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Antibacterial and Cytotoxicity Activities of Major Compounds from *Tinospora cordifolia* Willd. Growing on *Mangifera indica* L.

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Abstract

Objective: To investigate the major constituents of *Tinospora cordifolia* Willd. growing on *Mangifera indica*, and to evaluate the efficacy of their antibacterial and cytotoxicity activities.

Methods: The ethanolic stem extract of *T. cordifolia* was subjected to silica gel 60 column chromatography, thin layer chromatography and medium pressure liquid chromatography for *isolation of* the major compounds. Identification of purified compounds was achieved by spectroscopic methods.. The crude extract and purified compounds were screened for their antibacterial and cytotoxicity properties using standard procedures.

Results: Two alkaloids were purified and identified as Magnoflorin (**1**) and Tembetarine (**2**). These compounds showed high *antibacterial activity* against *Bacillus cereus* and *Staphylococcus aureus* with both MIC (32-64 µg/ml) and MBC (128-256 µg/ml). The cytotoxicity activity of the purified compounds and crude extract was determined using MTT colorimetric assay against L929 and HEK293 cell lines. This showed weak cytotoxicity activity with IC_{50} values of 1162.24 to 2290.00 µg/ml and 1376.67 to 2585.06 µg/ml towards L929 and HEK293 cell lines, respectively.

Conclusion: The major compounds present in ethanolic stem extract of *T. cordifolia* growing on *M. indica* were extracted, purified and identified. This study suggests that these compounds exhibit great potential for antibacterial activity with weak cytotoxicity activity. They may be useful for their medicinal functions.

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Introduction

Tinospora cordifolia Willd. is a herbaceous vine of the family Menispermaceae. It was known in Thailand as "Boraphet" and was a glabrous climbing shrub found throughout the tropical regions of south east Asia. In *Ayurveda*, *T. cordifolia* was known as the king of *medicinal plants* for treating various ailments. It was an antispasmodic, antiperiodic, antipyretic, antidiabetic, anti-oxidant, anti-allergic and anti-inflammatory agent[1-12]. The plant had been reported for intermittent fevers and infective conditions such as typhoid, malaria, filariasis, and leprosy[13-16]. It had anthelmintic properties[17,18], and had been prescribed for urinary disorders, skin diseases, and eye diseases[19,20]. It was also used to treat gout and rheumatoid arthritis[21-23], and had cardiotonic, hematinic, expectorant, antiasthmatic, and aphrodisiac actions[24], and considered as the drug of choice in clearing the microcirculatory process of human body and other body channels[25]. It was reported that *T. cordifolia* growing on *Azadirecta indica* A. Juss had more bioactive potential than *T. cordifolia* and other *Tinospora* sp.[26]. *There* were a *lot of T. cordifolia* growing on *Mangifera indica* in Nakhon Pathom, Thailand. There was no report on the compounds isolated from *T. cordifolia* growing on *M. indica*. The present study investigated the antibacterial and cytotoxicity activities of major compounds isolated from ethanolic stems extract of *T. cordifolia* growing on *Mangifera indica* and determined the best concentration of the major compounds and crude extract responsible for these activities.

Materials & Methods

Plant Material and Extraction Procedure

Stems of *T. cordifolia* growing on *M. indica* were collected from the environs of Nakhon Pathom, Thailand, between September, 2017 and January, 2018. The stems were washed thoroughly 2-3 times with running water, cut into small pieces, and air dried under shade. The dried stems were then crushed in a grinder to coarse powder. Five hundred grams of powdered materials were added to 2000 ml of ethanol. The solution thus obtained was kept in an air tight flask for 24 h. The suspension was filtered using filter paper. Filtrate was evaporated at 60°C and a powdered form was obtained. The crude extract was prepared by adding methanol to obtain a stock concentration of 6 g/ml.

