Diversity, Phylogeny, anticancer and antimicrobial potential of fungal endophytes associated with *Monarda citriodora* L.

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**Abstract**

**Background:** Present study focuses on diversity and distribution analysis of endophytic fungi associated with different tissues of the *Monarda citriodora* Cerv. ex Lag. (Lamiaceae/Labiatae). Anticancer and antimicrobial potential of isolated endophytes have also been investigated.

**Results:** A total of twenty eight fungal endophytes belonging to 11 different genera were isolated from this plant. All the endophytic fungi belonged to the Ascomycota phylum. The leaves were immensely rich in fungal species, while roots showed the highest tissue specific fungal dominance. Out of 28 fungal species, 72% endophytic extracts were found cytotoxic against one or more human cancer cell lines. The most prominent anticancer activity (IC₅₀ value <10 μg/mL) was shown by MC-14 L (*Fusarium oxysporum*), MC-14 F (*F. oxysporum*), MC-18 L (*Aspergillus fumigatus*), MC-24 L (*Cladosporium tenuissimum*), MC-25 L (*Fusarium* sp.), MC-26 F (*F. oxysporum*) extracts. 75% of the extracts showed antimicrobial activities in agar disc-diffusion assay and 27% in the tube dilution method (MIC <100 μg/mL) respectively against the tested pathogens. Extracts of MC-14 L (*F. oxysporum*) and MC-18 L (*A. fumigatus*) displayed broad spectrum antimicrobial activity.

**Conclusions:** These results indicated that *M. citriodora* harbors a rich fungal endophytic community with anticancer and antimicrobial activities. The isolated endophyte MC-24 L (*C. tenuissimum*) has the potential to be a source of novel cytotoxic/antimicrobial compounds. This is the first report of diversity of fungal endophytes isolated from *M. citriodora*.

**Keywords:** Endophytic fungi, *Monarda citriodora*, Antimicrobial activity, Tube dilution method, Cytotoxicity, MTT assay

**Background**

Endophytes are one of the most important parts of plant microbiota. These microbes reside inside the plants in variety of relationship but they do not cause any deleterious effect to them [1]. Endophytes are ubiquitous in nature and are found in every plant. They coexist inside the plant and are beneficial for better adaptability of the plants in their ecological niche [1–3]. During this process, endophytes interact with each other and their host plant changing the metabolic ability of the plant. In addition endophytes are known to produce a multitude of secondary metabolites of pharmaceutical importance [4, 5]. These metabolites belong to diverse structural classes and show variety of bioactivities including antifungal, antibacterial, antiviral, anticancer, antidiabetic, insecticidal, immunosuppressive, antioxidant etc. [6–9]. Some of the natural products such as cephalosporins, lovastatin, ivermectin, rapamycin, cyclosporine, paclitaxel etc. still find use as immunosuppressant and to control the infectious and parasitic diseases, lipid disorders, cancer and hypertension [10, 11].
Increasing incidence of cancer and development of superbug’s due to antibiotic resistance are the major challenges for researchers [12, 13]. Therefore, search for safer and novel drug based on natural product is of utmost importance. The success can be achieved by selecting the underexplored and/or unexplored biological resources. They are expected to be the producer of new chemical entities. Endophytic fungi belonging to unique environment are one of the promising sources of novel and natural drugs [10, 14].

*Monarda* commonly named as horsemint, beebalm or wild bergamot belongs to Lamiaceae (Labiatae), the mint family. It is vastly distributed, medicinal, aromatic and ornamental herb which grows annually or perennially [15, 16]. USA, Canada, and Mexico are its origin point but it also occurs in Europe and Asia [15, 17]. In India, it is growing in Shivalik hills since 1990. It consists of roughly thirty species and is being grown as garden, food and/or medicinal plant [18]. It is used as a flavoring agent in drinks, bakery and meat products. Its leaves were pulverized and sprinkled on meat to repel insects and to preserve the meat. Its decoction is used to cure catarrh, colds, toothaches, headaches, gastric disorders, nausea, menstrual pain, insomnia, to lessen fevers, soothe sore throat and to relieve flatulence. It is topically used for curing skin eruptions and infections. Its aroma is used in potpourri. Traditionally it has been used to treat variety of respiratory, digestive and skin disorders. Its use as febrifuge, diaphoretic, antirheumatic, carminative, sedative, diuretic and stimulant has also been reported [16]. The plant also has antiseptic, anti-oxidant and antifungal properties [19–22]. Major component of its essential oil, thymol, is now-a-days used in modern commercial mouthwash formulations [20, 22, 23]. Recently its essential oil is found to have anticancer property targeting PI3K pathway [24].

The economic importance of *M. citriodora* L. created an enthusiasm to study the endophytic community associated with this plant and their spatial distribution. They were also explored for antibacterial and anticancer activities. This attempt is novel as endophytes from *M. citriodora* have not been documented.

**Methods**

**Collection, identification and authentication of plant material**

Fully matured *M. citriodora* plants were collected randomly between March – April, 2013 from Shivalik hills, Jammu and Kashmir (32.73°N 74.87°E, altitude of about 1073 ft), India. The species was identified by taxonomist via leaf and flower morphology and preserved in the herbarium (accession no. 18554) and in the farm of IIIM as genetic resource.

**Isolation of endophytes**

The endophytic fungi were isolated from *M. citriodora* as described by Strobel and Daisy [1] with slight modifications. Different tissues (leaves, roots and flowers) of the disease free plants were carefully excised with a sterile scalpel. In first instance, these tissues were cleaned by thorough washing in running tap water, followed by de-ionized (DI) water. Clean tissue pieces were sterilized in series of solution: 70% ethanol; 1% sodium hypochlorite (v/v); again 70% ethanol for 1 min each. Finally they were again rinsed with sterile distilled water two times. After surface sterilization, tissues were dried on blotting sheets and cut into 1 cm square pieces. These sterile small pieces were placed on water and potato dextrose agar (PDA) plates containing streptomycin (250 μg mL⁻¹) to inhibit the bacterial growth. At the same time, water used for washing the tissues (100 μL) was also plated on the PDA to confirm the effectiveness of surface sterilization. The plates were incubated at 25 ± 2 °C after wrapping with parafilm and observed daily. The fungal mycelia which started growing from the tissues were subcultured on new PDA plates. The obtained endophytic fungal isolates were coded according to their tissue of origin (MC-1 L, MC-2 L, MC-3 L, etc. from leaves, MC-13R, MC-20R, from roots and MC-7 F, MC-14 F, MC-21 F, from flowers). These endophytes were stored in paraffin oil at 4 °C and were deposited in RN Chopra, Microbial Repository, IIIM.

**Characterization of endophytes**

**Morphology based identification of endophytes**

The endophytic fungi were characterized on the basis of the morphology observed during their growth on PDA. They were also examined microscopically to determine the structure of hyphae, conidia, conidiophores and their arrangement.

