

Bioactive Profile of the Wild Mushroom Trogia cantharelloides

V. Ravikrishnan, K. R. Sridhar, and M. Rajashekhar

Abstract

Wild mushrooms have become an integral part of the human diet, health, and industrial applications worldwide. However, many of them will not serve as food due to their unpalatable taste or poisonous or gastrointestinal problems. Trogia cantharelloides is one such mushroom not preferred as food by the tribals in the Western Ghats of India. This study provides baseline data on the T. cantharelloides obtained from the foothills of the southwest region of the Western Ghats of India. Biochemical components like organic acids, sugars, polyphenols, flavonoids, phytic acid, vitamins, trypsin inhibition activity, hemagglutinin activity, and antimicrobial potential of T. cantharelloides are addressed. The therapeutic potential of the bioactive compounds of T. cantharelloides was documented using Duke's phytochemical and ethnobotanical database (www.ars-grin.gov/cgi-bin/duke). Accordingly, a total of 15 compounds compiled along with their characteristics, biological activity, and applications. This study provides scope to explore the bioactive potential of non-edible mushrooms for their use in future health, therapeutic and industrial applications.

Keywords

Bioactive principles · Therapeutics · Antimicrobial potential · The Western Ghats

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5.1 Introduction

The current global estimate of macrofungi is between 2.2 and 3.8 million species (Hawksworth and Lücking 2017). About 2000 species are considered safe for human consumption, and 700 species possess therapeutic potential (Lima et al. 2012). Many mushrooms are inedible owing to their tough texture, tastelessness, and poisonous qualities (Krupodorova et al. 2012; Ivanova et al. 2014; Sevindik 2020). Although many mushrooms are inedible, they are an essential source of biologically active metabolites of health, pharmaceutical, and industrial value (Bal et al. 2017, 2019; Özaltun and Sevindik 2020). There are many reports on mushroom poisoning owing to misidentification in different parts of the world (Lima et al. 2012; Jo et al. 2014; Özaltun and Sevindik 2020).

Mushrooms of the genus *Trogia* are glass funnel mushrooms distributed in tropical and subtropical regions (Mortimer et al. 2014). *Trogia infundibuliformis* exits in Thailand and Laos on rotten woody materials. This species has been reported from Maharashtra (India) on twigs (Senthilarasu 2014). The occurrence of toxic amino acids in *Trogia venenata* has been reported by many researchers (Shi et al. 2012; Zhou et al. 2012; Xu et al. 2018; Yin et al. 2019). The *Trogia cantharelloides* is a widespread species recorded in Puerto Rico, Cuba, Thailand, and China. Sevindik (2020) has documented the antioxidant potential of 13 species of poisonous mushrooms belonging to 10 genera. Index Fungorum has recorded 108 species and 156 records of the genus *Trogia* (accessed on November 23, 2020). It is also one of the wild mushrooms in the foothills of the Western Ghats of India. However, it is not acceptable for edibility by the tribals and local dwellers for unknown reasons (Ravikrishnan 2019).

If a mushroom is inedible or toxic, or poisonous, it is likely not to follow its biochemical composition and bioactive components. This chapter aims to document biochemical composition (organic acids, sugars, polyphenols flavonoids, and phytic acid), vitamins (vitamin C and b-carotene), pigment (lycopene), nutritional (proximal, mineral, amino acids, and fatty acids), antinutritional activity (trypsin inhibition and hemagglutinin) and antimicrobial potential of *T. cantharelloides* as baseline data for future exploitation.

5.2 Biology of Trogia

The genus *Trogia (Marasmiaceae, Basidiomycota)* has been named after Swiss mycologist Jacob Gabriel Trog. This genus circumscribed by Fries (1835) and erected the type species *Trogia montagnei*. Another species, *Cantharellus aplorutis* has been described by the French mycologist Camille Montagne in 1834 (Bélanger 1846). Subsequently, British botanist Corner (1966) emended the genus to include 56 species. Currently, the Index Fungorum has 156 records consisting of 108 species.

Trogia spp. commonly grow on woody litter, possess clitocyboid gills devoid of partial veils with white, yellowish, or pinkish spores. They have cartilage-like stipe,

broad to depressed pileus without partial veil and volva. On drying, their fruit bodies become tough and could be rejuvenated during moist conditions. So far, eight species of *Trogia* are reported from the Indian Subcontinent (*T. benghalensis*, *T. cantharelloides*, *T. cyanea*, *T. grisea*, *T. infundibuliformis*, *T. liaceogrisea*, *T. montagnei*, and *T. subviridis*) (Graham 1915; Uppal et al. 1935; Manjula 1983; Natarajan et al. 2005; Kumar and Manimohan 2009; Senthilarasu 2014; Dutta et al. 2017; Ravikrishnan et al. 2017).

