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Phytochemical, Antimicrobial and Cytotoxic Activities of Gaultheria Trichophylla Royle

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Abstract: *Gaultheria trichophylla* fruit is used by the indigenous people to treat asthma, headache, and as an appetizer in the alpine and sub-alpine regions of Western Himalaya. No studies exist on the antimicrobial significance of this species. The current study describes the phytochemical composition, in vitro cytotoxic, and antimicrobial effects of different extracts of *Gaultheria trichophylla*. In antimicrobial assay, four different bacterial strains (*Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) as well as four fungal strains (*Helminthosporium solani*, *Fusarium solani*, *Aspergillus flavus*, and *Aspergillus fumigates*) were used. Qualitative phytochemical screening showed the existence of different active compounds. Quantitative phytochemical screening showed the existence of phenolic contents in the range from 3.27 ± 0.44 mg GE/g to 14.17 ± 088 mg GE/g, whereas flavonoids were from 8.08 ± 0.48 mg QE/g to 26.9 ± 0.23 mg QE/g. The elemental analysis quantified essential minerals of life importance such as Na (3.24 ± 0.05 μgg^{-1}), Mg (1.93 ± 0.08 μgg^{-1}), and Ca (1.83 ± 0.056 μgg^{-1}), while none of the heavy metal levels were high from the permissible limit of WHO. Cytotoxic assay showed moderate activity in terms of LC50 of (50 µg/mL) for methanolic extracts. Antifungal assay of methanolic and other extracts against different tested fungal strains showed a zone of inhibitions from 29 ± 1.154% to 86.66 ± 0.09%. As an antibacterial, the MIC values were from 7.5 mg/mL to 15 mg/mL for the tested extracts. The observed biological potentials were at the expense of its phytochemical composition, however, further confirmation in animal models and responsible phytochemical isolations in pure form is needed.

Keywords: *Gaultheria trichophylla*; extracts; pharmacognostic; phytochemicals; elemental; cytotoxic activity; antimicrobial activity

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1. Introduction

The genus *Gaultheria*, belonging to family Ericaceae, are berry producing shrubs found in many parts of the world [1]. Few species of Gaultheria (*G. trichophylla* Royle.) and others are confined to Western Himalayas in their distribution [2]. In Uttarakand, India, alpine and sub alpine regions of Western Himalaya *G. trichophylla* Royle are used as appetizers in the form of fruits [3]. In Chinese herbal medicine, *Gaultheria yunnanensis* is traditionally used to treat swelling, rheumatoid arthritis, and pain [4]. To treat headaches, *G. trichophylla* is applied to the forehead [5]. Violet fruits of *G. trichophylla* are used in small amounts daily to treat asthma in temperate Himalaya Himachal Pradesh [6]. Winter green oil naturally obtained from *Gaultheria* is used in drug and food industries to enhance the scent and flavor of toothpaste, mouthwash, and confectioners. It is also used medicinally to relieve muscle and nerve pain as a complementary medicine in aromatherapy. Industrial usage includes using it as an antibiotic and an insecticide [7].

Describing herbal drugs in a systematic manner is based on multiple approaches of pharmacognostic, taxonomic, and chemical analysis, including documentation of their biological and geographical source, cultivation, collection, processing, morphological, microscopic, and chemical characters [8]. The herbal industries and indigenous communities of the Indo-Pak Sub-Continent commonly face problems in proper authentication and identification of the herbal drugs. They are made misguided and deal with completely different taxa [9]. The raw materials used by the pharmaceutical industry and people are usually obtained from the market, which may be contaminated, substituted, or adulterated accidentally or deliberately [10]. The drug identification involves physical, chemical, biochemical, and biological features [11]. To verify the medicinal nature of a drug, multi-solvent similarity methods of cold and hot treatment are used to verify the drug effectively [12].

The interest in traditional Phytomedicine products is increasing day by day throughout the world. It is highly significant to know the nature and safety of such plants for consumption purposes. The plants' samples must be screened out for safe levels of heavy and toxic elements such as Cu, As, Cd, Pb, and Hg to meet the safety threshold of the WHO. The life-essential elements such as P, K, and Na are important for keeping normal health; therefore, in order to recommend them for nutrition, quantification of these elements is highly significant [13]. These minerals (Zn, Cu, Fe, Pb, Mn, Cr, Ni, Sr, and Co) are necessary in smaller amounts. The absence or excess of these elements may cause abnormalities to the human body [14]. Sodium is essential for keeping the balance of inside and outside fluid and in regulating the volume of blood [15]. Magnesium (Mg) contents are important to control circulatory diseases and improve the dietary quality of plants, animals, and humans [16]. Calcium (Ca) is an important component for the development of bones and teeth [17]. Other than medicinal values, the plants and their polyphenolic compounds have become the focus of current nutritional interest due to their health promoting effects [18]. Different plant tissues such as vegetables, fruits, seeds, leaves, roots, and floral parts are enriched with naturally occurring phytochemicals known as phenolic compounds [19]. The oxidative stress can be treated with flavonoids naturally taken as human foods like catechin, quercetin, hypersoid, myricetin, and rutin [20].