Isolation of the Compounds

The crude extract was dissolved in methanol to perform the bioautography assays[27]. The major compounds were isolated by silica gel 60 (230-400 mesh, Merck) column chromatography and eluted with chloroform : methanol (20:1, 15:1, 10:1, 7:1 and 5:1). Fractions were monitored by thin layer chromatography (TLC) (Kieselgel 60 F254, Merck), and spots were visualized under ultraviolet light and by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol. The combined fractions were eluted with 60-80% chloroform in methanol by medium pressure liquid chromatography (400 x 40 mm column, Merck LiChroprep Si 60, 25-40 mm, UV-detection, 254nm) to afford fraction (fr.) A (54 mg), fr. B (98 mg) and fr. C (45 mg). The fr. C had no activity, and fr. A had low activity against tested microorganisms. Final purification of fr. B was achieved by preparative TLC (Si gel 60, 0.5 mm, Merck) to afford compound **1** (28 mg) from fr. B and compounds **2** (20 mg). The structures of purified compounds have been identified using NMR and mass spectral data. Optical rotations were measured on a Jasco P-1020 automatic digital polarimeter (Jasco International Co., Ltd., Tokyo, Japan). UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were recorded using a Bruker Tensor 27 FT-IR (Bruker Optics GmbH, Ettlingen, Germany) spectrophotometer with KBr pellets. NMR spectra were carried out on either a Bruker DRX-500 or an AM-400 (Bruker BioSpin GmbH, Rheinstetten, Germany) spectrometer with the deuterated solvent as an internal standard. ESI-MS (including High resolution electrospray ionisation mass spectra (HRESI-MS)) was performed on an API-Qstar-Pulsar i mass spectrometer (MDS Sciex, Concord, ON, Canada). The chemical structures of these compounds were identical with Magnoflorine (1) and Tembetarine (2) and shown in Figure 1.

Antibacterial Assay

An *in vitro* plate assay technique was used to test the inhibitory effects of the crude extract and purified compounds on the tested bacteria using the paper disk method according to Clinical Laboratory Standard Institute (CLSI)[28]. Sterile paper discs (6 mm, Whatman 2017-006) were loaded with 50 µl of two-fold

dilution of 440 mg/ml of crude extract or 1 mg/ml of purified compounds. Four bacterial species were used in this study: *Staphylococcus aureus* ATCC 25932, *Bacillus cereus* ATCC 7064, *Escherichia coli* ATCC 10536, *Salmonella typhimurium* ATCC 23564 and *Pseudomonas aeruginosa* ATCC 27853 and methicillin-resistance *Staphylococcus aureus* SP6-106 (the clinical isolate). These bacteria were cultured in nutrient broth at 37°C for 24 h. Dilutions of bacterial suspensions were prepared using McFarland standard tubes (1 x 10^8 CFU/ml). The air-dried discs with various concentrations of the crude extract and purified compounds were placed on a lawn of bacterial spread on Muller Hinton agar. The plates were incubated at 37°C for 24 h. The diameter of the formed inhibition zones around each disc was recorded. The experiment was carried out in triplicate using gentamicin (30 unit/disk) (Oxoid, UK) as a reference for antimicrobial activity control.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations of the compound were tested against microorganisms in a 96-well microtiter plate by NCCLS microbroth dilution methods (NCCLS)[29]. The compound was twofold diluted from 0.5 µg/ml to 512 µg/ml, while the crude extract was twofold diluted from 0.13 mg/ml to 136.1 mg/ml, in nutrient broth supplemented with 10% glucose containing 0.01% phenol red as a colour indicator. Bacteria were adjusted to 10^5 CFU/ml for each microtiter plate. The microtiter plates were incubated at 37°C for 24 h. Microbial growth was determined by observing the change of colour in the wells (red to yellow when there is microbial growth). The lowest concentration that showed no change of colour was considered as the MIC, which was determined by inoculating onto nutrient agar plates 10 µl of medium from each of the wells from the MIC tests which showed no turbidity. The plates were incubated at 37°C for 24 h. Minimum bactericidal concentration (MBC) was defined as the lowest concentration of the test agent at which no microbial growth was observed on the plates.