Trogia buccinalis can produce enzymes to degrade pollutants like anthracene, pentachlorophenol, and polyvinyl chloride (Martins-Franchetti et al. 2010). *Trogia venenata* is a poisonous mushroom responsible for the death of 400 people in Yunnan Province of China (Zhou et al. 2012). This mushroom possesses cardiotoxic amino acids, which leads to arrhythmia. *Trogia cantharelloides* is also a widespread neotropics (Halling and Mueller 2002). However, the tribals residing in the foothill of the Western Ghats of India are not consuming this mushroom. *Trogia infundibuliformis* possess small- to medium-size basidiomes with infundibuliform, perforated, membranous, pileus split, decurrent, distant lamellae, stipe central to excentric arising from the white discoid base, while *T. cantharelloides* although it resembles *T. infundibuliformis* distinctly differs in possessing crowded lamellae with small spore size (Pegler 1983).

5.3 Harvest and Process

We sampled mushrooms from Someshwara wildlife sanctuary in Udupi District (13°29'N, 74°50'E) of Agumbe Ghat in the Western Ghats of Karnataka. The average temperature ranges from 23 to 24 °C with 97% humidity. This sanctuary is composed of semi-evergreen as well as moist mixed deciduous forests. The climatic conditions and secondary products of forests are the primary sources for the growth of mushrooms. The whole fruit bodies of Trogia cantharelloides (Mont.) Pat. [synonym: Panus cantharelloides Mont.; Pocillaria cantherelloides (Mont.) Kuntze] were harvested from three locations of the forests (Fig. 5.1) as replicates, stored in a cool pack, and transferred to the laboratory within 4–5 h for processing. The transported fruit bodies were spread on blotting papers to remove the debris. Later, we processed part of the fruit bodies (about 3-5) from triplicate samples for moisture content gravimetrically. The rest of the fruit bodies were rinsed in distilled water and blot dry, followed by drying $(58 \pm 2 \,^{\circ}\text{C})$ in a hot-air oven by spreading on aluminum foils until attaining the constant weight. Triplicate dried fruit bodies blend into coarse to a fine powder. The flour of fruit bodies was transferred into air-tight glass containers and preserved in a refrigerator for further analysis.



Fig. 5.1 Profuse growth of *Trogia cantharelloides* on the woody litter buried in humus in Someshwara wildlife sanctuary of the Western Ghats of India

5.4 Biochemical Composition

5.4.1 Bioactive Components

Among the biochemical components of *T. cantharelloides* assessed, the flavonoids were highest (14.7 mg/g), followed by total phenolics (11.1 mg/g) and tannins (3.2 mg/g) (Table 5.1). Total phenolics content was assessed according to the procedure by Rosset et al. (1982). Gallic acid served as standard to express total phenolics as mg of gallic acid equivalents per gram dry mass of the mushroom (mg GAEs/g). Total phenolics of the wild mushrooms are an essential component in defense against herbivores. It varies between geographic conditions (Okoro 2012;

Table 5.1 Biochemical	Constituent	Quantity
constituents of <i>Trogia</i> cantharelloides (dry mass	Total phenolics (mg GAEs/g)	11.1 ± 0.23
basis) ($n = 3$, mean \pm SD)	Tannins (mg CEs/g)	3.2 ± 0.05
	Phytic acid (mg/g)	0.7 ± 0.06
	Flavonoids (mg QEs/g)	14.7 ± 0.13
	Vitamin C (mg AAEs/g)	1.8 ± 0.12
	β-carotene (µg/g)	1.96 ± 0.02
	Lycopene (µg/g)	1.40 ± 0.03

Attarat and Phermthai 2015). Total phenolics is the major constituent in *T. cantharelloides* along with flavonoids. The phenolics can combat cardiovascular diseases (Visioli et al. 2000; Meng et al. 2002). The vanillin-HCl method was adapted to determine tannin content (Burns 1971). It was denoted as catechin equivalents in mg per gram dry mass of the sample (mg CEs/g). In addition to phenolics, the presence of tannins and phytic acid provides additional strength for antioxidant activity.

The procedures with KH_2PO_4 performed extraction and estimation of phytic acid as standard to determine phytate (Deshpande et al. 1982; Sathe et al. 1983). In addition to antioxidant activity, phytic acid helps preventing kidney stone formation and calcium deposition in arteries (Knekt et al. 2004; Ye and Song 2008). The content of flavonoids is detected by the standard curve of quercetin dihydrate. The flavonoid content is expressed as quercetin equivalents in mg per gram dry mass (mg QEs/g) (Jia et al. 1999). Flavonoids are known for health-promoting attributes like cardioprotective, hepatoprotective, anti-inflammatory, and anti-diabetes (Champ 2002; Tapas et al. 2008).

