Original Article

Evaluation of Bioactive Metabolites Isolated from Endophytic Fungus *Chaetomium cupreum* of the Plant *Mussaenda luteola*

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ABSTRACT

Background: Endophytic fungi are one of the most promising resources of natural bioactive products. In the present study, secondary metabolites were isolated from the endophytic fungus *Chaetomium cupreum* and evaluated *in vitro* for pharmaceutical potentials. **Objective:** To characterize the secondary metabolites obtained from the endophytic fungus *Chaetomium cupreum* from the plant *Mussaenda luteola* and to determine its antioxidant, cytotoxicity and anti-mycobacterial activities. **Methods:** Three secondary metabolites were isolated by column chromatography and solvent-solvent fractionation method from the ethyl acetate and methanol extract of *C. cupreum.* The structures were elucidated by spectroscopic studies includes FT-IR, NMR, MS analysis. Anti-mycobacterial activity of metabolites was determined by Microtitre Plate Alamar Blue Assay (MABA) against *Mycobacterium tuberculosis* H37Rv (ATCC27294) and also evaluated the antioxidant potentials using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay. The cytotoxicity test was performed against breast cancer cell line MCF-7 using MTT assay. **Results:** Three secondary metabolites were characterized as 6- (heptacosa- 18' Z enyl)- 2- (- 18'' hydroxyl- 1'' enyl- 19'' oxy)- 3 hydroxy benzoquinone (**1**), (3β –5α– dihydroxy –6β– phenyl acetyloxy – ergosta –7, 22 – diene) (**2**) and 2-dodecanol (**3**). The results of bio-assays revealed that the compound (**1**) and (**2**) exhibit significant inhibition of mycobacterium with MIC of 6.25μg/ml and 25μg/ml respectively, which is similar to the standard streptomycin drug. It also exhibit good DPPH scavenging potential of 72.07 ± 1.95 and 71.63 ± 1.40 % respectively. The cytotoxicity was tested against breast cancer cell line MCF-7 and found compound **1** and **2** had cytotoxicity of 52% and 49% respectively at 100μg/mL. **Conclusion:** The present study indicated that the isolated secondary metabolites from the endophytic fungus *C. cupreum* could be a potential lead molecule of antimicrobial agents.

Key words: Endophytic fungi, *Chaetomium cupreum,* Secondary metabolites, Antimycobacterial, Antioxidant.

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INTRODUCTION

The impact of natural product and natural product's chemistry has astonishing role in the process of drug discovery. The history of using natural sources for treatment of various health ailments has started long back.[1] It has been reported almost more than fifty percentages of the drugs using clinically were obtained from natural products. From 2005 to 2007 alone, a total of 13 drugs with natural origin were approved. Many of the compounds are current in clinical trial were obtained from plant, animal and microbial sources. These chemically diverse compounds serve as a drug for wide range of diseases like cancer, diabetes, inflammation, asthma, arthritis, ulcer, obesity, immunological disorders, infections and many others.[2]

Endophytes are currently known as a prominent source of therapeutically valuable natural products as it inhabits millions of higher plants in different geographic

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locations.[3] Endophytes may synthesize the metabolites similar to that of host plants which plays an important role in ecological aspect and also in education and industrial perspective.[4] Among all microorganisms, fungi are the most commonly isolated endophytes that are associated with host plant interactions and exhibit symbiosis.[5] The relationship between host plants and endophytes are complex but yet mutualism or commensalism i.e. both the organisms gets benefit from the relationship. The endophytic fungi acquire benefit by nutrient uptake, gaining of energy, shelter and protection from the host plant. The plants also obtain benefits from the endophytic fungi since it produce different bioactive metabolites and enzymes that play a vital role in plant growth and tolerance to various stresses.[6] The rich diversity of endophytes housed by plants in different ecosystems and synthesis of diverse fungal secondary metabolites creates a way for new drug discovery.[7] Endophytic fungi afford wide group of active compounds in which 51% were unidentified earlier.[8] The various metabolic pathways like polyketide pathway, amino acid and isoprenoid derivation are involved in the production of secondary metabolites. A survey reported that the novel compounds produced by endophytic fungi comprises of 51% which is comparatively greater than soil fungus that was 38% thus concludes the endophytes are the predominant source for bioactive compounds. Endophytes, rather than using as an alternate for producing plant metabolites, it is also known for broad variety of novel chemicals which gains more interest among chemists, microbiologist and ecologist.[5] *Chaetomium* is the prevalent genera of saprophytic ascomycetes comprising of more than 300 species universally. It is most frequently found in soil and exhibit inhibition against phyto pathogens. Also it is present in dung, straw, paper, bird feathers, seeds, plant debris, soil and air.[9] *Chaetomium* sp. is one among the plant fungal endophytes, which synthesis the novel metabolites such as xanthones, anthraquinones, depsidones, chaetoglobosins, rubrorotiorin, benzoquinone derivatives, azaphilones and also these compounds exhibits the significant pharmacological potentials like antibacterial antimalarial, anticancer, antioxidant and many others.[10,11] The genus *Mussaenda* comprises of more than 200 species, were distributed in tropical countries, south-east Asia and southern China.[12] *M. luteola* is an ornamental plant and also possess ethnopharmacological potentials which have been used in Chinese folk medicine as antipyretic, anti-inflammatory and antimicrobial.[13] The plant has been reported to produce several bioactive metabolites like iridoids, rutin, quercetin, flavonol, flavonoids, anthocyanins, triterpenes, triterpenoid saponins and many more.[14,15] In the present study, we isolated secondary metabolites from the endophytic fungus *C. cupreum* and evaluated the antimycobacterial, antioxidant and anti-proliferative potential.