Cytotoxicity Activity Assay

In order to evaluate the cytotoxicity activity of the crude extract and purified compounds, a cytotoxicity test was performed and the effects of the median inhibitory dose (IC_{50}) on the murine fibroblast cell (L929) and embryonic kidney cell (HEK293) lines were assessed. These cell line were obtained from the Korean Cell Line Bank (Seoul, Korea). Different concentrations (1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 µg/ml) of the crude extract and purified compounds were prepared and used in the cytotoxicity test. To measure cytotoxicity, 5 x 10^4 cells were seeded in 96-well plates and incubated in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum containing different concentrations of the test agents at 37°C for 24 h in 5% CO_2 incubator. The wells were washed with a serum-free medium. Vehicle control groups were added with double distilled water.

In the tetrazolium salt, 3-4,5 dimethylthiazol-2,5 diphenyl tetrazolium bromide (MTT) assay[30], yellow MTT is reduced to purple formazan in the mitochondria of viable cells. One hundred microliters of the MTT working solution (0.5 mg/ml) was added to each well and incubated at 37°C for 5 h. Next, the media were removed, wells were washed with phosphate buffer saline and 100 µl of DMSO was added to solubilize the formazan crystalline product. The absorbance was measured with a plate reader (Packard AS10000 Spectrocount, USA) at 590 nm. The production of formazan dye was proportional to the number of viable cells.

The inhibition of the cell lines cytotoxicity rates for each test agents with different concentrations was calculated according to the following equation:

$$\%\text{Inhibition} = 100 - [(Abs_{sample} - Abs_{blank}) / (Abs_{control} - Abs_{blank})] \times 100$$

Where Abs_{sample} is the absorbance of the test agent and $Abs_{control}$ is the absorbance of the control reaction (containing all reagents except the test agent). The %inhibition was plotted against sample concentration, and a linear regression curve was established in order to calculate the IC_{50}. Tests were carried out in triplicate. Correlation coefficients were optimized.

Results

Ethanolic extract from the stems of *T. cordifolia* was purified by column chromatography and TLC. In the active fraction, two major compounds were isolated and identified as follows.

Compound **1**: Magnoflorine (**1**): $C_{20}H_{24}NO_4$; white amorphous powder; UV(MeOH) l_{max} (log ε) = 225, 273, 309 nm; ^{1}H-NMR (d^6-DMSO): G(ppm) = 2.95 (s, 3H); 3.40 (s, 3H); 3.83 (s, 3H); 3.89 (s, 3H); 4.50 (m, 1H); 7.03 (b.s., 3H); 9.9 (m, 2H). ^{13}C-NMR (^{12}C-d^6-DMS0): 142.2 (1); 120.9 (1a); 120.0 (1b); 148.8 (2); 110.4 (3); 120.2 (3a); 23.2 (4); 60.0 (5); 68.2 (6a);29.8(7); 125.8(7a); 119.4(8); 111.4(9); 149.3 (10); 141.6(11); 119.7(11a);42.9(N-CH3);53.1 (N-CH,); 56.1 (2-OCH3); 55.9 (10-OCH,); MS (70eV, m/e (%)): 341 (1); 327(0.5); 284(0.5); 283(0.4); 270(2); 256(3); 142 (10); 128(50); 127(20); 58(100).