**Molecular identification**

Fungal endophytic isolates were finally identified by ITS based rDNA sequencing. Genomic DNA of the endophytes was extracted from the in vitro grown biomass of endophytes using the protocol described by Reader and Broda [25]. Approximately 1 g of dried mycelia was kept in liquid nitrogen and crushed to a fine powder. It was transferred to 10 mL extraction buffer and vortexed thoroughly. The samples were incubated in water bath set at 65 °C for 30 min followed by intermittent mixing. The tubes were centrifuged at 10000 g for 5-10 min followed by extraction with chloroform:isoamyl alcohol (24:1). Aqueous layer was collected and DNA was precipitated with 2.5–3 volume of absolute ethanol in presence of 1/10th volume of sodium acetate (3 M pH 5.2). Tubes were inverted slowly to mix the contents followed by centrifugation at 8000 g for 20 min at 4 °C. Consequently, white/transparent pellets were washed
with ice cold 70% ethanol followed by air drying. Dried pellets were dissolved in 20 μL of water (molecular biology grade). ITS sequences containing ITS1-5.8S-ITS2 spanning 500-600 bp were amplified with the universal primers ITS1 (5'-GGAGTAAAGTCTGTAACAGG-3') and ITS2 (3'-TCCTCCGCTTATTGATATGC-5') [26]. PCR reaction was set up in 50 μL containing DNA (1–10 ng), 1x PCR buffer (with 15 mM MgCl2), each dNTP (200 mM), each primer (10 pmol, Sigma, USA) and 1U Taq DNA polymerase (Bangalore Genei, India). Cycling parameters were 5 min at 94 °C followed by 30 cycles of 94 °C for 30 s, 55 °C for 1 min, 72 °C for 1 min and a final extension for 10 min at 72 °C. The PCR product (10 μL) was resolved using agarose gel electrophoresis at 100 V. The amplified product was purified using a Gel extraction Kit (Qiagen, USA) and sequencing reaction was set up in a 10 μL: 40–60 ng of purified PCR product, 3.2 pmol forward/reverse primer, Big Dye Terminator sequencing mix 8 μL (v. 3.1, Applied Biosystems). Samples were sequenced on an automated sequencing system (Applied Biosystems). Resultant sequences (KUs27781-KUS27806, KU680345, KU680346) were submitted to a Genbank and were blasted against the nucleotide database using blastn Tool of the US National Centre for Biotechnology Information (NCBI) for final identification of endophytes [27].

Phylogenetic evaluation of endophytic fungi
For phylogenetic evaluation, endophytic ITS DNA sequences of their nearest neighbors were aligned in Alignment Explorer of MEGA4 software [28] using ClustalW option. Trimming and verification of the sequence alignment were carried out using the MUSCLE (UPGMA) algorithm [29]. The Maximum Composite Likelihood and Neighbor-Joining methods were used to compute the evolutionary distances and history respectively [30, 31]. The robustness of the tree was assessed by bootstrap analysis with 1000 replication [32].

Fungal diversity evaluation
To evaluate and quantify the endophytic fungal diversity associated with M. citriodora, different indices such as Menhinick’s index \( D_{nm} \), Camargo’s index \( 1/D_{nm} \) or species richness, Fisher’s log series index \( \alpha \), Simpson’s index \( D \), Simpson’s diversity index \( 1-D \), the Shannon diversity index \( H \), and Margalef’s richness \( D_{mg} \) were calculated [33–36]. The similarity indices for fungal endophytic assemblages among the tested tissues were also evaluated using Sorensen’s index \( QS \), and Jaccard’s index using the following equation: \[ QS = 2a/(2a + b + c) \]; \[ JS = a/(a + b + c) \] respectively, where \( a \) means the number of common fungal species found in endophytic populations of two different tissues and \( b \) and \( c \) mean the number of endophytic fungal species specific to tissues under comparison [37].

Bioactivity evaluation
Fermentation and extraction
For the extraction of molecules, the endophytic fungal isolates (twenty eight) were cultured on a set of five potato dextrose agar plates for a period of 15 days at 25 ± 2 °C in an incubator (New Brunswick, USA). 5 mm mycelial plug of 10-day old culture was used as inoculum. After fifteen days, fungal growth of five petri dishes was homogenized thoroughly with 12.5 mL of methanol. Homogenate was extracted with one volume of methylene chloride (DCM) (HPLC grade). The extraction process was repeated four times. Solvent containing extract was stripped off in a rotary evaporator. The stock solutions of extracts (10 mg mL\(^{-1}\)) were prepared in dimethyl sulfoxide (DMSO) and were used to evaluate the anticancer and antimicrobial potential.

Cytotoxic activity
Endophytic extracts were tested for their cytotoxic effects using an MTT assay. It is a colorimetric assay to quantify the cell survival and proliferation. Four human cancer cell lines: HCT-116 (colorectal carcinoma), A-549 (lung), MCF-7 (breast), PC-3 (prostate) were procured from National Centre for Cell Sciences (NCCS), Pune, India for the present study. The MTT assay was performed as described by Pathania et al. [24]. Cells were cultured in RPMI-1640 medium with 10% fetal calf serum (FCS), and 100U penicillin/100 μg mL\(^{-1}\) streptomycin. Cells were incubated at 37 °C with 98% humidity and 5% CO\(_2\) environment (in CO\(_2\) incubator, Thermo Electron Corporation, USA). The cell density was adjusted to 10\(^5\) cells mL\(^{-1}\) of RPMI medium and 200 μL of these cells were plated in a 96-well plate. Fungal extracts in different concentrations (10–100 μg mL\(^{-1}\)) were added to these wells and were incubated for 48 h. After 48 h, the medium of the wells was replaced with a medium containing 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, 100 μg mL\(^{-1}\)) for additional 3 h. Subsequently, supernatant was aspirated out and 200 μL DMSO was used to dissolve the MTT-formazan crystals. An ELISA reader (Thermo Labs, USA) was used to measure the optical density of 96 well plate at 540 nm with 620 nm as reference wavelength. 5-FU, paclitaxel, and adriamycin drugs were used as positive controls while DMSO was used as negative control. Extracts were also evaluated on normal cell line (FR2) in parallel. Absorbance of treated versus untreated cells was recorded and percent growth inhibition was calculated. CurveFit software was used to calculate IC\(_{50}\) value.
Antimicrobial activity
The extracts prepared from the endophytes were evaluated for antimicrobial activity. For antimicrobial activity, six test bacteria [Bacillus subtilis (MTCC No. 121), Pseudomonas aeruginosa (MTCC No. 424), Salmonella typhimurium (MTCC No. 98), Escherichia coli (MTCC No. 118), Klebsiella pneumoniae (MTCC No. 109), and Staphylococcus aureus (MTCC No. 737)] and yeast [Candida albicans (MTCC No. 183)] were purchased from Microbial Type Culture Collection (MTCC).

Agar disc-diffusion assay
The endophytic extracts were tested for their antimicrobial and antifungal activity using agar disc-diffusion method [38, 39]. We used streptomycin and amphotericin B as positive controls while DMSO (0.5%) was kept as negative control. Each of the bacterial strain was grown in nutrient broth at 37 °C with 200 rpm for 16 h in an incubator. Cell density of the culture was adjusted to the McFarland standard turbidity (0.5), which is equivalent to 1.5 × 10^8 colony forming units (CFU) mL^{-1} [38]. It was further diluted to give approximately 10^3 CFU mL^{-1}. For antibacterial assay, Mueller Hinton agar plates (90 mm, containing 20 mL medium) were spreaded with 100 μL of these cells. Stock solutions of fungal extracts and antibiotics were prepared to a conc. of 10 mg mL^{-1} and 1 mg mL^{-1} respectively. Fungal extracts (20 μL) and antibiotics (5 μL) were applied on sterile discs (6 mm diameter) in the centre of plates. All plates were incubated overnight at 37 °C. For antifungal activity, yeast extract peptone dextrose agar was used. Zone of inhibition (ZI) in terms of the diameter of area showing no bacterial growth around the disc was recorded in mm. The assays were repeated thrice and the mean value of three replications was calculated.

