



## GC-MS analysis of *Pisolithus arrhizus* (Scop.) Rauschert, (1959), a non-edible wild mushroom in search of novel bioactive compounds collected from Western Ghats of Karnataka, India

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### Abstract

Mushroom is a macro fungus, belonging to the kingdom Mycota. Mushrooms are unique, because, they are neither animal nor plant. Mushrooms are heterotrophic organisms, they show a great diversity in morphology and preference for habitats. The present study aims to estimate the nutritional profiling of *Pisolithus arrhizus* (Scop.) Rauschert, (1959) a non edible wild mushrooms collected from Western Ghats of Karnataka, India. Sporomas of collected mushroom were analyzed for their proximate chemical composition (moisture, proteins, carbohydrates, fat, fiber and ash) major and minor mineral elements (N, P, K, Ca, Fe, Mg, Mn, Cu, Zn) and heavy metal contents (Pb, Cd, Cr, Ni) on dry weight basis. The result reveals that, *Pisolithus arrhizus* is rich in carbohydrates 47.87%, and low amount of fat 0.41% also contains moderate amount of protein 5.17%, fibre 13.14% followed by ash 17.92% and moisture 15.49%. The energetic contribution was found to be (215.85Kcal/100 g). Nitrogen (N) and potassium (K) are the most abundant minerals in *Pisolithus arrhizus* and showed higher concentration of N (3.19%) and K (1.56%), whereas Ca (0.10%) and Mg (0.08%) and studies mushroom were also assessed for their trace elements (Fe, Mn, Zn and Cu). Amongst these Fe and Zn was found to be highest (314.60ppm) and (36.10ppm). Heavy metals (Ni, Pb, Cr, Cd) concentration was analyzed by atomic absorption spectrophotometer (AAS), the Cr content was found to be highest 13.25%, followed by Ni 2.35%. Six metabolites identified through GC-MS analysis in methanolic extract of *P. arrhizus*. The major metabolites are Butanal, 3-methyl-, Benzene, methyl-, Benzene, 1,3-bis(1,1-dimethylethyl)-, and 1-Hexanol, 2-ethyl- covering a peak area of 3.34%, 2.87%, 2.24% and 1.99%.

**Keywords:** nutrition, minerals, heavy metals, GC-MS, *Pisolithus arrhizus*, Western Ghats

### Introduction

Fungi are regarded as being the second largest group of organisms, in the biosphere after the insects. Known fungal species constitute only about 5% of their species in the world. Out of about 70,000 described species of fungi. It has been suggested that, around 14,000 to 15,000 species produce fruiting bodies of sufficient size and suitable structure to be considered as macro-fungi (mushrooms). There are about 5,000 of the species are considered to possess, varying degrees of edibility and more than 2,000 species from 31 genera are regarded as prime edible mushrooms. But, only 100 species of them are experimentally grown, 50 species are economically cultivated, around 30 species are commercially cultivated, and only about 6 species to have reached an industrial scale of production in many countries. Furthermore, about 1,800 are recognized for medicinal properties. The number of poisonous mushrooms is relatively small (approximately 10%). Of these, some 30 species are considered lethal (Chang, 2008, Chang and Miles, 2004) <sup>[1]</sup>.

Ectomycorrhizal fungi, such as mushrooms, are the important component of forest ecosystem growing on the most abundant bio-molecules of our nature, i.e., cellulose. Both the edible and non-edible mushrooms are regarded as macro fungi with a unique epigeous or hypogeous fruiting body and are large enough to be seen through naked eyes. Along with their role in nutritional and medicinal aspects, these ectomycorrhizal fungi have been used as a source of food for tribal peoples and act as mycorrhizal partner with

the forest trees. Fungi are key functional components of forest ecosystem (Brown *et al.* 2006) <sup>[2]</sup> and they have received less attention than animals and plants although they are universal and highly diverse in nature (Pipenbring 2007) <sup>[3]</sup>.