Food poisoning is measured as one of the major general reasons for illness and death in developing countries. Different causal agents are responsible for food spoilage or food borne diseases such as *Staphylococcus aureus*, Bacillus cereus, as well as members of Gram-negative bacteria like *Salmonella typhi*, *Escherichia coli*, and *Pseudomonas aeruginosa* [21]. Global reduction in wheat yield is caused by a serious disease (HLB) called leaf blotch disease of Helminthosporium [22]. The soyabean yield and quality is seriously reduced due to *Fusarium solani*, which causes root rot disease [23]. Fungus such as *Aspergillus flavus* causes invasive aspergillosis which is symbolized in intensive care patients by complications such as severe influenza pneumonia [24]. A filamentous, saprophytic fungus known as *Aspergillus fumigatus* causes several pulmonary ailments in birds, humans, and other mammals [25].

Phytotherapy is the use of specific medicinal plants and their isolated compounds of bio-active nature for health care and treatment of diseases [26]. To cure such diseases, the plant produces certain chemical compounds called secondary metabolites. Synthetic chemotherapeutic agents are associated with a number of side effects, whereas the plant secondary metabolites are comparatively safe and associated with low or no side effects. Therefore, scientists are trying to isolate antimicrobial agents from plant origin. In doing so, initially the plant extracts are tested for antimicrobial spectrum. Researchers in different parts of the world have studied and screened out the effects of plant extracts on microorganisms [27]. Extracts obtained from plants are often used in phytochemical research. Different bioassays are used to monitor efficacy of extracts and fractions. In the screening of cytotoxic and biological activities an economical and rapid test, brine shrimp (*Artemia salina*) test (BST), has been used [28].

Several plant species have been evaluated for their antimicrobial activity in the past twenty years [29]. Some plants tested for antimicrobial activity confirmed positive results [30]. Therefore, it is time to develop novel antimicrobial agents particularly resistant to conventional antimicrobials [31]. Developments of new drug sources involves exploration of different plants to be tested as therapeutic agents, as being living organisms, they also suffer from different diseases [32]. Most of the species of *Gaultheria* are edible and useful for the health of humans due to their good level of polyphenol; however, extensive studies are required for the toxicity testing, nutrients, and chemical compounds on the Gaultheria [33]. The species of genus Gaultheria grows very slowly, therefore, they are difficult to cultivate [34].

Due to the increasing demands of the huge population of the world and the alarming problem of the drug resistance developed by microbes, the present study is aimed to determine antimicrobial activities of the selected plant and correlate the observed biological potential with major phytochemical groups: phenolics and flavonoids.

2. Materials and Methods

2.1. The Plant Material

Plant materials in the form of fresh fruits for the current study were collected from the alpine zone of Miandam valley (35°4′ N and 72° 29–32′ E) District Swat KP Pakistan. The plant was identified by Professor Dr. Mushtaq Ahmad in the Department of Plant Sciences, Plant Taxonomy Lab Quid-i-Azam University Islamabad Pakistan. A voucher specimen with voucher number ISL-112304 was deposited in the national herbarium of the Department of Plant Sciences Quid-i-Azam University Islamabad Pakistan. Plant materials were shade dried at room temperature and subjected to a grinder for the formation of a coarse powder drug. The powder drug was stored in a light-resistant and air-tight bottle. Plant material in powdered form of (2 kg) was macerated in 10 L n-hexane, chloroform, ethyl acetate, butanol, water, and methanol with gentle shaking for one week. Using Whatman filter paper (No. 45), the extract was filtered. The obtained filtrate was allowed to evaporate and 36 g to 50 g of extracts was obtained for all solvents. The extracts of each solvent was taken in amounts of 5 g for different analyses, while the rest of the amount of each extract was stored in the refrigerator at 2–4 °C for further use [35].

2.2. Pharmacognostic Studies

Pharmacognostic tests, i.e., solubility and fluorescence tests (cold treatment and hot treatment) for the crude herbal powder of *Gaultheria trichophylla* were carried out using standard methods [36]. HPLC grade chemicals and solvents were used for the different studies of pharmacognostic tests. Standard procedures for solubility and florescence analysis were adopted [8]. The powdered drug at about 1 g was separately mixed with 5 mL of nineteen different solvents for determination of various physicochemical properties under cold and hot treatment. Extracts, powdered drugs, and crude herbal parts were studied out following the procedure [37]. For comparing of color analysis, the Indigo Company (Pakistan) paint chip card was used.

