.307.

Parveen, Z., Siddique, S., Shafique, M., Jabeen, S., Khannum, R., 2011. Volatile constituents, antibacterial and antioxidant activities of essential oil from *Nardostachys jatamans*i DC. Roots. Pharmocologyonline. 3, 329–337.

Pei, R.S., Zhou, F., Ji, B.P., Xu, J., 2009. Evaluation of combined antibacterial effects of eugenol, cinnamaldehyde, thymol, and carvacrol against *E. coli* with an improved method. J. Food Sci. 74, 379–383. https://doi.org/10.1111/j.1750-3841.2009.

Prasch, S., Bucar, F., 2015. Plant derived inhibitors of bacterial efflux pumps: an update. Phytochem. Rev. 14, 961–974. https://doi.org/10.1007/s11101-015-9436-y.

Rao, V.S., Rao, A., Karanth, K.S., 2005. Anticonvulsant and neurotoxicity profile of *Nardostachys jatamansi* in rats. J. Ethnopharmacol. 102, 351–356. https://doi.org/10.1016/j.jep.2005.06.031.

Razack, S., Kumar, K.H., Nallamuthu, I., Naika, M., Khanum, F., 2015. Antioxidant, biomolecule oxidation protective activities of *Nardostachys jatamansi* DC and its phytochemical analysis by RP-HPLC and GC-MS. Antioxidants. 4 (1), 185–203. https://doi.org/10.3390/antiox4010185.

Rosato, A., Sblano, S., Salvagno, L., Carocci, A., Clodoveo, M.L., Corbo, F., Fracchiolla, G., 2020. Anti-biofilm inhibitory synergistic effects of combinations of essential oils and antibiotics. Antibiotics 9, 637. https://doi.org/10.3390/antibiotics9100637.

Rubiolo, P., Sgorbini, B., Liberto, E., Cordero, C., Bicchi, C., 2010. Essential oils and volatiles: sample preparation and analysis. A review. Flavour Fragr. J. 25, 282–290. https://doi.org/10.1002/ffj.1984.

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. Ind. Crops Prod. 154,. https://doi.org/10.1016/j.indcrop.2020.112569 112569.

Singh, R.K., Vaishali Panda, S.K., Murthy, P.N., Panigrahi, G., Sharma, P.K., 2014. Evaluation of anti-inflammatory potential of *Nardostachys jatamansi* rhizome in experimental rodents. J. Coast Life Med. 2 (1), 38–43. https://doi.org/10.12980/JCLM.2.2014J10.

Sharma, N., Sharma, A.R., Patel, B.D., Shrestha, K., 2016. Investigation on phytochemical, antimicrobial activity and essential oil constituents of *Nardostachys jatamansi* DC. in different regions of Nepal. J. Coast. Life Med. 4 (1), 56–60.

Singh, V., Rana, N., Ali, M., 2018. GC-MS analysis and anti-microbial activities of volatile oil of *Nardostachys jatamansi* D.C. rhizomes obtained from Haridwar region, Uttrakhand. Pharmacogn J. 10 (2), 230–234.

Srivastava, J., Chandra, H., Nautiyal, A.R., Kalra, S.J., 2014. Antimicrobial resistance (AMR) and plant-derived antimicrobials (PDA_ms) as an alternative drug line to control infections. 3. Biotech 4 (5), 451–460. https://doi.org/10.1007/s13205-013-0180-y.

Subashini, R., Yogeeta, S., Gnanapragasam, A., Devaki, T., 2006. Protective effect of *Nardostachys jatamansi* on oxidative injury and cellular abnormalities during doxorubicin-induced cardiac damage in rats. J. Pharm. Pharmacol. 58, 257–262. https://doi.org/10.1211/jpp.58.2.0014.

Thielmann, J., Muranyi, P., Kazman, P., 2019. Screening essential oils for their antimicrobial activities against the foodborne pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*. Heliyon. 5. https://doi.org/10.1016/j.heliyon.2019.e01860. e01860.

Vaze, K., 2003. Lesser known essential oils of India: their compositions and uses. Fragrance Flavour Assoc. India 3, 47–58.

Wayne, P.A., 2003. NCCLS – National committee for clinical laboratory standards. Performance standards for antimicrobial susceptibility testing: Eleventh informational supplement. Document M100–S11. National Committee for Clinical Laboratory Standard.

Wojtunik, K.A., Ciesla, L.M., Waksmundzka-Hajnos, M., 2014. Model studies on the antioxidant activity of common terpenoid constituents of essential oils by means of the 2,2-diphenyl-1-picrylhydrazyl method. J. Agri Food Chem. 62 (37), 9088–9094. https://doi.org/10.1021/jf502857.

Wu, S.-Q., Li, R., Jiang, Z.-T., Wang, Y., Tan, J., Tang, S.-H., 2020. Evaluation of antioxidant active ingredients of spikenard essential oil by ultra-fast gas chromatography electronic nose and radical scavenging mechanism. Ind. Crops Prod. 151, 112489.

Yahiaoui, F., Benameur, Q., Ben-Mahdi, M.H., 2017. Antibacterial activity of *Mentha pulegium* essential oil against avian isolated ESBL, producing bacteria and its synergistic potential with antibiotics. Int. J. Pharm. and Pharma 9, 35–41. https://doi.org/10.22159/ijpps.2017v9i6.17092.

Yang, X.-N., Khan, I., Kang, S.C., 2015. Chemical composition, mechanism of antibacterial action and antioxidant activity of leaf essential oil of *Forsythia koreana* deciduous shrub. Asian Pac. J. Trop. Med. 8 (9), 694–700.

Yap, P.S., Lim, S.H., Hu, C.P., Yiap, B.C., 2013. Combination of essential oils and antibiotics reduce antibiotic resistance in plasmid-conferred multidrug resistant bacteria. Phytomedicine 20, 710–713. https://doi.org/10.1016/j.phymed.2013.02.013.