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Research paper

Evaluation of leaves of *Goniothalamus wynaadensis* Bedd. for inhibition of metabolic viability of cancer cells & antimicrobial efficacy

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ABSTRACT

Introduction: *Goniothalamus wynaadensis* Bedd. is a less explored medicinal plant belonging to the family Annonaceae. This species has been traditionally used by tribes of Wayanad, Kerala, in India, and also Indonesian tribes for joint-related ailments and also as a deodorizer. The aim of the study was to analyze leaf extracts of the plant for their antimicrobial and anticancer properties along with phytochemical screening.
Methods: MTT assay was used to assess the cytotoxic potential of plant extracts against MDA-MB-231 and A-549. Antimicrobial potential of plant extracts was analyzed via liquid broth turbidometry assay and well in agar plate methods. Identification tests were carried out for phytochemical screening.
Results: Antimicrobial assay showed EC_{50} values for ethyl acetate extract 0.82 mg/mL against *Escherichia coli*, 0.82 mg/mL against *Salmonella typhi*, & 0.88 mg/mL against *Staphylococcus aureus* which are quite significant. Other solvent extracts also displayed antimicrobial activities at varying dosage. MTT assay was performed at the dose concentrations of 1 µg/mL, 5 µg/mL & 25 µg/mL, 50 µg/mL and 100 µg/mL to confirm the cytotoxicity of the extracts. The ethyl acetate extract displayed EC_{50} values of 4.96 µg/mL against A-549 lung cancer cell line and 2.50 µg/mL against MDA-MB-231 breast cancer cell line. Other extracts were also found to be cytotoxic.
Conclusion: Ethyl acetate & water extracts have demonstrated potent activity against *Salmonella typhi, Escherichia coli, & Staphylococcus aureus* bacteria as well as, showing cytotoxicity against tested cancer cell lines. These results can be explored to identify individual phyto-molecules from the extracts with anticancer & antimicrobial potential.

1. Introduction

From ancient times even before modern research and the development of treatment technologies, mankind utilized plants as herbal formulations and employed various other traditional regional methods of medications to treat various ailments [1,2]. The bridging gap between the modern era of the medicinal system and the old traditional approaches as a means of treatment has been defined as Complementary Alternative Medicine (CAM). The ethno-medicinal system is when natural resources used in the CAM system are studied and used to extract the active molecules which could be of therapeutic significance [3,4]. By collecting traditional information on plant species or genera of interest, we learn their medicinal importance, which later via explorative and investigational studies may give many new potent medicinally important molecules in the field of drug discovery. Here, the present study is also an effort to explore the pharmacological importance of *Goniothalamus wynaadensis* Bedd. This plant has come under the nearly threatened species category, and yet no scientific study about its pharmacological significance or ethnomedicinal importance has been reported to date.

Goniothalamus wynaadensis Bedd. is a species endemic to the southern region of India and belongs to the Annonaceae family [5,6]. It is dispersed in the Southern – Western Ghats of India, particularly in Wayanad and Kannur region of Kerala state and in Kodagu district in Karnataka state in India, [5,7]. The regional vernacular name of *Goniothalamus wynaadensis* Bedd. is “Aanapanal”. In combination with other plants, *G. wynaadensis* Bedd. has been reported to be part of the traditional herbal system [8]. The bark juice is reported to be traditionally used by tribes, native to Mananthavady region in Kerala for joint-related ailments viz., arthritis [9,10]. In Indonesian tribes, its flowers are known to be used as a deodorizer [11]. *G. wynaadensis* Bedd. has been reported as part of herbal formulations in combination

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with other plant species for skin diseases, leukaemia, lung cancer, breast cancer, prostate cancer, diabetes and gynaecological disorders [8,12–14]. The *Goniothalamus* genus is distributed in paleotropical regions; dominating in the Eurasia zone and it is known for its medicinal properties like febrifuge, insect repellent, stomachic, antimalarial, for treating cholera, postpartum protective remedy, anticancer, antimicrobial activities and others [15–17]. Several species belonging to this genus have been reported with different chemical, analytical & biological studies [17–19]. The *Goniothalamus* species are an important source for phytochemicals like acetogenins, anthraquinones, flavonoids, terpenoids, alkaloids and specifically styryl lactones, which are found in abundance in this genus [15,19–21]. Styryl lactones are interesting molecules with anticancer, trypanocidal, anti-inflammatory, anti-fertility, mosquito repellent, antimalarial, and antimicrobial properties.

There is so far, only one report on the *G. wynaadensis* Bedd. by Ajithabai et al., where a few phytochemical constituents are reported without specifying any bioactivity [7]. The present communication reports about the antimicrobial efficacy of leaf extracts of *G. wynaadensis* Bedd. against gram-positive & gram-negative bacteria and, their potential to affect the cell viability of A-549 and MDA-MB-231 cancer cell lines for the first time. Classification of phytochemical constituents into broad classes of natural products based on chemical testing is also reported.

2. Materials and methods

2.1. Plant material collection

Goniothalamus wynaadensis Bedd. leaves were collected from wild plants growing in the local regions of Kerala (in Wayanad) including Peria forest, Chembra hills, and Thamarasserey garden (Western Ghats) during the non-flowering season. The voucher specimen was deposited at M.S. Swaminathan Research Foundation and Community Agrobiodiversity Centre, Puthoorvayal, Kalpetta, Wayanad, Kerala, India. The specimen was confirmed as *G. wynaadensis* Bedd. with the local name' Anapanal' and was issued Herbarium Specimen number MSSH WAYANAD No.0462 (supplementary information Fig. 2).

