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Phenotypical, Mycochemicals, Proximate Composition and Antifungal Activity of *Phylloporia ribis* (Schumach) Ryvarden from India

Talluri Ribka[1], Praveen Kumar Nagadesi[2*], Vasavi Ponnuru[1], Vijayadurga Thatha[3] and Akella V.K.S.N. Pratyusha[4]

[1]*Department of Botany, Post Graduate Wing, Andhra Loyola College, Vijayawada, Andhra Pradesh, India*
[2]*Department of Botany, St Joseph's College, Lalbagh road, Bangalore, Karnataka, India*
[3]*Department of Botany, C. R. Reddy College for Women's, Vatluru, Andhra Pradesh, India*
[4]*Department of Chemistry, Post Graduate wing, Andhra Loyola College, Vijayawada, Andhra Pradesh, India*

ABSTRACT

Herbal medicines were popular in the treatment of many diseases due to green medicine is safe, easily available and less side effects. So the mushrooms belonging to Hymenochaetace members like Phellinus and Phylloporia were used in preparation of crude drugs/folk medicines. The sporophores of Phylloporia members were collected from Andhra Pradesh, India. The sporophore was used for phenotipical identification, Mycochemical evaluation, physicochemical properties, and Antifungal activity against plant pathogens. Based on the phenotypical or morphological characters the sporophore was identified as Phylloporia ribis (Schumach.) Ryv.

The extracts of P. ribis sporophore powder contain Carbohydrates, Proteins, Amino acids, Lipids, Alkaloids, Glycosides, Cardiac glycerides, Flavonoids, Phenols, Terpinoids, Steroids, Sterols, saponins, Tannins, and Phosphate. The best solvent for extraction is methanol when compared to ethanol and water. The wide range of bioactive compounds constituents would be useful for immunity boosters as food supplements, drug discovery and development of various new formulations. The proximate composition evaluation is useful for standardization of P. ribis in powder form. That will help to identify the genuine specie in adulteration test. The methanolic extract of p. ribis sporophore powder shown best antifungal activity when compared to water extract. Except 5% concentration all methanolic extracts of P. ribis shown 100% inhibition of Aspergillus niger causing soft rot of carrot. For the first time Mycochemical bio active compounds, proximate composition evaluation, antifungal activity of P. ribis were reported from India.

Keywords: Mycochemical; Immunity boosters; Antifungal; *Phylloporia ribis*; Nutritional value

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INTRODUCTION

Medicinal mushrooms have been proposed as a novel therapy for several diseases for the survival of patients. So, mushrooms contain powerful compounds that enhance and balance your body's ability to fight disease and stay healthy. They have been used medicinally since at least 3000 BCE. Eastern medical practitioners have known for more than 5,000 years that mushrooms contain powerful immune-boosting compounds and protective properties. Mushrooms are reported to have antimicrobial, anti-inflammatory, cardiovascular-protective, antidiabetic, hepatoprotective, and anticancer properties. It is well-established that mushrooms are adept at immune modulation and affect hematopoietic stem cells, lymphocytes, macrophages, T cells, dendritic cells (DCs), and natural killer

(NK) cells [1]. *Hymenochaetaceae* have genus like *Phellinus Quél.* (1886:172), *Inonotus P. Karst*. (1879:39), *Coltricia Gray* (1821:644), *Phylloporia Murrill* (1904:141) and *Cyclomyces Kunze* ex Fr. (1830:512) [2]. The species diversity of genus *Phylloporia* was well-studied worldwide. It is widespread in European forest regions, North America forest, and Asia forests [3]. *Phylloporia Murrill* was introduced in the *Hymenochaetaceae* Donk, as an unusual polypore species. *P. parasitica Murrill* growing on the underside of living leaves in Columbia was studied by Murrill [4]. The species concept in *Hymenochaetaceae* family with *Hymenomycetes* genus was studied by Parmasto [5]. The taxonomy of several pairs of closely related *Hymenomycetes* species like *Phylloporia ribis* and *P. ephedrae* was described and A new combination of *P. ephedrae* (Voronich.) Parm was proposed as new taxa [5]. Basidiocarps of *Phylloporia* are used as a source of natural medicine in China [6]. In China *P. ribis* is used as edible fungus [6]. It was found that fruiting bodies of *P. ribis* have been used as food and functional ingredients for the treatment of pharyngitis, laryngitis, tonsillitis, and hyperglycemia [7]. But in India the preliminary work on medicinal mushroom like *P. ribis* was done. So in the present study aims at Mycochemical bioactive compounds, proximate composition and antifungal activity of *P. ribis* sporophore powder were tested.