2.3. Phytochemical Studies

A standard procedure, according to [38], was adopted with slight modification by using silica gel 60 GF 254 instead of silica gel 60 GF 245 for qualitative estimation of flavonoids. For qualitative analysis of different phytochemicals, protocol was followed as given against each phytochemical in the respective table given in Section 3.2. Using methodology given by [39], amount of total phenolic contents (TPC) and [40] were used for total flavonoids content present in each extract.

2.4. Elemental Analysis

Elemental analysis was performed according to the standard procedure of [41] with the help of the Shimadzu AA-670 atomic absorption spectrophotometer using the relation: Nutrient cation in plants = (ppm in extract − Blank) × A/W × dilution factor.

2.5. Cytotoxic Activity

Methanolic extracts of plant was tested for cytotoxic potential according to protocol of [42]. For cytotoxic evaluation different concentrations of methanolic extracts were used such a: 1, 5, 10, 50, 100, 250, 500, and 750 µg/mL, respectively. Using computer software, graph pad prism LC_{50} values were determined.

2.6. Antifungal Activity

Antifungal activity for various plant extracts was carried out using the procedure of [43] by agar tube dilution method. Four fungal pathogenic strains, e.g., *Helminthosporium solani*, *Fusarium solani (0300)*, *Aspergillus flavus (0064)*, and *Aspergillus fumigatus (66)* were used in current assay. The concentrations used were 5, 10, and 15 mg/mL. Positive and negative control test tubes containing Terbinafine and DMSO were respectively inoculated.

2.7. Antibacterial Activity

Using the standard procedure as adapted by [44], antibacterial evaluation for plant extracts were carried out. Four pathogenic bacterial strains were used, such as Gram positive i.e., *Bacillus subtillis* ATCC6633, and *Staphylococcus aureus* ATCC6538 as well as Gram negative i.e., *Escherichia coli* ATCC15224 and *Pseudomonas aeruginosa* ATCC13048. Briefly, about 0.75 mL of the broth culture containing 10^8 colony forming units (CFU) per ml of the test strain was added to 75 mL of nutrient agar medium at 45 °C, mixed well, and then poured into a 14 cm sterile petri plate. Three concentrations viz. 7.5 mg/mL, 15 mg/mL, and 30 mg/mL were used for methanolic extract and other extracts. Roxithromycin 1 mg/mL and Cefixime-USP 1 mg/mL were used for positive control and one (DMSO) was used as a negative control treatment for reference in every Petri plate.

2.8. Statisctical Analysis

Using software Statistix 8.1 version, data was subjected to one-way ANOVA for the analysis of variance. All means significantly different from one another have different English alphabets while those means which were non-significantly different from one another share the same English alphabets at HSD (2.45) and $p < 0.05$.

3. Results

3.1. Pharmacognostic Studies

Pharmacognostic tests, i.e., solubility and fluorescence test in cold conditions and hot conditions, were carried out for crude herbal parts of *Gaultheria trichophylla* in order to distinguish the herbal powder drug from its close relatives. The results and observations revealed from cold treatment are given in Table 1 and those under hot treatment are given in Table 2.

Table 1. Physicochemical properties under cold treatment.

S.No	Treatment	Under Visible Light	Under UV	On the Filter Paper Under Visible Light	On the Filter Paper Under UV Light	Solubility
1	Dried leaf powder	Pinkish green	Pinkish red	-	-	-
2	Powdered drug + 50% KOH	Woven slats	Milky green	Celery seed	Woven slats	Semi sol
3	Powdered drug + 10% aq. $FeCl_3$	Dark brown	Brownish black	Spiny green	Revival mahogany	Soluble
4	Powdered drug + dH_2O	Transparent	Milky green	Egg shell	Spiny green	Semi sol

Table 1. *Cont.*

S.No	Treatment	Under Visible Light	Under UV	On the Filter Paper Under Visible Light	On the Filter Paper Under UV Light	Solubility
5	Powdered drug + HCl Conc	Woven slats	Brownish green	Glinted white	Spiny green	Soluble
6	Powdered drug + HCl 50%	Silk knot	Hidden green	Gardenia	Violet	Soluble
7	Powdered drug + H_2SO_4 Conc	Brownish black	Greenish brown	Chocolate	Greenish brown	Soluble
8	Powdered drug + H_2SO_4 50%	Dark cotton	Light brown	Hidden green	Willow branch	Semi sol
9	Powdered drug + HNO_3 Conc	Golden	Brownish green	Glinted white	Revival mahogany	Soluble
10	Powdered drug + HNO_3 50%	Golden	Hidden green	Glinted white	Violet	Soluble
11	Powdered drug + CH_3OH Conc	Willow branch	Yellowish red	Gardenia	White	Soluble
12	Powdered drug + CH_3OH 50%	Transparent	Single blade	Gardenia	Hidden green	Soluble
13	Powdered drug + $CHCl_3$ Conc	Glinted white	Milkish red	Green whimsy	Sky shy	Soluble
14	Powdered drug + $CHCl_3$ 50%	Light leaf	Spring cut	Gardenia	Light leaf	Soluble
15	Powdered drug + C_2H_5OH Conc	Transparent	Milkish red	Gardenia	White	Soluble
16	Powdered drug + C_2H_5OH 50%	Transparent	Greenish whimsy	Gardenia	Hidden green	Soluble
17	Powdered drug + CH_3COOH Conc	Light golden	Milky pink	Gardenia	White	Soluble
18	Powdered drug + CH_3COOH 50%	Caramel cream	Spring cut	Gardenia	White	Soluble
19	Powdered drug + C_6H_6 Conc	Spiney green	Dark red	Gardenia	White	Soluble
20	Powdered drug + C_6H_6 50%	Light lichens	Bands of red and green	Light lichens	Red and spots of white lilac	Soluble