2.2. Extraction

The leaves of *Goniothalamus wynaadensis* weighing 4.5 kg were collected and shade dried for 2 weeks. These were further powdered fine using a heavy-duty grinder to the weight of 2.5 kg. Further, for sequential extraction, the plant sample was soaked in different solvents of increasing polarity order & collected as following extracts: Hexane (Hex-G), Chloroform (CLFM-G), Ethyl Acetate (EA-G), Methanol (MeOH-G), Methanol-Water (7:3) (MW-G) and Water (Wtr-G). All the extracts were dried under reduced pressure by evaporating solvents using rota-vapour (Heidolph, Germany). Extracts were labelled as Hex-G for hexane, CLFM–G for Chloroform, EA-G for ethyl acetate, Meth-G for methanol, MW-G for methanol-water (7:3), and Wtr–G for water.

2.3. Microbial strains & culture media

Three microbes were selected for antimicrobial study; namely *Escherichia coli (E. coli) (gram –ve), Staphylococcus aureus (S. aureus) (gram + ve) & Salmonella typhi (S. typhi) (gram + ve)*. Bacterial cultures were obtained from the Biochemistry Department, Gulbarga University, Kalaburagi. All the obtained bacterial cultures were subcultured & freshly grown in Luria-Bertoni media (LB media) 24 h preceding each experiment & stored at a temperature below 4°C.

2.4. Preparation of test samples & well in agar plates

The stock solution was prepared using 10 mg of each plant extract in 1 mL of Dimethyl sulfoxide (DMSO) and was further subjected to serial dilution. Sterilized well borer of 5 mm was used for making holes in the agar plate. The concentration of 0.2 mg/mL to 1 mg/mL per well in agar plate was maintained by taking 2 µL to 10 µL and for liquid broth 20 µL to 100 µL in every 10 mL of inoculated liquid media [22,23].

2.5. Chemicals for antimicrobial assay

Neomycin antibiotic was used as standard reference antibiotic (positive control) for turbidometric assay as well as for well in agar plate method. DMSO was used as a solvent for dissolving all extracts to prepare stock solutions.

2.6. Antimicrobial assay methodology

The assay was conducted following two methods; liquid broth dilution test (turbidometric assay) and well in agar plate method, as per the CLSI guidelines [22–24]. Bacterial inoculum was standardized to $1.5 \times 10^{\hat{}8}$ CFU (colony forming unit) equivalent to 0.5 McFarland Standard required for susceptibility tests on microbial growth [22,25].

2.7. Broth-based turbidometric assay (TB)

The turbidometric assay was carried out based on the methodology reported by Othman et al. (2011) along with some alterations [22,26]. The media was autoclaved, cooled to room temperature & then inoculated with bacterial strains, and kept for incubation overnight so that the growth of bacteria should reach in log phase. The next day fresh LB media was prepared; transferred to test tubes (10 mL each) and autoclaved. 100 µL from strain cultures were added to each test tube leaving one test tube as blank (to observe growth without strains in culture) for LB media. DMSO was taken as solvent control, one test tube kept as control with strain culture, one test tube contained the positive control Neomycin 3 µg/mL separately. To the rest of the test tubes 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL & 1 mg/mL concentrations from each extract were added. All the test tubes were kept incubated at 37°C. The optical density at 660 nm on *UV/VIS spectrometer* – PerkinElmer *Lambda* 25 was observed after 18 – 20 hours of incubation. Survival index (SI) was calculated which was used to quantify EC_{50} value [22,27,28].

$$SI = \frac{OD_{660} \text{ of the sample at a corresponding time point} \times 100}{OD_{660} \text{ of mid-log of control bacterial growth}}$$

Survival index represents the bacterial growth in the tested sample to the percentage of growth of controls at the mid-log phase. Effective concentration (EC_{50}) values that inhibit 50 % of microbial growth were calculated with the help of SI values.

2.8. Well in agar plate method

Bacterial colonies were cultured in liquid broth medium one night before the experiment. LB – agar medium was prepared and poured into plates. All the plates were cultured with respective strains and divided each into eight sections including DMSO. A sterile cotton swab was used for inoculating the plate with bacterial strain culture and labelled. Streaking with bacterial culture by sterile cotton swab was done on the surface of the LB-agar plate with the rotation of plate to perform uniform spreading of inoculums. Plates were left to solidify for 10 min; later holes were bored in plates with well borer and, test sample extracts were pipetted into holes [23,24].

2.9. Cell culture and sample extracts preparation

A-549 (metastatic lung cancer cell line), MDA-MB-231 (metastatic breast cancer cell line), were obtained from NCL, Pune and sub-cultured

freshly before conducting the assay. Cell culture media was prepared from Gibco's DMEM media with 10 % Foetal Bovine Serum (FBS) and 1 % penicillin-streptomycin at the 37°C and 5 % CO_2 in a humid atmosphere. Stock solutions of 1 mg/mL of sample extracts were prepared in DMSO. Further, with serial dilution, test solutions were prepared to 25 µg/mL, 50 µg/mL, and 100 µg/mL concentrations; to finally take the doses of concentrations 1 µg/mL, 5 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL.

2.10. MTT cytotoxicity assay

MTT assay was conducted in 96 well plates, which were cultured & incubated in the aforementioned media while each well was seeded with 8,000–10,000 cells. Cell counting was done with an automated cell counter before starting the cell treatment. The assay was done in triplicate with cell treatment being done in triplicate dose concentrations (1 µg/mL, 5 µg/mL & 25 µg/mL) followed by another experiment in increased dose concentrations (50 µg/mL and 100 µg/mL) and incubated for 48 h. Camptothecin was used as a positive control. After 48 h, media from each cell was discarded and treated with (0.5 mg/mL of 1x PBS) MTT dye adding 100 µL in each well and incubated at room temperature for 4 h to allow the formation of formazan crystals. After that MTT solution was discarded from each well; 100 µL of DMSO again in each well was added to dissolve formazan crystal. The absorbance was read spectrophotometrically using a microplate reader at 570 nm. The results were then represented as mean ± S.D. obtained from three independent experiments.