Western Ghats of India is one of the 25 biodiversity hotspots. It is also referred to as Sahyadri (Benevolent mountains), running north to south along the Western edge of deccan plateau. Western Ghats is globally known as a region of huge ecological importance, due to their rich biodiversity, including, high cultural, geological and aesthetic values. Karnataka state is rich in biodiversity elements, their diversity and distribution and covers 60% of Western Ghats. The average elevation of Western Ghats is about 1200 meters MSL (Metres above mean sea level) and receives annual rainfall between 3000 and 4000mm. (Bandi M 2020) <sup>[4]</sup>. *Pisolithus arrhizus* is a widespread earth-ball like fungus, and commonly called as dead man's foot. This puffball black viscous gel is used as a natural dye for clothes. *P. arrhizus* is a major component in mycorrhizal fungus mixtures that are used in gardening as powerful root stimulators and it is inedible.

### Materials and Methods

#### Collection and authentication

Sporocarp of *Pisolithus arrhizus* (Scop.) Rauschert were collected from Koppa, Chikkamagaluru district, Karnataka between the August and October 2017 (Fig.1). Collected sporocarp was studied for their morphological and anatomical characters (Fig-2). Classical taxonomy was followed for the identification <sup>[1]</sup>.

## Nutritional analysis.

### Proximate Composition of mushrooms Samples

The mushroom sporocarps was subjected for proximate chemical composition (moisture, ash, proteins, carbohydrates, fat and fiber) using the AOAC procedures (1990, 1998) [5, 6]. The crude protein content of the samples was estimated by macro-Kjeldahl method [3]. The crude fat was determined by extracting 2g of powdered mushrooms sample with petroleum ether, using a Soxhlet apparatus. The ash content was determined by placing 2g of mushrooms sample in silica crucibles for incineration at  $525^{\circ}\text{C} \pm 20^{\circ}\text{C}$  for 6 hours. Total carbohydrates were calculated by difference. The analysis was conducted at Padmashree Research Institute, Kengeri, Bangalore. Energy value was estimated based on of crude protein, fat and carbohydrate using the formula given below: Energy = 4 x Weight of proteins + carbohydrates + 9 x Weight of Fat (Kcal)

### Determination of mineral elements (N, P, K, Ca, Mg, Fe, Mn, Zn and Cu) and heavy metals (Pb, Cd, Cr, Ni).

The mineral elements in *Pisolithus arrhizus* were evaluated. Total nitrogen in wild mushrooms sample was determined by Kjeldahl method, the element phosphorus (P) was estimated by vanado- molybdate yellow colour method and potassium (K) by flame photometer. Atomic absorption spectrophotometer (AAS) was used for the determination of calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) in dried fruiting bodies of wild mushrooms (Bhargava and Raghupathi, 1993) [7]. The values of elements N, P, K, Ca, Mg were expressed in terms of

percentage and Fe, Mn, Zn and Cu were calculated as parts per million (ppm). Heavy metals (Pb, Cd, Cr, Ni) were determined in the di acid digest of mushroom sample using Atomic Absorption Spectrometer (AAS). Two grams of mushroom sample was taken in 100ml conical flask. Then add 40ml DTPA extracting solution, shaken for 2hrs (120 oscillation/min), filter the solution, and the filtrate obtained was used for the analysis and the values following the methods (AOAC 1985, 1990) [5, 6], HLS tendon (2009) [8].

### Preliminary myco-chemical screening

The preliminary myco-chemical screening was performed for testing the presence or absence of various chemical groups (alkaloids, flavonoids, terpenoids, Cardiac glycosides, Phenolics, Steroids, tannins, saponins, Quinones and Tannin) present in the crude extracts (Kokate, 2005) [9].

### GC-MS Analysis

The methanolic extracts were investigated for the potent volatile constituents by Gas Chromatography coupled with Mass Spectroscopy (GC Model: Thermo Trace GC Ultra, MS Model: Thermo DSQ II, at Padmashree Research Institute, Kengeri, Bangalore). The spectrum of the unknown components was compared with the spectrum of the known components in library (NIST and WILEY) to identify the compounds, followed by the method given by (Subavathy and Thilaga 2016) [10], Naik *et al.* (2019) [11].