Compound **2**: Tembetarine (**2**): $C_{20}H_{26}NO_4$; white amorphous powder; UV(MeOH) l_{max} (log ε) = 210 (4.45); 228sh (4.2); 284 (3.8). 'H-NMR (d^6-DMSO): G (ppm) = 3.16 (s, 3H); 3.36 (s, 3H); 3.80 (s, 6H); 4.73 (m, 1H); 6.00 (s, 1H); 6.45-7.10 (m, 4H); 8.99 (s, 2H). ^{13}C-NMR (^{12}C-d^6-DMS0): 71.1(1); 50.3 (N-CH,); 54.5(N-CH3); 50.5(3); 22.9(4); 123.2(4a); 111.6(5); 146.6(6); 146.4(7); 114.7(8); 119.0(8a); 36.7~; 128.5 (1'); 116.3(2'); 144.6(3'); 147.7(4'); 112.1(5'); 120.2 (6'); 55.9(6-OCH3); 55.6(4'-OCH3). MS (70 eV; m/e (%)): 343(6); 206(3); 192(100); 177(20); 149(8); 148 (7); 142(17); 128(7); 127(8); 58(75). Compounds **1** and **2** were identified as magnoflorine (**1**) and tembetarine (**2**), respectively. Their 'H- and ^{13}C-NMR spectral data were identical with those of magnoflorine and tembetarine previously reported by Pachaly and Schneider[31].

The crude extract from the stems of *T. cordifolia* showed a dark brown color. The crude extract yield was 12.0 g/kg while the percentage yields of the purified compounds **1** and **2** were about 0.47% and 0.33% (w/w), respectively. The antibacterial activity of the crude extract and purified compounds is summarized in Table 1. Various concentrations of crude extract and purified compounds were tested using agar disc diffusion assay. A zone of inhibition >8 mm in diameter was interpreted as sensitive. All of the susceptible strains were sensitive to the crude extract at 30 mg/disc. The crude extract showed the highest activity against *B.* cereus ATCC 7064, MRSA SP6-106 and *S. aureus* ATCC 25932 at 30 mg/disc with the average zones of inhibition being 14.33 ± 2.25 mm, 15.73 ± 2.77 mm and 18.28 ± 3.68 mm, respectively. However, this crude extract showed low activity against *E. coli* ATCC 10536 and *S. typhimurium* ATCC 3564 at 30 mg/disc with the average zones of inhibition 8.33 ± 2.88 mm and 8.43 ± 2.17 mm, respectively, and also showed moderate activity against *P. aeruginosa* ATCC 27853 at 30 mg/disc with the average zones of inhibition 10.27 ± 2.65 mm. Compounds **1** and **2** showed the highest activity against *B. cereus* ATCC 7064, MRSA SP6-106 and *S. aureus* ATCC 25932 at 50 µg/disc with the zones of inhibition ranging from 16.34 ± 4.53 mm to 19.67 ± 3.68 mm. They also showed moderate activity against *P. aeruginosa* ATCC 27853, *E. coli* ATCC 10536 and *S. typhimurium* ATCC 3564 at 50 µg/disc with the zones of inhibition ranging from 8.66 ± 2.31 mm to 10.50 ± 2.83 mm. Sensitive results were not obtained with discs containing 3.75-7.5 mg/disc of the crude extract and 1-5 µg/disc of compounds **1** and **2** against *E. coli* ATCC 10536 and *S. typhimurium* ATCC 3564, and 7.5 mg/disc of the crude extract and 1 µg/disc of compounds **1** and **2** against *P. aeruginosa* ATCC 27853.

Adopting a classification based on MIC values proposed by Kuete[32], and Kuete and Efferth[33], the antibacterial activity of a plant extract is considered significant when the MICs are below 100 µg/ml, moderate when 100 ≤ MIC ≤ 512 µg/ml, and weak if MIC > 512 µg/ml. Consequently, where the activity of the crude extract showed MIC values greater than 512 µg/ml, it was therefore considered a weak inhibitor against all the test microorganisms. Compounds **1** and **2** showed the lowest MIC (32 µg/ml) against *B. cereus* ATCC 7064 (Table 2). These were followed by the MIC values (64 µg/ml) against MRSA SP6-106 and *S. aureus* ATCC 25932. Compounds **1** and **2** had high MIC values (512 µg/ml) against *P. aeruginosa* ATCC 27853, *E. coli* ATCC 10536 and *S. typhimurium* ATCC 3564. Compounds **1** and 2 showed the lowest MBC (128-256 µg/ml) against *B. cereus* ATCC 7064, MRSA SP6-106 and *S. aureus* ATCC 25932 whereas these compounds had high MBC values (>512 µg/ml) against *P. aeruginosa* ATCC 27853, *E. coli* ATCC 10536 and *S. typhimurium* ATCC 3564. The crude extract had no inhibitory activity in MBC against *P. aeruginosa* ATCC 27853, *E. coli* ATCC 10536 and *S. typhimurium* ATCC 3564.

