Chemical and Bioactive Profiling, and Biological Activities of Coral Fungi from Northwestern Himalayas

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Ramaria Fr. and Clavaria L. are the two major genera of coral mushrooms within families Gomphaceae and Clavariaceae, respectively. Besides having important role in forest ecology, some species of these are reported to possess high nutraceutical and bioactive potential. There is a hidden diversity of coral mushrooms in Northwestern Himalayas. Present studies describe the detailed biochemical profiling and antioxidant, and antibacterial activities of twelve coral mushroom species. Biochemical profiling of nutrients and nutraceuticals was done with standard techniques and by using HPLC, UPLC and GC. Experiments were also conducted to check the toxic metals detection. Antioxidant activities were calculated using EC50 values from mushroom extracts. Antibacterial activities were checked on six pathogenic bacterial strains through minimum inhibition concentrations. Although, differences were observed in the net values of individual species but all the species were found to be rich in protein, macro and micro minerals, carbohydrates, unsaturated fatty acids, essential amino acids, phenolics, tocopherols, anthocynadins and carotenoids. All the species showed significant antioxidant and antibacterial activities. These species are reported to free from heavy toxic metals. Present studies will open the way for their large scale commercial exploitations and use in pharmaceutical industries as antioxidant, antibacterial and nutraceutical constituents.

Wild mushrooms are used from ancient times and have a long history as nutritious tasty food items with low calorific values and high in proteins, vitamins, iron, zinc, selenium, sodium, chitin, fibres and minerals1–3. Besides high nutritional value these have well known medicinal utility due to well established therapeutic potential4–10. Fruitbodies and mycelium of some species are reported to contain important bioactive compounds with high antioxidant potential11. Extracts from the wild fungi are considered as important remedies for the prevention and cure of many diseases from decades in several parts of the world12,13. Immunomodulating molecules extracted from mushrooms are used nowadays to improve immune function in cancer patients during radio and chemotherapy and help to prolong survival times in many type of cancers14. Role of the wild edible mushrooms in lowering blood pressure and free cholesterol in plasma is well understood15. The bioactive compounds extracted from wild mushrooms have antioxidant16, antitumor, and antimicrobial properties17. The nutraceuticals analyzed in these mushrooms are dietary fibres, polyunsaturated fatty acids, immunomodulatory proteins, polysaccharides, amino acids, keto acids, minerals, antioxidative vitamins, and other antioxidants18–20.

The genera Ramaria (Basidiomycetes, Agaricimycetes, Gomphales) and Clavaria (Basidiomycetes, Agaricimycetes, Agaricales) are worldwide in distribution. Some of the species of both these genera are used by people of native places for culinary purposes. Most of the species are not studied for detailed profiling of nutritional and nutraceutical compounds. The Northwestern Himalayan regions of India include the states of Himachal Pradesh (30°22 to 33°12 N latitude and 75°45 to 79°04 E longitude), Uttarakhand (28°43 N to 31°28 N latitude and 77°34 E to 81°03 E longitude), and some parts of Jammu and Kashmir (34°8 N and 77°34 E). The regions in these states have extensive areas under forest and hidden diversity of coral mushrooms. Few species of these coral mushrooms are used by local inhabitants for culinary purposes from ancient times while exact

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composition and nutraceutical utility of many species is still unknown to till date. There are very few reports about the exploration of scattered species of these genera from Northwestern Himalayas. The knowledge of culinary status of these species is restricted to aged people. During the frequent surveys to Northwestern Himalayas, six species of coral mushrooms belonging to genus Ramaria viz., R. botrytis, R. rubripersanens, R. flava, R. flavescens, R. aurea, R. stricta and six species belonging to genus Clavaria viz., C. fragilis, C. coralloides, C. purpurea, C. vermicularis, C. amoena and C. rosea were collected. Informations about their culinary status were collected from local inhabitants. All the species were studied for their detailed biochemical profiling and biological activities for the first time.