Table 2. Physicochemical properties under hot treatment.

S.No	Treatment	Under Visible Light	Under UV	On the Filter Paper under Visible Light	On the Filter Paper under UV Light	Solubility
1	Dried leaf powder	Pinkish green	Pinkish red	-	-	-
2	Powdered drug + 50% KOH	Woven slats	Light brown	Celery seed	Violet	Semi sol
3	Powdered drug + 10% aq. $FeCl_3$	Green brown	Brownish black	Spiney green	Revival mahogany	Soluble
4	Powdered drug + dH_2O	Light cotton	Milky green	Egg shell	Spiney green	Semi sol
5	Powdered drug + HClConc	Light brown	Pinkish brown	Glinted white	Spiney green	Soluble
6	Powdered drug + HCl 50%	Silk knot	Hidden green	Gardenia	Violet	Soluble
7	Powdered drug + H_2SO_4 Conc	Brownish black	Dark brown	Chocolate	Dark green	Soluble
8	Powdered drug + H_2SO_4 50%	light golden	Light brown	Hidden green	Willow branch	Soluble
9	Powdered drug + HNO_3 Conc	Dark golden	Pinkish brown	Glinted white	Violet	Soluble
10	Powdered drug + HNO_3 50%	Golden	Light brown	Glinted white	Violet	Soluble
11	Powdered drug + CH_3OH Conc	Marsh green	Pinkish red	Gardenia	White	Soluble
12	Powdered drug + CH_3OH 50%	Cotton	Single blade	Gardenia	Hidden green	Soluble
13	Powdered drug + $CHCl_3$ Conc	Woven slats	Milkish red	Green whimsey	Sky shy	Soluble
14	Powdered drug + $CHCl_3$ 50%	Celery seed	Hidden green	Gardenia	White lilac	Soluble
15	Powdered drug + C_2H_5OH Conc	light golden	Milkish red	Gardenia	White	Soluble
16	Powdered drug + C_2H_5OH 50%	Cotton	Single blade	Gardenia	Hidden green	Soluble
17	Powdered drug + CH_3COOH Conc	light golden	Milkish red	Gardenia	White	Soluble
18	Powdered drug + CH_3COOH 50%	Silk knot	Hidden green	Gardenia	White	Soluble
19	Powdered drug + C_6H_6 Conc	Spiney green	Dark red	Gardenia	White	Soluble
20	Powdered drug + C_6H_6 50%	Light lichens	Bands of red and green	Light lichens	Red and spots of white lilac	Soluble

3.2. Qualitative Analysis

Qualitative analysis of methanolic extracts showed the presence of different active constituents in *G. trichophylla* as given in Table 3. Using Mayer's reagent and Dragendroff's reagent test, alkaloid presence was confirmed through Dragendroff's reagent. Qualitative estimation of flavonoids through TLC showed the presence of four flavonoids (Figure 1).

Table 3. Qualitative estimation of phytochemical and their respective protocols.

S.NO	Test	Result	Protocol Used
1	Flavonoid	+ +	[38,45]
2	Saponin	+ +	[38]
3	Tannins	+ +	[46]
4	Phenolic	+ +	[45]
5	Terpenoids	+ +	[38]
7	Alkaloids	+ +	[38]

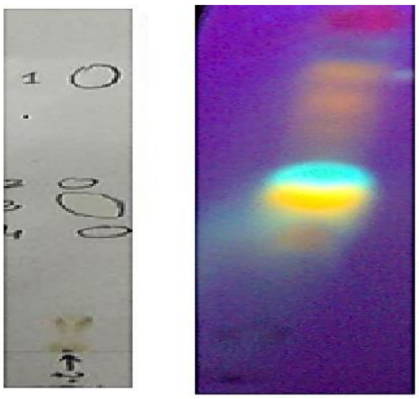

Figure 1. Qualitative estimation of flavonoids with TLC.