Table 1. Antibacterial activity of the crude extract and purified compounds on the tested microorganisms.

Tested agents/concentrations		Diameters of inhibition zones on tested microorganisms (mm)					
		S.a.[a]	B.c.	E.c.	S.t.	P.a.	MRSA
Crude extract	3.7 mg/disc	7.33 ± 1.67	7.84 ± 1.72	NZ	NZ	NZ	7.71 ± 1.84
	7.5 mg/disc	8.50 ± 1.33	8.86 ± 1.64	NZ	NZ	7.62 ± 1.56	8.92 ± 2.53
	15 mg/disc	10.28 ± 2.67	13.16 ± 2.54	7.72 ± 1.77	7.35 ± 1.24	8.65 ± 2.51	12.62 ± 2.86
	30 mg/disc	14.33 ± 2.25	18.28 ± 3.68	8.33 ± 2.88	8.43 ± 2.17	10.27 ± 2.65	15.73 ± 2.77
Compound 1	1 mg/disc	8.22 ± 1.14	8.57 ± 2.13	NZ	NZ	NZ	8.74 ± 1.62
	5 mg/disc	8.87 ± 1.67	9.20 ± 2.38	NZ	NZ	8.24 ± 2.52	9.16 ± 2.33
	10 mg/disc	13.21 ± 5.08	14.67 ± 3.92	8.34 ± 2.16	8.30 ± 2.62	9.11 ± 2.77	14.10 ± 2.36
	50 mg/disc	16.34 ± 4.53	18.88 ± 3.97	8.66 ± 2.31	8.72 ± 2.35	10.50 ± 2.83	18.21 ± 3.33
Compound 2	1 mg/disc	7.14 ± 1.77	8.64 ± 2.65	NZ	NZ	NZ	8.56 ± 2.21
	5 mg/disc	8.38 ± 2.83	9.50 ± 2.76	NZ	NZ	8.08 ± 2.85	8.85 ± 2.38
	10 mg/disc	12.50 ± 3.44	13.50 ± 3.33	8.21 ± 2.37	8.16 ± 2.13	8.88 ± 2.84	12.54 ± 2.82
	50 mg/disc	16.35 ± 3.88	19.67 ± 3.68	8.72 ± 2.63	8.81 ± 2.47	9.50 ± 2.77	18.51 ± 3.55
Gentamicin	10 mg/disc	23.76 ± 1.74	22.88 ± 1.33	21.45 ± 1.61	20.74 ± 1.84	21.35 ± 1.46	23.58 ± 1.66

[a]S.a.: *S. aureus* ATCC 25932

B.c.: *B. cereus* ATCC 7064

E.c.: *E. coli* ATCC 10536

S.t.: *S. typhimurium* ATCC 23564

P.a.: *P. aeruginosa* ATCC 27853 and

MRSA; methicillin-resistance *S. aureus* SP6-106.

The data were presented as Mean ± Standard deviation (SD). NZ = No inhibition zone.

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of crude extract, purified compounds on tested microorganisms.