Tube dilution method
For quantitative antimicrobial and antifungal activity, endophytic extracts were evaluated by tube dilution method [39]. Different dilutions of fungal extracts (12.5–100 μg mL^{-1}) and antibiotics (6.5–50 μg mL^{-1}) were prepared from the stock solutions. For each pathogen, 100 μL of 10^4 CFU mL^{-1} was mixed with 900 μL of each dilution of fungal extracts and antibiotics. Three replicates of each sample were processed. Appropriate positive, negative and blank controls (virgin media) were also prepared in triplicate. All tubes were incubated overnight at 37 °C. The lowest inhibitory/bactericidal concentrations (MIC/MBC) were recorded. The assays were replicated thrice and the mean values were calculated.

Statistical analysis
All the collected parameters were examined with ANOVA and TUKEYS post hoc analysis using Graph Pad Prism software.

Results
Identification and characterization of the endophytic fungi
Endophytic fungi were isolated from healthy and symptomless tissues (leaves, roots and flowers) of M. citriodora to access their diversity, phylogeny and bioactive potential. Twenty eight endophytes were isolated. Absence of any growth on PDA, plated with water obtained from last rinse of tissues suggests that efficient surface sterilization has been performed. Endophytes were identified by their colony morphology and microscopic examination (Additional file 1: Figure S1 Table S1). Further their molecular identification was carried out by ITS based rDNA sequence analysis. Details of the fungal endophytes, their isolation source, GenBank accession numbers, and closest sequence homolog are given in Table 1.

The isolated endophytic fungi belonged to 11 different genera. The maximum numbers of endophytes (18 isolates) were hosted by leaves followed by flowers (8 isolates) and finally roots (2 isolates) (Table 1). All the endophytic fungi belonged to the Ascomycota phylum. Out of these, 46.5% isolates belonged to class Sordariomycetes followed by Eurotiomycetes (28.5%), and Dothideomycetes (25%). The Sordariomycetes were represented by the orders Hypocreales, Xylariales, Glomerellales, and Sordariales with most of them being distributed in leaves and flowers. Eurotiomycetes were isolated from leaves alone and were represented by the order Eurotiales, whereas Dothideomycetes were isolated from all tissues and were represented by the order Pleosporales and Capnodiales. Fusarium spp., Aspergillus spp., and Cladosporium spp. were dominant over other species. In leaves, Aspergillus spp. (27%) occurred with the highest frequency followed by Fusarium spp. (22%), Cladosporium spp. (11%) while Fusarium spp. (62.5%) was dominant in flowers. The Fusarium spp. were found associated with both leaves and flowers, where as Cladosporium sp. was found associated with leaves and roots. Endophytes specifically isolated from the leaves tissues were Curvularia aeria, Neosartorya hiratsukae, Muscodor yucatanensis, Neurospora sp., Colletotrichum boninense, Alternaria alternata, Penicillium commune, Fusarium spp., Aspergillus spp., and Cladosporium spp. whereas Gibberella internedia, Fusarium spp., Cladosporium cladosporioides, and Colletotrichum gloeosporioides were found specific to flowers. Roots harbor only Alternaria carthami and Cladosporium sp.

Phylogenetic evaluation
The phylogenetic tree describes the taxonomic relationship between the fungal endophytic species recovered from various tissues of M. citriodora (Fig. 1). In the phylogenetic tree, the sequences were clustered into four groups representing Sordariomycetes, Eurotiomycetes,
and Dothideomycetes classes. Sequences belonging to order Pleosporales and Capnodiales were clustered separately. Paludomyces mangrovei ATCC26191 was laid down separately as out group. In the bootstrap analysis with 1000 replicates, bootstrap values (the percentage of clustering the associated taxa together) were computed and shown next to the branches in the phylogenetic tree indicating the stability of tree/subtree.

**Fungal diversity analysis**

The diversity of the endophytic fungal populations isolated from different tissues was evaluated by various indices such as Fisher alpha diversity index (α), Shannon index H, Simpson’s diversity index (1-D), Margalef richness index (Dm) (Table 2). The species richness (Dmn) was highest in the leaves (4.24) followed by the flowers (2.82) and roots (1.41). The tissue-specific fungal dominance was the highest in the roots (0.71), followed by flowers (0.47) and leaves (0.24). The most dominant species isolated from the leaves and flowers was *Fusarium oxysporum* sp. (Pi = 0.14) followed by *Fusarium sp.* (Pi = 0.07), whereas the most dominant species isolated from the leaves and roots was *Cladosporium* sp. with a relative proportion Pi = 0.07. The rest of the species were less dominant (Pi = 0.03). The Shannon, Simpson’s (0.94) and Margalef indices respectively revealed a high certainty and consistency of endophytic fungal species in the roots (0.69) and highly diverse and taxonomically rich fungal endophytes in leaves. Species evenness was uniform in leaves and roots, whereas slightly lower (0.88) in flowers. Global beta diversity indices were recorded as follows: Whittaker diversity index: 1.7692, Harri-son diversity index: 0.076923, Cody diversity index: 4, Routledge diversity index: 0.076923, Wilson-Shmida diversity index 3.6923, Mourell diversity index: 0.16054, Harrison
diversity index 2: 0.021739, Williams’s diversity index: 0.33333. Sorenson’s and Jaccard’s (Jc) similarity indices were mentioned in Table 3. These indices indicate the significant diversity of endophytes within and between the different tissues of *M. citriodora*.

**Bioactivity evaluation**

**Anticancer activity**

Anticancer activities of the endophytic extracts at the 100 μg mL⁻¹ concentration were found against four human cell lines (HCT-116, MCF-7, PC-3, and A-549) while they didn’t inhibit the growth of normal cells (Table 4). Nearly 72% of the extracts showed cytotoxic activity against one or the other human cancer cell line with percent growth inhibition ranging from 51–100%. In the present study, sensitivity of cell lines against tested extracts was in the following order: PC-3 > MCF-7 > HCT-116 > A-549 cell lines. Thirty nine percent of the extracts showed >50% growth inhibition on all the tested human cancer cell lines.
Out of twenty eight extracts, the extracts showing highest percentage of growth inhibition for all the tested human cancer cell lines were assayed at four different concentrations to check the IC$_{50}$ values (Table 5). Seven extracts were found to be active at least against one of the tested cell lines with IC$_{50}$ of <20 μg mL$^{-1}$. Six extracts showed anticancer activity against A-549 and MCF-7 cell lines each, whereas only five and four extracts were active against HCT-116, and PC-3 cell lines respectively with IC$_{50}$ <20 μg mL$^{-1}$.

With IC$_{50}$ <10 μg mL$^{-1}$, six extracts showed cytotoxic activity against one of the tested cell lines, whereas two extracts MC-14 L (Fusarium oxysporum), MC-24 L (Cladosporium tenuissimum) were active against all the tested cell lines. With same IC$_{50}$ value, four extracts were recorded cytotoxic against HCT-116 cell line, six against A-549 and three against PC-3 and MCF-7 cell lines.

