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ORIGINAL ARTICLE

Chemical composition, antioxidant and antimicrobial activities and characterization of bioactive compounds from essential oil of *Cinnamomum tamala* grown in north-western Himalaya

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Abstract The essential oil (EO) obtained from the fresh and dried leaves of *Cinnamomum tamala* was analysed by gas chromatography/mass spectrometry. EO from fresh leaves showed the presence of 21 compounds, whereas, EO from the dried leaves of *C. tamala* showed the presence of 20 compounds. In vitro assays namely scavenging ability against 1,1-diphenyl-2-picrylhydrazyl, reducing power and chelating ability on Fe^{2+} ions were used to determine the antioxidant potential of EO of *C. tamala*. With regard to antifungal activity, EO from dried leaves was more effective against *Alternaria alternata* and *Curvularia lunata* than the EO from fresh leaves. Similarly, EO from *C. tamala* leaves also showed potent antibacterial activity against two Gram negative and two Gram positive bacteria namely, *Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Furthermore, bioactive molecule from *C. tamala* EO having antifungal and antioxidant activity was isolated and characterized using bioautography, preparative thin layer chromatography and GC/MS analysis and was determined as eugenol. Its minimum inhibitory amount against *A. alternata* and *C. lunata* was determined using bioautography assay and was found to be 9.5 and 8.2 μg respectively.

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Abbreviations

BHT	Butylated hydroxytoluene
GC/MS	Gas chromatography/mass spectrometry
TLC	Thin layer chromatography
PTLC	Preparative thin layer chromatography
MIA	Minimum inhibitory amount
DPPH	1,1-diphenyl-2-picrylhydrazyl
EO	Essential oil
MIC	Minimum inhibitory concentration

Introduction

Based on their use in traditional medicine, a good number of modern drugs have been isolated from natural sources. Traditional medicine is a mean for health care for more than 65 % of world population (Nabavi et al. 2014). Different chemical compounds present in medicinal plants may act individually, additively or in synergy to improve health. As per one report published by World Health Organization (WHO), about 80 % of world population use medicinal plants to cure human diseases (Serrentino 1991). Raw materials or preparations containing phyto-chemicals with significant antioxidant activities and health benefits have been produced from some endemic species of medicinal plants (Exarchou et al. 2002). It has been reported that phenolic compounds, such as flavonoids, phenolic acids, tannins and phenolic diterpenes are primarily responsible for the antioxidant effect of plant products (Pietta 2000). The antioxidant properties of plant raw materials are attributed to the presence of polyphenols in them (Hamrouni et al. 2013). Further more, the

antioxidant activity of polyphenols is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators and reductants (Hamedo 2015).

Similarly, there has been a considerable interest to use plants and spices for the elimination of microorganisms because of increasing antibiotic resistance (Kunin 1993; Rahman 1999). There are reports of the active principles of essential oils from various plants with antibacterial or antifungal activity. The antimicrobial activity of essential oils is assigned to a number of small terpenoids and phenolic compounds (thymol, carvacrol, eugenol) which also in pure form demonstrate high antibacterial activity (Hamedo 2015).

Cinnamomum tamala is one of the species of the genus *Cinnamomum* and is native to India. It is a moderate sized evergreen tree belonging to family Lauraceae. In northern India, the plants are raised from seeds sown in the nursery beds in March–April. The ease with which essential oils can be obtained from this plant makes it ideal for cash crop farming (Gutteridge and Shelton 1994). Different species of genus *Cinnamomum* has been reported to possess excellent antibacterial, antitermite, antimite, antifungal and antioxidant properties (Chen et al. 2002).

The objective of this study was to evaluate the antioxidant and antimicrobial activity of *C. tamala* essential oil in vitro and to isolate and characterize the bioactive compound.

Materials and methods

Collection and identification of plant material

Fresh leaves from *C. tamala* were collected and identified at Herbal Garden and Herbarium Research Institute in ISM, Joginder Nagar, District Mandi (HP), India.