MATERIALS AND METHODS

General experimental procedures

UV-Vis spectral measurement was performed for the column fractions ($CHCl_3$) on a Shimadzu UV-2401 range between 200-800 nm at room temperature. NMR spectroscopic analysis of sample was performed using Bruker Avance 400 NMR spectrometer (operating at 400 MHz for ^1H and 100 MHz for ^{13}C, $CDCl_3$ as solvent). ESI-MS was analysed by using Micromass ZQTM 4000 mass spectrometer. FT-IR spectrum analysis was done in KBr pellets on a Shimadzu, FT-IR 8300 between 4000 and 400cm^{-1}. Column Chromatography (CC) was carried out on silica gel (60-120 mesh) and all solvents used for analysis and experiments were bought from Himedia chemicals.

Sample collection and isolation of endophytic fungus

The plant samples were collected from Vellore district, Tamil Nadu, India. The endophytic fungus was isolated from the surface sterilized leaves of *M. luteola* by imprint method.[16] The leaf segments were impregnated aseptically in Potato Dextrose Agar (PDA) plate added with antibiotic streptomycin (30µg/mL) and incubated at 30°C for 2 weeks. From the emerging cultures pure strains was isolated and identified as *Chaetomium cupreum* on the basis of colony morphology and 18s rRNA sequencing (GenBank ID: KY806554).

Cultivation

Mass cultivation of the fungus for the isolation of metabolites was carried out in 1 L Potato Dextrose Broth (PDB, Himedia, India) and kept for incubation at room temperature under static conditions for 30 days. Following cultivation, the culture was filtered to give the filtrate and mycelia.

Extraction and metabolite isolation

The filtrate of fungal broth was extracted with Ethyl cetate (EtOAc) and the fungal mycelium (mat) was extracted with Methanol (MeOH). The obtained ethyl acetate extract (1.267g) was fractionated by silica gel column chromatography (Chang *et al.* 2017) using $CHCl_3$: MeOH gradient elution (100:0; 80:20; 60:40; 40:60; 20:80; 0:100) to afford eighteen fractions (Fraction 1-18). The fractions were determined for maximum wavelength,

λ_{max} using UV–Vis spectrophotometer. The column fraction of 8, 9, 10 and 11 showed the similar TLC profile, so these fractions were pooled together and dried. The combined dried fraction was washed with diethyl ether and the soluble diethyl ether fraction was collected and evaporated to dryness to obtain compound 1 (51.63mg). The same above procedure was followed for mat methanol extract (1.127g) of *C. cupreum* and 12 fractions (Fraction 1-12) were obtained. The column fraction of 2, 3 and 4 showed similar TLC profile, so these fractions were combined and dried fraction was washed with hexane and the soluble hexane fraction was collected and evaporated to dryness to obtain compound 2 (24.26mg). The hexane insoluble fraction was dissolved with chloroform to retrieve compound 3 (44.56 mg). The isolated compounds were subjected for spectral studies for identification.

Bioassay Studies

Microplate Alamar blue assay

Anti-mycobacterial potential of isolated compounds were performed by microplate alamar blue assay.[17,18] using the virulent strain *M. tuberculosis* H37Rv (ATCC27294). Briefly, compounds were dissolved in DMSO and diluted with Middlebrook 7H9 broth supplemented with 0.2% (v/v) glycerol, 1.0 g of casitone per litre and 10% (v/v) OADC (Oleic acid albumin dextrose). Serial twofold dilutions of the sample were prepared directly in a sterile 96-well flat-bottom microtiter plate. The concentration range tested for the compound was 0.8 to 100µg/ml. All wells were added to each 100 µL volume of the inoculum except media well alone (control). Pyrazamide, ciprofloxacin and streptomycin were used as positive controls. The plate were sealed and kept for 7 days incubation at 37°C. After the period of incubation 30µL of resazurin reagent was added to each well and re-incubated the plate for overnight. The growth of bacteria was indicated by change of colour from blue to pink due to reduction. The lowest concentration in which the drug prevents the colour change is Minimum Inhibitory Concentration (MIC) of the tested compounds.