Results and Discussion

Nutrients Profiling. Among the twelve coral mushrooms, some coral mushrooms viz., Ramaria botrytis, R. flava, R. flavescens, R. stricta, Clavaria fragilis and C. vermicularis are traditionally used by the native people of Northwestern Himalayan regions for culinary purposes from ancient times (Table 1). The detailed composition of nutrients of coral mushrooms from Northwestern Himalayas is presented in Table 2. Protein contents in all the species varied between 10.81 ± 1.2 – 21.65 ± 0.2%. Protein contents of Ramaria botrytis and Clavaria fragilis were found to be higher than other species. Minimum percentage of protein contents was documented in R. stricta (10.81 ± 1.2%). All the species of coral mushrooms contained low percentage of fat ranged from 0.22 – 1.49%. Ramaria botrytis contained least percentage of crude fat (0.22%) and R. flava contained highest percentage of fat content (1.49%) in all the twelve species. Coral mushrooms belonging to genus Clavaria contained lower percentage of fat as compared to Ramaria species. In general coral mushrooms of both genera from Northwestern Himalayas were found to be higher in protein and low in fat. However differences were observed in net values of individual species. Crude fibres ranged from 0.28 – 1.33% in all the species with highest percentage in Ramaria botrytis and lowest in R. stricta. Ash content varied between 0.23 – 1.27%. Carbohydrates were found to be in higher percentage as compared to other nutritional components and their percentage was ranged from 40.50 – 50.26%. Amongst twelve species of Ramaria and Clavaria, Ramaria stricta contained minimum percentages of all the nutrients analyzed. Nutrient contents of Ramaria stricta were found to be in lesser amount as compared to other species. Six minerals were analyzed from these species viz., Fe, Na, K, Ca, Mg and Cu. Ramaria botrytis and Clavaria fragilis contained higher amounts of the minerals and least values for these were detected in Ramaria stricta. All the species were found to be rich in Mg. Its highest amount was detected in Ramaria botrytis 15.7 ± 3.12 mg/100g and lowest amount in R. stricta 8.4 ± 2.15 mg/100g. Results obtained for preliminary studies to check the toxicity level due to presence of heavy metals showed negative results for all the species. This means that the tested species of Himalayan coral mushrooms do not contain any heavy metals which cause toxicity in many wild edible mushroom species due to the fact that mushrooms accumulate toxic heavy metals.

All the species contained glucose and rhamnose as the principal sugars. However, other sugars viz., xylose, mannose, galactose and fructose were also detected in trace amounts from all the species. Ramaria stricta contained lowest percentage of glucose (50.12 ± 2.54%) whereas R. botrytis contained highest percentage of glucose (69.11 ± 3.9%). There are previous reports on documentation of culinary edible species from native regions of Northwestern Himalayas but there are no reports on the detailed chemical and bioactive profiling of present species of coral mushrooms. Nutrients content of Ramaria botrytis were documented similar to the previously analyzed wild edible R. brevispora (21.1%), R. formosa (20.3%). But higher than wild coral and common edible mushroom Lyophyllum decastes (18.31%)21. Moreover, nutrient profiling of presently tested species are very much similar to the composition of several commercially cultivated edible and highly prized medicinal mushroom species analyzed from Himalayan regions viz., Clavaria cinerea, Agaricus bisporus, Boletus edulis, Morchella esculenta, Cordyceps sinensis, and Lentimula edodes22–26. Results obtained for nutritional composition of presently evaluated species are in conformity with other species of wild and commercially cultivated mushrooms with high protein and carbohydrate contents and low fat levels which directly making them nutritionally valuable27. Although the nutritional compositions of all the twelve species differed but the values are significantly higher than previously worked out species of related genera viz., Clavaria rosea, Agaricus arvensis, Lepiota leucothites, Amanita caesarea, Gymnopilus junonius, Coprinus atramentarius, Hygrocybe coccinea, Hygrophorus pustulatus and Lactarius pubescens collected from these regions28. Presence of small percentage of crude fibres is making these coral fungi important in nutritional point of view. Only few species viz., Ramaria brevispora and Clavaria rosea have been analyzed for the nutritional composition from Indian regions29. The results for the nutritional analysis of these species are comparable with previously documented species of medicinal mushrooms belonging to related and different genera from Northwestern Himalayas28.

Bioactive Profiling. The detailed fatty acid composition of all the twelve species is presented in Table 3. The major fatty acids found in all the coral mushrooms were linoleic acid (C18:2), followed by Paullinic acid (C20:1) and palmitic acid (C16:0). Polyunsaturated fatty acids were the main group of fatty acids documented in all the species. Ramaria botrytis and Clavaria fragilis contained lower percentages of saturated fatty acids and higher polyunsaturated fatty acids as compared to other species. C18:2 trans-linoleic acid percentages in presently evaluated coral mushrooms was documented much higher than wild edible Pleurotus ostreatus, Lactarius salmonicolor, Flammulina velutipes, Russula anthropina and Boletus reticulatus30. In all the twelve species of coral mushrooms unsaturated fatty acids were predominated over saturated fatty acids. Unsaturated fatty acids were ranged from 65 – 70% of total fatty acids.