Sample preparation

Cinnamomum tamala leaves were divided into two parts. One part of the fresh leaves was used for extraction of essential oil and rest was dried in shade. Dried leaves were further used for the extraction of essential oil.

Extraction of essential oil

Essential oil from fresh as well as dried leaves was extracted through hydro-distillation using Clevenger-type apparatus (Guleria et al. 2008). The yield of the EO was determined and expressed in percentage.

GS/MS analysis

The mass spectrometer used for GC–MS analysis was GC–MS 4000 (Varian, USA) under the following conditions: capillary column, HP-5 MS (30 m × 0.25 mm; film thickness 0.25 μm); temperature program, 50 °C (held for 5 min) raised to 280 °C at a rate of 3 °C/min; injector temperature, 280 °C; carrier gas, helium, at flow rate of 1.0 ml/min. The MS scan parameters included electron impact ionization voltage of 70 eV and a mass range of 40–500 m/z. The identification of the components was based on the comparison of their mass spectra with those stored in NIST05 library or with mass spectra from literature (Adams 2007). Furthermore, in addition to MS, retention times (Rts) of compounds present in EO were authenticated by running standards.

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

This method was performed as described by Bozin et al. (2006). 1 ml of various dilutions of test material were mixed with 1 ml of a 90 μM DPPH methanolic solution and final volume was made to 4 ml with methanol. After an incubation of 1 h in the dark at 25 °C, the absorbances at 517 nm were recorded as A_{sample}, using a UV/VIS spectrophotometer (Labomed, USA). A blank experiment was also performed using the same procedure to a solution without the test material and the absorbance was recorded as A_{blank}. The free radical scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

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

Antioxidant activities of test compounds were expressed as IC_{50}, defined as the concentration of test material required to produce a 50 % decrease in initial DPPH concentration. BHT was used as standard antioxidant compound.

Reducing power assay

The reducing power was determined according to the method of Oyaizu (1986). Each test sample was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium ferricyanide and the mixture was 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. Finally the absorbance was measured at 700 nm against a blank. BHT was used as a control. The test sample concentration

providing 0.5 of absorbance (IC_{50}) was calculated from the graph of absorbance versus concentration.

Chelation power on metal ions

Chelating effect was determined according to the method of Dinis et al. 1994. The essential oil (100 μl) was mixed with 2.9 ml of methanol and 60 μl of 2 mM $FeCl_2$ and the reaction was allowed to proceed for 30 s. After 120 μl of 5 mM ferrozine was added, the solution was mixed, left to stand for 10 min at room temperature, and then the absorbance of the mixture was determined at 562 nm in UV/VIS spectrophotometer (Labomed, USA). The ratio of inhibition of ferrozine Fe^{2+} complex formation was calculated as follows:

$$\% \text{ inhibition } = [(\text{absorbance of control} - \text{absorbance of sample})/ \text{absorbance of control}] \times 100.$$

Quercetin was used as control.

Determination of antifungal activity

Antifungal activity was studied by using contact assay in vitro, which produces hyphal growth inhibition. Briefly, potato dextrose agar (PDA) plates were prepared, using 9 cm diameter glass petri dishes. Test sample was added to the sterilized potato dextrose agar containing 0.001 % Tween 20 v/v and the mixture was poured in 9 cm glass petri plates. 5 mm bit of test fungus was placed in the centre of the dishes. The plates were then incubated in the dark at 26 °C. The extension diameter (mm) of hyphae from the centres to the sides of dishes was measured every 24 h till the growth of fungus in the plate without test component (control) reached the edge of the plate. Growth inhibition of treatment against control was calculated as percentage, using the formula [(C − T/C) × 100] where C is the hyphal extension (mm) of control and T is the hyphal extension (mm) of plate treated with essential oil solution.