Cytotoxicity Assay against Breast Cancer Cell Line MCF-7

Cytotoxicity was tested against breast cancer cell line by MTT colorimetric assay.[19] Breast cancer cell line MCF-7 were cultured in Dulbecco's Modified Eagle's Medium added with 10% Fetal Bovine Serum and 10µg/mL streptomycin in a humidified CO_2 (5%) atmosphere at 37°C. The extract with various concentrations were seeded in 96-well plates comprising of a final volume of 100µL/ well and incubated for 24 hr. Afterwards, 10µL of MTT solution was added into the wells and incubated for 1-4 h at 37°C. The formation of formazan crystals the MTT solution was discarded and 100µL of solubilisation solution was then added to each well to dissolve formazan crystals. The absorbance was read at 570nm.

DPPH radical scavenging assay

Free radical scavenging potential of isolated compounds from endophytic fungus was evaluated using DPPH method as described.[20] The absorbance was measured at 517nm and ascorbic acid was used as a standard. Each analysis was done in triplicate.

RESULTS

The endophytic fungus *C. cupreum* was isolated from the leaves of *M. luteola*. The fungus was identified based on morphological and molecular studies. A crude ethyl acetate extract of *C. cupreum* was subjected to silica gel column to yield three compounds.

Compound (1): "6- (heptacosa- 18' Z enyl)- 2- (- 18'' hydroxyl- 1'' enyl- 19'' oxy)- 3 hydroxy benzoquinone" (Figure 1). Brownish yellow oily; HRESIMS m/z 911.079 (calcd for $C_{61}H_{112}O_4$, 909.539 g/mol) NMR spectrum data ($CDCl_3$, ^1H 400 and ^{13}C 100 MHz) see Table 1. FT-IR (KBr) ν_{max} signal bands were 3298, 2960, 2873, 1722, 1612, 1450, 1368, 1201, 1045, 947, 879, 790, 731, 574, 482 cm^1.

Compound (2): (3β–5α– Dihydroxy –6β– phenyl acetyloxy – ergosta –7, 22 – diene) (Figure 2) Oily yellow: EIMS: m/z 550.021 (calcd for $C_{36}H_{52}O_4$, 548.88 g/mol). ^{13}C NMR (100 MHz) and ^1H NMR (400 MHz) spectrum data showed in Table 2. FT-IR (KBr) ν_{max} signal bands were 3298, 2922, 2852, 1616, 1381, 1286, 1234, 1066, 916, 763, 705, 555, 484 cm^1.

Compound (3): 2- Dodecanol (Figure 3) was isolated as whitish oil and the molecular formula was proposed as $C_{12}H_{26}O$ based on HR-EIMS showing an ion at m/z 187.923 (calcd. for 186.340); ^{13}C NMR ($CDCl_3$, 100MHz) spectral data: δ 14.14 (C-1), δ 33.84 (C-2), δ 31.44 (C-3), δ 29.71 (C-4), δ 29.52 (C-5), δ 30.20 (C-6), δ

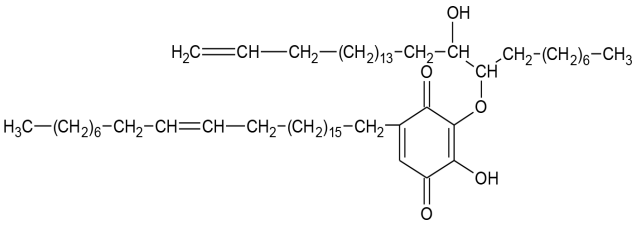

Figure1: Compound 1- "6- (heptacosa- 18' Z enyl)- 2- (- 18'' hydroxyl- 1'' enyl- 19'' oxy)- 3 hydroxy benzoquinone".