3.3. Quantitative Estimation of Phytochemical

Quantitative estimation of phytochemical (total phenolics and total flavonoids) revealed that maximum concentration of total phenolics (14.17 ± 0.88 mg/g) were recorded in GTHE and (13.54 ± 0.55 mg/g) in GTME while the lowest contents (3.27 ± 0.44 mg/g) were reported in GTWE. Similarly, estimation of the total flavonoid contents revealed maximum concentration of the total flavonoids contents (26.9 ± 0.23 mg/g) in GTME and (26.8 ± 3.04 mg/g) in GTHE while the lowest contents (8.08 ± 0.48 mg/g) were reported in GTWE (Figure 2).

3.4. Elemental Analysis

Elemental analysis of different macro and micro nutrients suggests that Na contents were found in higher concentrations (3.2418 ± 0.055 $\mu g g^{-1}$), however, appreciable amounts of Mg (1.9301 ± 0.088 $\mu g g^{-1}$) and Ca (1.835 ± 0.056 $\mu g g^{-1}$) were also reported. The lowest quantity was reported for K (0.0107 ± 0.060 $\mu g g^{-1}$). Elemental analysis suggests that *G. trichophylla* is a rich source of important macronutrients such as Na, Mg, and Ca, respectively. The heavy metals reported in the current study were under the stipulate quantity of the WHO (Figure 3).

3.5. Cytotoxic Assay

Cytotoxic effects of methanolic extracts were checked out using brine shrimps assay in a control and a normal environment (Table 4). The LC_{50} was calculated for methanolic extracts of *G. trichophylla* were (50 µg/mL). Results of cytotoxic activity showed that moderate death rate of the brine shrimps might be expected due to the biologically active components in the methanolic extracts of *G. trichophylla*.

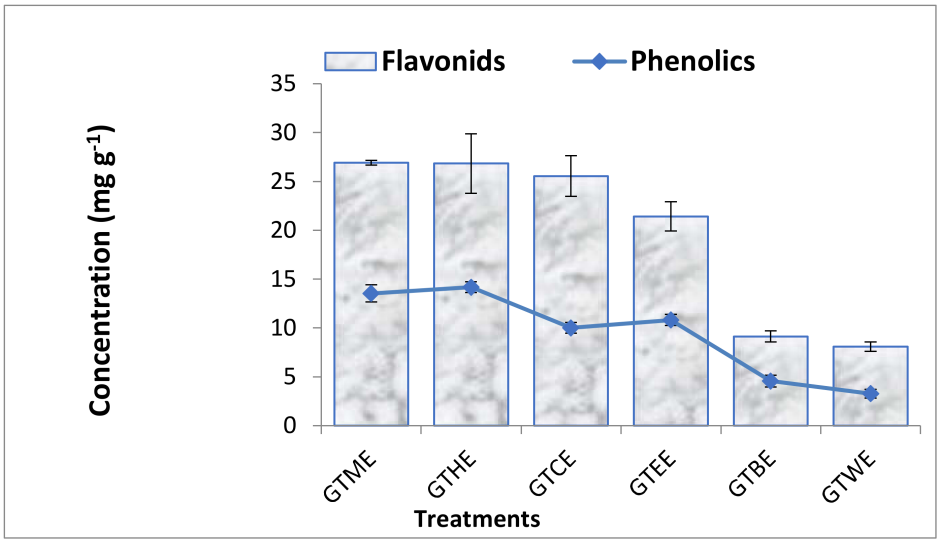

Figure 2. Phytochemical results of quantitative concentration of total phenolics and flavonoids. **Key:** *Gaultheria trichophylla* methanolic extract (GTME), n-hexane extract (GTHE), chloroform extract (GTCE), ethyl acetate extract (GTEE), butanol extract (GTBE), and water extract (GTWE).

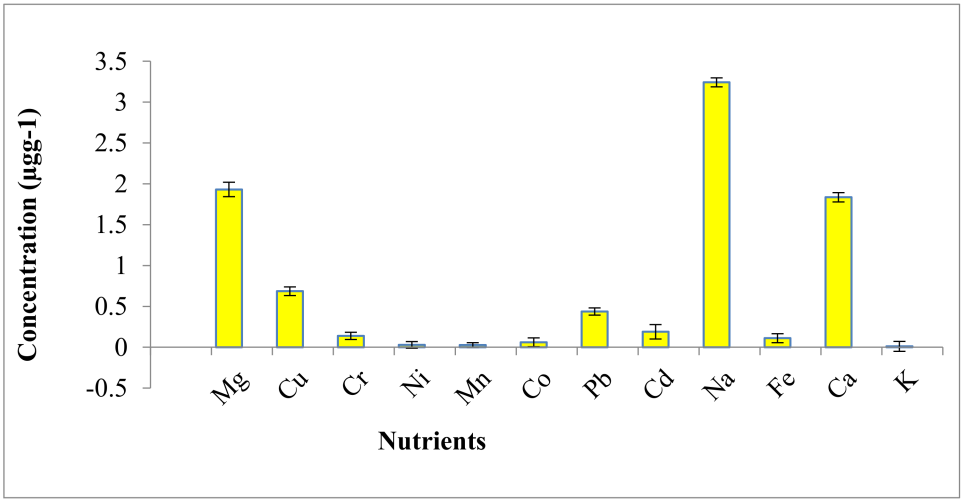

Figure 3. Elemental analysis results for quantitative concentration of different macro and micro nutrients.