2.11. Phytochemical screening test

All the solvent extracts were subjected to 11 different phytochemical screening tests to analyze the presence of secondary metabolites in *Goniothalamus wynaadensis* Bedd. leaves. Out of these, nine tests were found positive. These tests were for identification of alkaloids, flavonoids, tannins, carbohydrates, glycosides, saponins, cardiac glycosides, triterpenoids, resins, phenols and steroids [29–31]. Standard methods opted to perform a screening test for identifying various secondary metabolites are given in Table 1.

2.12. Statistical analysis

All the data is expressed in mean ± S.D. Statistical analysis was performed using Origin Lab program with one way ANOVA. The significant difference was considered as $p < 0.05$.

3. Results

3.1. Broth dilution turbidometric assay

Using the optical density (OD) values of extracts at different dose-concentrations; the survival index (SI) of bacteria was calculated based on the formula aforementioned in the materials and methods section. *E. coli* (Fig. 1) is most effectively being inhibited by extracts with no interference by solvent DMSO. A maximum of 42 % of bacterial survival was observed for ethyl acetate (EA-G) extract at 1 mg/mL (Fig. 1) of concentration against *E. coli*. Water (Wtr-G) extract was observed to reduce the bacterial survival to 45 % similarly by methanol (MeOH-G) extract survival index (SI) appeared to decline to 46 %. The methanol-water (MW-G) extract & hexane (Hex-G) extract shrunk the bacterial growth to 52 % and 56 % at 1 mg/mL concentration. In case of *Gram + ve* bacteria *S. aureus* (Fig. 3), polar extracts MW-G and Wtr-G showed 46 % and 43 % SI, while in EA-G and Hex-G extracts diminished the *S. aureus* survival to 48 % and 49 %. Similarly, CLFM-G & Meth-G extracts showed SI until 54 % and 52 %. *S. typhi* another *gram –ve* bacteria (Fig. 2) was interestingly affected by most of the extracts. The EA-G, Wtr-G & CLFM-G extracts have shown SI against *S. typhi* of 43 %, 47 % & 49 % and MeOH-G extracts have displayed 51 % bacterial survival. Ethyl acetate was most potent among all the extracts with lowest EC_{50} values for *E. coli* (0.83 mg/mL), *S. typhi* (0.82 mg/mL), and third most potent for *S. aureus* (0.88 mg/mL) while Wtr-G extract was most highly toxic for *S. aureus* with EC_{50} values of 0.68 mg/mL (Supplementary information Table 1).

Table 1
List of various phytochemical screening tests performed on extracts of *Goniothalamus wynaadensis* Bedd.

S.no.	Phytoconstituents	Tests	Identification
1.	Alkaloids	Wagner's test	Reddish-brown precipitate
		Dragondroff's test	Orange-red precipitate
2.	Carbohydrates	Fehling's test	Red precipitate
		Molisch test	Red violet ring
3.	Flavonoids	Conc. H_2SO_4 test	Orange to crimson color
		Lead acetate test	Yellow precipitate
		NaOH test	Yellow color
4.	Glycosides	Borntrager's test	Rose pink-red color
		Modified Borntrager's test	Pink-red colour
5.	Steroids	Liebermann – Burchard test	Violet – blue ring at the interface
		Salkwoski test	Red-brown color
6.	Cardiac Glycosides	Keller – Kelliani's test	Blue color
		Legal test	Deep red color
7.	Tannins	Ferric Chloride test	Blue-Violet color
		Lead acetate	white precipitate
8.	Proteins	Biuret test	Pink Color
		Ninhydrin test	Blue-violet color
		Xanthoprotic test	Yellow-orange color
9.	Saponins	Froth test	Honey comb-like froth
10.	Triterpenoids	Liebermann – Burchard test,	Bluish-green translucent color
		Salkwoski test	Reddish-brown color
11.	Phenols	Ferric chloride test	Blue, violet color
		Lead acetate	white precipitate
		bromine test	white precipitate

3.2. Well in agar plate method

In addition to macro-dilution liquid broth assay, well in agar plate method supported the observations & confirmed the analysis of the antimicrobial activity of extracts at the similar concentrations from (0.2–1) mg/mL witnessing the inhibition against bacterial strains. All the extracts created clear visible zones of inhibition against bacterial strains showing similarity to broth dilution turbidometric test. All the extracts appeared active in case of *E.coli*, while for *S. typhi*, the appearance of inhibition zones was selectively observed in Hex-G, CLFM-G, EA-G, and MW-G in line with the results of the turbidometric assay. EA-G, MeOH-G, CLFM-G, and Wtr-G extracts gave clear activity for *S. aureus* with visible zones of inhibition.

3.3. Inhibition of cytotoxicity of cancer cell lines

MTT assay was performed on A-549 and MDA-MB-231 cells with all 6 extracts at the dose – concentrations of (1, 5, 25, 50, and 100) µg/mL. On 48 h of incubation and later treating the plates with MTT dye, absorbance reading was taken at 570 nm and results were calculated in the form of percentage viability of cells. The anticancer molecule camptothecin was used as a reference for the study. A non-uniform pattern of inhibition observed, as the polarity of extracts increased. In Hex-G and EA-G extract, potent uniform inhibition was observed with least viability at 25 µg/mL. While MW-G and Wtr-G extract observed to be more active at 5 µg/mL (Figs. 4 and 5). A set of comparatively more interesting outcomes were observed for breast cancer cells with *G. wynaadensis* Bedd. extracts. Hex-G extract maintained uniform pattern in results and was found more cytotoxic; inhibiting 79 %, and 82 % of