Table 1: NMR spectral data of compound 1 (CDCl3, 400 and 100 MHz).					
Position	**δ C NMR**	**δ H (J in Hz)**	**Position**	**δ C NMR**	**δ H (J in Hz)**
1	130.89 (C)	-	32	19.73 (CH_2)	1.202 -1.423*
2	128.81 (C)	-	33	14.19 (CH_3)	0.879
3	173.97 (C)	-	1'	123.99 (CH_2)	4.980
4	128.81 (CH)	5.359	2'	124.48 (CH)	5.345
5	130.89 (C)	-	3'	68.15 (CH_2)	2.328
6	174.38 (C)	-	4'	37.45 (CH_2)	1.626
7	68.18 (CH_2)	2.347	5'	34.12 (CH_2)	1.202 -1.423*
8	20.54 (CH_2)	1.626	6'	34.01 (CH_2)	1.202 -1.423*
9	23.79 (CH_2)	1.202 -1.423*	7'	31.44 (CH_2)	1.202 -1.423*
10	38.74 (CH_2)	1.202 -1.423*	8'	29.47 (CH_2)	1.202 -1.423*
11	34.16 (CH_2)	1.202 -1.423*	9'	29.26 (CH_2)	1.202 -1.423*
12	32.78 (CH_2)	1.202 -1.423*	10'	27.32 (CH_2)	1.202 -1.423*
13	30.41 (CH_2)	1.202 -1.423*	11'	24.92 (CH_2)	1.202 -1.423*
14	30.32(CH_2)	1.202 -1.423*	12'	27.55 (CH_2)	1.202 -1.423*
15	29.14 (CH_2)	1.202 -1.423*	13'	28.93 (CH_2)	1.202 -1.423*
16	29.67 (CH_2)	1.202 -1.423*	14'	29.61 (CH_2)	1.202 -1.423*
17	28.93 (CH_2)	1.202 -1.423*	15'	30.37 (CH_2)	1.202 -1.423*
18	27.10 (CH_2)	1.202 -1.423*	16'	31.94 (CH_2)	1.202 -1.423*
19	27.46 (CH_2)	1.202 -1.423*	17'	34.87 (CH_2)	1.626
20	24.96 (CH_2)	1.202 -1.423*	18'	70.28 (CH)	3.665
21	23.75 (CH_2)	1.202 -1.423*	19'	69.44 (CH)	3.573
22	45.40 (CH_2)	1.202 -1.423*	20'	63.34 (CH_2)	2.347
23	68.34 (CH_2)	2.328	21'	37.11 (CH_2)	1.202 -1.423*
24	124.48 (CH)	3..714	22'	30.05 (CH_2)	1.202 -1.423*
25	123.99 (CH)	3.616	23'	24.48 (CH_2)	1.202 -1.423*
26	65.03 (CH_2)	2.347	24'	22.70 (CH_2)	1.202 -1.423*
27	45.15 (CH_2)	1.202 -1.423*	25'	20.15 (CH_2)	1.202 -1.423*
28	34.53 (CH_2)	1.202 -1.423*	26'	19.12 (CH_2)	1.202 -1.423*
29	29.71 (CH_2)	1.202 -1.423*	27'	14.05 (CH_3)	0.921
30	22.99 (CH_2)	1.202 -1.423*	OH-2	-	6.979
31	20.35 (CH_2)	1.202 -1.423*	OH-18'	-	5.578

Note: * - overlapping

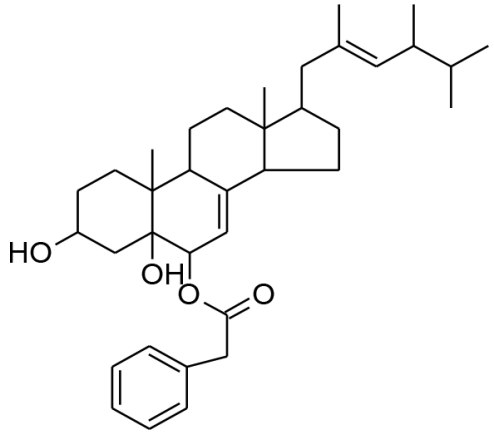

Figure 2: Compound 2- (3β–5α– Dihydroxy –6β– phenyl acetyloxy – ergosta –7, 22 – diene).

29.17 (C-7), δ 22.71 (C-8), δ 29.37 (C-9), δ 30.58 (C-10), δ 31.94 (C-11) and δ 19.78 (C-12). ^{1}H NMR ($CDCl_3$, 400MHz) spectrum showed a triplet at δ0.86 (J=7.2Hz), multiplet at δ 1.25 -1.90 and δ 2.26 – 2.30.

Anti-mycobacterial assay

Results for the investigation of anti-mycobacterial potential of isolated compounds against *M. tuberculosis* H37Rv is shown in Figure 4. It was observed that compound 1 and 2 showed significant inhibition of *M. tuberculosis* H37Rv with MIC of 25µg/mL and 6.25µg/mL respectively. Compound (3) does not have inhibition against *Mycobacterium*.