**Antimicrobial activity**

The antimicrobial potential of the isolated endophytes was investigated against some human pathogens by agar disc-diffusion and tube dilution method. Crude DCM extracts containing metabolites of these endophytes inhibited the growth of pathogens (Table 6). About 75% of extracts showed antimicrobial activity against tested pathogens with a zone of inhibition (ZI) ranging from 6–35 mm. Six (21%), 14 (50%), 7 (25%), 11 (39%), 10 (36), and 9 (32%) extracts presented significant antimicrobial activity against *E. coli*, *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, *S. typhimurium*, and *B. subtilis* respectively. Extracts of MC-14 L (*Fusarium oxysporum*) and MC-18 L (*Aspergillus fumigatus*) displayed ZI (9–35 mm) against all the tested human pathogens including *C. albicans*. Extract of MC-18 L (*Aspergillus fumigatus*) displayed better ZI (24–35 mm) than the extract of MC-14 L (*Fusarium oxysporum*) against bacterial pathogens. Extracts of MC-2 L (*Fusarium chlamydomysporum*) and MC-24 L (*Cladosporium tenuissimum*) showed antimicrobial activity against all the tested bacterial strains with a ZI 10–26 mm.

The antimicrobial activity of the isolated endophytes was also evaluated by tube dilution method (Table 7). Only eight extracts (27%) displayed antimicrobial potential with the MIC value ranging from 10–100 μg mL$^{-1}$. In the present study, 24.13%, 13.8% and 10.3% extracts showed antimicrobial activity against *S. aureus*, *E. coli*, and *C. albicans* respectively. Out of eight active extracts, four were found to be active against *S. typhimurium*. Extracts of MC-14 L (*Fusarium oxysporum*), and MC-24 L (*Cladosporium tenuissimum*) showed more potent antimicrobial activity than streptomycin, whereas MC-18 L (*Aspergillus fumigatus*) and streptomycin showed equal antimicrobial potency against *S. typhimurium*. Similarly, Extracts of MC-14 L (*Fusarium oxysporum*), MC-14 F (*Fusarium oxysporum*) and streptomycin were found to be as active against *S. aureus*. MC-14 L (*Fusarium oxysporum*) extract inhibited the growth of all the pathogens used in the study (MIC/MBC 10–25 μg mL$^{-1}$) and was as active as streptomycin against *E. coli*. It inhibited *K. pneumoniae*, and *B. subtilis* with an MIC of 12.5 μg mL$^{-1}$. Extracts of MC-18 L (*Aspergillus fumigatus*) and MC-24 L (*Cladosporium tenuissimum*) showed a broad range of antibacterial activity as it inhibited the growth of all the bacterial pathogens used in the study (MIC/MBC 10–50 μg mL$^{-1}$). Extracts of MC-24 L (*Cladosporium tenuissimum*) inhibited *K. pneumoniae*, *S. aureus*, *E. coli*, and *P. aeruginosa* with an MIC of 12.5 μg mL$^{-1}$. Only three extracts MC-14 L (*Fusarium oxysporum*), MC-14 F (*Fusarium oxysporum*) and MC-16 L (*Neurospora sp.*) showed antimicrobial activity against *C. albicans* with an MIC value 25–100 μg mL$^{-1}$.

**Discussion**

Aromatic and medicinal plants, producers of ethnopharmacologically important secondary metabolites and essential oils being used in food preservation and in reducing the dose of antibiotic for the treatment of bacterial infections are the legitimate targets to isolate the endophytic fungi. These fungi are utilized in

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**Table 2** Tissue specific diversity indices of endophytic fungi isolated from *Monarda citriodora*

| Indices                | Leaves | Roots | Flowers |
|------------------------|--------|-------|---------|
| Taxa_S                 | 18     | 2     | 6       |
| Individuals            | 18     | 2     | 8       |
| Simpson’s Dominance_D  | 0.055  | 0.5   | 0.218   |
| Simpson_1-D            | 0.944  | 0.5   | 0.781   |
| Shannon_H              | 2.890  | 0.693 | 1.667   |
| Evenness_e^H/S         | 1      | 1     | 0.883   |
| Brilloquin             | 2.022  | 0.346 | 1.102   |
| Menhinick richness index| 4.243  | 1.414 | 2.121   |
| Margalef richness index| 5.882  | 1.443 | 2.404   |
| Equitability_J         | 1      | 1     | 0.930   |
| Fisher_alpha diversity index| 0  | 0    | 10.91   |
| Berger-Parker dominance| 0.055  | 0.5   | 0.375   |
| Chao-1                 | 171    | 3     | 16      |

**Table 3** Jaccard’s (Jc) and Sorensen’s similarity indices for endophytic fungi of *Monarda citriodora*

| Jaccard’s index/ Sorensen’s index | Leaf | root | Flower |
|-----------------------------------|------|------|--------|
| Leaf                              | 1.0  | 0.1  | 0.16   |
| Root                              | 0.05 | 1.0  | 0.0    |
| Flower                            | 0.09 | 0.0  | 1.0    |
management of plant diseases. This is the first report on
the diversity, phylogeny and bioactive potential of endo-
phytic fungi associated with *M. citriodora*, an aromatic
and medicinal plant. All the fungal endophytes recov-
ered in the present study represented the phylum Asco-
ymycota, which is one of the most diverse and ubiquitous
phyla of eukaryotes covering approximately 8% of the
Earth’s landmasses [40, 41]. The most prevalent class of
fungi was Sordariomycetes followed by Eurotiomycetes
and Dothideomycetes. The endophytes of *M. citriodora*
were compared with the endophytes of *Ocimum sanctum*
because they both belong to same family and both are
aromatic plants. Endophytes associated with *Oci-