Estimated the vitamin C content according to Roe (1954) with ascorbic acid as standard, and its content was noted as ascorbic acid equivalents in mg per gram of the dry mass (mg AAEs/g) (Table 5.1). Vitamin C is present in substantial quantity in *T. cantharelloides*, and it is a potent antioxidant as well as radical-scavenger; however, it will be vulnerable to increased temperature (Gregory III 1996; Podmore et al. 1998). The β -carotene and lycopene contents are assessed by the method outlined by Barros et al. (2007) (Table 5.1). Carotenoids in mushrooms are also known for their antioxidant activity (Barros et al. 2007). A substantial amount of vitamin C is found in *T. cantharelloides* compared to β -carotene and lycopene. All these components are known as potential antioxidants and radical scavengers.

5.4.2 Sugars, Organic Acids, and Polyphenols

Soluble sugars of *T. cantharelloides* are assessed using an amino column with acetonitrile and water (3:1) as the mobile phase (Reis et al. 2012). Three soluble sugars found with the highest quantity of arabinose in methanol extract (2.5 mg/g) below the detectable level in aqueous extract (Table 5.2). The second highest soluble sugar was glucose in aqueous extract (1.7 mg/g), and it was 0.9 mg/g in methanol extract. Trehalose was higher in methanol extract than aqueous extract (0.4 vs. 0.3 mg/g). Turfan et al. (2018) opine that the soluble sugar composition of wild mushrooms is controlled by many factors (e.g., genetic, stage of growth, and conditions of harvest).

Organic acids were evaluated based on the protocol by Pereira et al. (2013). Four organic acids were detected with the highest quantity of succinic acid in aqueous extract (24.4 mg/g), while it was below the detectable level in methanol extract (Table 5.2). Like succinic acid, acetic acid (2.3 mg/g) and tartaric acid (0.9 mg/g) were found only in methanol extract. Pyruvic acid is detected in aqueous and methanol extracts (2.3 and 1.3 mg/g, respectively). Organic acids in mushrooms

Soluble sugar	mg/g
Arabinose (A)	BDL
Arabinose (M)	2.5 ± 0.00
Glucose (A)	1.7 ± 0.14
Glucose (M)	0.9 ± 0.02
Trehalose (A)	0.3 ± 0.02
Trehalose (M)	0.4 ± 0.00
Organic acid	
Acetic acid (A)	BDL
Acetic acid (M)	2.3 ± 0.09
Pyruvic acid (A)	1.3 ± 0.10
Pyruvic acid (M)	0.4 ± 0.01
Succinic acid (A)	24.4 ± 0.80
Succinic acid (M)	$\begin{array}{c} 2.3 \pm 0.09 \\ \hline 1.3 \pm 0.10 \\ 0.4 \pm 0.01 \\ 24.4 \pm 0.80 \\ \hline BDL \\ BDL \\ 0.9 \pm 0.03 \\ \hline \end{array}$
Tartaric acid (A)	BDL
Tartaric acid (M)	0.9 ± 0.03
Polyphenols	
Methyl catechol	0.5 ± 0.01
Ethyl catechol	3.2 ± 0.00
	Arabinose (A) Arabinose (M) Glucose (A) Glucose (M) Trehalose (A) Trehalose (M) Organic acid Acetic acid (A) Acetic acid (A) Pyruvic acid (A) Succinic acid (A) Succinic acid (A) Tartaric acid (A) Polyphenols Methyl catechol

principally serve as flavoring agents and antioxidants (Vaughan and Geissler 1997; Silva et al. 2004). These components are not susceptible to changes depending on the mushroom processing and storage conditions (Cámara et al. 1994).

Polyphenols were estimated based on the method by Dasgupta et al. (2015). Among polyphenols, the ethyl catechol was highest (3.2 mg/g), followed by methyl catechol (0.5 mg/g) (Table 5.2). Like fruits and vegetables, mushrooms are also a source of many polyphenols that possess considerable therapeutic value and correlated with antioxidant activities (Barros et al. 2009; Ren et al. 2014; Lin et al. 2015; Smolskaité et al. 2015).