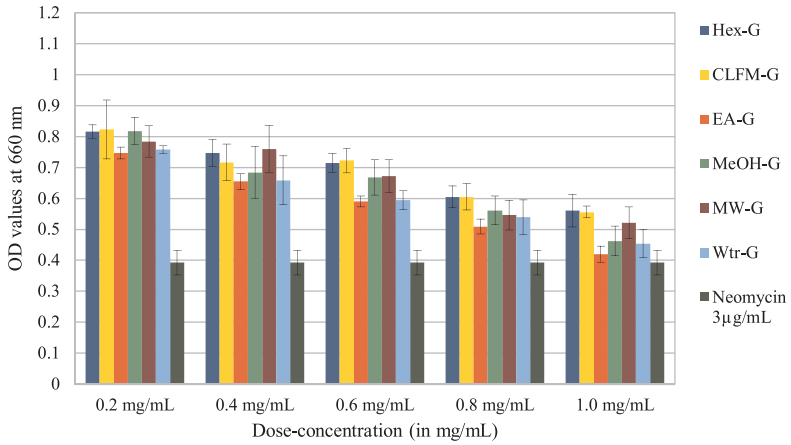


Fig. 1. Bar graph representation of *E. coli* survival index at concentrations from (0.2–1) mg/mL based on broth dilution turbidometric assay. Error bars are showing ± SD; each experiment as three separate events was carried out in triplicate.

A-549 cells and 78 % and 81 % of MDA-MB-231 cells at a dose concentration of 5 and 25 µg/mL. At 5 µg/mL majority of extracts expressed inhibition of 70 % approximately in case of both cell lines (Figs. 4 and 5). The CLFM-G, EA-G, MeOH-G, MW-G, and Wtr-G extracts were all found to be more cytotoxic at 5 µg/mL concentrations. However, to ensure the consistency of the obtained results, further, high dose concentrations of 50 µg/mL and 100 µg/mL from same extract stock solutions were analysed on both cancer cell lines (Fig. 6). It was approximately for all the extracts 90 % inhibition (Fig. 6) was observed; outcomes ensured the potent cytotoxic nature of plant extracts.

It is noteworthy here; in literature review of Goniothalamus majority of cytotoxic styryl lactones, alkaloids have been isolated from hexane, ethyl acetate, methanol extracts. To further support this statement, phytochemical screening results were also found to provide similar observations.

3.4. Phytochemical screening of extracts from leaves

The chemical screening test tabulated in Table 1 were performed on all the solvent extracts after evaporating the solvent from extracts [30–32] to identify the presence of types of secondary metabolites in leaves of *Goniothalamus wynaadensis* Bedd. The presence is depicted by (+) sign and absence is shown by (-) sign in Table 4. As per the results of these tests; leaves showed the presence of alkaloids, carbohydrates, phenols, flavonoids, triterpenoids, and tannins. It also showed the presence of steroids, and glycosides.

4. Discussion

This is the first article, reporting any pharmacological study performed on *G. wynaadensis* Bedd. The present analysis is performed following macro-dilution based turbidometric assay and well in agar method. The bacterial strain samples were obtained from the biochemistry department of Gulbarga University, Kalaburagi. Further subculturing of bacterial strains was performed based on the predetermined growth rate of strains that is; inoculation of bacteria to the main test broth was done during the log phase growth of bacterial strains. Bacterial strains of 100 µL were incorporated to 10 mL of LB media broth solution in each test tube; while adding the varying concentrations of extracts from respective stock solutions. The resulting turbidity of the solutions was analyzed after intervals of 18 – 20 hours with the help of Optical Density (OD) values readings at 660 nm of wavelength. The OD values for all bacterial strains, obtained at 0.2 mg/mL of concentration are depicted as the minimal concentration at which inhibition can be observed. However, a further increase in concentration was observed to be enhancing the growth inhibitory effect. These observations were further confirmed by calculating the bacterial survival index to identify the effective concentration (EC_{50}) values [27,28]. The antimicrobial effect of *G. wynaadensis* leaves extracts were analysed against Neomycin (a broad-spectrum antibiotic, active against both gram + ve and gram –ve bacteria) at single dose concentration 3 µg/mL. Neomycin actively inhibits *E. coli*, *S. aureus* and *S. typhi* [33–36].

Although; 0.4 mg/mL & 0.6 mg/mL were certainly arresting the

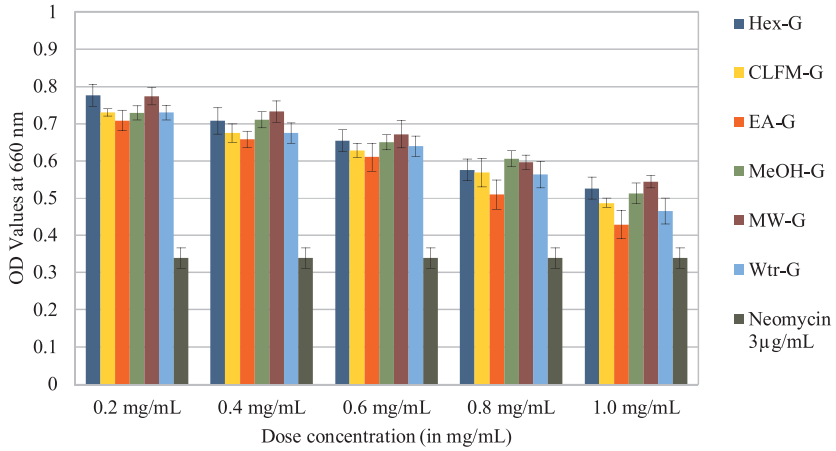


Fig. 2. Bar graph representation of *S. typhi* survival index at concentrations from (0.2–1) mg/mL based on broth dilution turbidometric assay. Error bars are showing ± SD; each experiment as three separate events was carried out in triplicate.