| S.No. | Tissue Cell line type | Conc (μg mL⁻¹) | Percent Growth inhibition |
|---|---|---|---|
| 1 | MC-1 L | 100 | A549: 13 ± 0.8, 0 ± 0; HCT116: 13 ± 0.3, 16 ± 0.5 |
| 2 | MC-2 L | 100 | 35 ± 1, 22 ± 0.3, 52 ± 0, 10 ± 0 |
| 3 | MC-3 L | 100 | 33 ± 0.5, 12 ± 0.4, 20 ± 0.4, 41 ± 0.4 |
| 4 | MC-4 L*** | 100 | 67 ± 1, 65 ± 0.2, 91 ± 0.2, 79 ± 0.3 |
| 5 | MC-5 L | 100 | 0 ± 0, 47 ± 0, 76 ± 0, 51 ± 0.4 |
| 6 | MC-6 L | 100 | 27 ± 0.5, 35 ± 0.5, 54 ± 0.5, 56 ± 0.4 |
| 7 | MC-8 L*** | 100 | 98 ± 1, 99 ± 0.4, 94 ± 0.4, 95 ± 0.4 |
| 8 | MC-9 L | 100 | 22 ± 0.8, 10 ± 0.2, 14 ± 0.3, 48 ± 0.2 |
| 9 | MC-10 L | 100 | 47 ± 0, 18 ± 0.4, 45 ± 0.4, 49 ± 0 |
| 10 | MC-12 L | 100 | 34 ± 1, 29 ± 0.6, 37 ± 0.2, 58 ± 0 |
| 11 | MC-14 L*** | 100 | 96 ± 1, 100 ± 0.2, 94 ± 0.3, 100 ± 0.2 |
| 12 | MC-15 L | 100 | 24 ± 0.8, 51 ± 0.8, 86 ± 0, 59 ± 0.5 |
| 13 | MC-16 L*** | 100 | 59 ± 0, 47 ± 0.2, 88 ± 0.2, 61 ± 0.2 |
| 14 | MC-17 L | 100 | 48 ± 1, 23 ± 0, 23 ± 0.5, 25 ± 0 |
| 15 | MC-18 L*** | 100 | 100 ± 0, 93 ± 0.5, 100 ± 0.2, 90 ± 0.2 |
| 16 | MC-20 L*** | 100 | 77 ± 1, 82 ± 0.5, 77 ± 0.2, 63 ± 0.5 |
| 17 | MC-24 L*** | 100 | 77 ± 1, 99 ± 0.4, 88 ± 0.4, 96 ± 0 |
| 18 | MC-25 L*** | 100 | 88 ± 1, 100 ± 0.2, 94 ± 0.3, 99 ± 0.4 |
| 19 | MC-13R | 100 | 40 ± 1, 20 ± 0.6, 35 ± 0.2, 50 ± 0.5 |
| 20 | MC-20R | 100 | 10 ± 1, 1 ± 0.4, 26 ± 0.2, 5 ± 0 |
| 21 | MC-7 F*** | 100 | 83 ± 1, 87 ± 0, 92 ± 0.5, 74 ± 0 |
| 22 | MC-14 F*** | 100 | 99 ± 0, 100 ± 0.4, 91 ± 0.5, 100 ± 0 |
| 23 | MC-17 F | 100 | 53 ± 1, 28 ± 0, 0 ± 0, 7 ± 0 |
| 24 | MC-21 F | 100 | 0 ± 0, 8 ± 0.3, 11 ± 0.3, 17 ± 0 |
| 25 | MC-22 F*** | 100 | 84 ± 1, 75 ± 0.3, 88 ± 0.4, 80 ± 0.2 |
| 26 | MC-23 F | 100 | 0 ± 0, 9 ± 0.2, 0 ± 0, 33 ± 0.4 |
| 27 | MC-25 F | 100 | 45 ± 1, 90 ± 0.2, 77 ± 0, 81 ± 0.6 |
| 28 | MC-26 F | 100 | 94 ± 0, 87 ± 0.5, 97 ± 0.5, 91 ± 0.2 |
| | Paclitaxel | 1 μM | 79 ± 1 |
| | 5-Fluorouracil | 20 μM | 45 ± 0 |
| | Adriamycin | 1 μM | 46 ± 0.7 |
| | Mitomycin | 1 μM | 63 ± 0 |

*** Highly Significant at *P* < 0.05
suggesting that distinct micro-environments of tissues are responsible for shaping their micro-biota differently. Thus, tissue specificity was evident among the endophytes of the *M. citriodora*. Leaves supported the wider diversity of endophytes followed by flowers and roots. This might be because of greater surface area of leaves exposed to outer environment. These results well corroborate with the previous studies of endophytic fungi isolation on various Indian medicinal plants [42, 43].

In present study, dark septate fungi such as *Curvularia aeria* and *Alternaria alternata* were isolated from leaves contrary to their common habitat i.e. root tissue [44, 45].

For searching the safer and novel drug based on natural product, endophytic extracts were screened for cytotoxic and antibacterial activity. Nearly 72% of the extracts showed cytotoxic activity against one of the tested cell lines while 39% of the extracts were active against all the tested cell lines with >50% growth inhibition. $IC_{50} < 20 \mu g mL^{-1}$ (cytotoxicity criteria given by National Cancer Institute for the screening the crude plant extracts) was shown by 25% of extracts [46]. In comparison, Carvalho et al. [47] have reported that three out of sixteen endophytic extracts isolated from the plant *Strzyphonindron adstringens* (Mart.) Coville (Fabaceae), had $IC_{50} 20 \mu g mL^{-1}$ against MCF-7 cell line, whereas Zhao et al. [48] ascertained the cytotoxicity of the extract of endophyte *Hypocreila lixii* R18 ($IC_{50} = 29.8 \mu g mL^{-1}$) against PC-3 cell line. This was found to be less cytotoxic than four extracts [MC-14 L (*Fusarium oxysporum*), MC-24 L (*Cladosporium tenuissimum*), MC-25 L (*Fusarium oxysporum*), MC-26 F (*Fusarium oxysporum*)] used in the present study against the same cell line.

Overall MC-8 L (*Aspergillus oryzae*), MC-14 L (*Fusarium oxysporum*), MC-18 L (*Aspergillus fumigatus*), MC-24 L (*Cladosporium tenuissimum*), MC-25 L (*Fusarium oxysporum*), MC-14 F (*Fusarium oxysporum*), MC-26 F (*Fusarium oxysporum*) extracts were found most promising in terms of cytotoxic activity ($IC_{50} < 10 \mu g mL^{-1}$) against different human cancer cell lines.

The cytotoxic molecules beavvericin ($IC_{50} = 1.42 \mu g mL^{-1}$) and bikaverin ($IC_{50} = 0.161 \mu g mL^{-1}$) were isolated from an endophytic *Fusarium oxysporum* [49]. Till now approximately more than 500 compounds have been isolated from *Fusarium* genera. Out of which, 26 compounds have cytotoxic activity. On the basis of natural product dictionary records, only one cytotoxic molecule is known from *Fusarium oxysporum* while no cytotoxic molecule is reported from *Cladosporium tenuissimum*. Thus there is lot of scope of isolating new cytotoxic compounds from these endophytes [MC-14 L (*Fusarium oxysporum*), MC-24 L (*Cladosporium tenuissimum*).

The antimicrobial potential of the endophytes isolated from *M. citriodora* was investigated against human pathogens by agar disc-diffusion and tube dilution methods. About 75% extracts showed antimicrobial activity against the tested pathogens with a zone of inhibition (ZI) ranging from 6-35 mm which is a higher percentage in comparison to 48.3% active endophytic bacterial extracts isolated from *Aloe vera* with the ZI 6–12 mm [50]. This indicates that endophytes from *M. citriodora* have better antimicrobial potential than endophytic bacteria from *Aloe vera*. In present study, 50% and 25%, extracts showed antimicrobial activity against *S. aureus* and *E. coli* respectively. This is in contrast to Guimaraes et al. [51] who screened 39 endophytic fungal extracts and found that 5.1%, 25.6% and 64% extracts to be active against *S. aureus*, *E. coli* and *C. albicans* respectively. Hazalin et al. [52] screened 300 endophytic extracts against human pathogens, out of which only 8% extracts were active. Wiyakrutta et al. [53] also screened 360 endophytic extracts against *Mycobacterium tuberculosis* and found 90 (25%) extracts to be active. The highest