5.4.3 Bioactive Compounds

The GC-MS/MS assessed the chemical composition of mushroom with the Scion 436-GC Bruker model coupled with a triple quadrupole mass spectrophotometer. The relative percentage of each component estimated by comparison of average peak area to the total areas with software MS Work Station 8. The National Institute of Standard and Technology (NIST) Version # 2.0 library database employed to identify the chemical components. The spectrum of the unknown component compared with the spectrum of the known component stored in the NIST library. Particulars of each compound ascertained by Srinivasan and Kumaravel (2016). Therapeutic potential of each compound (NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library Version # 2.0; Gaithersburg, MD, USA)

established based on the Dr. Duke's phytochemical and ethnobotanical databases (www.ars-grin.gov/cgi-bin/duke).

The GC-MS/MS analysis of a crude extract of T. cantharelloides showed up to 16 bioactive compounds of applied value (Table 5.3). Those compounds include purine nucleobase, cyclic purine nucleotide, indonel galactonic acid derivative, fatty acid methyl esters, saturated fatty acids, triterpene, g-lactam, and phytosterol. Details of bioactive compounds present in T. cantharelloides include: purine derivatives (adenosine 3,5-cyclic monophosphate and 6H-purin-6-one,1,7-dihydro-); growth hormone (indole); squalene; 2-pyrrolidinone; 2-acetamide-2-deoxygalactono-1,4lactone; ergosterol; saturated fatty acids (tetradecanoic and n-hexadecanoic acids); ethyl ester (9,12-octadecadienoic acid); fatty acid methyl esters (hexadecanoic, 9,12octadecadienoic, stearic, dodecanoic and 9-octadecenoic acids). Many are useful in anti-inflammatory, immunostimulant, health protection: cytoprotective, anticarcinogenic, antitumor, hypercholesterolemic, antioxidant, antiandrogenic, antiangiogenic, and diuretic. Several compounds possess antibacterial, antifungal, nematicidal, and antiviral properties. Some of them are industrially valued potential, such as nutraceuticals, flavors, and lubricants.

5.5 Nutritional and Antinutritional Attributes

5.5.1 Nutritional Components

The nutritional profile of *T. cantharelloides* (proximal qualities, minerals, amino acids, and fatty acids) was evaluated by Ravikrishnan et al. (2017). The carbohydrate content was highest (86.7%), followed by crude fiber (11.1%), crude protein (9.5%), and total lipids (2.3%) with a calorific value of 1720 kJ/100 g. Among the minerals, phosphorus was the highest (260 mg/100 g), followed by potassium (12.6 mg/100 g), calcium (1.3 mg/100 g), and magnesium 1.2 mg/100 g). The rest of the minerals (iron, copper, sodium, selenium, and zinc) were <1 mg/100 g. The Na/K ratio of *T. cantharelloides* (<1) obeys the NRC-NAS standard (NRC-NAS 1989; USDA 1999), and it is a favorable ratio to combat the blood pressure (Yusuf et al. 2007).

Among dispensable and indispensable amino acids of *T. cantharelloides* (mg/100 g protein) similar to other edible mushrooms, the glutamic acid was highest (12.9 mg) followed by glycine (9.1 mg), serine (8.6 mg), alanine (7.8 mg), arginine (7 mg), lysine (6.9 mg), leucine (6.6 mg), proline (6.3 mg), valine (5.7 mg), arginine, threonine (4.9 mg each), and isoleucine (4.5 mg). The rest of the amino acids were: <4 mg/100 g protein (histidine, tyrosine, methionine, cystine, and phenylalanine).

The fatty acid profile of *T. cantharelloides* consists of ten saturated and six unsaturated fatty acids. Among saturated fatty acids (g/100 g total lipids), palmitic acid content was the highest (10.2 g), followed by stearic acid (2.7 g) and lignoceric acid (1.7 g). Rest of the saturated fatty acids (capric, lauric, myristic, pentadecanoic, heptadecanoic, behenic, and tricosanoic acids) were <1 g. Among the unsaturated fatty acids (g/100 g total lipids), the linoleic acid content was highest (34.6 g),