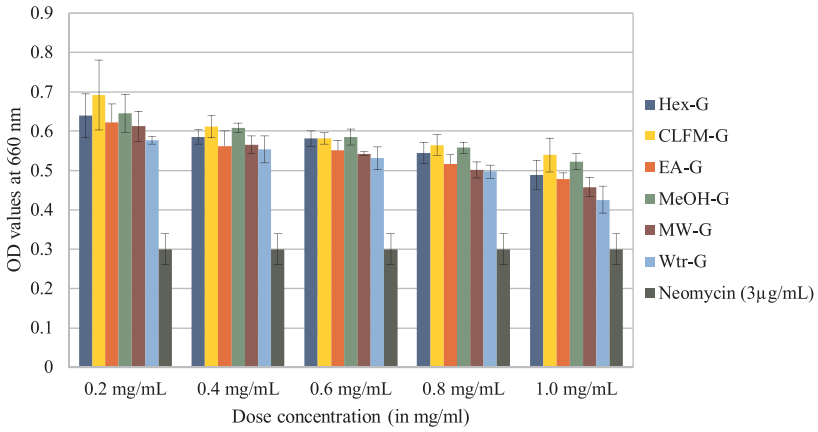
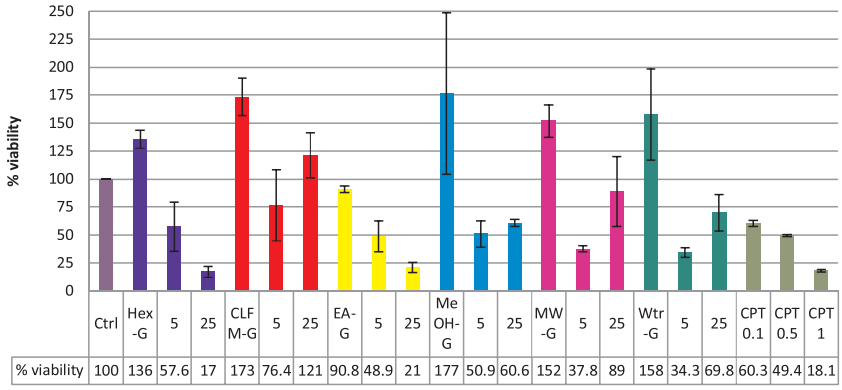
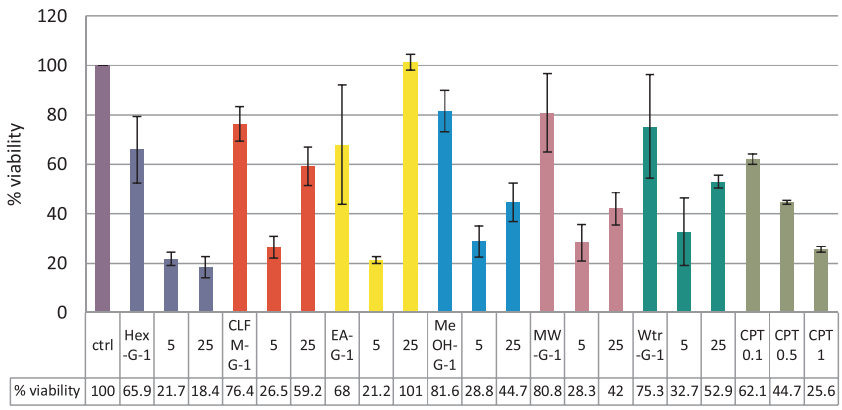


Fig. 3. Bar graph representation of *S. aureus* survival index at concentrations from (0.2–1) mg/mL based on broth dilution turbidometric assay. Error bars are showing ± SD; each experiment as three separate events was carried out in triplicate.



	Ctrl	Hex -G	5	25	CLF M-G	5	25	EA-G	5	25	Me OH-G	5	25	MW -G	5	25	Wtr -G	5	25	CPT 0.1	CPT 0.5	CPT 1
% viability	100	136	57.6	17	173	76.4	121	90.8	48.9	21	177	50.9	60.6	152	37.8	89	158	34.3	69.8	60.3	49.4	18.1

Fig. 4. Percentage viability of A-549 cells at (1, 5, 25) µg/mL calculated from absorbance taken at 570 nm. CPT is camptothecin positive control taken in study at 0.1 µm, 0.5 µm, and 1 µm.



	ctrl	Hex -G-1	5	25	CLF M-G-1	5	25	EA-G-1	5	25	Me OH-G-1	5	25	MW -G-1	5	25	Wtr -G-1	5	25	CPT 0.1	CPT 0.5	CPT 1
% viability	100	65.9	21.7	18.4	76.4	26.5	59.2	68	21.2	101	81.6	28.8	44.7	80.8	28.3	42	75.3	32.7	52.9	62.1	44.7	25.6

Fig. 5. Percentage viability of MDA-MB-231 cells at (1, 5, 25) µg/mL calculated from absorbance taken at 570 nm. CPT is camptothecin posiive control taken in study at 0.1 µm, 0.5 µm, and 1 µm.

growth; on further enhancing the concentration (until inhibition rate was brought close enough to the Neomycin) of all extracts till 1 mg/mL; inhibition was found comparable to the standard control Neomycin at 3 µg/mL. *E. coli* strain was affected most by EA-G extract of leaves as well as from Wtr-G and Meth-G extracts of *G. wynaadensis* leaves with a maximum inhibition of bacterial growth being 58 % at 1 mg/mL (SI value = 42 %). *S. aureus* growth was substantially arrested at 1 mg/mL of dose from Wtr-G extract, EA-G, and (7:3) MW-G extracts of leaves; with 57.5 % inhibition (SI value – 42.5 %) by Wtr-G extract, 52 % inhibition by EA-G extract and 55 % inhibition by MW-G extract. *G. wynaadensis* Bedd. leaves extracts showed significant inhibition over *S. typhi*; with three extracts recording less than 50 % bacterial survival at 1 mg/mL of concentration. All extracts effectively inhibited the *S. typhi* strain with CLFM-G (51 %), EA-G (57 %), and Wtr –G (53 %) inhibition at highest dose concentration taken at 1 mg/mL (Table 2). EC_{50} values (Supplementary Information Table 1) calculated from survival index values identifies EA-G (EC_{50} = 0.82 mg/mL) as most potent extract against *E. coli* and *S. typhi*, while for *S. aureus* Wtr-G (EC_{50} = 0.68 mg/mL) and MW-G (EC_{50} = 0.79 mg/mL) were most potent extracts.