Table 4. Results of cytotoxic activity for methanolic extracts of *G. trichophylla*.

S.No	Conc. (µg/mL)	No. of Live	No. of Dead	Percent Activity	LC_{50}
1	1	8	2	20	
2	5	7	3	30	
3	10	7	3	30	
4	50	5	5	50	50
5	100	5	5	50	µg/mL
6	250	4	6	60	
7	500	1	9	90	
8	750	0	10	100	

3.6. Antifungal Assay

The current antifungal study of *G. trichophylla* extracts was tested out against fungal strains (Table 5). More than 70% growth of *Helminthosporium solani* was inhibited by GTME (71 ± 2.8), while the lowest inhibition was recorded for GTHE (48.5 ± 3.75). In the case of

Fusarium solani, maximum inhibition was recorded for GTEE (72.5 ± 2.59) while lowest for GTME (58 ± 0.57). The results for *Aspergillus flavus* revealed maximum inhibition for GTME (78 ± 2.886) and lowest for GTEE (29 ± 1.154). The antifungal activity against *Aspergillus fumigates* revealed maximum inhibition for GTEE (61.5 ± 0.29) and lowest for GTCE (44 ± 1.54) at $p < 0.05$.

Table 5. Results of antifungal activities for different extracts of *Gaultheria trichophylla*.

	% Inhibition of Various Fungal Species			
Treatment	***Helminthosporiumsolani***	***Fusarium solani***	***Aspergillus flavus***	***Aspergillus fumigatus***
Control	86.33 ± 0.88 ^{a}	77.3 ± 1.2 ^{a}	84 ± 1.52 ^{a}	86.66 ± 0.09 ^{a}
GTME	71 ± 2.87 ^{b}	58 ± 0.57 ^{bc}	78 ± 2.886 ^{b}	59 ± 5.19 ^{bc}
GTHE	48.5 ± 3.75 ^{d}	70 ± 1.154 ^{ab}	35 ± 4.04 ^{c}	53.5 ± 3.17 ^{c}
GTCE	57 ± 2.89 ^{c}	67.5 ± 0.29 ^{b}	37 ± 0.577 ^{c}	44 ± 1.54 ^{d}
GTEE	58.5 ± 0.29 ^{c}	72.5 ± 2.59 ^{ab}	29 ± 1.154 ^{cd}	61.5 ± 0.29 ^{b}
GTBE	53 ± 4.04 ^{cd}	65.5 ± 3.75 ^{b}	37 ± 0.577 ^{c}	55 ± 0.58 ^{c}
GTWE	68 ± 4.04 ^{b}	72 ± 0.57 ^{ab}	30.5 ± 1.44 ^{cd}	53 ± 4.04 ^{c}

Key: Means significantly different from one another have different English alphabets while those not significantly different from one another share the same English alphabets at HSD (2.45) and $p < 0.05$.

3.7. Antibacterial Assay

The antibacterial activity was performed for various extracts of *G. trichophylla* to check out the zone of inhibition (Table 6). Gram positive bacteria such as *Bacillus subtillis* was inhibited by MIC value of GTME (15 mg/mL), GTEE (7.5 mg/mL), GTBE (7.5 mg/mL), and GTWE (15 mg/mL), respectively. MIC values for *Staphylococcus aureus* include 7.5 mg/mL for methanolic and other extracts. In case of Gram-negative bacteria GTCE, GTEE, GTBE, and GTWE (7.5 mg/mL) showed MIC against *Escherichia coli*; however, GTME and GTHE inhibited the growth of *Escherichia coli* with MIC 15 mg/mL and 30 mg/mL. The bacterial strain *Pseudomonas aeruginosa* was inhibited by 7.5 mg/mL of GTME, GTHE, GTCE, GTEE, GTBE, and GTWE; however, no inhibition was recorded, for the extracts were symbolized with ND (not detected) against the said strains. Figure 4 shows the zone inhibition recorded for the tested extract against the bacterial strain used.