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timeCompound13.87Adenosine 3,5-cyclic13.87Adenosine 3,5-cyclic 3.74 $6H$ -Purin-6-one,1,7- 3.74 $6H$ -Purin-6-one,1,7- 7.07 Indole 7.07 Indole 7.07 Indole 27.79 Squalene 3.61 2 -Pytrolidinone 3.61 2 -Pytrolidinone 3.61 2 -Acetamide-2- 3.61 2 -Acetamide-2- 7.66 2 -Acetamide-2- $3.4.9$ Ergosterol 12.64 Tetradecanoic acid 12.64 Tetradecanoic acid 12.64 Tetradecanoic acid 15.11 Hexadecanoic acid 16.03 n -Hexadecanoic acid 16.03 n -Hexadecanoic acid 17.43 $9,12$ -Octadecadienoic acid, 17.43 $9,12$ -Octadecadienoic acid, 17.89 Stearic acid, methyl ester 17.89 Stearic acid, methyl ester 17.53 9-octadecenoic acid, methyl 17.53 9-octadecenoic acid, methyl	Compound	Retention		Nature of the		Peak
13.87Adenosine 3.5-cyclicCyclic purine 3.74 $6H$ -Purin-6-one,1,7-Purine 3.74 $6H$ -Purin-6-one,1,7-Purine 7.07 $6H$ -Purin-6-one,1,7-Purine 7.07 $6H$ -Purin-6-one,1,7-Purine 7.07 $1dole$ $1ndole$ 7.07 $5qualene$ $1ndole$ 7.07 $5qualene1ndole27.795qualene1ndole3.612-Pyrrolidinone1rdole3.612-Pyrrolidinone1rdole3.612-Acetamide-2-Galactonic acid3.49Ergosterol14-lactam3.49Ergosterol2412.64Tetradecanoic acid2trated fatty12.64Tetradecanoic acid2trated fatty12.64Tetradecanoic acid2trated fatty12.64Tetradecanoic acid2trated fatty12.64Tetradecanoic acid2trated fatty12.64Tetradecanoic acid2trated fatty12.64Tetradecanoic acid2trated fatty12.6412-Octadecalienoic acid, methyl2trated fatty12.6412-Octadecanoic acid2trated fatty12.64$	number	time	Compound	compound	Biological activity/uses/applications	area (%)
3.74 $6H-Purin-6-one,1,7-$ Purine 7.07 IndoleIndole 7.07 IndoleIndole 27.79 SqualeneTriterpene 27.79 Squalene γ -lactam 3.61 2 -Pyrrolidinone γ -lactam 12.64 $Ergosterol\gamma-lactam16.03n-Hexadecanoic acid\gamma-lactam16.03n-Hexadecanoic acid\gamma-lactam16.03n-Hexadecanoic acid\gamma-lactam16.03n-Hexadecanoic acid\gamma-lactam16.03n-Hexadecanoic acid\gamma-lactam16.03n-Hexadecanoic acid\gamma-lactam17.439, 12-Octadecadienoic acid\gamma-lactam17.439, 12-Octadecadienoic$	1	13.87	Adenosine 3,5-cyclic monophosphate	Cyclic purine nucleotide	Second messenger	1.44
7.07IndoleIndole27.79SqualeneTriterpene27.79SqualeneTriterpene3.612-Pyrrolidinone γ -lactam3.612-Pyrrolidinone γ -lactam3.612-Acetamide-2-Galactonic acid7.662-Acetamide-2-Galactonic acid9.12.64ErgosterolPhytosterol12.64Tetradecanoic acidSaturated fatty12.64Tetradecanoic acidSaturated fatty13.10Hexadecanoic acidSaturated fatty13.409.12-Octadecadienoic acid, methylFatty acid17.439.12-Octadecadienoic acid, methyl esterIntelyl ester17.89Stearic acid, methyl esterIntelyl ester17.539-octadecenoic acid, methyl esterIntelyl ester17.539-octadecenoic acid, methyl esterIntelyl ester17.539-octadecenoic acid, methyl esterIntel	7	3.74	6H-Purin-6-one,1,7- dihydro-	Purine nucleobase	Anti-inflammatory and cytoprotective	1.29
27.79SquateneTriterpene 3.61 2-Pyrrolidinone γ -lactam 3.61 2-Pyrrolidinone γ -lactam 3.61 2-Actamide-2-Galactonic acid 7.66 2-Actamide-2-Galactonic acid 34.49 ErgosterolPhytosterol 12.64 Tetradecanoic acidPhytosterol 12.64 Tetradecanoic acidSaturated fatty 12.64 n -Hexadecanoic acidSaturated fatty 12.61 n -Hexadecanoic acidSaturated fatty 12.64 n -Hexadecanoic acidSaturated fatty 16.03 n -Hexadecanoic acidSaturated fatty 15.11 n -Hexadecanoic acidSaturated fatty 18.40 $9,12$ -Octadecadienoic acid, Patty acid ethyl 17.43 $9,12$ -Octadecadienoic acid, methyl ester 17.43 $9,12$ -Octadecadienoic acid, methyl ester 17.89 Stearic acid, methyl ester 17.89 Stearic acid, methyl ester 10.18 Dodecanoic acid, methyl ester 17.53 9 -octadecenoic acid, methyl ester	3	7.07	Indole	Indole	Anticariogenic, cancer preventive, anti-acne, antibacterial, anti-Salmonella, anti-streptococci	0.44
3.61 2 -Pyrrolidinone γ -lactam 7.66 2 -Acetamide-2-Galactonic acid 7.66 2 -Acetamide-2-Galactonic acid 34.49 ErgosterolPhytosterol 34.49 ErgosterolPhytosterol 12.64 Tetradecanoic acidSaturated fatty 12.64 Tetradecanoic acidSaturated fatty 12.64 Tetradecanoic acidSaturated fatty 12.64 n -Hexadecanoic acidSaturated fatty 12.64 n -Hexadecanoic acidSaturated fatty 16.03 n -Hexadecanoic acidPatty acid ethyl 15.11 Hexadecanoic acid methylPatty acid 17.43 $9, 12-Octadecadienoic acid, methyl esterPatty acid17.439, 12-Octadecadienoic acid, methyl esterPatty acid17.89Stearic acid, methyl esterPatty acid17.539-octadecenoic acid, methylPatty acid$	4	27.79	Squalene	Triterpene	Cancer preventive, diuretic, chemopreventive, and immunostimulant	0.69