In the well in agar method; the pattern appeared as inhibition zones were closely correlated with the results observed in liquid broth turbidometric assay. As the concentration was increased, the inhibitory

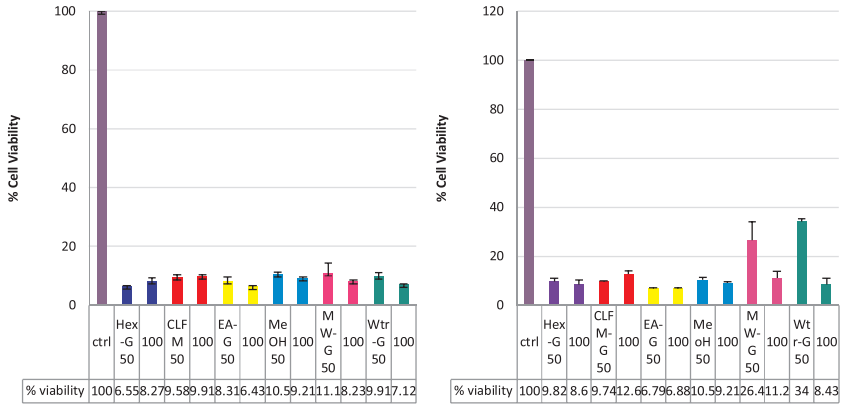


Fig. 6. Maximum Cytotoxicity observed from all the extracts at 50 µg/mL and 100 µg/mL for MDA-MB-231 and A-549 Cells.

Table 2
Survival index value & standard deviations calculation for *E. coli*, *S. typhi*, *S. aureus* bacterial strain from OD values observed at 660 nm for various extracts from *G. wynaadensis* Bedd. Leaves.

E. coli Survival index			*S. typhi* Survival Index			*S. aureus* Survival Index		
Hex-G (mg/mL)	SI value (%)	± SD	Hex-G (mg/mL)	SI value (%)	± SD	Hex-G (mg/mL)	SI value (%)	± SD
0.2	81.61	0.0221	0.2	77.62	0.0296	0.2	63.96	0.0559
0.4	74.68	0.0440	0.4	70.73	0.0353	0.4	58.55	0.0181
0.6	71.5	0.0303	0.6	65.4	0.0287	0.6	58.14	0.0195
0.8	60.47	0.0356	0.8	57.57	0.0294	0.8	54.48	0.0269
1.0	56.09	0.0528	1.0	52.65	0.0298	1.0	48.84	0.0370
EA-G	SI value (%)	± SD	EA-G	SI value (%)	± SD	EA-G	SI value (%)	± SD
0.2	74.63	0.0189	0.2	70.8	0.0276	0.2	62.17	0.0481
0.4	65.42	0.0254	0.4	65.78	0.0217	0.4	56.23	0.0381
0.6	58.97	0.0179	0.6	60.99	0.0376	0.6	55.23	0.0245
0.8	50.95	0.0241	0.8	50.94	0.0397	0.8	51.72	0.0236
1.0	41.95	0.0258	1.0	42.92	0.0379	1.0	47.88	0.0149
CLFM-G	SI value (%)	± SD	CLFM-G	SI value (%)	± SD	CLFM-G	SI value (%)	± SD
0.2	82.29	0.0952	0.2	72.99	0.0096	0.2	69.15	0.0892
0.4	71.68	0.0580	0.4	67.55	0.0249	0.4	61.18	0.0286
0.6	72.24	0.0385	0.6	62.81	0.0191	0.6	58.16	0.0138
0.8	60.56	0.0426	0.8	56.8	0.0384	0.8	56.47	0.0266
1.0	55.63	0.0184	1.0	48.71	0.0123	1.0	53.93	0.0431
MeOH-G	SI value (%)	± SD	MeOH-G	SI value (%)	± SD	MeOH-G	SI value (%)	± SD
0.2	81.77	0.0443	0.2	72.93	0.0195	0.2	64.53	0.0483
0.4	68.36	0.0846	0.4	71.04	0.0218	0.4	60.84	0.0123
0.6	66.75	0.0563	0.6	65.05	0.0203	0.6	58.59	0.0200
0.8	56.07	0.0467	0.8	60.6	0.0210	0.8	55.81	0.0139
1.0	46.19	0.0479	1.0	51.27	0.0274	1.0	52.24	0.0199
MW-G	SI value (%)	± SD	MW-G	SI value (%)	± SD	MW-G	SI value (%)	± SD
0.2	78.36	0.0499	0.2	77.39	0.0226	0.2	61.23	0.0384
0.4	75.97	0.0759	0.4	73.25	0.0283	0.4	56.54	0.0228
0.6	67.2	0.0528	0.6	67.17	0.0375	0.6	54.31	0.0048
0.8	54.65	0.0479	0.8	59.64	0.0197	0.8	50.23	0.0203
1.0	52.09	0.0514	1.0	54.41	0.0172	1.0	45.83	0.0242
Wtr-G	SI value (%)	± SD	Wtr-G	SI value (%)	± SD	Wtr-G	SI value (%)	± SD
0.2	75.76	0.0126	0.2	73.01	0.0198	0.2	57.7	0.0099
0.4	65.85	0.0792	0.4	67.52	0.0271	0.4	55.4	0.0343
0.6	59.36	0.0302	0.6	63.96	0.0275	0.6	53.1	0.0285
0.8	53.91	0.0559	0.8	56.37	0.0358	0.8	49.71	0.0167
1.0	45.45	0.0455	1.0	46.52	0.0343	1.0	42.57	0.0346