Fig 1: Location where *Pisolithus arrhizus* sporocarp was collected

## Result and discussion

**Taxonomic notes:** KU/AB/RN/SERB-10

*Pisolithus arrhizus* (Scop.) Rauschert, (1959)

MycoBank No: 303705

## Scientific classification

**Kingdom:** Fungi

**Division:** Basidiomycota

**Class:** Agaricomycetes

**Order:** Boletales  
**Family:** Sclerodermataceae  
**Genus:** *Pisolithus*  
**Species:** *P. arhizus*

**Description**

**Habitat:** solitary widely scattered found in road sides on sandy or gravelly soil.

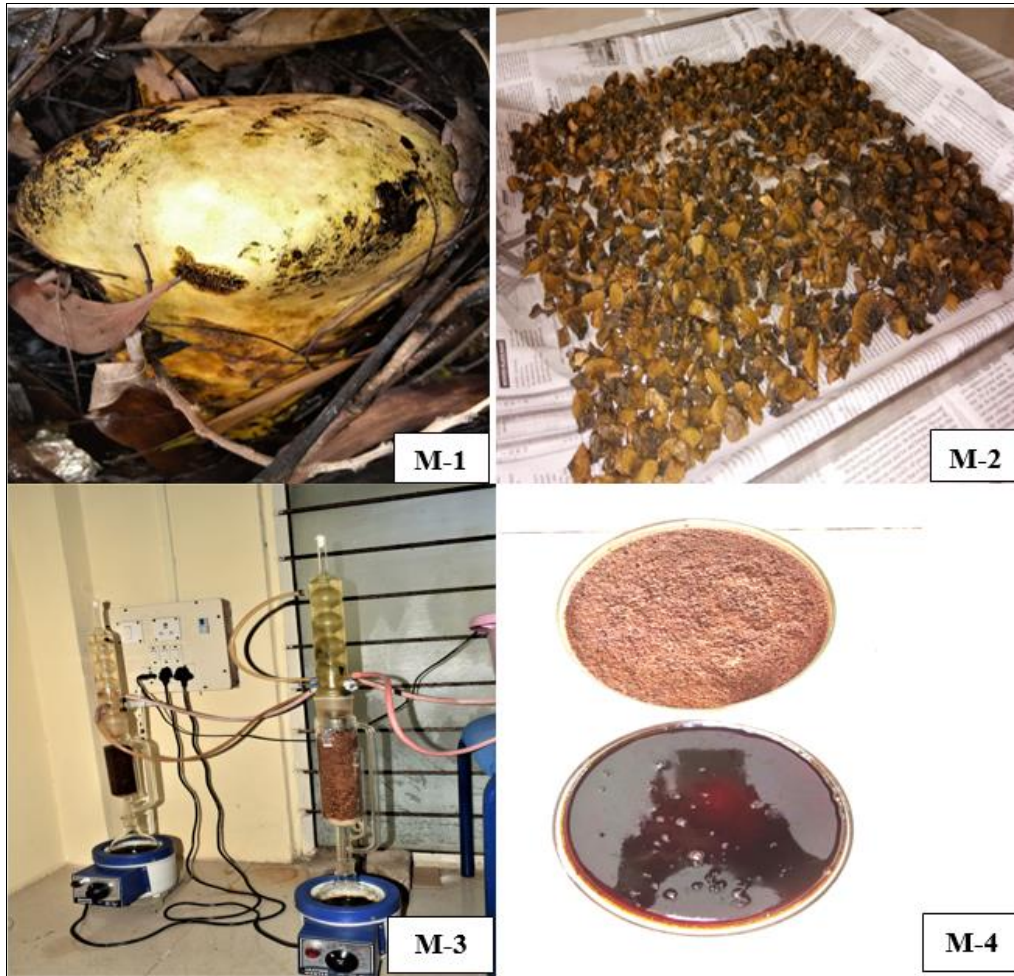
**Mushroom color:** greenish-yellow, yellow, brownish.

**Fruiting body:** tuberoid to spherical, with a roundish head, 4 - 18 cm height, 4 - 12 cm wide with internal, passively

discharged spores. Gleba with many small (c. 5 x 2 mm) and firm chambers.

**Stipe:** 0.5 - 10 x 2.5 - 10 cm. Peridium (outer surface) smooth, buffish to brown color, Flesh tough and hard, with a staining yellow pigment.