7.66 2 -Acetamide-2- deoxygalactono-1,4-lactoneGalactonic acid derivative 34.49 ErgosterolPhytosterol 34.49 ErgosterolPhytosterol 12.64 Tetradecanoic acidSaturated fatty acid 12.64 Tetradecanoic acidSaturated fatty 12.64 Tetradecanoic acidSaturated fatty acid 16.03 n -Hexadecanoic acidSaturated fatty 17.49 9.12 -Octadecadienoic acid, methyl esterMethyl ester 17.43 9.12 -Octadecadienoic acid, methyl esterMethyl ester 17.89 Stearic acid, methyl esterMethyl ester 10.18 Dodecanoic acid, methyl esterMethyl ester 17.53 9 -octadecenoic acid, methyl esterMethyl ester 17.53 9 -octadecenoic acid, methyl esterMethyl ester 17.53 9 -octadecenoic acid, methyl esterMethyl ester	5	3.61	2-Pyrrolidinone	γ-lactam	Serve as intermediate/precursor in pharmaceutical drugs and used in inkjet cartridges	3.12
34.49ErgosterolPhytosterol 34.49 Tetradecanoic acidPhytosterol 12.64 Tetradecanoic acidSaturated fatty 16.03 n -Hexadecanoic acidSaturated fatty 16.03 n -Hexadecanoic acidacid 18.40 $9,12$ -Octadecadienoic acid,Fatty acid ethyl 18.10 $9,12$ -Octadecadienoic acid,Fatty acid ethyl 17.43 $9,12$ -Octadecadienoic acid,Fatty acid 17.43 $9,12$ -Octadecadienoic acid,Fatty acid 17.43 $9,12$ -Octadecadienoic acid,Fatty acid 17.43 $9,12$ -Octadecadienoic acid,methyl ester 17.89 Stearic acid, methyl esterFatty acid 17.89 Stearic acid, methyl estermethyl ester 10.18 Dodecanoic acid, methyl estermethyl ester 17.53 9 -octadecenoic acid, methyl estermethyl ester 17.53 9 -octadecenoic acid, methyl estermethyl ester 17.53 9 -octadecenoic acid, methyl estermethyl ester	6	7.66	2-Acetamide-2- deoxygalactono-1,4-lactone	Galactonic acid derivative	N-acetylglucosaminidase inhibitor	0.16
12.64Tetradecanoic acidSaturated fatty acid16.03 n -Hexadecanoic acidSaturated fatty acid16.03 n -Hexadecanoic acidSaturated fatty acid18.40 $9,12$ -Octadecadienoic acid, ethyl esterFatty acid ethyl15.11Hexadecanoic acid methylFatty acid15.11Hexadecanoic acid methylFatty acid17.43 $9,12$ -Octadecadienoic acid, esterFatty acid17.43 $9,12$ -Octadecadienoic acid, methyl esterFatty acid17.89Stearic acid, methyl estermethyl ester10.18Dodecanoic acid, methyl estermethyl ester10.18Dodecanoic acid, methyl estermethyl ester17.53 9 -octadecenoic acid, methyl estermethyl ester17.53 9 -octadecenoic acid, methyl estermethyl ester17.53 9 -octadecenoic acid, methyl estermethyl ester	7	34.49	Ergosterol	Phytosterol	Antitumor, antiangiogenic, anti-flu, and antiviral	3.75
16.03 <i>n</i> -Hexadecanoic acidSaturated fatty18.409,12-Octadecadienoic acid,Fatty acid ethyl18.409,12-Octadecadienoic acid,Fatty acid ethyl15.11Hexadecanoic acid methylFatty acid15.12Hexadecanoic acid methylFatty acid17.439,12-Octadecadienoic acid,Fatty acid17.439,12-Octadecadienoic acid,Fatty acid17.89Stearic acid, methyl estermethyl ester17.89Stearic acid, methyl estermethyl ester10.18Dodecanoic acid, methyl estermethyl ester10.139-octadecenoic acid, methylFatty acid17.539-octadecenoic acid, methyl estermethyl ester17.539-octadecenoic acid, methylFatty acid17.539-octadecenoic acid, methylFatty acid17.539-octadecenoic acid, methylFatty acid	8	12.64	Tetradecanoic acid	Saturated fatty acid	Cancer preventive, nematicidal, hypercholesterolemic, antioxidant, lubricant, and antifungal	8.58
18.409,12-Octadecadienoic acid, ethyl esterFatty acid ethyl ester15.11Hexadecanoic acid methyl esterFatty acid 	6	16.03	<i>n</i> -Hexadecanoic acid	Saturated fatty acid	Flavoring agent, antiandrogenic, hypocholesterolemic, and antioxidant	0.38
15.11Hexadecanoic acid methyl esterFatty acid methyl ester17.439, 12-Octadecadienoic acid, methyl esterFatty acid 	10	18.40	9,12-Octadecadienoic acid, ethyl ester	Fatty acid ethyl ester	Anti-inflammatory agent	55.57
17.439, 12-Octadecadienoic acid, methyl esterFatty acid methyl ester17.89Stearic acid, methyl esterFatty acid methyl ester10.18Dodecanoic acid, methylFatty acid 	11	15.11	Hexadecanoic acid methyl ester	Fatty acid methyl ester	Nutrient, energy storage and membrane stabilizer	4.84
17.89Stearic acid, methyl esterFatty acid10.18Dodecanoic acid, methylFatty acid10.18Dodecanoic acid, methylFatty acid17.539-octadecenoic acid, methylFatty acidesteresterEster	12	17.43	9, 12-Octadecadienoic acid, methyl ester	Fatty acid methyl ester	Personal care products	0.75
10.18Dodecanoic acid, methylFatty acidesterestermethyl ester17.539-octadecenoic acid, methylFatty acidester, (E)-ester, (E)-methyl ester	13	17.89	Stearic acid, methyl ester	Fatty acid methyl ester	Flavoring agent and industrial value	1.67
17.53 9-octadecenoic acid, methyl Fatty acid ester, (E)- methyl ester	14	10.18	Dodecanoic acid, methyl ester	Fatty acid methyl ester	Flavoring agent	2.92
	15	17.53	9-octadecenoic acid, methyl ester, (E)-	Fatty acid methyl ester	Industrial value	1.75