effect also appeared to enhance with maximum clear zones appearing at 1 mg/mL concentration. It was observed in the case of active extracts, that the inhibition zones forming at the (0.8 and 1) mg/mL concentrations were as clear as those formed by Neomycin 30 µg disc (Table 3). *E. coli and S. aureus* growth were more effectively restricted by EA-G, Wtr-G & MW-G than by Meth-G, Hex-G. For *S. typhi* clear zone of inhibitions were observed by all extracts however EA-G, CLFM-G & Wtr-G appeared more efficient in inhibiting bacterial growth (Supplementary information - Fig. 1). The earlier studies reported on other species of Goniothalamus genus confirm the antimicrobial effect against *S. aureus*, *E coli*, and *S. typhi*; including a few being active against MRSA species [37–39]

The 48 h treatment with extracts resulted in a considerable cytotoxic effect against MDA-MB-231 cell line and A-549 cell lines (Figs. 4 and 5). EA-G, MeOH-G, and Hex-G extracts appear to be more cytotoxic; while MW-G and Wtr-G extracts were found to be active in a comparatively non-uniform trend. The relatively non-polar EA-G extract showed the strong cytotoxic effect at 25 µg/mL with percentage cell viability of 20 % only. In a similar inhibition pattern, Hex-G extract produced maximum inhibition of 83 % (i.e., 17.03 % cell viability) at

Table 3
Zone of inhibition values obtained from well in agar (WA) method for *E. coli*, *S. typhi*, *S. aureus* (# numbers specified in brackets represents the code used for labelling on petridishes).

(a) Inhibition zone diameter values (in mm) for *E. coli* (**NI = No inhibition, *NU = Non-Uniform zone)

E. coli

Method	Sample/ Inhibition Diameter (in mm)					
WA	Antibiotic/extract samples					
Concentration	Hex-G	CLFM-G	EA-G	MeOH-G	MW-G	Wtr-G
30 µg (Neomycin)	18.5 ± 0.5 (8)[#]	20.0 ± 0.5 (8)	17.0 ± 0.1 (7)	18.0 ± 0.4 (8)	20.0 ± 0.6 (8)	19.0 ± 1.0 (7)
1 mg/mL	16.2 ± 1.1 (6)	16.0 ± 1.0 (6)	18.5 ± 1.5 (6)	17.0 ± 0.5 (5)	12.0 ± 0.5 (6)	13.0 ± 1.0 (6)
0.8 mg/mL	14.0 ± 0.4 (5)	14.0 ± 0.2 (5)	16.0 ± 0.5 (5)	15.2 ± 0.4 (6)	11.0 ± 1.2 (5)	12.0 ± 0.5 (5)
0.6 mg/mL	12.0 ± 0.2 (3)	12.0 ± 0.2 (4)	12.5 ± 0.3 (3)	13.0 ± 0.5 (4)	9.5 ± 0.5 (4)	11.0 ± 0.5 (4)
0.4 mg/mL	9.6 ± 0.3 (4)	9.0 ± 0.7 (3)	NU* (8)	9.5 ± 0.5 (3)	8.0 ± 0.5 (3)	10.5 ± 0.2 (3)
0.2 mg/mL	8.0 ± 0.2 (2)	7.0 ± 0.1 (2)	9.0 ± 0.8 (2)	7.0 ± 1.0 (2)	7.5 ± 0.7 (2)	9.5 ± 0.5 (2)

(b) Inhibition zone diameter values (in mm) for *S. typhi*

S. typhi

Method	Sample/ Inhibition Diameter (in mm)					
WA	Antibiotic/extract samples					
Concentration	Hex-G	CLFM-G	EA-G	MeOH-G	MW-G	Wtr-G
30 µg (Neomycin)	17.0 ± 0.1 (n)[#]	18.5 ± 0.5 (n)	19.5 ± 0.5 (n)	18.5 ± 1.0 (n)	NU* (n)	16.5 ± 0.5 (n)
1 mg/mL	13.5 ± 0.3 (6)	18.0 ± 0.5 (6)	19.0 ± 0.5 (6)	12.5 ± 0.5 (6)	13.0 ± 0.5 (6)	16.0 ± 0.5 (6)
0.8 mg/mL	12.0 ± 0.5 (5)	17.5 ± 0.5 (5)	18.5 ± 0.5 (5)	10.0 ± 0.2 (5)	9.0 ± 0.5 (5)	12.0 ± 0.5 (5)
0.6 mg/mL	11.0 ± 0.3 (4)	15.0 ± 1.0 (4)	16.5 ± 0.5 (4)	9.5 ± 0.4 (4)	8.0 ± 0.5 (4)	11.5 ± 0.3 (4)
0.4 mg/mL	10.0 ± 0.5 (3)	13.0 ± 0.5 (3)	14.5 ± 0.5 (3)	8.5 ± 0.2 (3)	6.5 ± 0.5 (3)	10.0 ± 1.0 (3)
0.2 mg/mL	NI** (2)	11.0 ± 0.5 (2)	13.0 ± 0.5 (2)	6.5 ± 0.5 (2)	5.0 ± 0.5 (2)	8.5 ± 0.5 (2)