Amino acids profiling showed the presence of eleven amino acids (essential and non essential) viz., aspartic acid, arginine, alanine, proline, tyrosine, valine, leucine, lysine, isoleucine, methionine and glutamic acid. All the amino acids were detected in appreciable amounts from all the species of coral mushrooms. However, in some species of coral mushrooms belonging to genus Clavaria amino acids viz., proline, lysine and isoleucine were not detected. The details of the amino acid profile are presented in Table 4. Glutamic acid was documented in higher
Recent studies have documented for ABTS radical scavenging activities in which EC50 were ranged from 0.39–0.92 mg/mL. Lowest content of total phenolic compounds and total flavonoid contents found in the extracts. Similar results were shown for DPPH radicals accept electrons or hydrogen radicals to form stable diamagnetic molecules. Higher EC50 values were shown for DPPH radical scavenging activity in all the twelve species showed differences in effectiveness. Amongst all, the species showed higher EC50 values and lower DPPH radical scavenging activity and vice versa. EC50 values obtained for DPPH radical scavenging activity in all the twelve species showed differences in effectiveness. Amongst all, the species showed lowest EC50 values and higher EC50 values obtained for DPPH radical scavenging activity and vice versa. EC50 values obtained for DPPH radical scavenging activity in all the twelve species showed differences in effectiveness. Amongst all, the species showed lowest EC50 values and higher EC50 values obtained for DPPH radical scavenging activity and vice versa. EC50 values obtained for DPPH radical scavenging activity in all the twelve species showed differences in effectiveness. Amongst all, the species showed lowest EC50 values and higher EC50 values.

Table 1. Details of collections and culinary status of coral mushroom species collected from Northwestern Himalayas.

| Species               | Location               | Altitude (m) | Culinary Status                                                                 |
|----------------------|------------------------|--------------|---------------------------------------------------------------------------------|
| Ramaria botrytis     | Kalatope (Himachal Pradesh, India) | 2300         | Edible: Used by native people for culinary purposes                             |
| R. rubriperrunens     | Sonmarg (Jammu Kashmir, India)   | 2800         | Unknown: People are not aware about culinary potential                           |
| R. flava              | Janjehli (Himachal Pradesh, India) | 2400         | Edible: Used by native people for culinary purposes                             |
| R. flavescens         | Maclodelganj (Himachal Pradesh, India) | 2200         | Edible: Used by native people for culinary purposes                             |
| R. aurea              | Nainital (Uttrakhand, India)   | 2600         | Unknown: People are not aware about culinary potential                           |
| R. stricta            | Kalatope (Himachal Pradesh, India) | 2300         | Edible: Used by native people for culinary purposes                             |
| Clavaria fragilis     | Janjehli (Himachal Pradesh, India) | 2400         | Edible: Used by native people for culinary purposes                             |
| C. comaloides         | Kumaoon (Uttrakhand, India)    | 2800         | Unknown: People are not aware about culinary potential                           |
| C. purpurea           | Maclodelganj (Himachal Pradesh, India) | 2200         | Unknown: People are not aware about culinary potential                           |
| C. vermicularis       | Sonmarg (Jammu Kashmir, India)   | 2800         | Edible: Used by native people for culinary purposes                             |
| C. amoenus            | Nainital (Uttrakhand, India)    | 2600         | Unknown: People are not aware about culinary potential                           |
| C. rosea              | Nainital (Uttrakhand, India)    | 2600         | Unknown: People are not aware about culinary potential                           |

percentage as compared to other amino acids. The higher percentage of this was documented from Ramaria botrytis (3.17%) and Clavaria fragilis (3.15%).