Determination of antibacterial activity

Antimicrobial activity of essential oils was determined by agar–well diffusion method (Feyza et al. 2009). Four test bacteria namely *Bacillus subtilis* MTCCCC 2389, *Staphylococcus aureus* MTCC7443), *Micrococcus luteus (*MTCC 4821) and *Pseudomonas aeruginosa* MTCC2642) were used in the study. 20 μl of the essential oil (diluted with DMSO, 1:1) was added to the well in the agar plate previously seeded with test organism. The plates were incubated at 37 °C for 24 h and the net zone of inhibition (mm)

was determined. Chloramphenicol was used as positive control.

Determination of MIC by broth dilution method

Minimum inhibitory concentration of the essential oils was determined using micro dilution method (NCCLS 2002). Essential oil diluted with DMSO (1:1) was dissolved in nutrient broth to achieve the required concentrations (62.5–2000 μg/ml). The test bacterial suspension was added into each tube to yield bacterial density of 10^6 cfu/ml and the inoculated tubes were incubated at 37 °C for 24 h. 50 μl of 0.2 mg/ml *p*-iodonitrotetrazolium violet (INT) was then added in each tube and incubated for 30 min at 37 °C. Development of pink colour in the tube indicated the bacterial growth. MIC corresponded to the lowest concentration at which no bacterial growth was observed.

Qualitative antifungal and antioxidant activity assay by bioautography

Bioautographic evaluation was conducted using pre-coated aluminium silica gel TLC plates (5 × 20 cm, 0.25 mm thickness, silica gel G 60 F254, Merck, Darmstadt, Germany). A 5 μl of 1:10 dilution of test essential oil in ethyl acetate was spotted on two plates (one plate was used for antibacterial and another for antioxidant activity screening) and allowed to dry for a few minutes. The plates were developed with hexane: ethyl acetate (9:1 v/v) solvent mixture in a pre-saturated glass chamber and air-dried. For determining the antifungal activity against *A. alternata* and *C. lunata*, aliquots of 25–50 ml of inoculum spray solution (ca. 3 × 10^5 conidia/ml) were prepared for test fungi with liquid potato dextrose. Plates were sprayed lightly to a damp appearance three times with spore suspension and incubated for 4 days in a dark moist chamber at 25 °C. Fungal growth inhibition appeared as clear zones against a dark background (Sridhar et al. 2003). For determining the antioxidant activity, the developed air-dried plate was sprayed with 0.2 % DPPH in methanol and was further air-dried for 30 min. Appearance of yellow spots on a purple background on the TLC plate showed the presence of antioxidant compound (Mimica-dukic et al. 2003).

Isolation of antifungal/antioxidant constituent from *C. tamala* essential oil

A streak of test essential oil was applied manually on a PTLC glass plate (20 × 20 cm, 1 mm thickness) and allowed to dry for a few minutes. After air drying the plate was developed using the same solvent mixture as described earlier and two sets of plates were used in parallel. One of the plates from each set was used to perform direct

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bioautography with test pathogens or sprayed with DPPH as described previously to visualize the antifungal and antioxidant compounds. Bands that showed antifungal or antioxidant activity were scrapped off from the second plate of each set of experiment. The bioactive molecule was then set free from silica gel by elution with ethyl acetate and then centrifuged ($12000 \times g$, 15 min) to remove the silica gel and the supernatants were collected.

Antifungal activity of bioactive molecule isolated from *C. tamala*

Different amounts of bioactive molecule Ct_1 were loaded onto the TLC plate and bioautography was performed as described earlier using *A. alternata* and *C. lunata* as test pathogens. Antifungal activity was determined as MIA of active compound required for the inhibition of fungal growth on TLC plate.

Identification of bioactive compound

The isolated bioactive compound corresponding to Ct_1 having antifungal and antioxidant activity was determined using GC/MS analysis as described earlier.

Statistical analysis

All experiments were carried out in triplicate. Data are expressed as mean ± standard deviation.