EXPERIMENTAL SECTION

Collection and Phenotypical Identification

The sporophore was collected from Andhra Pradesh, India, during the rainy season (July-September) of the years 2017 to 2019. Field characters like habit, host, name of locality and other macro-morphological characteristics were recorded for sample specimens. For Phenotipical identification of sporophore, different Macroscopic features like abhymenial, hymenial surfaces, context, and pore tubes of species were examined. Microscopic features like hyphae, basidiospores and pilear crust were observed by preparing crush mounts and free-hand sections in water, 5% KOH solution, and staining was done with cotton blue (1%, in lacto phenol), Congo red (1%, in distilled water), phloxine (1%, in distilled water), and Melzer's reagent [8-12]. Voucher specimen of *P. ribis* (ALC 30) has been deposited at the herbarium of the Museum of Botany Department, Andhra Loyola College, Vijayawada, Andhra Pradesh, India (ALC).

Extraction of Bioactive Compounds

The sporophore of *P. ribis* was initially rinsed thrice in distilled water and dried on paper toweling and samples was cut into fine pieces and powdered. For preparing the extracts methanol, ethanol and water was used as solvents. For every 1 gram of powder, 50 ml of solvent was used and was subjected to extraction using maceration. After the completion of extraction, the supernatant was filtered through Whatman No. 1 filter paper and the filtrates stored at 4°C for further use to perform various assays for determination of bioactive mycochemicals and antifungal activity.

Mycochemical Tests

The screening of bioactive mycochemicals in fresh sporophores of *P. ribis* is tested by using standard methods of Indian Pharmacopoeia followed by [13-19].

Test for Carbohydrates

Molisch's test: To a small amount of the extract few drops of Molisch's reagent was added followed by the addition of conc. H_2SO_4 along the sides of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 ml of distilled water. Formation of red or dull violet colour at the inter phase of two layers indicates the presence of carbohydrates. First yellow then brick red precipitate was observed.

Fehling's test: The extract was treated with 5 ml of Fehling's solution (A and B) and kept in boiling water bath for 5-10 min. The formation of yellow or red colour precipitate indicates the presence of reducing sugar

Test for Proteins

Biuret test: Test sample (3 ml) was mixed with 4% NaOH and few drops of 1% $CuSO_4$ solution were added. Violet or pink color not appeared. To 3 ml of the extract few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple colour. On addition of alkali, it becomes dark violet.

Tests for Amino Acids

Ninhydrin test: Test sample (3 ml) and 3 drops of 5% ninhydrin solution were heated in boiling water for 10 mins. Purple color appeared.

Test for Lipids

Brown bag test: Certain kinds of paper such as a piece of brown paper bag can readily absorb lipids and can be used to test for the presence of lipids.

Emulsion test: Suspended the sample in ethanol which allows lipids to dissolve in it, and then decanted the liquid into water. Since lipids do not dissolve in water, it falls out of the solution as cloudy white emulsion.

Test for Glycosides

Free content of the sugar extract was determined. The sample was hydrolysed with mineral acid (dilute hydrochloric or dilute sulphuric acid). Again the total sugar content of the hydrolysed extract was determined. Increase in the sugar content indicated the presence of glycoside in the extract.

$$\text{Glycoside} + H_2O \rightarrow \text{Aglycon (genin)} + \text{Glycon (sugar)}$$

Liebermann's test: We added 2.0 ml of acetic acid and 2 ml of chloroform with whole aqueous plant crude extract. The mixture was then cooled and we added H_2SO_4 concentrated. Green color showed the entity of aglycone, steroidal part of glycosides.

Legal's test: Aqueous or alcoholic sample extract was mixed with 1 ml of pyridine sodium nitroprusside was added. Pink to red color appeared.

Test for Cardiac Glycosides

Keller-Kiliani test: A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% $FeCl_3$ mixture was mixed with the 10 ml aqueous plant extract and 1 ml H_2SO_4 concentrated. A brown ring formed between the layers which showed the entity of cardiac glycosides.