Tocopherol contents in all the species are detailed in Table 5. α-tocopherol and β-tocopherol were found to be present in all the species. However, α-tocopherol was not documented in Ramaria aurea and R. stricta. Tocopherol contents were ranged from 0.01–1.71 μg/g in all the species. Ramaria botrytis and Clavaria fragilis contained maximum amount of all the three isomers as compared to other species. Results obtained for β-carotene, lycopene, phenolic compounds, ascorbic acid, and anthocyanidins composition of all the species is presented in Table 5. β-carotene in all the species ranged from 0.55–0.92 μg/100 g. Highest amount of this was detected in Ramaria botrytis (0.92 μg/100 g) and lowest in R. stricta (0.57 μg/100 g). Lycopene content in all the species ranged from 0.28–0.49 μg/100 g. Highest amount of this was detected in R. botrytis (0.49 g/100 g) and lowest in R. stricta (0.28 μg/100 g). Phenols were detected in significant amounts from all the species (40.32–56.35 mg/g). The highest amounts of phenolic compounds were detected in R. botrytis (56.35 mg/g) and lowest in R. stricta (40.32 mg/g). Ascorbic acid content of all the species ranged from (0.42–0.89 mg/100 g). Anthocyanids ranged from 13.11–22.92 mg/100 g in all the twelve species. Highest content of these were detected in Ramaria botrytis (22.92 mg cyanidin chloride/100 g extract) and least in R. stricta (13.11 mg cyanidin chloride/100 g extract). Each species differed in net amounts of all these components with other species. High percentage of UFA showed the medicinal importance of these coral mushrooms as UFA increase the HDL cholesterol and decrease LDL cholesterol, triacyl-glycerol, lipid oxidation, and LDL susceptibility to oxidation. All the three isomers viz., α-, β-, and γ-tocopherol were documented from all the twelve species of coral mushrooms. However γ-tocopherol was not detected in four species of coral mushrooms viz., Ramaria aurea, R. stricta, Clavaria amoenus and C. rosea. β-tocopherol was detected in higher amount as compared to α- tocopherol and γ-tocopherol from all species of coral mushrooms. The composition of studied coral mushrooms is comparable to previously analyzed edible mushrooms reported from Northwestern Himalayas. Ramaria botrytis and Clavaria fragilis contained significantly higher amounts of tocopherol as compared to other species. The high levels of these two compounds correspond with higher oxidative activities and play a vital role in human body as α-tocopherol was considered the most active form of vitamin E in humans and it was reported to exhibit the highest biological activities. All the coral mushrooms contained phenolic compounds in higher amounts than other bioactive compounds. Phenolic compounds of Clavaria and Ramaria analyzed presently were documented in much higher amount than Clavaria and Ramaria species evaluated from the forests of Western Ghat in Souther part of India.

Presence of high phenolic compounds accounts for the high antioxidant properties of all these species. Besides, their direct antioxidant activities, phenolic compounds are capable to promote other activities like anti-proliferation, cell cycle regulation, and induction of apoptosis. Other bioactive compounds viz., β-carotene, lycopene, and ascorbic acids were detected in low amounts. Traces of anthocyanidins were also detected from all these species. The presence of these functional medicinal compounds in all the studied coral mushrooms is making them important for their pharmaceutical uses.

**Biological Activities.** Antioxidant and Antibacterial activities. Antioxidant properties of all the species were expressed as EC50 values. The details are presented in Table 6. Stabilities of DPPH radicals are widely used to evaluate the antioxidant activities of proton-donating substances according to their hydrogen-donating ability. DPPH radicals accept electrons or hydrogen radicals to form stable diamagnetic molecules. Higher EC50 values indicate the lower effectiveness for antioxidant properties and vice versa. EC50 values obtained for DPPH radical scavenging activity in all the twelve species showed differences in effectiveness. Amongst all, the species Ramaria botrytis and Clavaria fragilis showed lowest EC50 values and higher in R. stricta. Ramaria botrytis and Clavaria fragilis showed higher DPPH radical scavenging activity and R. stricta showed lower DPPH radical scavenging activities than other species. This could be explained by the DPPH radical-scavenging activity being related to the content of total phenolic compounds and total flavonoid contents found in the extracts. Similar results were documented for ABTS radical scavenging activities in which EC50 were ranged from 0.39–0.92 mg/mL. Lowest EC50 values were obtained for Ramaria botrytis and Clavaria fragilis showing high antioxidant activities of these...
Nutritional profiling of coral mushroom species collected from Northwestern Himalayas. Values are expressed as mean ± SE and different letters represent the significant difference in each column with P<0.05 according to Tukey’s test.