Results and discussion

Essential oils are valuable natural products consisting of many compounds that are used as raw materials in spices, food, perfumes, cosmetics, aromatherapy and phytotherapy. This has attracted the attention of many scientists and encouraged screening of plants in order to study the biological activities of their oils or chemical constituents. Each of the constituents contributes to the benefit or adverse effects of these oils (Lahlou 2004). The recent upsurge in antibiotic-resistant reports has increased the popularity of the essential oils as naturally occurring antimicrobial agents and therefore, plant essential oils may offer a great potential alternative for the treatment of many infectious diseases that could not be cured by current drugs (Lahlou 2004; Cosentino et al. 1999). A number of small terpenoids and phenolic compounds such as thymol, carvacrol, and eugenol are responsible for the antimicrobial activity of essential oils (Karapinar and Aktung 1987; Conner 1993). In the present study, yield of the yellow coloured oil obtained from the dried and fresh leaves of *C. tamala* was 0.6 and 0.15 percent respectively.

GC/MS analysis of the EO extracted from the fresh leaves of *C. tamala* revealed the presence of 21 compounds. In totality 93.82 percent compounds were identified in EO from fresh leaves consisting of a complex mixture of monoterpenes (25.04 %), sesquiterpenes (0.14 %) and phenylpropanoids (68.64 %) (Table 1). Similarly, EO from the dried leaves of *C. tamala* showed the presence of 20 compounds. In this case, total 94.42 percent compounds were identified in EO from dried leaves consisting of a complex mixture of monoterpenes (23.9 %), sesquiterpenes (0.20 %) and phenylpropanoids (70.32 %) (Table 2).

The DPPH radical scavenging activity of the EO extracted from fresh and dried leaves of *C. tamala* is shown in Table 3. The results showed higher DPPH radical scavenging activity in the essential oil from the dried leaves (IC_{50} value of 1.6 ± 0.1 mg/ml) than that obtained from the fresh leaves of *C. tamala* (IC_{50} value of 1.7 ± 0.1 mg/ml). Neutralization of DPPH free radical either by transfer of an electron or hydrogen atom may be

Table 1 Chemical composition of EO estimated using GC–MS from extracts isolated from fresh leaves of *Cinnamomum tamala*

S. no.	Compound	Class	RT (min)	Percent[a]
1	α-thujene	Monoterpene	13.600	0.48
2	α-pinene	Monoterpene	14.039	4.01
3	Camphene	Monoterpene	14.973	1.56
4	Benzaldehyde	Phenylpropanoid	15.697	2.7
5	β-pinene	Monoterpene	16.496	2.12
6	α-phellandrene	Monoterpene	18.004	1.12
7	3-carene	Monoterpene	18.135	0.33
8	Cymene	Monoterpene	18.716	0.18
9	Limonene	Monoterpene	19.250	2.34
10	Eucalyptol	Monoterpene	19.451	0.31
11	Linalool	Monoterpene	22.903	9.04
12	Borneol	Monoterpene	26.852	1.37
13	4-terpinenol	Monoterpene	27.215	0.11
14	*p*-cymene-8-ol	Monoterpene	27.487	0.12
15	α-terpineol	Monoterpene	27.914	0.61
16	Cinnamaldehyde	Phenylpropanoid	29.178	0.35
17	Eugenol	Phenylpropanoid	31.743	52.54
18	Bornyl acetate	Monoterpene	32.153	1.21
19	Bergamiol	Monoterpene	36.168	0.13
20	β-caryophyllene	Sesquiterpene	38.353	0.14
21	Cinnamyl acetate	Phenylpropanoid	39.267	13.05

RT retention time

Monoterpenes = 25.04 %

Sesquiterpenes = 0.14 %

Phenylpropanoids = 68.64 %

Total identified = 93.82 %

[a] Data is average of three replicates

Table 2 Chemical composition of EO estimated using GC–MS from extracts isolated from dried leaves of *Cinnamomum tamala*