Test for Steroids

Salkowski's test: Sample: Sample (2 ml) was mixed with 2 ml of concentrated Sulphuric acid, it was well shaken then chloroform layer appeared red and acid layer shown greenish yellow fluorescence.
Lieberman-Buchard reaction: Sample (2 ml) was mixed with chloroform. 1-2 ml of acetic anhydride was added and 2 drops concentration sulphuric acid was added from the sides of the tube. First red then blue and finally green colour appeared.

Tests for Sterols

The sample was treated with 5% potassium hydroxide solution appearance of pink colour indicated the presence of sterols.

Test for Saponin

Foam test: To 1 ml of the extracts 5 ml distilled water was added and shaken vigorously. Formation of foam indicated presence of saponins.

Phosphate Test

Ammonium molybdate test: A small amount of the sample is acidified with concentrated nitric acid, to which a little ammonium molybdate is added. The presence of phosphate ions is indicated by the formation of a bright yellow precipitate layer of ammonium phosphomolybdate. The appearance of the precipitate can be facilitated by gentle heating

Silver nitrate test: To a small amount of sample add silver nitrate solution. Silver phosphate is formed as a yellow precipitate by the reaction between a soluble silver compounds, such as silver nitrate with a soluble orthophosphate.

Proximate composition: To establish standards for their identity, quality, and purity of hymenophore powder the proximate composition was carried out for *P. ribis* collected from ALC, Vijayawada, Andhra Pradesh, India. The pulverized sporophore of *P. ribis* was used for the standardization of physicochemical parameters in triplicate. Foreign matter, moisture content, extractive values, ash values [20,21] dry matter [22], absorption properties, foaming properties [23], emulsion values [24], dispersibility [25], flow characteristics, swelling index [26] were determined.

Antifungal Test

To test the antifungal activity of *P. ribis*, the method described by Nagadesi and Arya [27] was used. The *Aspergillus niger* was causing soft rot of carrot, *A. oryzae* was causing mold in paddy seeds, *Mucor racemosus* causing soft rot if bitter guard, *Rhizopus stolonifer* was causing soft rot in lady's finger and *R. artocarpi* causing fruit rot in Jack fruit was isolated and used for antifungal test. The fungal extracts were mixed with appropriate volume of medium (PDA) to obtain concentrations ranging from 5 to 25% in the final volume of 100 ml of medium. This 100 ml medium was dispensed into 100 mm Petri plates with triplicates. Plant pathogenic fungi were placed in the centre of each plate. Control sets were also prepared without fungal extract. The plates were incubated at 25 ± 20°C and growth of colony was measured after 7 days of inoculation. The radial growth of mycelium was measured at two points along the diameter of the plate and mean of these two readings was taken as the diameter of the colony. The growth of colony in control sets was compared with that of various treatments and difference was converted into percent inhibition by following formula

$$\text{Percent inhibition} = \frac{\text{Diameter of control set} - \text{diameter of treated set}}{\text{Diameter of control set}} \times 100$$

RESULTS AND DISCUSSION

Phenotypical Identification

The sporophore was found on the living tree trunk of *Peltophorum roxburghii* (G.Don) Degener causing bunt rot. The sporophore is identified as *P. ribis* (Schumach.) Ryvarden, belonging to *Hymenochaetaceae* family. The description of the sporophore was given below *Phylloporia ribis (Schumach*.) Ryvarden, Basidiocarps perennial, sessile, woody, semicircular often encircling the stem on which it is growing, often several basidiocarps fused to become imbricate with overlapping pilei, pilei flat and up to 21.4 × 13.2 × 11,5 cm in size; upper surface is sulcate, concentric bands with depressions, dark brown when young, black when it becomes old, smoother with pits and small tubercles, soft and compressible, in actively growing basidiocarps have yellowish brown wavy margin, the upper part distinctly separated from the context proper by a black zone (Plate I Figure 1(A)); Hymenium surface dark rusty brown, pores small and circular,7-9 per mm, invisible to the naked eye; tube layers concolorous, up to 1 cm thick and indistinctly stratified (Plate I Figure 1(B)); Context dense and shiny when broken, dark reddish brown, thicker than the upper soft tomentum which is separated by a black zone, in compound basidiocarps this black zone may occur irregularly in the context due to late fusion of previous separate basidiocarps. Hyphal system monomitic; generative hyphae with simple septa, thick-walled, in the tomentum sparingly branched with scattered septa, yellow to pale rusty brown, 3.25-6.5 µm in diameter, in the context and trama golden brown coloured and up to 9,7 µm in diameter, in the subhymenium hyaline and thin-walled, 3.25 µm in diameter.