Tested micro-organisms	Antibacterial activity of the tested agents							
	Crude extract (mg/ml)		Compound 1 (mg/ml)		Compound 2 (mg/ml)		Chloramphenicol (mg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S.a.[a]	17.01	68.05	64	256	64	256	4	512
B.c.	8.51	68.05	32	128	32	128	8	>512
E.c.	>136.1	>136.1	512	>512	512	>512	4	>512
S.t.	>136.1	>136.1	512	>512	512	>512	8	>512
P.a.	68.05	>136.1	512	>512	512	>512	8	>512
MRSA	17.01	68.05	64	256	64	256	4	512

[a]S.a.; *S. aureus* ATCC 25932
B.c.; *B. cereus* ATCC 7064
E.c.; *E. coli* ATCC 10536
S.t.; *S. typhimurium* ATCC 23564
P.a.; *P. aeruginosa* ATCC 27853
MRSA; methicillin-resistance *S. aureus* SP6-106.

Table 3. IC_{50} of the crude extract, purified compounds against normal cell lines after 24 h using the MTT assay.

	IC_{50}[a] values of crude extract, purified compounds on tested cell lines (μg/ml)	
Test microorganisms	L929[b] cells	HEK293[c] cells
Crude extract	2290	2585.06
Compound 1	1162.24	1376.67
Compound 2	1245.33	1642.81

[a]IC_{50} values represent the concentration causing 50% growth inhibition. They were determined by linear regression analysis.
[b]L929, murine fibroblast cell line.
[c]HEK293, human embryonic kidney cell line.

To evaluate the cytotoxicity activity of the crude extract and purified compounds against L929 and HEK293, the cell lines were incubated with different doses of two-fold dilution (1-512 µg/ml) of the crude extract and purified compounds. After 24 h of incubation, cell viability was determined by MTT assay. The crude extract and purified compounds induced cell cytotoxicity in a concentration-dependent manner. The corresponding IC_{50} was calculated, and the results are presented in Table 3. The cytotoxicity activity of the crude extract and purified compounds was observed and showed weak cytotoxicity activity with IC_{50} values of 1162.24 to 2290.00 µg/ml and 1376.67 to 2585.06 µg/ml towards L929 and HEK293 cell lines, respectively.

Discussion

Medicinal properties of plant derived compounds are known to show curative activity against several bacteria and it is not surprising that the medicinal plant extracts are used traditionally by herbalists to treat bacteria related ill-health.

T. cordifolia also exerted considerable antibacterial effect against tested pathogens. This plant has been extensively subjected to chemical investigations, and a number of chemical constituents belonging to different groups such as trepenoids, alkaloids, diterpenoid lactones, sesquiterpenoids, lignans, flavonoids, tannins, cardiac glycosides, steroids have been reported, which may account for the antimicrobial property of these agents[34-52]. Our research findings regarding the major compounds of *T. cordifolia* from Nakhon Pathom, Thailand, differ from previous reports in the literature regarding *T. cordifolia* from other geographical regions. Bisset and Nwaiwu[53] reported that the major quaternary alkaloids in *Tinospora* species were generally the protoberberine bases berberine and palmatine. Nagaprashanthi *et al.*[54] found that the hydroalcoholic extract of *T. cordifolia* grew over *Azadirachta indica* (neem plants) has potential antimicrobial activity similar to *Azadirachta indica*, and also has higher potential antimicrobial activity than the hydroalcoholic extract of *T. cordifolia* climbing on fencing. This may explain why the host plants (*T. cordifolia*) will acquire the medicinal properties when they survive on neem plants and their extracts contain more of the active compounds. In this study, therefore, the extract of *T. cordifolia* growing on *M. indica* from Nakhon Pathom, Thailand, was found to have a significantly different chemical composition from the extract of *T. cordifolia* from other geographical locations. This property could be attributed to exchange of bioactive constituents from *M. indica* to *T. cordifolia*, but further studies are warranted to elucidate the exact mechanisms responsible for the observed bioactive compounds. In addition, variations in the chemical composition of the extracts are known to differ considerably due to the existence of different subspecies. They might also be attributable to other factors such as climate, different regional geographic and seasonal conditions, metabolism of plants, stage of maturity and extraction conditions[55]. Alkaloids like berberine, palmatine, tembetarine, magnoflorine, choline, tinosporin, columbin, isocolumbin, tetrahydropalmatine have been isolated from stem and root extracts of this plant[53-59]. They present numerous biological activities such as being emetic, anticholinergic, antitumor, diuretic, sympathomimetic, antiviral, antihypertensive, analgesic, antidepressant, muscle relaxant, anti-inflammatory, antimicrobial, and antiulcer[60]. The alkaloids have proton-accepting nitrogen atom and one or more proton-donating amine hydrogen atoms, which form hydrogen bonds with proteins, enzymes, and receptors. Furthermore, they, generally, have functional groups such as phenolic hydroxyl. The later might explain the exceptional bioactivity of the alkaloids[61]. In this study, major bioactive *compounds;* magnoflorine *and* tembetarine *were isolated from T. cordifolia*, and have antibacterial activity especially on Gram-positive bacteria. The biological activities, such as antioxidant, anti-a-glucosidase, a-tyrosinase inhibitory, anti-inflammatory, and anticancer activities, of magnoflorine have been reported. Magnoflorine from *Aristolochia debilis* stems showed significant antioxidant activity as a DPPH free radical scavenger, considerable a-tyrosinase inhibitory effect and also showed considerable anti-inflammatory activity in high dosage[62]. Hung *et al.*[63] also have reported that, magnoflorine plays a role in protecting high-density lopoprotein (HDL) under oxidative stress. Patel and Mishra[64] indicated that magnoflorine from *T. cordifolia* stems inhibited a-glucosidase activity. For anticancer activity, the cytotoxic effect of *T. cordifolia* stem extracts against