Table 2: NMR spectral data of compound 2 (CDCl3, 400 and 100 MHz).					
Position	**δ C NMR**	**δ H (J in Hz)**	**Position**	**δ C NMR**	**δ H (J in Hz)**
1	25.63 (CH_2)	1.254 (m)	20	29.70 (CH_2)	2.340 (m)
2	31.92 (CH_2)	1.333 (m)	21	24.96 (CH_3)	1.231 (d, J=6.6)
3	34.12 (CH)	3.657 (m)	22	130.23 (C)	-
4	33.09 (CH_2)	1.277 (m)	23	128.7 (CH)	4.169 (dd, J=8.4, 15.0)
5	44.74 (C)	-	24	39.09 (CH)	2.850 (m)
6	51.49 (CH)	3.611 (br, J=5.3)	25	29.60 (CH)	2.391 (m)
7	127.91 (CH)	4.180 (br, J=5.3)	26	21.11 (CH_3)	0.831 (d, J=7.8)
8	130.23 (C)	-	27	22.58 (CH_3)	0.859 (d, J=7.8)
9	36.90 (CH)	2.311 (m)	28	20.30 (CH_3)	0.88 (d, J=6.6)
10	30.03 (C)	-	1'	179.25 (C=O)	-
11	34.00 (CH_2)	1.292 (m)	2'	29.46 (CH_2)	2.340 (m)
12	31.45 (CH_2)	2.311 (m)	3'	130.23 (C)	-
13	42.83 (C)	-	4'	130.03 (CH)	7.535 (d, J=8.8)
14	29.53 (CH)	1.283 (m)	5'	129.74 (CH)	7.514 (d, J=8.6)
15	26.93 (CH_2)	1.277 (m)	6'	127.91 (CH)	7.119 (d, J=8.65)
16	27.21 (CH_2)	2.320 (m)	7'	130.03 (CH)	7.535 (d, J=8.8)
17	42.83 (CH)	2.328 (m)	8'	129.74 (CH)	7.514 (d, J=8.6)
18	(CH_3)	0.836 (s)	OH-3	-	4.180 (d, J=5.4)
19	(CH_3)	0.852 (s)	OH-5	-	4.115 (d, J=5.5)

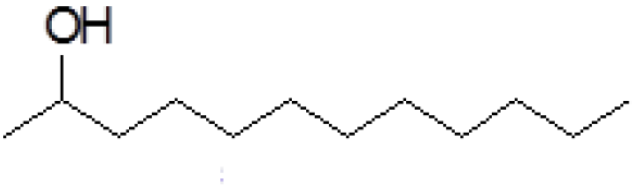

Figure 3: Compound 3- 2- Dodecanol.

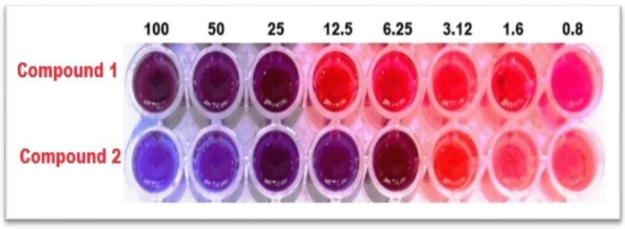

Figure 4: Antimycobacterial activity of compound 1 and 2 from endophytic fungus *C. cupreum* of *M. luteola* against *Mycobacterium tuberculosis* H37Rv (Microplate Alamar blue assay).

Cytotoxicity assay

The various concentrations of the isolated compounds 1, 2 and 3 were tested for cytotoxicity activity against breast cancer cell line MCF-7. The results (Table 3) revealed that the compound 1 and 2 showed highest cytotoxicity of 52% and 49% respectively at concentration 100µg/mL. The viability reduces in a dose dependent manner. Compound 3 do not showed cytotoxicity.

Antioxidant assay

The isolated compounds (1) and (2) showed significant DPPH scavenging potential of 72.07±1.95 and 71.63± 1.40 respectively at the concentration of 100µg/mL. The percentage inhibition of DPPH and ascorbic acid

Table 3: Cytotoxicity of Isolated Compounds against Breast Cancer Cell Line MCF-7.		
Conc. µg/ml	**% of cell viability**	
	C1	**C2**
25	84.90±1.15	89.10±0.69
50	72.15±0.80	77.33±0.41
75	60.94±0.69	64.06±0.72
100	48.91±0.86	51.93±0.21
Control	100 % viable cells	