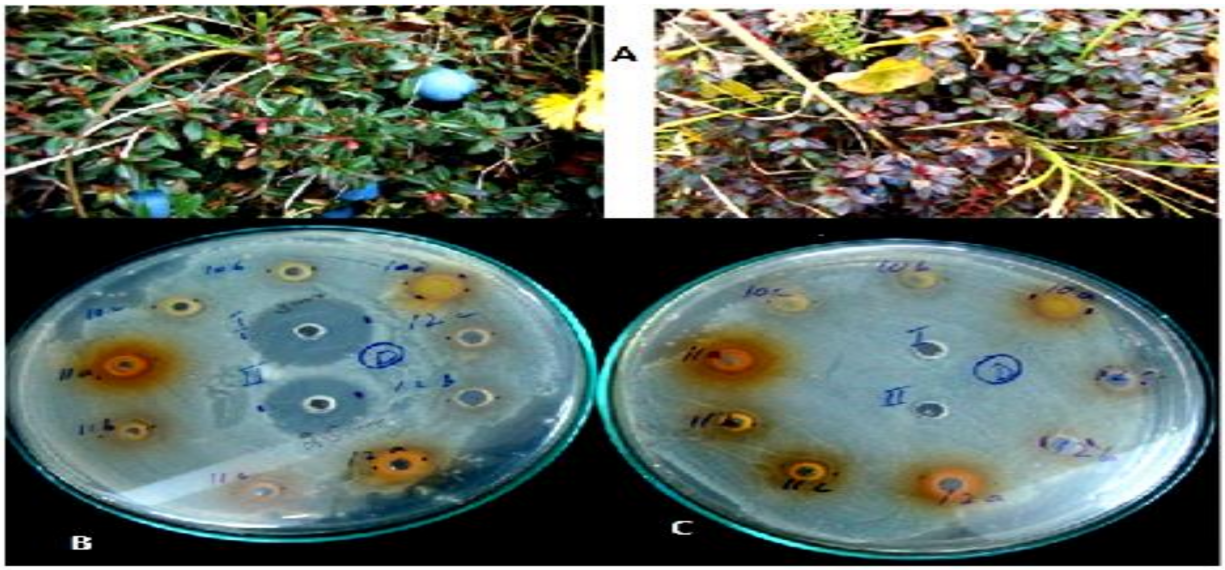

Figure 4. *Gaultheria trichophylla* (**A**). Growing in its natural habitat (**B**), and (**C**) Clear zone of inhibition of antibacterial activities.

Table 6. Antibacterial activities of various fractions of *Gaultheria trichophylla*.

Samples	*Bacillus subtillis*			*Staphylococcus aureus*			*Escherichia coli*			*Pseudomonas aeruginosa*		
	30 mg/mL	**15 mg/mL**	**7.5 mg/mL**	**30 mg/mL**	**15 mg/mL**	**7.5 mg/mL**	**30 mg/mL**	**15 mg/mL**	**7.5 mg/mL**	**30 mg/mL**	**15 mg/mL**	**7.5 mg/mL**
GTME	14.5 ± 0.5^{d}	11.5 ± 0.408^{e}	ND	15.5 ± 0.25^{cd}	13 ± 0^{d}	11.5 ± 0.5^{e}	13 ± 1^{de}	10.5 ± 0.25^{e}	ND	12.75 ± 0.75^{de}	11.5 ± 0.25^{e}	10 ± 1^{ef}
GTHE	ND	ND	ND	13 ± 0.5^{d}	11 ± 1^{e}	10 ± 1^{ef}	11.5 ± 0.5^{e}	ND	ND	13.5 ± 0.5^{d}	13.5 ± 0.5^{d}	9.5 ± 0.5^{f}
GTCE	ND	ND	ND	16 ± 0.5^{c}	14 ± 2^{cd}	12.5 ± 1.5^{de}	16 ± 0^{c}	11.5 ± 0.25^{e}	11 ± 0^{ce}	11.5 ± 0.5^{e}	11 ± 0.5^{e}	13.5 ± 0.5^{d}
GTEE	16 ± 1^{c}	13 ± 0.816^{de}	11.5 ± 0.5^{e}	20.5 ± 0.75^{b}	14.5 ± 0.5^{cd}	13.5 ± 0.5^{d}	17.5 ± 0.5^{bc}	13.5 ± 0.25^{de}	12 ± 1^{c}	20.5 ± 2.5^{b}	11 ± 1.5^{e}	11 ± 1^{e}
GTBE	14.5 ± 0^{d}	11.5 ± 0.408^{e}	9.5 ± 0.5^{f}	16.5 ± 0.25^{c}	14.5 ± 0.5^{cd}	12 ± 1^{de}	16.5 ± 1.5^{c}	12 ± 0^{de}	10.5 ± 0.5^{e}	17.5 ± 1.5^{bc}	14.5 ± 0.25^{c}	13.5 ± 0.5^{d}
GTWE	14 ± 2^{d}	11.5 ± 0.408^{e}	ND	17.5 ± 0.25^{bc}	15.5 ± 0.5^{cd}	13 ± 0^{d}	14.5 ± 0.5^{d}	11 ± 0.5^{e}	10.5 ± 1.5^{e}	13 ± 0^{d}	12.5 ± 0.75^{de}	13 ± 1^{d}
PC-I	27.5 ± 0.5^{a}	27.5 ± 0.5^{a}	27.5 ± 0.5^{a}	27.5 ± 0.5^{a}	27.5 ± 0.5^{a}	27.5 ± 0.5^{a}	27.5 ± 0.5^{a}	27.5 ± 0.5^{a}	27.5 ± 0.5^{a}	27.5 ± 0.5^{a}	27.5 ± 0.5^{a}	27.5 ± 0.5^{a}
PC-II	18.5 ± 0.5^{b}	18.5 ± 0.5^{b}	18.5 ± 0.5^{b}	18.5 ± 0.5^{b}	18.5 ± 0.5^{b}	18.5 ± 0.5^{b}	18.5 ± 0.5^{b}	18.5 ± 0.5^{b}	18.5 ± 0.5^{b}	18.5 ± 0.5^{b}	18.5 ± 0.5^{b}	18.5 ± 0.5^{b}