### Table 5 Anticancer activity ($IC_{50} (\mu g mL^{-1})$) of the extracts prepared from endophytic fungi isolated from *M. citriodora* against different human cancer cell lines

| S.NO. | Cell line type | IC50 (\(\mu g mL^{-1}\)) |
|-------|---------------|---------------------------|
|       | Tissue Code   | Colon                     | Lung          | Prostate     | Breast       |
| 1.    | MC-8 L        | 38.0 ± 0.08             | 24.3 ± 0.03   | 26.4 ± 0.06  | 19.7 ± 0.1   |
| 2.    | MC-14 L       | <10 ± 0.0               | <10 ± 0.0     | <10 ± 0.0    | <10 ± 0.0    |
| 3.    | MC-18 L       | 9.0 ± 0.02              | 4.8 ± 0.02    | 27.7 ± 0.06  | 19.6 ± 0.02  |
| 4.    | MC-24 L       | <10 ± 0.0               | <10 ± 0.0     | <10 ± 0.0    | <10 ± 0.0    |
| 5.    | MC-25 L       | <10 ± 0.0               | <10 ± 0.0     | 9.9 ± 0.02   | 12.7 ± 0.02  |
| 6.    | MC-7 F        | 32.2 ± 0.2              | 23.6 ± 0.02   | 32.3 ± 0.04  | 26 ± 0.04    |
| 7.    | MC-14 F       | 62.8 ± 0.5              | <10 ± 0.0     | 23.3 ± 0.02  | 42.7 ± 0.3   |
| 8.    | MC-26 F       | 11.1 ± 0.01             | <10 ± 0.0     | 11.7 ± 0.01  | 8.0 ± 0.02   |

Paclitaxel in nM: 120 ± 0.2, <10 ± 0.0, 65 ± 0.3, 777 ± 0.1

*Note: same letters as superscript depicts significant difference at \(P < 0.05\) between \(IC_{50}\) values of two cell lines (Additional file 1: Figure S2)*

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number of active extracts (50%) against *S. aureus* in the present study indicates that endophytic fungi isolated from *M. citriodora* might be used to produce potent antimicrobial compounds.

The antimicrobial potential of the endophytic extracts was evaluated by tube dilution method. Only eight extracts (27%) displayed antimicrobial activity against the tested human pathogens with an MIC value ranging from $10^{-1}$ to $100 \mu g L^{-1}$. On comparing the two methods, it was found that upon using agar disc-diffusion, twenty one extracts were found to be active, whereas by using tube dilution method only eight extracts were found to be active with MIC value $\leq 100 \mu g L^{-1}$. Extract of MC-14 L (*Fusarium oxysporum*), which displayed ZI between 11–15 mm, showed better antimicrobial activity with an MIC/MBC value 12.5–25 $\mu g L^{-1}$ against all the tested pathogens, whereas MC-18 L (*Aspergillus fumigatus*), which displayed better ZI (24–35 mm), showed little bit lower MIC value (12.5–50 $\mu g L^{-1}$). Results suggest that difference in antimicrobial potency may be because a more diffusible but less active extract may give a bigger zone of inhibition than a non-diffusible but more active extract.

In present study, extracts inhibited the human pathogens with an MIC value of 12.5 $\mu g L^{-1}$ whereas in another study crude extracts of endophytes *Papulaspora immersa* and *Arthrinium arundinonis* isolated from *Smallanthus sonchifolius* inhibited

### Table 6 Zone of inhibition (mm) of the extracts prepared from endophytic fungi isolated from *M. citriodora* against different human pathogens

| Endophyte   | K. pneumoniae | S. aureus | E. coli | *P. aeruginosa* | S. typhimurium | B. subtilis | C. albicans |
|-------------|---------------|-----------|---------|-----------------|----------------|-------------|-------------|
| MC-1 L      | -             | -         | -       | -               | -              | -           | -           |
| MC-2 L***   | 10 ± 0.2      | 21 ± 0.2  | 22 ± 0.3| 21 ± 0          | 22 ± 0.2       | 23 ± 0.2    | -           |
| MC-3 L      | -             | -         | -       | -               | -              | -           | -           |
| MC-4 L      | -             | 12 ± 0.6  | 14 ± 0.5| 12 ± 0.2        | 15 ± 0         | 10 ± 0.2    | 6 ± 0.4     |
| MC-5 L      | 10 ± 0.3      | -         | -       | -               | -              | -           | -           |
| MC-6 L      | -             | -         | -       | -               | -              | -           | -           |
| MC-8 L      | -             | 9 ± 0.5   | -       | 10 ± 0.3        | -              | -           | -           |
| MC-9 L      | -             | -         | -       | 25 ± 0.5        | -              | -           | -           |
| MC-10 L     | -             | 15 ± 0.3  | -       | -               | 10 ± 0         | -           | -           |
| MC-12 L     | 15 ± 0.3      | -         | 14 ± 0.2| 12 ± 0.3        | 16 ± 0.3       | -           | 27.5 ± 0.2  |
| MC-14 L     | 13 ± 0.5      | 12 ± 0.2  | 13 ± 0.6| 12 ± 0          | 15 ± 0.3       | 11 ± 0.5    | 11 ± 0.2    |
| MC-15 L     | -             | 14 ± 0.5  | -       | -               | -              | -           | -           |
| MC-16 L     | -             | 17 ± 0    | 30      | 12 ± 0.2        | 18 ± 0.5       | 10 ± 0      | -           |
| MC-17 L     | -             | -         | -       | -               | -              | -           | 10 ± 0      |
| MC-18 L***  | 35 ± 1        | 24 ± 0    | 35 ± 0.3| 28 ± 0.5        | 32 ± 0.2       | 27 ± 0.3    | 9 ± 1       |
| MC-20 L     | -             | -         | -       | -               | -              | -           | -           |
| MC-24 L     | 10 ± 0.2      | 22 ± 1    | 17 ± 0.2| 26 ± 0.5        | 17 ± 0.4       | 15 ± 0      | -           |
| MC-25 L     | -             | -         | -       | -               | -              | 10 ± 0.4    | -           |
| MC-13R      | -             | 10 ± 0.2  | -       | -               | -              | 10 ± 0.7    | -           |
| MC-20R      | -             | 10 ± 0.3  | -       | 10 ± 0.4        | -              | -           | -           |
| MC-7 F      | -             | -         | -       | 12 ± 0.4        | -              | -           | -           |
| MC-14 F     | -             | 12 ± 0.4  | -       | 10 ± 0.3        | -              | -           | 9 ± 1       |
| MC-17 F     | -             | 15 ± 0.4  | -       | -               | -              | -           | -           |
| MC-21 F     | -             | -         | -       | -               | -              | -           | -           |
| MC-22 F     | -             | -         | -       | 17 ± 0.4        | 13 ± 0.2       | -           | -           |
| MC-23 F     | -             | 16 ± 0    | 22 ± 0.3| -               | 12 ± 0.5       | -           | -           |
| MC-25 F     | -             | -         | -       | -               | -              | -           | -           |
| MC-26 F     | -             | -         | -       | -               | -              | -           | -           |
| Streptomycin | 22.5 ± 0.4    | 18 ± 0.5  | 18.7 ± 0| 25 ± 0.2        | 20 ± 0.5       | 18 ± 0.7    | -           |
| Amphotericin B | -         | -               | -       | -               | 24.6 ± 0.2     | -           | -           |

*** Highly Significant at $P < 0.05$
the human pathogens with an MIC value of 90–280 μg mL⁻¹ [54]. Akinsanya et al. [50] reported that the MIC and MBC values of the endophytic bacterial extracts isolated from Aloe vera range from 0.625 to 20 mg mL⁻¹ against some of the bacterial indicators, which is quite low when compared to the present study. In another study, two endophytic extracts were found to be active against Microsporum gypseum with an MIC < 12.5 μg mL⁻¹ [55].