S. no.	Compound	Class	RT (min)	Percent[a]
1	α-thujene	Monoterpene	13.621	0.47
2	α-pinene	Monoterpene	14.059	4.53
3	Camphene	Monoterpene	14.993	1.73
4	Benzaldehye	Phenylpropanoid	15.725	1.44
5	β-pinene	Monoterpene	16.515	2.05
6	α-phellandrene	Monoterpene	18.027	0.95
7	3-carene	Monoterpene	18.158	0.32
8	Cymene	Monoterpene	18.741	0.18
9	Limonene	Monoterpene	19.273	2.23
10	Eucalyptol	Monoterpene	19.471	0.45
11	Linalool	Monoterpene	22.921	7.9
12	Borneol	Monoterpene	26.876	1.21
13	Terpinen-4-ol	Monoterpene	27.238	0.13
14	*p*-cymene-8-ol	Monoterpene	27.513	0.11
15	α-terpineol	Monoterpene	27.939	0.52
16	Cinnamaldehyde	Phenylpropanoid	29.203	0.34
17	Eugenol	Phenylpropanoid	31.764	62.83
18	Bornyl acetate	monoterpene	32.179	1.12
19	β-caryophyllene	Sesquiterpene	38.389	0.20
20	Cinnamyl acetate	Phenylpropanoid	39.292	5.71

RT retention time

Monoterpenes = 23.9 %

Sesquiterpenes = 0.20 %

Phenylpropanoids = 70.32 %

Total identified = 94.42 %

[a] Data is average of three replicates

responsible for observed antioxidant activity of the essential oils (Naik et al. 2003; Guleria et al. 2013). Furthermore, most natural antioxidant compounds often work synergistically with each other to produce a broad spectrum antioxidant effect that creates an effective defence system against free radical attack (Madsen and Bertelsen 1995). DPPH is a free radical compound that has been widely used to determine the free radical scavenging capacity of various samples (Dandapat et al. 2014). Hydrogen donating ability of the antioxidants is responsible for their DPPH radical scavenging activity and when an antioxidant scavenges the free radical the DPPH solution becomes light yellow (Pieme et al. 2014).

The reducing power of the EO obtained from fresh and dried leaves of *C. tamala* is shown in Table 3. Higher reducing power was observed in the EO extracted from the dried leaves (IC_{50} of value 2.75 ± 0.098 mg/ml) as compared to the fresh leaves (IC_{50} of value 2.8 ± 0.1 mg/ml) of *C. tamala.* In this assay, the yellow colour of the test solution changes to green depending on the reducing power of test specimen. The reductants present in the solution leads to the reduction of the Fe^{3+} ferricyanide complex to the ferrous form. The ability of essential oils to reduce Fe(III) may be attributed to hydrogen donation from phenolic compounds (Shimada et al. 1992; Duh 1998). The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity (Meir et al. 1995).

Metal chelating capacity is considered as one of the important mechanism of antioxidant activity. The ferrous ions are the most powerful pro-oxidants among various species of transition metals present in food system (Yomauchi et al. 1998). The chelating power on metal ions of the EO obtained from fresh and dried leaves of *C. tamala* is depicted in Table 3. It is evident from the results that EO derived from dried leaves of *C. tamala* showed higher metal ion chelating activity (88.2 ± 1.2 % at 4.95 mg/ml) as compared to EO obtained from fresh leaves (85.0 ± 1.4 % at 4.95 mg/ml). Furthermore, decrease in the absorbance of ferrozine Fe^{2+} complex was dose dependent. This study is in conformity with the observation made in the literature that iron binding to phenolic antioxidants can cause reduced interaction of iron with oxygen molecules by changing the redox potential, thus converting Fe^{2+} ion to Fe^{3+} and thereby slowing down oxidative damage (Singh et al. 2007a, b).