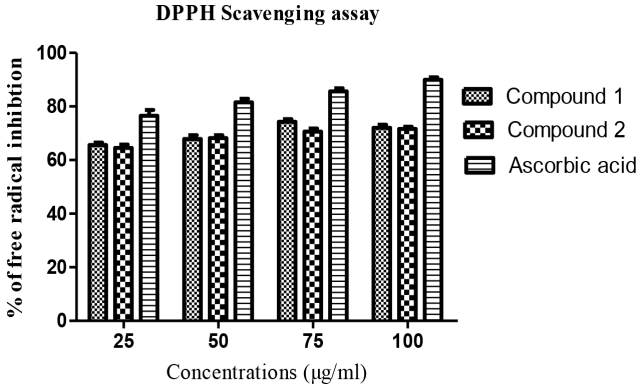

Figure 5: DPPH free radical scavenging activity of isolated compounds 1 and 2 from *C. cupreum*.

were shown in Figure 5. Compound (3) does not have significant inhibition against DPPH free radical.

DISCUSSION

Endophytic fungi are the major resources for exploiting new and novel secondary metabolites with many therapeutically applications.[5] In the present study three metabolites were isolated from the ethyl acetate fraction of endophytic fungus *C. cupreum* by repeated chromatography on Si gel column and evaluated for anti-mycobacterial and antioxidant potential.

Compound (1): The intense IR absorption maxima ν_{max} at 3298 represents the presence of OH stretch, 2960 and 2873 shows the presence of C-H stretch, 1722 and 1616 were assigned to C=O stretching, 1450 and 1368 shows the presence of C-H, 879 and 947 were assigned to C=C bending. The peak at 482- 790 reveals the presence of C-H group. The ^{1}H NMR spectrum indicated the presence of one meta coupled aromatic protons at δ.5359 (t, J= 2.3 Hz, 1H), it also showed the signals due to a long alkyl side chain at around δ 1.202~1.423, a terminal methyl at δ 0.879, 0.921 (t, J= 6.8 Hz, 3H) were assigned to C-33 and C-27' position respectively. The signals due to hydroxyl groups were observed at δ 6.979 (OH-2) and δ 5.578 (OH-18') were assigned to C-2 and C-18' position.

The ^{13}C NMR spectra of compound 1 exhibited sixty carbon peaks. The peaks at δ 173.97 and δ 174.38 represents the presence of keto (C=O) groups at C-3 and C-6 position and peaks of δ 138.89, 128.81 also assigned to C-1, C-5 and C-2, C-4 position of benzene ring and these data resembles to the benzoquinone structure. The two peaks at δ123.99 and δ124.48 may be due to the presence of a pair of sp^2 hybridized carbon atoms assigned to C-24, C-25 and C-1', C-2'. The peaks at δ 19.73 and δ 19.12 are attributing to the two methyl (CH_3) groups which are assigned to C-33 and C-27' respectively. From the obtained ^{1}H and ^{13}C NMR spectral data (Table 1) of compound 1 could be assigned as "6- (heptacosa- 18' Z enyl)- 2- (- 18'' hydroxyl- 1'' enyl- 19'' oxy)- 3 hydroxy benzoquinone" (derivatives of benzoquinone).

Compound (2): The IR absorption maxima ν_{max} at 3298 shows the presence of OH stretch, 2922 and 2852 shows the presence of C-H stretch, 1616 shows C=O, 1286 shows the presence of C-O (aromatic ester). The ν_{max} from 484- 763 reveals the presence of C-H. ^{13}C NMR spectrum data (Table 2) showed the presence of 36 carbon signals which are recognized as six methyl, nine methylene, fourteen methine, six quartenary carbons and one carbonyl group (C=O; -C-O). Among them one methine [δ_c 34.12 (d, C-3)] and one quartenary carbon [δ_c 44.74 (s, C-5)] assigned to those bearing hydroxyl group and eleven olefinic carbons [δ_c 127.91 (d. C-7), δ_c 130.23 (s, C-8), δ_c 130.23 (s, C-22), δ_c 128.7 (d, C-23), δ_c 179.25 (s, C- 1'), δ_c 130.23 (s, C-3'), δ_c 130.03 (d, C- 4'), δ_c 129.74 (d, C-5'), δ_c 127.91 (d, C-6'), δ_c 130.03 (d, C-7') and δ_c 129.74 (d, C-8')] were observed. It also possessing fifteen degree of unsaturation (seven degree of unsaturation found in sterol moiety and eight degree of unsaturation was showed in the benzene derivatives which attached to the sterol moiety).