**Spores:** globose, with spines, warts or crests (to 2.3 µm high), thick-walled, 7 - 11 x 7 - 10 µm, brown. Basidia 6 - 14 µm long, spherical to barrel-shaped holobasidia with four sterigmata, clamp connections present and Edibility: Inedible



**Fig 2:** M1- Habitat and sporocarp of *Pisolithus arhizus* (Scop.) Rauschert; M2- Dried sample; M3- Soxhlet extraction; M4- Crude methanolic extract.

**Nutritional profile.**

**Macro nutritional composition**

The macro nutritional composition depicting the ash value, moisture content, fat, protein, fibre and carbohydrates were evaluated and estimated the energetic value (on dry weight basis) of the studied wild mushroom samples (Table-1). The carbohydrate content was found to be highest 47.87%, followed in order by moisture 15.49%, ash 17.92%, fiber

13.14% and protein 5.17%. The energetic contribution found to be 215.85 (Kcal/100g) (Figure-3).

The present study confirmed that, wild mushrooms are rich sources of carbohydrates and protein, and low amounts of fat. Mushrooms are most important for modern medicine and they represent an unlimited source of compounds which are modulators of tumour cell growth.

**Table 1:** Proximate Analysis for wild mushrooms

Sl no	Name of the mushroom	Moisture%	Fat%	Ash %	Fiber%	Protein%	Carbohydrate %	Energy (Kcal/100g)
1.	<i>Pisolithus arhizus</i> (P)	15.49	0.41	17.92	13.14	5.17	47.87	215.85

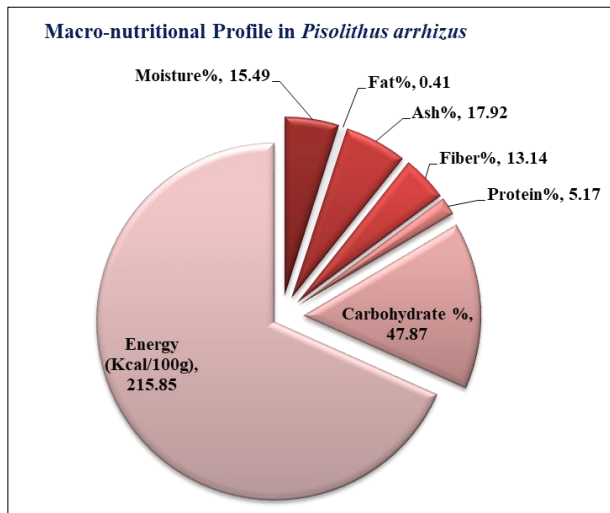


Fig 3: Total percentage of macro-nutritional profile in *Pisolithus arrhizus*.

**Determination of mineral elements**

**Major mineral**

The mineral elements *Pisolithus arrhizus* were evaluated and the result indicated in (Table-2). The element phosphorus (P) was estimated by vanado- molybdate yellow colour method and potassium (K) by flame photometer. Atomic absorption spectrophotometer (AAS) was used for the determination of calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) in dried fruiting bodies of macrofungi (Bhargava and Raghupathi, 1993) [7]. The result reveals that, the percentage of major elements viz., N found to be abundant 3.19%, followed by K 1.56%, where as P, Ca & Mg found to be very less 0.14%, 0.10%, & 0.08%. These data revealed that the studied mushroom had higher quantities of N and K compared to P, Ca, Mg (Fig-4). This suggests that the wild mushrooms were rich in N, K compared to P, Ca and Mg.

Table 2: Major mineral elements in wild mushrooms

Sl no	Name of the mushroom	N %	P %	K %	Ca %	Mg %
1.	<i>Pisolithus arrhizus</i>	3.19	0.14	1.56	0.10	0.08

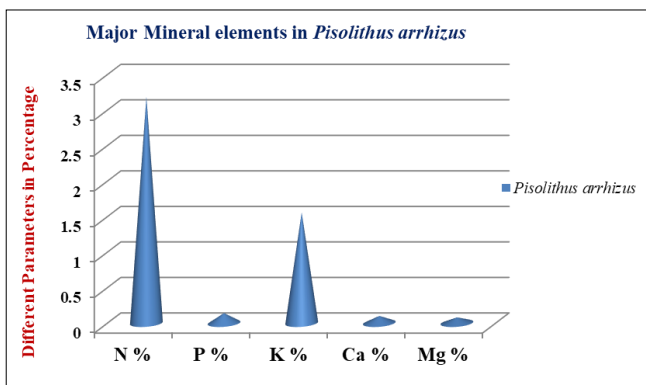


Fig 4: The percentage of major mineral elements in three wild mushrooms. (N > K > P > Ca > Mg).