Setae in hymenium were absent, Basidia clavate, 4-sterigmate, 12.5-16.6 × 6.5-7.7 µm simple-septate at the base. Basidiospores usually abundantly present, ellipsoid, thin-walled, pale yellow to hyaline, negative in Melzer's reagent, 3-4.5 × 2.5-3 µm.

Habitat: Found on the living tree trunk at the base of *Peltophorum rouxbergii* causing white rot, from Andhra Loyola College, Krishna district, Andhra Pradesh, India, collected by N. Praveen Kumar, Accession no: ALC 30. 20-8-2017.

Phylloporia species are mostly parasitic on lianas and roots of shrubs and trees, which are difficult to identify in the Neotropics. As a result, the extent of host specialization of *Phylloporia* species is unknown [28]. In the present study the *P. ribis* is causing bunt rot at the base of the living tree. *P. ribis* was reported growing strictly on the roots of living Crataegus species of *Ribes*, *Lonicera* and *Symphoricarpos*, which could produce white rot and heartrot in living hardwoods [29]. In the present study the *P. ribis* is found on living tree trunk at the base of *P. rouxbergii*, which causing white rot. *P ribis*. It is distributed throughout East Asia also and, is a white-rot fungus that prefers to live on stumps of *Rosa polyantha* and *Weigela subsessilis* [30]. In the present study the *P. ribis* was reported from Andhra Pradesh, India.

Mycochemical Bioactive Compounds

The mycochemical screening of three different extracts showed great variation in terms of bioactive compounds (Table 1).

Table 1: Mycochemical composition of *Phylloporia ribis* in different solvent extracts

S.No	Mycochemicals	Mycochemical test	Ethanol	Methanol	Water
1	Carbohydrates	Molisch's test	+++	++++	+++
		Fehling's test	+++	+++	++
2	Proteins	Biuret test	+++	+++	+++
3	Amino acids	Ninhydrine test	+++	++	++
4	Lipids	Brown Bag test	++	+++	+
		Emulsion test	++	+	--
5	Alkaloids	Wagner's test	-	+++	++
		Mayers test	-	+	+
6	Glycosides	Liebermann's Test	-	+++	++
		Legal's test	-	++	--
7	Cardiac glycerides	Keller-Kiliani Test.	+++	++	++
8	Flavonoids	Lead acetate solution test	++++	++++	++
9	Phenols	Ferric chloride test	++	+++	--
10	Terpinoids	Saloweski test	+++	++	++
11	Steroids	Lieberman-Buchard reaction	++	+++	+
		Salkowski's test	++	++++	+
12	Sterols	KOH test	+	++	+
13	Saponins	Forthing Test	+	+++	++
14	Tannins	Ferric chloride test	-	+++	++
15	Phosphates	Ammonium molybdate test	+++	+++	++
		Silver nitrate test	+	++	++

+=Present, ++=moderately present, +++ (or) ++++=Excellent

The extracts of *P. ribis* contain Carbohydrates, Proteins, Amino acids, Lipids, Alkaloids, Glycosides, Cardiac glycerides, Flavonoids, Phenols, Terpinoids, Steroids, Sterols, saponins, Tannins, and Phosphate.

The best solvent for extraction is methanol when compared to ethanoland water. The methanolic extract of *P. ribis* showed excellent concentration of Carbohydrates, Proteins, Lipids, Alkaloids, Glycosides, Flavonoids, Phenols, Steroids, saponins, Tannins, and Phosphate. The ethanolic extract of *P. ribis* showed excellent concentration of Carbohydrates, Proteins, Amino acids, Cardiac glycerides, Flavonoids, Terpinoids. The water extract of *P. ribis* showed the excellent concentration of Carbohydrates, Proteins (Plate I Figure (1C-1E)).
The optimum polysaccharide extraction method of *P. ribis* (Schumach.:Fr.) Ryvarden (PrR) was studied and also compared the polysaccharide content of PrR collected in different years The polysaccharide content of annual and biennial PrR was 4.48% and 4.01% respectively. It is concluded that content of PrR polysaccharide decreased with the increase of years [31]. In the present study the *P. ribis* shown excellent concentration of carbohydrates and proteins. A comprehensive screening shows that ergothioneine is the most abundant antioxidant in the wild macrofungus *P. ribis* Ryvarden [32].