cancer prostate (PC-3), colon (Colo-205, HCT-116), lung (A546, NCIH322) and breast cancer (T47D) cell lines have been reported [65], and magnoflorine has shown selected cytotoxicities against the murine leukemia (P388) cell line[66]. The antibacterial properties of *T. cordifolia* have been investigated by researchers world wide[54,67-69]. Kumar *et al.*[70] have reported that, the crude extract from *T. cordifolia* leaves had antibacterial effect on *E. coli*. However this research found that the crude extract from *T. cordifolia* stems had low antibacterial activity against this microorganism. Variations in the chemical composition of the compounds are known to differ considerably not only due to the existence of different part of plants or subspecies, but might also be attributed to other factors such as climatic, geographic and seasonal condition of the regions, metabolism of plants, stage of maturity and extraction conditions[55]. With regard to the purified compounds, magnoflorine and tembetarine exhibited antibacterial activity that varied between bacterial species (MIC = 32 – 512 µg/ml). This differs from the report of Mushtaq *et al.*[71]. They found that magnoflorine isolated from *Aquilegia fragrans* exhibited weak antibacterial activity against various mastitis pathogens such as *S. aureus*, and *Staphylococcus equorum* with MIC values of 500 µg/ml.

Tinospora and *Aristolochia* plant overdoses may have serious renal side-effects. Testing the cytotoxicity of the crude extract and purified compounds was carried out on L929 and HEK293 cells. The crude extract and purified compounds showed no toxicity on both cells even at high dosage. That means, they had no cytotoxicity on L929 and HEK293 cells. This was similar to the findings of Li and Wang[62], that magnoflorine had no toxicity on HEK293 and HT-29 cells at the concentration of 400 µg/ml.

Conclusion

The results obtained in this study thus suggest that the major bioactive *compounds (compound **1**;* magnoflorine *and compound **2**;* tembetarine*) isolated from T. cordifolia* have antibacterial activity especially on Gram-positive bacteria. These compounds showed bactericidal effects against *B. cereus* ATCC 7064, MRSA SP6-106 and *S. aureus* ATCC 25932 with both MIC and MBC at a concentration of 32-64 µg/ml and 128-256 µg/ml, respectively. Compounds **1** and **2** showed weak cytotoxicity activity against normal fibroblast L929 and embryonic kidney HEK293 cells and may be useful for their medicinal functions.

Conflict of Interest

The authors have declared that no competing interest exists.

Acknowledgements

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