**Trace elements**

The determination of the composition of trace elements in *Pisolithus arrhizus* has given in (table-3). The result reveals that, the Fe content found to be abundant (314.60ppm) followed by Zn and Mn (36.10ppm) and (34.15ppm) where as Cu observed lowest (8.70ppm). These results indicated

that, *Pisolithus arrhizus* contains higher amount of Iron and Zn than Manganese and Cu (Fig-5). It may be appropriate to mention here that the soils where mushrooms have present are rich in these minerals.

Table 3: Trace mineral elements in wild mushrooms

Sl no	Name of the mushroom	Fe (P.P.M)	Mn (P.P.M)	Zn (P.P.M)	Cu (P.P.M)
	<i>Pisolithus arrhizus</i>	314.60	34.15	36.10	8.70

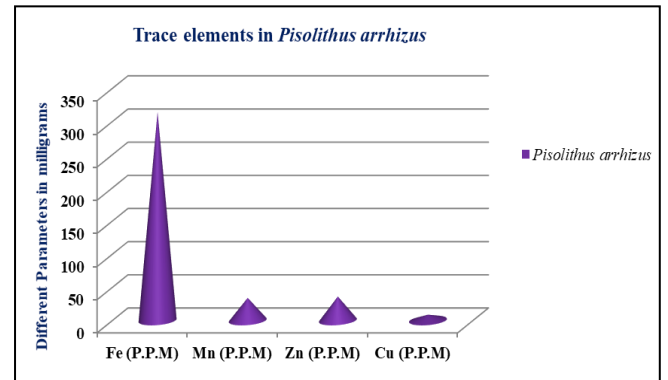


Fig 5: The trace mineral elements (mg) present per kg of *Pisolithus arrhizus* (Fe > Zn > Cu > Mn).

**Heavy metals**

The heavy metals content in *Pisolithus arrhizus* were determined and indicated in table (table-4)., and the result reveals that the Cromium (ppm) content obtained highest (13.25ppm) followed by Nickle (2.35ppm) and Lead (1.25ppm) and Cadmium observed lowest and which is found to be (0.10ppm). The data presented to suggests that the studied richer in Cromium and Nickle compare to Cadmium and Lead. (Fig-6).

Table 4: Heavy metals in edible wild mushrooms

Sl no	Name of the mushroom	Pb (P.P.M)	Cd (P.P.M)	Cr (P.P.M)	Ni (P.P.M)
	<i>Pisolithus arrhizus</i>	1.25	0.10	13.25	2.35

**Mycochemical extraction**

The extraction was done to separate the soluble mycochemicals leaving behind the residue. Hence, in the current study, twelve mushrooms were extracted by soxhlet method using certain solvents of low, medium and high polarities. The solvents used were petroleum ether, chloroform and methanol. It was observed that methanol extraction produced maximum yield of mycochemicals, about 8.98% respectively. Polar compounds like sugars, amino acid and glycoside compounds (Houghton and Raman, 1998) [12] and terpenoid, saponin, tannins, polyphenol (Cowan, 1999) [13] can be dissolved in methanol.

**Preliminary mycochemical analysis**

The results of the mycochemical screening of fruiting body of studied mushroom extract are summarized in (Table-5) The result indicated the presence of almost all the secondary metabolites viz., Alkaloids, Flavonoids, Cardiac glycosides, Phenolic Compounds, Steroids, Tannin, lignin, Quinones, Saponins and Terpenoids, In *Pisolithus arrhizus* mushroom extract. The myco-chemical alone or in synergistic effect may have tremendous therapeutic potential in curing various ailments.