The comparison of ^{1}H NMR data (Table 2) with that of known sterol isolated from the endophytic fungus *Chaetomium globosum*[21] showed signals due to six methyl groups [δ_H (3H, S, H_3-18), (3H, S, H_3-19)], a methyl Δ^{22}- sterol side chain [δ_H 0.83 (3H, d, J=7.8 Hz, H3-26), δ_H 0.85 (3H, d, J=7.8 Hz, H3-27), δ_H 0.88 (3H, d, J=6.6 Hz, H3-28), δ_H 1.231 (3H, d, J=6.6 Hz, H3-21)] and two hydroxyls [δ_H 4.115 (1H, 5- OH), 4.180 (1H, 3-OH)]. The NMR spectrum data patterns of compound 2 were similar to the sterol moiety ergosterol except for some change in ring B steroidal nucleus. The major difference was that the hydroxyl group at position C-6 was esterified with the phenyl acetic acid. The compound 2 carried the ergosterol type of derivatives [3α, 5β- dihydroxy, 6β- phenyl acetoxy- ergosta- 7-22 diene]. ^{13}C and ^{1}H NMR spectra of compound 2 were very similar to those of many fungal metabolites like globosterol, ergosterol including its co-occurring derivatives.[22]

Compound (3) was isolated as whitish oil and the molecular formula was proposed as $C_{12}H_{26}O$ based on HR-EIMS showing an ion at m/z 187.923 (calcd. for 186.340); ^{13}C NMR ($CDCl_3$, 100MHz) spectrum showed the presence of two methyl groups (CH_3) and ten methylene groups (CH_2). δ 14.14 (C-1), δ 33.84 (C-2), δ 31.44 (C-3), δ 29.71 (C-4), δ 29.52 (C-5), δ 30.20 (C-6), δ 29.17 (C-7), δ 22.71 (C-8), δ 29.37 (C-9), δ 30.58 (C-10), δ 31.94 (C-11) and δ 19.78 (C-12). ^{1}H

NMR ($CDCl_3$, 400MHz) spectrum showed a triplet at δ0.86 (J=7.2Hz) for methyl groups (CH_3), a *multiplet* at δ 1.25 -1.90 and δ 2.26-2.30 for methylene groups (CH_2) revealed that it was a straight alkyl chain (C_{12}). The ^{13}C, ^{1}H NMR and MS data of compound 3 were corresponding to 2- dodecanol.

Tuberculosis (TB) is a deadly contagious disease caused by *Mycobacterium* sp., mostly *Mycobacterium tuberculosis*. The *Mycobacterium* strains show intrinsic drug resistance to most of the antibiotics.[23] An effective anti TB drug must kill and prevents the growth of drug resistant mycobacteria.[24] Previously it was reported 92 fungal strains from Thailand medicinal plants were found to inhibit the development of *M. tuberculosis* H37Ra strain with MIC of 0.0625-200 μg/mL.[25]

In this study the results for antimycobacterial potential revealed that the compound 1 and 2 showed significant inhibition of *M. tuberculosis* H37Rv with MIC of 25μg/ml and 6.25 μg/mL respectively (Figure 2). The MIC value of compound 2 was found similar to the standard streptomycin drug. Also compound 1 and 2 had potential scavenging ability of 72.07± 1.95 and 71.63± 1.40 respectively at the concentration of 100 μg/mL (Figure 5). The compound 3 (2- dodecanol) do not have any inhibition against *M. tuberculosis* and DPPH free radical. All long-chain alcohols displayed an antimycobacterial activity with MICs ranging from 5 to 40μg/ml and efficacy increasing with chain length.[26] Linear (C20) aliphatic compound (eg. Phytol) showed optimum mycobacterial inhibition at MIC of 2mcg/ml.[27] Compound 1 has two linear alky chains more than C20 attached to benzoquinone moiety, due to structural steric hindrance may decrease the mycobacterial inhibition compare to C20 alkyl chain but compound 1 had significant inhibition with MIC of 25mcg/ml. Free methyl group and hydroxyl group at C3, C5 and C6 in steroid metabolites were responsible for bacterial growth inhibition. Ergosterol showed MIC of 32mcg/ml against Mycobacterium strain[27] and ergosterol ester derivatives (2) exhibit MIC of 6.26mcg/ml. The increase of antibacterial potential is may be due to additional of acidic phenyl acetoxy moiety. Four ergosterol derivatives, (22E,24R)-19(10–>6)-abeo-ergosta-5,7,9,22-tetraen-3β-ol, (22E,24R)-ergosta-4,7,22-trien-3-one, (22E,24R)-ergosta-4,6,8(14),22-tetraen-3-one, (22E,24R)-ergosta-7,22-dien-3β, 5α,6β-triol, (22E,24R)-6-acetoxy-ergosta-7,22-dien-3β,5α,6β-triol and (22E,24R)-3,6-diacetoxy-ergosta-7,22-dien-3β,5α,6β-triol were isolated from an endophytic fungi *Colletotrichum* sp. of *Ilex canariensis* were exhibit significant antimicrobial potentials against the fungus *Microbotryum violaceum*, the alga *Chlorella fusca* and the bacteria *Escherichia coli* and *Bacillus megaterium*.[28]