followed by oleic acid (18.5 g), docosahexaenoic acid (9.8 g), and linolelaidic acid (1.2 g). Palmitoleic and linolenic acids were <1 g. The ratio of total unsaturated and saturated fatty acids was as high as 4.

5.5.2 Antinutritional Qualities

Trypsin inhibition activity is measured based on Kakade et al. (1974). The control consists of all reagents without the mushroom extract—calculated trypsin inhibition units (TIu) per mg of dry mass. The TIu was very low in *T. cantharelloides* (0.08/ mg). Many mushrooms are devoid of trypsin inhibition activity (e.g., Ghate and Sridhar 2017). Hemagglutination activity is determined based on Occenã et al. (2007) by heparinized human erythrocytes (A+, B+, AB+, and O+) to express hemagglutination unit per gram (Hu/g). There was no hemagglutination activity against B+ erythrocytes, while it was up to 200 Hu/g in the other three erythrocytes. Like trypsin inhibition activity, many mushrooms are devoid of hemagglutinin activity (Ghate and Sridhar 2017). It is likely the antinutritional factors diminished or eliminated in mushrooms on cooking or by heat treatment.

5.6 Antimicrobial Potential

Four Gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*), five Gram-negative bacteria (*Enterobacter aerogenes*, *Escherichia coli*, *Haemophilus influenzae*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*), and one yeast (*Candida albicans*) were procured from the Microbial Type Culture Collection (MTCC), Chandigarh, India. Bacterial strains were maintained on Muller–Hinton agar medium, while the *C. albicans* were on Sabouraud dextrose agar medium. Dispensed overnight grown culture of each strain (200 ml) into sterile Muller–Hinton broth (20 ml for bacteria), Sabouraud dextrose broth (20 ml for yeast), and incubated at 37 °C to obtain 105 CFU/ml. Powder of (5 g) *T. cantharelloides* (from triplicate samples) extracted in 25 ml of methanol on a rotary shaker (120 rpm) up to 24 h followed by oven drying (50 \pm 2 °C). The dried methanol extract dissolved in 20% dimethyl sulfoxide (DMSO) to get a stock solution (2 mg/ml), and the DMSO was used as solvent control to test its inhibitory effect.