**Table 5:** Mycochemicals in *Pisolithus arrhizus*

Mycochemical group	Pet. ether extract	Chloroform extract	Methanol extract
Alkaloids	-	-	+
Flavonoids	+	-	+
Cardiac glycosides	+	+	+
Phenolic Compounds	-	-	+
Steroids	-	-	+
Tannin	-	-	-
lignan	-	-	+
Quinones	-	-	-
Saponins	+	-	-
Terpenoids	-	+	+

**Characterization of metabolites using GC-MS**

Analysis of GC-MS chromatogram of methanolic extract of *Pisolithus arrhizus* showed 6 volatiles respectively in varying proportions (Fig-7). Six metabolites identified through spectrogram in methanolic extract of *P. arrhizus*,

the major metabolites are Butanal, 3-methyl-, Benzene, methyl-, Benzene, 1,3-bis(1,1-dimethylethyl)-, and 1-Hexanol, 2-ethyl- covering a peak area of 3.34%, 2.87%, 2.24% and 1.99% (Table-6).

**Table 6:** GC-MS analysis of *Pisolithus arrhizus* (Scop.) Rauschert, sporocarp methanolic extract

Sl. No.	Area %	Chemical name	Properties
1.	3.34	Butanal, 3-methyl-	Food additives and Flavoring Agents, Flavor Profile are Burnt, Cocoa, Floral, Malt, reported uses: non-alcoholic beverages 17 ppm, alcoholic beverages 100 ppm, ice cream, ices, etc 7.6 ppm, candy 52 ppm, baked goods 24 ppm, gelatins& puddings 46 ppm, chewing gum 300 ppm, herbicides, pharmaceuticals, fragrances. (PubChem-Isoamyl alcohol/ C <sub>5</sub> H <sub>12</sub> O) ( <a href="https://pubchem.ncbi.nlm.nih.gov/compound/31260">https://pubchem.ncbi.nlm.nih.gov/compound/31260</a> )
2.	2.87	Benzene, methyl-	Binding affinity to Dengue virus, hypocholesteremic agent, Xa inhibitor, used for thromboembolic diseases, antineoplastic agents, antitumor, phosphodiesterase inhibitors and inodilators, Cytotoxic to cancer cell, Antimicrobial, Antihypertensive, Insecticidal, Immunomodulator, Antifungal, Anticoccidial, Antiproliferative, Anti-allergic, Antidiabetic, Inhibitor of acetylcholinesterase, Inhibition of butyrylcholinesterase, histone deacetylase inhibitors, used treatment for Alzheimer's disease, Cytoprotective, Anti-juvenile, herbicidal, Antiinflammatory, anti-HIV-1, antigonococcal agents, Antiviral, Antifeedant, Anticoagulant, Larvicidal, antiulcer,antithrombotic, Air fresheners &fragrant,manufacturing of drugs, manufacturing of fertilizers, Food additive& flavoring agent, fuel additives, (PubChem-Toluene/ C <sub>7</sub> H <sub>8</sub> ) ( <a href="https://pubchem.ncbi.nlm.nih.gov/compound/1140">https://pubchem.ncbi.nlm.nih.gov/compound/1140</a> )
3.	0.85	Cyclohexanol, 4-(1,1-dimethylethyl)-, trans-	Airfresheners, fragrance,Food additive, colorings, flavors, Pesticide. (PubChem-4-Tert-Butylcyclohexanol/ C <sub>10</sub> H <sub>20</sub> O) ( <a href="https://pubchem.ncbi.nlm.nih.gov/compound/7391">https://pubchem.ncbi.nlm.nih.gov/compound/7391</a> )
4.	1.99	1-Hexanol, 2-ethyl-	Antifreezing agents, Binding agents, used in paint,manufacturing of plastics, manufacturing of drugs, Colorant, Food additive & flavoring agent, Fragrant, fuel additives,Fungicide, lubricant, manufacturing of fertilizers, manufacturing of Crude oil, crude petroleum, refined oil products, fuel oils, drilling oils, rubber. (PubChem-2-Ethylhexanol/ C <sub>8</sub> H <sub>18</sub> O) ( <a href="https://pubchem.ncbi.nlm.nih.gov/compound/7720">https://pubchem.ncbi.nlm.nih.gov/compound/7720</a> )
5.	0.99	Undecane, 2-methyl-	No activity reported
6.	2.24	Benzene, 1,3-bis(1,1-dimethylethyl)-	No activity reported