Present study along with previous studies have indicated that Fusarium spp. are the most dominant species among endophytes producing bioactive compounds such as pentaketide (CR377: 2-methylbutyraldehyde-substituted-α-pyrone), beauvericin, subglutinol A and B [56–58]. These molecules were found active against C. albicans, and methicillin-resistant S. aureus. The Fusarium spp. were isolated from Selaginella pallescens, Cinnamomum kanehirae, Tripterygium wilfordii plants respectively [56–58].

In terms of antimicrobial activities extracts of MC-14 L (Fusarium oxysporum), MC-18 L (Aspergillus fumigatus), MC-24 L (Cladosporium tenuissimum) and MC-14 F (Fusarium oxysporum) were found promising. Only three antibacterial and 5 antifungal molecules are known from Fusarium oxysporum while no antibacterial and antifungal molecule is known from Cladosporium tenuissimum. Thus these endophytes [MC-14 L (Fusarium oxysporum), MC-24 L (Cladosporium tenuissimum)] could be a source of new antimicrobial compounds.

### Table 7 Antimicrobial activity (MIC/MBC values in μg mL⁻¹) of the extracts prepared from endophytic fungi isolated from M. citriodora against different human pathogens

| Endophyte | K. pneumoniae | S. aureus | E. coli | P. aeruginosa | S. typhimurium | B. subtilis | C. albicans |
|-----------|---------------|-----------|---------|---------------|----------------|------------|-------------|
| MC-1 L    | -             | -         | -       | -             | -              | -          | -           |
| MC-2 L    | >100          | 25        | 100     | 100           | 100            | >100       | -           |
| MC-3 L    | -             | -         | -       | -             | -              | -          | -           |
| MC-4 L    | -             | >100      | >100    | >100          | >100           | >100       | -           |
| MC-5 L    | >100          | -         | -       | -             | -              | -          | -           |
| MC-6 L    | -             | -         | -       | -             | -              | -          | -           |
| MC-8 L    | -             | 100       | -       | 100           | -              | -          | -           |
| MC-9 L    | -             | -         | >100    | -             | -              | -          | -           |
| MC-10 L   | -             | >100      | -       | >100          | >100           | -          | -           |
| MC-12 L   | >100          | -         | >100    | >100          | >100           | -          | -           |
| MC-14 L   | 12.5          | 12.5 (MBC) | 12.5  (MBC) | 25 (MBC) | 12.5 (MBC) | 12.5  | 25          |
| MC-15 L   | -             | >100      | -       | -             | -              | -          | -           |
| MC-16 L   | -             | 100       | >100    | >100          | >100           | >100       | 100         |
| MC-17 L   | -             | -         | -       | -             | -              | -          | -           |
| MC-18 L   | 50            | 25        | 25      | 25            | 12.5           | 12.5       | >100        |
| MC-20 L   | -             | -         | -       | -             | -              | -          | -           |
| MC-24 L   | 12.5          | 12.5      | 12.5    | 12.5          | 12.5 (MBC)     | 25         | -           |
| MC-25 L   | -             | -         | -       | -             | -              | >100       | -           |
| MC-13 R   | -             | >100      | -       | -             | -              | >100       | -           |
| MC-20 R   | -             | >100      | -       | >100          | -              | -          | -           |
| MC-7 F    | -             | -         | -       | >100          | -              | -          | -           |
| MC-14 F   | -             | 12.5 (MBC)| -       | 25            | -              | -          | 25          |
| MC-17 F   | -             | >100      | -       | -             | -              | -          | -           |
| MC-21 F   | -             | -         | -       | -             | -              | -          | -           |
| MC-22 F   | -             | -         | -       | 100           | >100           | -          | -           |
| MC-23 F   | -             | >100      | >100    | -             | >100           | -          | -           |
| MC-25 F   | -             | -         | -       | -             | -              | -          | -           |
| MC-26 F   | -             | -         | -       | -             | -              | -          | -           |
| Streptomycin | 12.5 (MBC)   | 12.5 (MBC)| 12.5  (MBC) | 12.5 (MBC) | 12.5 (MBC) | 12.5 |
| Amphotericin | 12.5 (MBC)   | 12.5 (MBC)| 12.5  (MBC) | 12.5 (MBC) | 12.5 (MBC) | 6.25(MBC)|
Conclusion
In conclusion, this is the first report of diversity of fungal endophytes isolated from *M. citriodora*. These results indicated that *M. citriodora* harbors a rich fungal endophytic community with anticancer and antimicrobial activities. Extracts of MC-14 L (*Fusarium oxysporum*) and MC-24 L (*Cladosporium tenuissimum*) were found to have potent cytotoxic and antimicrobial properties thus could be a source of novel cytotoxic/antimicrobial compounds highlighting their potential use in the development of drugs (anti-cancer/antimicrobial), but needs to be further investigated at molecular and mechanistic level.

Additional file

**Table S1.** Morphological characteristics of endophytes isolated from *Monarda citriodora*. Figure S1. Microscopic images of endophytes isolated from *Monarda citriodora*. L. The ITS sequences from endophytic isolates are available via the following links: http://www.ncbi.nlm.nih.gov/nucleotide/KUJS27781; KUJE68345; KUJE68346. Rest of raw datasets of bioactivity used and/or analysed during the current study can be available from the corresponding author on reasonable request. (DOC 2192 kb)

Abbreviations

K_{50}: Concentration of extract at which 50% growth inhibition of cell line occurs; MBC: Minimum bactericidal concentration; MIC: Minimum inhibitory concentration; MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; ZI: Zone of inhibition.

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Availability of data and materials

Most of the data generated or analysed during this study are included in this published article and its Additional file 1.

Authors’ contributions

MK designed the study, analyzed the data and written the research paper. SP carried out the isolation and molecular characterization of endophytes, fermentation, extraction and the antimicrobial experiments. SV carried out the anticancer experiments while SKS analyzed the anticancer data. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

*“Not applicable”.*

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References

1. Strobel GA, Daisy B. Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev. 2003;67:491–502.

2. Lu C, Shen Y. Harnessing the potential of chemical defenses from antimicrobial activities. BioEssays. 2004;26:808–13.

3. Liu J, Wang Z, Zhou S. Response of endophytic bacterial communities in banana tissue culture plantlets to Fusarium wilt pathogen infection. J Gen Appl Microbiol. 2008;54:83–92.

4. Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. J Nat Prod. 2007;70:461–77.

5. Koehn FE, Carter GT. The evolving role of natural products in drug discovery. Nat Rev Drug Discov. 2003;2:206–20.

6. Derman AL. Pharmacologically active secondary metabolites of microorganisms. Appl Microbiol Biotechnol. 1999;52:455–63.

7. Tan RX, Zou WX. Endophytes a rich source of functional metabolites. Nat Prod Rep. 2001;18:448–59.

8. Staniek A, Woerdeman HG, Kuyser O. Endophytes: exploring biodiversity for the improvement of natural product based drug discovery. J Plant Interact. 2008;3:75–93.

9. Strobel GA. Endophytes as sources of bioactive products. Microbes infect. 2003;5:535–44.

10. Clardy J, Walsh C. Lessons from natural molecules. Nature. 2004;432:829–37.

11. Stiele A, Strobel GA, Stiele D. Taxol and taxane production by Taxomyces andreanum, an endophytic fungus of Pacific yew. Science. 1993;262:214–6.