Also it has been reported that non-flavonoids polyphenolics can reduce iron and then form Fe^{2+}-polyphenol complexes that are inert (Laughton et al. 1987). Antioxidants causes the chelation of transition metal ions which possesses the ability to catalyse H_2O_2 decomposition, is an important mechanism of antioxidant activity (Manian et al. 2008). Higher metal ion chelating activity of EO derived from dried leaves as compared to EO obtained from fresh leaves of *C. tamala* may be attributed to the presence of

Table 3 Antioxidant activity of EO obtained from fresh and dried leaves of *Cinnamomum tamala*

S. no.	Component[a]	DPPH radical scavenging radical activity IC_{50} (mg/ml)	% Chelating effect	Reducing power IC_{50} (mg/ml)
1.	EO from fresh leaves	1.7 ± 0.1	85.0 ± 1.4	2.80 ± 0.100
2.	EO from dried leaves	1.6 ± 0.1	88.2 ± 1.2	2.75 ± 0.098

[a] Values are mean ± standard deviation of three parallel measurements

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Table 4 Percent antifungal index and antifungal activity values of the EO from *Cinnamomum tamala*

Pathogen	% Antifungal index		Antifungal activity [IC$_{50}$ (µg/ml)]	
	EO from fresh leaves	EO from dried leaves	EO from fresh leaves	EO from dried leaves[a]
Alternaria alternata	25.00 ± 1.15	28.42 ± 1.2	2932 ± 72.27	2403.7 ± 19.0
Curvularia lunata	29.49 ± 1.5	34.86 ± 1.5	2417.3 ± 23.0	2117.7 ± 19.80

[a] Values are mean ± standard deviation of three parallel measurements

Table 5 Antibacterial activity of essential oil from fresh leaves of *Cinnamomum tamala*

Microorganisms	Zone of inhibition (mm)		Minimum inhibitory concentration (µg/ml)
	Essential oil[a]	Chloramphenicol	Essential oil
Bacillus subtilis MTCC2389	23.3 ± 2.88	18 ± 0.57	550
Micrococcus luteus MTCC4821	20.1 ± 1.97	19 ± 1.02	>1000
Staphylococcus aureus MTCC7443	20.0 ± 0.00	20 ± 0.89	>1000
Pseudomonas aeruginosa MTCC2642	19.6 ± 0.57	17 ± 0.78	>1000

[a] Values are mean ± standard deviation of three parallel measurements
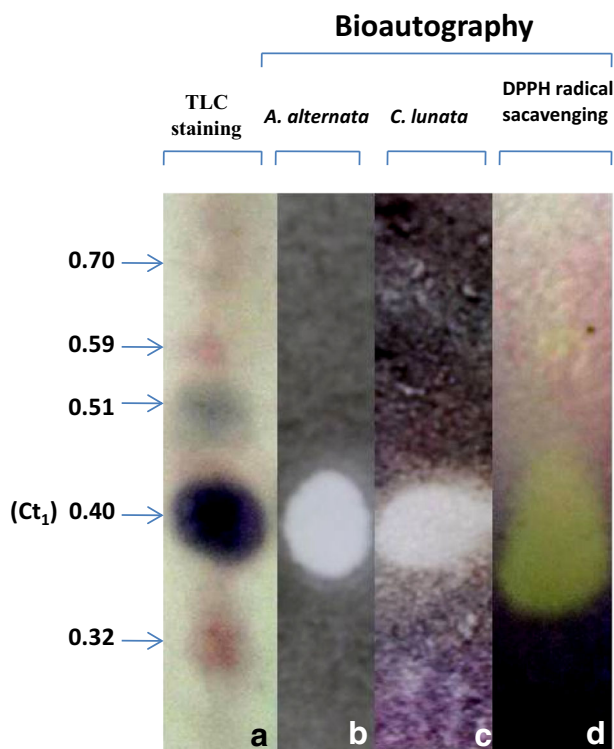
Fig. 1 TLC of essential oil from *Cinnamomum tamala* leaves. **a** Detection with vaniline/sulphuric acid reagent; **b** bioautography with *Alternaria alternata* spores; **c** bioautography with *Curvularia lunata* spores; **d** detection of free radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (0.5 mM). Ct$_1$ corresponds to the component of the essential oil having antifungal and free radical scavenging activity
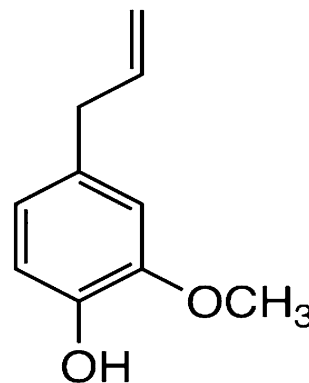
Fig. 2 Chemical structure of eugenol

certain phenolic compounds with properly oriented functional groups.