**Discussion**

Mushrooms form an essential nutritional and pharmacological source in the global food chain as they are rich in proteins, carbohydrates and fibre. However, many strains of mushrooms contain toxins that cause human sickness and death. Mushrooms are important constituent of diet in many ethnic tribes in India. Conventional mycological knowledge of most Indian tribal groups is extensive and profound. Ethnic Indian tribes are known to use nearly 283 species of wild mushrooms, of 2000 species recorded all over the worldwide Purkayastha et al, (1985)<sup>[14]</sup>. In India around 100 species of mushrooms are proven poisonous to humans (J.H Diaz 2005)<sup>[15]</sup>. Hepato-toxicity is caused mainly by mycotoxins viz., gyromitrin and amatoxin synthesized by a number of Amanita species and some members of the Conocybe, Galerina, and Lepiota genera Pegler et al. (1995)<sup>[16]</sup>, Enjalbert et al. (2002)<sup>[17]</sup>. Common poisonous macrofungal species identified from hilly terrains in South India are *Chlorophyllum molybdites*, *Omphalotus*

*olivascens*, and *Mycena pura*. But human poisonings are rare as ethnic tribes are practiced in identifying poisonous from non-poisonous mushrooms Purkayastha and Chandra (1985)<sup>[14]</sup>, Kumar and Kaviyarasan (2012)<sup>[18]</sup>, Sarma et al. (2010)<sup>[19]</sup>. Mushroom poisoning in humans has been described since time immemorial (“Rigveda” at least 3500 BC and “Atharvaveda” at least 1500 BC). (Jha and Tripathi 2012)<sup>[20]</sup>. Thetoxic nature is because of neurotoxins, protoplasmic poisons and gastrointestinal irritants. In India, mushrooms are widely consumed, especially in the hilly regions of Jammu and Kashmir, Uttarakhand, Himachal Pradesh, and North-eastern states SDS Greval (1950)<sup>[21]</sup>.

**Conclusion**

Wild mushrooms are rich sources of protein, carbohydrates and low amounts of fat. Generally mushrooms are vital for modern medicine and they represent an infinite source of compounds which are modulators of tumour cell growth. The non-edible mushroom *Pisolithus arrhizus* (Scop.)

Rauschert were analyzed in this study for their the macronutritional composition depicting the rich source of carbohydrates, fibre & protein and low amount of fat. mineral contents confirmed the sources of major minerals (N, P, K, Ca & Mg) and trace (Fe, Mn, Zn, Cu) mineral elements. The studied mushroom were also assessed for heavy metals and showed permissible limits. Hence, further studies are recommended to measure the effective toxicity and as sources of novel molecules for integration of this non edible wild mushrooms into pharmaceutical industries.

After the present investigation, it can be concluded that *Pisolithus arhizus* (Scop.) Rauschert. sporocarp methanolic extract with rich full of useful mycochemicals. GC–MS analysis of methanolic extract revealed the presence of 6 compounds in that 4 compounds were known for its medicinal properties, most of them were food additive and flavoring agents followed by antioxidant, antihypercholesterolemic, anti-inflammatory agents, anti-viral, anti-cancer etc..

The overall study on antimicrobial, GC–MS analysis reports that *Pisolithus arhizus* (Scop.) Rauschert. sporocarp species contains many active compounds which by their with good nutritional and rich with bioactive compounds. Hence, it is finally concluded that *Pisolithus arhizus* (Scop.) Rauschert. sporocarp can be explored for potential nutritional and bioactive potentials with rich full of useful mycochemicals.

#### Acknowledgment

The authors are grateful to Science and Engineering Research Board SERB (EEQ/2016/000669) Government of India, New Delhi, for funding through granting research project and also thankful to Kuvempu University, Jnana Sahyadri, Shankaraghata, Karnataka for providing Academic and Administrative support.

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