(c) Inhibition zone diameter values (in mm) for *S. aureus*

S.aureus

Method	Sample/ Inhibition Diameter (in mm)					
WA	Antibiotic/extract samples					
Concentration	Hex-G	CLFM-G	EA-G	MeOH-G	MW-G	Wtr-G
30 µg (Neomycin)	19.5 ± 0.5 (n)[#]	16.0 ± 0.2 (n)	18.5 ± 0.5 (n)	15.5 ± 0.5 (5)	11.0 ± 1.0 (n)	NU* (n)
1 mg/mL	13.0 ± 0.2 (6)	15.0 ± 0.5 (6)	19.5 ± 0.5 (6)	14.0 ± 1.5 (6)	12.0 ± 0.5 (6)	14.5 ± 0.5 (6)
0.8 mg/mL	11.0 ± 0.5 (5)	13.0 ± 1.5 (5)	19.0 ± 0.5 (5)	10.0 ± 0.5 (5)	10.0 ± 0.5 (5)	13.0 ± 0.2 (5)
0.6 mg/mL	8.5 ± 0.2 (4)	13.0 ± 0.5 (4)	13.5 ± 0.5 (4)	9.0 ± 0.1 (4)	8.0 ± 0.2 (4)	11.0 ± 0.3 (4)
0.4 mg/mL	7.0 ± 0.5 (3)	11.0 ± 0.8 (3)	11.5 ± 0.5 (3)	8.0 ± 0.2 (3)	7.0 ± 0.1 (3)	10.0 ± 0.2 (3)
0.2 mg/mL	6.0 ± 0.2 (2)	8.5 ± 0.5 (2)	10.5 ± 0.5 (2)	7.5 ± 0.4 (2)	5.0 ± 0.1 (2)	6.0 ± 0.5 (2)

Table 4
Secondary metabolites screening test output in various solvent fractions of leaves of *G. wynaadensis* Bedd.

Secondary metabolites	Hexane	Chloroform	Ethyl acetate	Methanol	Methanol: Water	Water
Alkaloid	+	+	+	+	+	+
Carbohydrate	+	–	+	+	–	–
Flavonoids	+	+	+	+	+	+
Glycosides	–	–	–	+	+	–
Steroids	+	+	+	–	–	+
Cardiac glycosides	+	+	+	+	–	–
Tannins	–	–	–	–	–	–
Proteins	–	–	–	–	–	–
Saponins	–	–	–	+	+	–
Resins	–	–	–	–	–	+
Triterpenoids	+	–	+	+	+	+
Phenols	+	+	+	+	+	+

25 µg/mL. The polar extracts too were arresting cell growth at the dose-concentration of 5 µg/mL with maximum cell viability of 50 % observed in MeOH-G extract, 37.7 % in MW-G extract (7:3), and 34.3 % in Wtr-G extract. These results although were obtained in a somewhat non-uniform pattern. Hence, the cytotoxic behaviour of other closely related species from the same Goniothalamus genus was compared and verified. The obtained results accorded with the studies reported by Abdelwahab et. al, 2009, on *G. umbrosus*, and Iqbal et, al. 2015, on *G. sesquipedalis* [21,37,40]. Further, in the process of supporting the observations of lower concentrations (1, 5 & 25) µg/mL and identifying the dose-concentration required for maximum inhibition, the assay was repeated at higher concentrations [37,40]. This analysis represented that apart from MW-G and Wtr-G, all extracts were highly cytotoxic at 50 µg/mL concentration (Fig. 6). On collectively observing all values with the help of Origin graph software, all dose concentrations were plotted individually for each extract against the percentage viability to calculate the relative EC_{50} values of these extracts against both the cancer cell lines (Supplementary Information Table 2). The Hex-G and EA-G extracts were found to be more potent for MDA-MB-231 triple-negative breast cancer cell lines. While EA-G, MW-G, and Wtr-G extracts have shown cytotoxicity for A-549 Lung cancer cell lines.

As per the results of phytochemical screening, the *G. wynaadensis* Bedd. leaves appear to be enriched with alkaloids, phenolic compounds, and steroids. The positive results of identification of phenolic compounds, steroids, alkaloids in extracts could be correlated to anti-bacterial effects of extracts. Goniothalamus species are reported to be rich in alkaloids, styryl lactones, furnaopyrones & acetogenins etc. [18,41–45]. Therefore, resulting active extracts will be further explored for identification of active molecules which may be solely responsible for exerting anti-bacterial effects or cytotoxic effects against cancer cell lines.

5. Conclusion

The chemical study conducted on leaf extracts of *Goniothalamus wynaadensis* Bedd. depicts the presence of alkaloids and phenolic

compounds exclusively. Positive lead acetate test specifically confirms the presence of lactone derivatives in the leaves, which may be responsible for the cytotoxic activity of extracts. The antimicrobial study performed by broth dilution turbidometric assay and, well in agar plate method on *gram + ve* and *gram -ve* bacteria confirms for considerable antimicrobial properties displayed by ethyl acetate extract (EA-G), as well as water (Wtr-G) and methanol-water extract (MW-G) at the dose of 1 mg/mL by the leaves of *G. wynaadensis* Bedd. Antimicrobial data obtained by both the above methods were found to be complementing and was found to be on par with Neomycin at extracts dose 1 mg/mL. All the solvent extracts were found to possess cytotoxic potential against both A549 & MDA-MB-231 cancer cell lines with a slightly better effect against triple-negative metastatic MDA-MB-231 cells. These encouraging bioassay results can further be explored to identify & structure elucidate individual phyto-molecules from the species for their anticancer & antimicrobial potentials to identify probable new phytomedicines.

Author's contribution

Harish Holla planned the research work and manuscript correction. Akanksha Sharma & Praveen Sharma performed all the experimental work, Akanksha Sharma also wrote the manuscript. Sandeep Singh & T. B. Karegoudar guided the biological experiments.

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Declaration of Competing Interest

The authors declare there are no conflict of Interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eujim.2019.101000.

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