The antibacterial and antifungal activity of the mushroom extracts were evaluated using the well-diffusion method (Bauer et al. 1966). Bacterial culture (0.1 ml; 105 CFU/ml) of 24 h old was inoculated on Muller–Hinton agar and spread out. Similarly, spread the yeast culture on the Sabouraud dextrose agar. Agar well was cut, and 200 μ g of the mushroom extract loaded into each well, and the DMSO served as control. Each plate comprised of three wells (experimental) with a standard antibiotic disc (Vancomycin, 30 μ g/disc for bacteria; Fluconazole, 25 μ g/disc for yeast). The plates incubated at 37 \pm 1 °C (18–24 h) with bacteria and for yeast at

	Diameter of inhibition (mm)		
	T. cantharelloides	Vancomycin	Fluconazole
	(200 µg/well)	(30 µg/disc)	(25 µg/disc)
Gram-positive bacteria			-
Bacillus cereus MTCC430	11.3	19.7	-
Bacillus subtilis MTCC441	17.0	25.7	-
Staphylococcus aureus MTCC96	12.0	22.0	-
Streptococcus pneumoniae MTCC655	15.7	26.0	-
Gram-negative bacteria			
Enterobacter aerogenes MTCC424	NI	20.0	-
Escherichia coli MTCC443	09.3	22.0	-
Haemophilus influenzae MTCC3826	16.0	21.7	-
Proteus vulgaris MTCC1771	09.0	18.7	-
Pseudomonas aeruginosa MTCC424	07.7	31.0	-
Yeast			
Candida albicans MTCC183	08.7	-	26.0

Table 5.4 Antimicrobial activity of *Trogia cantharelloides* and standard antibiotics (n = 3, mean) (*NI* no inhibition, - not tested)

26 \pm 1 °C (48–72 h). The plates were examined for inhibition to measure the inhibition diameter using a dial caliper.

The standard DMSO did not inhibit tested bacteria and yeast. The crude extract of *T. cantharelloides* showed antimicrobial activity against Gram-positive and Gramnegative bacteria and *C. albicans* (Table 5.4). The zone of inhibition was highest against *H. influenzae* (16 mm), followed by *B. subtilis* (17 mm) and *S. pneumoniae* (15.7 mm). Although the extract of *T. cantharelloides* used for the test was about seven-fold higher than the standard antibiotic vancomycin, none showed higher activity than the vancomycin. The *C. albicans* showed an 8.7 mm inhibition diameter at 200 mg/well, lower than the standard antibiotic fluconazole (25 mg/disc, 26 mm).

Lindequist et al. (2005) opined that wild mushrooms possess antimicrobial compounds to survive in their natural habitats. Managing Gram-negative bacteria is more complex than Gram-positive bacteria (e.g., cell wall inhibiting antibiotics). Inhibition of Gram-positive bacteria, Gram-negative bacteria, and *C. albicans* by the extracts of *T. cantharelloides* indicated its broad-spectrum antibiosis. However, the inhibition ability of *T. cantharelloides* against bacteria and yeast was below the standard antibiotics. It is interesting to note that *T. cantharelloides* can inhibit a broad range of pathogenic microorganisms.

5.7 Conclusions

Several wild mushrooms are not edible owing to their unpalatable or toxic, or poisonous properties. However, many of them have value-added bioactive components of health, pharmaceutical, and industrial importance. The T. cantharelloides, although not consumed by the tribals and local dwellers of the foothills of the Western Ghats of India, possesses many biochemical, nutritional, and bioactive components in substantial quantities. The carbohydrate content was highest in the fruit bodies, followed by crude fiber and crude protein. The Na/K ratio (<1) obeys the NRC-NAS standards and is favorable to combat blood pressure. It possesses many essential amino acids (e.g., threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine) as well as essential fatty acids (e.g., linoleic, linoleic, and docosahexaenoic acids). It possesses reasonable quantities of flavonoids, phenolics, vitamin C, carotenoids, and succinic acid. Numerous bioactive components of health combating potential found in this neglected mushroom anti-inflammatory, immunostimulant, cytoprotective, anticarcinogenic, (e.g., antitumor, hypercholesterolemic, antioxidant, antiandrogenic, antiangiogenic, and diuretic). It is endowed with broad-spectrum antibiosis against pathogenic bacteria, fungi, nematodes, and viruses. Owing to the potential bioprospective qualities, T. cantharelloides may be a future bioresource of health, pharmaceutical, and industrial values.

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