In the present study the *P. ribis* showed the antioxidant compounds like Phenols flavonoids terpinoids. The effect of *P. ribis* glucan (PRG) administration against immune injury due to free radical formation was evaluated in mice. The results showed that glucan administration significantly increased thymus and spleen indices, spleen lymphocyte proliferation and NK cells activity, as well as CD8 T cell numbers, and decreased CD4+CD8+[33]. In the present study the *P. ribis* had shown excellent concentration of carbohydreates proteins glycosides and phenols so this should be useful for immunomodulator.

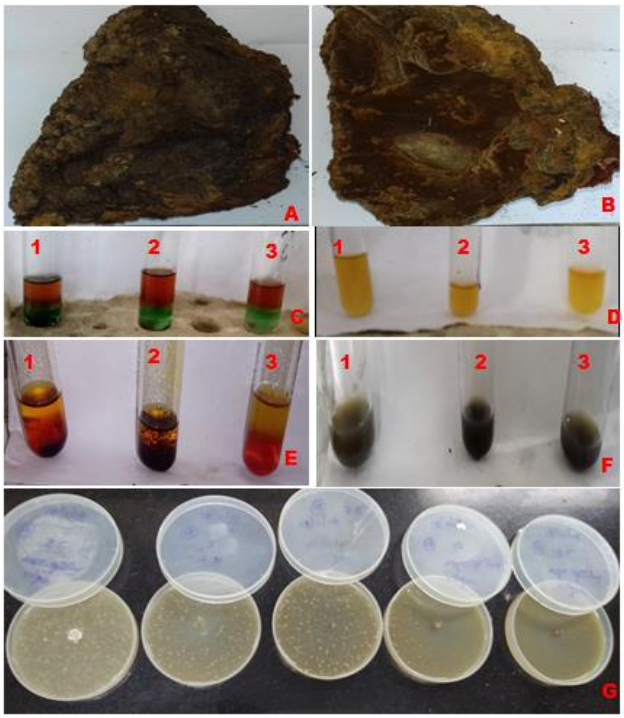

Plate I: Figure 1 (A): The sporophore upper surface of *Phylloporia ribis;* Figure (B): the hymenium surface of *Phylloporia ribis* having small, circular pores*;* Figure (C): 1. Ethanol extract 2. Methanol extracts 3. Water extract of *P. ribis* showing presence of carbohydrates; Figure (D): 1. Ethanol extract 2. Methanol extracts 3. Water extract of *P. ribis* showing presence of Proteins*;* Figure (E): 1. Ethanol extract 2. Methanol extracts 3. Water extract of *P. ribis* showing presence of Glycosides*;* Figure (F): 1. Ethanol extract 2. Methanol extracts 3. Water extract of *P. ribis* showing presence of Tannins*;* Figure (G): the antifungal activity of Methanol extract of *P. ribis* sporophore powder on Plant pathogen *Aspergillus niger*

Proximate Composition

The hymenophore powder of *P. ribis* shows the proximate composition in Table 2. Foreign matter present in sporophore powder of *P. ribis* is 0.5% whereas moisture content in sporophore powder of *P. ribis* is 7.5%. The

higher the dry matter in sporophore of *P. ribis* indicates presence of less moisture. The extractive value of *P. ribis* powder shows higher ethanol soluble value when compared to water soluble content. The ash content of *P. ribis* sporophore powder is 4.89%, showing higher acid insoluble ash when compared to water soluble ash. The absorption capacity of *P. ribis* sporophore powder shown higher water absorption when compared to oil absorption. The emulsion formation capacity of *P. ribis* sporophore powder shown low dispersibility when compared to emulsion stability. The flow properties of *P. ribis* sporophore powder of *P. ribis* shown tapped density is more when compared to bulks density. The foam formation capacity of *P. ribis* sporophore powder shown higher swelling capacity when compared to foaming capacity.