12. Pizari P, Parkin DM, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in 1990 Internationally. J Cancer. 1999;83:18–29.

13. Petersen PJ, Wang TZ, Dushin RG, Bradford PA. Comparative in vitro activities of AC98-6446, a novel semisynthetic glycopeptidolide derivative of the natural product mannopeptimycin alpha, and other antimicrobial agents against gram-positive clinical isolates. Antimicrob Agents Chemother. 2004;48:79–46.

14. Pupo MT, Guimaraes DO, Furtado NAJC, Borges WS. Microbial natural products: a promising source of bioactive compounds. In: Taft CA, editor. Modern Biotechnology in Medicinal Chemistry and Industry. Kerala: Research Signpost; 2006. p. 51–78.

15. Bailey LH. Manual of cultivated plants. New York: Macmillan; 1977.

16. Duke JA. Farmacia verde, ed. All, Bucuresti, 2007.

17. Collins JE, Bishop CD. Evaluation of *Monarda citriodora* var citriodora as an alternative Western European non-food oil crop. In: Proceedings of the Second European symposium on Industrial Crops and Products Pisa, Italy; 1993.

18. Scora R. Study of the essential leaf oils of the genus *Monarda* (*Labiatae*). Amer J Bot. 1967;54:466–52.

19. Collins JE, Bishop CD. Evaluation of *Monarda citriodora* var citriodora and *Pelargonium* citridorsum (*Labiatae*) for use as an essential oil source. J Essent Oil Res. 1999;11:299–304.

20. Bishop CD, Thornton IB. Evaluation of the antifungal activity of the essential oil of *Monarda citriodora* var citriodora. J Essent Oil Res. 2008;3:75–89.

21. Bishop CD, Thornton IB. Evaluation of the fungitoxic activity of *Monarda citriodora* var citriodora grown in the United Kingdom. J Essent Oil Res. 1994;6:27–9.

22. Zhan Guo L, Xu HL, Wei L. Chemical Composition of Antibacterial Activity of Essential Oil from *Monarda citriodora* Flowers. Adv Med Res. 2011;183:185–90.

23. Collins JE, Bishop CD, Deans SG, Svoboda KP. Composition of the essential oil from the leaves and flowers of *Monarda citriodora* var citriodora grown in the United Kingdom. J Essent Oil Res. 1994;6:27–9.

24. Pathania AS, Guru SK, Verma MK, Sharma C, Abdullah ST, Malik F, Chandra S, Katoh M, Bhushan S. Disruption of the PI3K/AKT/mTOR signaling cascade and induction of apoptosis in HL-60 cells by an essential oil from *Monarda citriodora*. Food Chem Toxicol. 2013;62:246–54.

25. Baeder U, Broda P. Rapid preparation of DNA from filamentous fungi. Lett Appl Microbiol. 1985;1:17–20.

26. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T, editors. PCR protocols: a guide to methods and applications. San Diego: Academic; 1990. p. 315–22.
27. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997;25:3389–402.

28. Tamura K, Dudley J, Nei M, Kumar S. MEAGAR: Molecular Evolutionary Genomics Analysis (MEGA) software version 4.0. Mol. Bio. Evol. 2007;24:1569–9.

29. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004;32:1792–8.

30. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406–25.

31. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA. 2004;101:11305–10.

32. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 1985;39:783–91.

33. Suryanarayanan TS, Kuresvan TS. Endophytic fungi of some halophytes from an estuarine mangrove forest. Mycol Res. 2000;104:1465–7.

34. Hoffman M, Gunatilaka A, Ong J, Shimabukuro M, Arnold AE. Molecular analysis reveals a distinctive fungal endophyte community associated with foliage of Montane oaks in southeastern Arizona. J Ariz Nev Acad Sci. 2008;3:907–10.

35. Tao G, Liu ZY, Hyde KD, Liu XZ, Yu XN. Whole rDNA analysis reveals novel endophytic fungi in Bletilla Ochracea (Orchidaceae). Fungal Divers. 2008;33:101–22.

36. Kusari P, Kusari S, Spittler M, Kayser O. Endophytic fungi harbored in Canna sativa: diversity and potential as biocontrol agents against host plant specific phytopathogens. Fungal Divers. 2013;60:137–51.

37. Osono T, Moti A. Distribution of philosphere fungi within the capacity of giant dogwood. Mycoscience. 2004;45:161–8.

38. McFarland J. Standardization of bacterial culture for the disc diffusion assay. J Amer Med Assoc. 1987;49:1176–7.

39. Rios JL, Recio MC, Villar A. Screening methods for natural products with antimicrobial activity: a review of the literature. J Ethnopharmacol. 1988;23:127–49.

40. Schol CL, Sunji GH, Lopez GF, Townsend JP, Mladkoveska J, et al. The Ascomycota Tree of life: A phyllum wide Phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Syst Biol. 2009;58:234–39.

41. Chowdhary K, Kaushik N. Fungal endophyte diversity and bioactivity in the Indian Medicinal plant Ocimum sanctum L. PlosOne in press 2015; doi:10.1371/journal.pone.0141444.

42. Kaul S, Ahmed M, Zargar K, Sharma P, Dhar MK. Prospecting endophytic fungal assemblage of Digitalis lanata Ethn. (Foxglove) as a novel source of digitoxin: a cardiac glycoside. Biotech. 2013;3:335–40.

43. Mishra A, Gond SK, Kumar A, Sharma VK, Verma SK, Khawar RN, et al. Season and tissue type affect fungal endophyte communities of the Indian medicinal plant Tinospora cordifolia more strongly than geographic location. Microb Ecol. 2012;64:388–98.

44. Reininger V, Gruning C, Seiber TN. Host species and strain combination determine growth reduction of spruce and birch seedlings colonized by root associated dark septate endophytes. Environ Microbiol. 2012;14:1064–76.

45. Yuan Z, Zhang C, Lin F, Kubicek CP. Identity, diversity and molecular phylogeny of the endophytic mycobiota in rare wild rice roots (Oryza granulata) from a nature reserve in Yunnan, China. Appl Environ Microbiol. 2010;76(5):1642–52.

46. Lee CC, Houghton P. Cytoxicity of plants from Malaysia and Thailand used traditionally to treat cancer. J Ethnopharmacol. 2005;100:237–43.

47. Carvalho CR, Goncalves VN, Pereira CB, Johann S, Galliza IV, Alves TMA, Canniabis sativa (Mart.) Coville adstringens. Environ Microbiol. 2004;76(5):1642–52.

48. Zhao J, Li C, Wang W, Zhao C, Luo M, Mu F, Fu Y, Yu Y, Yao M. Hypocea lixia, novel endophytic fungi producing anticoagulant agent, isolated from pigeon pea (Cajanus cajan [L.] Millsp.). J Appl Microbiol. 2013;115:102–113.

49. Zhan J, Burns AM, Liu MX, Faeth SH, Leslie GAA. Search for cell motility and angiogenesis inhibitors with potential anticoagulant activity: beetle venom and other constituents of two endophytic strains of Fusarium oxysporum. J Nat Prod. 2007;70:1227–32.

50. Akinanya MA, Goh JK, Lim SP, Ting ASY. Diversity, antimicrobial and antioxidant activities of cultivable bacterial endophyte communities in Aloe vera. FEMS Microbiol Lett. 2015;362:fnv184.