Means significantly different from one another have different English alphabets while those not significantly different from one another share the same English alphabets at HSD (2.45) and $p < 0.05$.

4. Discussion

In the south west district of China, *Gaultheria yunnanensis* (Franch.) Rehd. from the family Ericaceae is widely used herbal medicine to treat rheumatoid arthritis, trauma, swelling pains, chronic tracheitis, cold, and vertigo. Analgesic and antipyretic effects of this plant are due to its volatile oil called "wintergreen oil" [47]. From the pharmacognostic evaluation it can be concluded that the powder drug of *G. trichophylla* can be differentiated and authenticated on the basis of fluorescence and solubility analysis. The various solvents (C_6H_6 and HNO_3) used in the current study for pharmacognostic characterization may be recommended as a reference for future studies. Qualitative study of phytochemicals suggested the presence of various active constituents, while an appraisable quantity of phenolics and flavonoids were also reported in a quantitative estimation of different extracts. The current findings of the total phenolic and flavonoids contents also agreed with [24]. Flavonoids estimation with TLC showed the presence of four flavonoids. The presence of phytoconstituents in *G. trichophylla* such as rutin, quercetin, and gallic acid was also confirmed using HPLC techniques by [48]. According to [47], some salicylate derivatives, organic acids, lignans, diterpenoids, triterpenoids, sterols, coumarins, and flavonoids have been isolated and identified from the roots of *G. yunnanensis*. From the study of [4], two methyl salicylate glycosides were naturally isolated from *G. yunnanensis*. These results also showed consistency with those of [49]. Elemental analysis of the current work revealed important nutritional minerals which are parallel to the findings of [50].

The outcomes of cytotoxic activity revealed moderate cytotoxic activity. Using extracts of *G. trichophylla* [48] and working on fruits of *Gaultheria pumila* [51] reported excellent anticancer activity against the human cancerous cell line. Conclusion of results against said fungal strains revealed that methanolic extracts of *G. trichophylla* were found more efficient fallowed by ethyl acetate and n-hexane extracts. According to [52,53] antifungal properties are due to saponins.

Antibacterial activities conclude that methanolic, acetate, and n-hexane extracts were found more efficient against all the aforementioned bacterial strains. The same extracts of the *Toddalia asiatica* leaf and stem were found active against gram positive and gram-negative bacterial strains according to [54]; similar results were also reported by [43]. Inhibitory effects against various pathogenic bacterial strains of plant extracts can be compared with phenolic composition [55,56]. Crude catechins were found more efficient against gram positive bacteria [51].

A classification for antifungal activities based on MIC values of extracts was proposed by [57]. Extracts considered to be strong inhibitors have MIC values of 500 ug/mL, while extracts having MIC values of 600–1500 ug/mL are considered as moderate inhibitors, and extract considered as weak inhibitors have MIC values above 1600 ug/mL. In this contest, our current study showed antifungal activity of all fractions against the said pathogen; however, our scheme was slightly modified and the fractions that inhibited growth of fungus by more than 70% were considered as strong inhibitors, those having 60–70% were considered as moderate, and below 50% were considered as weak inhibitors. The outcomes of current work defended the use of investigated plant in the alpine and sub-alpine region of Western Himalaya as ethno-medicine.

5. Conclusions

In this study, the selected plant was initially screened out for its phytochemical composition. In the form of extracts, its antibacterial, antifungal, and cytotoxic potentials were evaluated. The essential metal composition was also evaluated. Appreciable amounts of major phytochemical groups along with essential metals were detected in the extract samples. The observed antimicrobial activities suggested that the selected plant extracts contained therapeutic agents that need to be isolated in pure form. Due to the endemic nature of *G. trichophylla*, it is important to conserve the plant using ex-situ and in-situ techniques.

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