The *Phellinus pachyphloeus* showed negligible foreign matter and low moisture content as 13.67% [34], in the present study the *P. ribis* showed very low amount of foreign matter and moisture content as 7.5%. It also shows high dry weight as 86.33% [34]. In the present study also the *P. ribis* showed high dry weight as 82.9%. It also shown good flow characteristics and high dispersibility (85.67%) [34]. In the present study, the *P. ribis* shown moderate flow characteristics and low dispersibility. It also has shown high alcohol soluble extractives [34]. In the present study also high alcohol soluble extractives was observed. It also has shown high water absorption capacity [34]. In the present study the *P. ribis* shown high water absorption capacity. It also had shown good emulsion properties [34]. In the present study also the *P. ribis* shown high emulsion character. It also shown high content of water soluble ash (3%) [34] In the present study the *P. ribis* shown low water soluble ash content.

Table 2: Proximate composite evaluation in sporophore of *Phylloporia ribis*

S. No	Parameter	Properties	*Phylloporia ribis*
1	Physical parameters	Foreign matter (%)	0.5
2		Moisture content (%)	7.5
3		Dry matter (%)	82.9
4	Extractive values (%)	Ethanol soluble extractives	2.25
		Water soluble extractives	1.87
5	Ash content (%)	Total ash	4.89
		Acid insoluble ash	1.56
		Water soluble ash	1.21
6	Absorption properties (ml/g)	Oil absorption capacity	5.98
		Water absorption capacity	48.56
7	Emulsion properties (%)	Emulsifying capacity	34.45
		Emulsion stability	25.43
		Dispersibility (%)	20
8	Flow properties	Bulk density (g/ml)	0.56
		Tapped density (g/ml)	0.78
9	Foaming properties (%)	Foaming capacity	15.67
		Foaming stability	Oct-25
		Swelling Index (%)	50

Antifungal Activity

The antifungal activity of *P. ribis* on plant pathogenic fungi was shown in Table 3. The methanolic extract of *P. ribis* shown best antifungal activity when compared to water extract. The 20%, 25% concentration of methanolic

extract of *P. ribis* shown 100% inhibition of plant pathogenic fungi tested. The 25% concentration of water extract of *P. ribis* has shown 100% inhibition of plant pathogenic fungi. Except 5% concentration all methanolic extracts of *P. ribis* shown 100% inhibition of *Aspergillus niger* (Plate I Figure 1(G)).

Table 3: Antifungal activity of *Phylloporia ribis* in different plant pathogenic fungi

Fungi	Methanol					Water				
	5%	10%	15%	20%	25%	5%	10%	15%	20%	25%
Aspergillus niger	86.1	100	100	100	100	45.4	62.3	73.5	91.3	100
A. oryzae	89.5	94.6	100	100	100	59.5	68.6	83.6	93.7	100
Mucor racemosus	78.3	92.2	100	100	100	38.3	57.2	72.5	89.6	99.2
Rhizopus stolonifer	87.6	96.2	99.3	100	100	43.8	60.6	76.9	93.5	100
Rhizopus artocarpi	83.8	94.9	100	100	100	60.5	70.2	83.4	90.1	98.9

The live-cell filtrates of *T. viride* producing CuNPs shown better controlling on plant pathogenic fungi tested when compared to live-cell filtrates of *A. niger*. The 20% and 25% concentrations of a bio-controlling agent like *A. niger* and *T.viride* filtrates showed 100% inhibition of plant pathogenic fungi [35].In the present study also the methanolic extract of *P. ribis* with 20% and 25% concentration shown 100% inhibition of plant pathogenic fungi.

CONCLUSION

The present study suggests that, the *Hymenochaetaceae* members are valuable source in herbal medicines. Green medicine is safe, easily available and less side effects, so the different sporophores powders were used in preparation of crude drugs/folk medicines. The India sporophores of *Phylloporia ribis* contain Carbohydrates, Proteins, Amino acids, Lipids, Secondary metabolites and Phosphate. The present of different Mycochemical bioactive components within the *P. ribis* suggest that it is edible, rich in nutrients and the secondary metabolites may be used in promoting the immunity through diet, drug discovery and development of various new formulations. In the present study, the *P. ribis* should be utilized as a suitable antifungal agent against plant pathogens causing soft rot in Vegetables and fruits. The proximate composition evaluation is useful for standardization of *P. ribis* in powder form. That will help to identify the genuine specie in adulteration test. For the first time mycochemical bio active compounds, proximate composition evaluation, antifungal activity of *P. ribis* were reported from India. The studies should be performed further in order to isolate the bioactive principles in pure form which were responsible for Immunity booster, drug development, treatment of different pestilence, antioxidant, anti-inflammatory, antibiotic nature and antimicrobial activity**.**

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