In the present study, antifungal activity of EO from *C. tamala* leaves was evaluated using two agriculturally important phytopathogenic fungi namely *A. alternata* and *C. lunata*. Comparison of antifungal indices and IC$_{50}$ values of EO obtained from *C. tamala* revealed that *C. lunata* was more sensitive to EO of *C. tamala* as compared to *A. alternata*. Antifungal activity of EO from fresh and dried leaves of *C. tamala* is depicted in Table 4. On the basis of the results of the antifungal test, it is evident that the antifungal index of EO from fresh and dried leaves of *C. tamala* against *A. alternata* was 25 ± 1.15 % and 28.42 ± 1.2 % and IC$_{50}$ values were 2932 ± 72.27 µg/ml and 2403.7 ± 19 µg/ml respectively. Antifungal index of

EO from fresh and dried leaves of *C. tamala* against *C. lunata* was 29.49 ± 1.5 % and 34.86 ± 1.5 % respectively. Similarly, IC_{50} values of EO from fresh and dried leaves of *C. tamala* against *C. lunata* were 2417.3 ± 23 µg/ml and 2117.7 ± 19.8 µg/ml respectively. Results obtained from antibacterial activity assays of the EO from fresh leaves of *C. tamala* are presented in Table 5. The oil was tested for antibacterial activity against two Gram-negative and two Gram-positive bacteria and was found effective against all the tested bacterial strains. The oil was highly inhibitory against *Bacillus subtilis* (MTCC 2389) followed by *Micrococcus luteus* (MTCC4821), *Staphylococcus aureus* (MTCC7443) and *Pseudomonas aeruginosa* (MTCC 2642). Minimum inhibitory concentration (MIC) of EO from *C. tamala* leaves was 550 µg/ml against *Bacillus subtilis* MTCC2389. Furthermore, antifungal compound of the EO from *C. tamala* leaves was observed on the TLC plate using *A. alternata* and *C. lunata* as test organisms in bioautography (Fig. 1b, c). It was further isolated using repeated preparative TLC. Similarly, for the isolation of antioxidant compound, $DPPH^{.}$ was used in direct bioautography assay. When bioautography was performed for observing antifungal activity one inhibition zone at R_f value 0.40 was observed. Intriguingly, this compound also showed antioxidant activity in direct bioautography test (Fig. 1d). The compound corresponding to R_f value 0.40 was marked as Ct_1. Pure Ct_1 was further subjected to direct bioautography for determination of MIA against *A. alternata* and *C. lunata*. The results showed that Ct_1 possessed stronger fungitoxic activity against *C. lunata* (MIA = 8.2 ± 0.02 µg) than *A. alternata* with MIA value of 9.5 ± 0.01 µg. The bioactive constituent of the *C. tamala* EO corresponding to Ct_1 was identified as eugenol using GC/MS analysis (Fig. 2).

In the present study we investigated the antimicrobial and antioxidant activity of EO from fresh and dried leaves of *C. tamala*. To the best of our knowledge this may be assumed as first report on isolation and identification of the bioactive compound responsible for antifungal and antioxidant activity of *C. tamala* grown in north-western Himalaya. The results demonstrate the antifungal potential of bioactive molecule namely eugenol from *C. tamala* EO against *A. alternata* and *C. lunata*. In addition, EO from fresh and dried leaves of *C. tamala* showed significant antioxidant and antifungal activity which was higher in EO from dried leaves as compared to EO from fresh leaves. Seasonal variation in EO content of *C. tamala* and level of bioactive molecule may be investigated in future for ascertaining its commercial value.

Compliance with ethical standards

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