Breast cancer is the second largest frequently occurring cancer among women.[29] It occurs due to germ line or somatic mutations and exists as a non-metastatic disease.[30] MCF-7 is the most frequently used breast cancer cell line which was found at Michigan Cancer Foundation in 1973. Since this cell line is a hormone sensitive and express oestrogen receptor, it is highly used for breast cancer studies.[31] Taxol, podophylotoxin, camptothecine, vinblastine, Torreyanic acid are some of the anticancer drugs originated from endophytic fungi of medicinal plants.[32] In this study, Compound 1 and 2 showed cytotoxic potential against MCF-7 cell line at various concentrations in a dose dependent manner. Compounds 1, 2 and 3 were reported here for the first time from the endophytic fungus *Chaetomium cupreum* of the plant *M. luteola*.

CONCLUSION

Endophytic fungi have proven to be a promising source of bioactive natural products. The present study results indicate that the isolated compounds from the endophytic fungus *C. cupreum* had a significant antimycobacterial and antioxidant potential. Furthermore, toxicity testing and high yield production of bioactive compounds is necessary for using it as an active lead compound for drug development process.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

CC: Column Chromatography; **$CHCl_3$:** Chloroform; **DMSO:** Dimethyl Sulfoxide; **DPPH:** 2;2-Diphenyl-1-1-Picryl Hydrazyl; **EtOAc:** Ethyl Acetate; **FT-IR:** Fourier Transform Infra-Red; **MABA:** Microtitre Alamar Blue Assay; **MeOH:** Methanol; **MIC:** Minimum Inhibitory Concentration; **MS:** Mass Spectroscopy; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide; **NMR:** Nuclear Magnetic Resonance; **OADC:** Oleic Acid Albumin Dextrose; **PDA:** Potato Dextrose Agar; **PDB:** Potato Dextrose Broth; **rRNA:**

Ribosomal Ribo-nucleic acid; **UV-Vis:** Ultraviolet-Visible.

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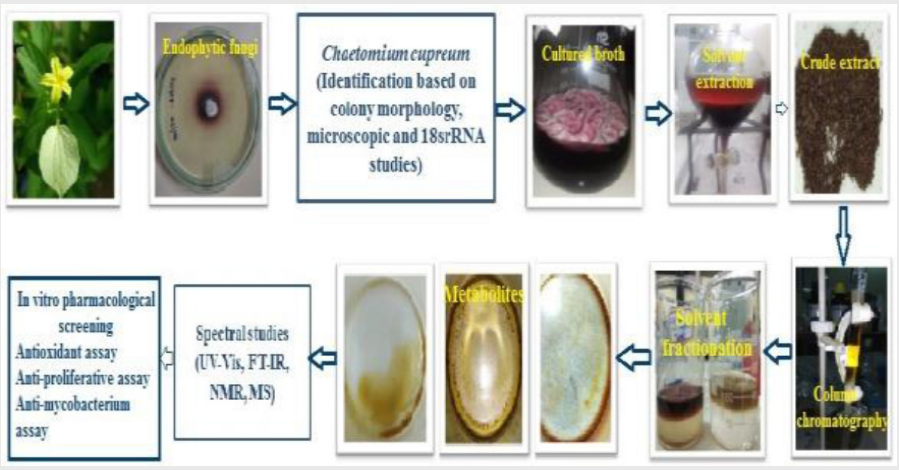
SUMMARY

Endophytic fungus was isolated from the plant *Mussaenda luteola* and it was identified as *Chaetomium cupreum* species based on colony morphology, microscopic examination and 18s rRNA sequencing. The fully-grown culture broth was extracted with ethyl acetate and the fungal mat was extracted with methanol to obtain crude. The crude extract was subjected to column chromatography and solvent- solvent fractionation method for purification process. The isolated metabolites were structurally elucidated by spectral studies (FT-IR, NMR, MS). Three compounds were obtained from *C. cupreum*- Benzoquinone derivative from ethyl acetate fraction of broth, ergosterol derivative and 2-dodecanol from methanolic mat extract. All three metabolites were screened for pharmacological potentials such as antioxidant, anti-proliferative and anti-tuberculosis.

PICTORIAL ABSTRACT



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