| Fatty acids | Ramaria botrytis | R. rubripersicans | R. flavida | R. flavescens | R. aurea | R. stricta | Clavaria fragilis | C. coralloides | C. purpurea | C. vermicularis | C. amaena | C. rosea |
|-------------|-----------------|-----------------|------------|---------------|---------|----------|-----------------|----------------|-----------|---------------|-----------|---------|
| C9:0        | 0.09 ± 0.00     | 0.08 ± 0.00     | 0.07 ± 0.00 | 0.072 ± 0.00  | 0.054 ± 0.00 | 0.043 ± 0.00 | 0.08 ± 0.00     | 0.06 ± 0.00     | 0.06 ± 0.00 | 0.07 ± 0.00     | 0.06 ± 0.00 | 0.07 ± 0.00     |
| C10:0       | 0.47 ± 0.01     | 0.36 ± 0.02     | 0.32 ± 0.00 | 0.30 ± 0.01   | 0.26 ± 0.02  | 0.22 ± 0.00  | 0.46 ± 0.00     | 0.41 ± 0.01     | 0.42 ± 0.00 | 0.43 ± 0.01     | 0.47 ± 0.01 | 0.39 ± 0.00     |
| C12:0       | 0.38 ± 0.00     | 0.27 ± 0.00     | ND         | 0.38 ± 0.08   | 0.07 ± 0.06  | ND         | 0.38 ± 0.00     | 0.32 ± 0.00     | 0.36 ± 0.00 | 0.23 ± 0.00     | 0.30 ± 0.00 | 0.30 ± 0.00     |
| C16:0       | 2.19 ± 0.03     | 2.11 ± 0.02     | 1.95 ± 0.08 | 1.82 ± 0.03   | 1.69 ± 0.02  | 1.5 ± 0.02   | 2.13 ± 0.03     | 1.98 ± 0.03     | 1.89 ± 0.00 | 1.78 ± 0.03     | 1.19 ± 0.03 | 1.68 ± 0.03     |
| C16:1       | 1.11 ± 0.00     | 1.09 ± 0.00     | 1.07 ± 0.00 | 1.04 ± 0.00   | 0.91 ± 0.00  | 0.91 ± 0.00  | 1.10 ± 0.00     | 1.05 ± 0.00     | 1.01 ± 0.00 | 0.99 ± 0.00     | 0.88 ± 0.00 | 0.89 ± 0.00     |
| C17:0       | 0.49 ± 0.00     | 0.42 ± 0.00     | 0.37 ± 0.00 | 0.39 ± 0.00   | 0.29 ± 0.00  | 0.22 ± 0.00  | 0.47 ± 0.00     | 0.41 ± 0.00     | 0.43 ± 0.00 | 0.36 ± 0.00     | 0.37 ± 0.00 | 0.40 ± 0.00     |
| C18:1       | 2.48 ± 0.00     | 1.95 ± 0.00     | 1.72 ± 0.00 | 1.68 ± 0.00   | 1.25 ± 0.00  | 1.12 ± 0.00  | 2.46 ± 0.00     | 1.99 ± 0.00     | 1.58 ± 0.00 | 1.98 ± 0.00     | 1.70 ± 0.00 | 1.44 ± 0.00     |
| C18:2       | 8.28 ± 0.00     | 6.54 ± 0.00     | 5.79 ± 0.00 | 5.28 ± 0.00   | 4.94 ± 0.00  | 3.93 ± 0.00  | 8.18 ± 0.00     | 7.18 ± 0.00     | 7.23 ± 0.19 | 7.68 ± 0.16     | 7.12 ± 0.00 | 6.28 ± 0.00     |
| C20:0       | 4.20 ± 0.02     | 3.18 ± 0.03     | 3.1 ± 0.17  | 2.90 ± 0.24   | 2.78 ± 0.30  | 2.39 ± 0.00  | 4.15 ± 0.00     | 3.93 ± 0.25     | 3.85 ± 0.00 | 3.99 ± 0.22     | 3.20 ± 0.22 | 3.43 ± 0.22     |
| SFA         | 3.13 ± 0.24     | 2.82 ± 0.14     | 2.34 ± 0.14 | 2.57 ± 0.26   | 2.07 ± 0.30  | 1.76 ± 0.11  | 3.05 ± 0.30     | 2.77 ± 0.30     | 2.73 ± 0.20 | 2.63 ± 0.22     | 1.92 ± 0.22 | 2.39 ± 0.24     |
| MUFA        | 4.08 ± 0.21     | 3.46 ± 0.22     | 3.16 ± 0.22 | 3.01 ± 0.24   | 2.38 ± 0.27  | 2.20 ± 0.11  | 4.03 ± 0.22     | 3.45 ± 0.44     | 3.02 ± 0.23 | 3.37 ± 0.22     | 2.70 ± 0.35 | 3.03 ± 0.40     |
| PUFA        | 12.48 ± 1.12    | 9.72 ± 0.88     | 8.89 ± 0.99 | 8.18 ± 0.83   | 7.72 ± 0.88  | 6.32 ± 0.99  | 12.33 ± 1.34    | 11.11 ± 1.57    | 11.08 ± 1.14 | 11.67 ± 1.12    | 10.32 ± 1.12 | 9.71 ± 1.13     |

species. Higher EC50 values were obtained for Ramaria stricta showing lowest ABTS radical scavenging activities of this species. Higher effectiveness in ferrous ion chelating activity and scavenging ability on nitric oxide was detected in Ramaria botrytis and low effectiveness was detected in R. stricta. In general, all the twelve coral mushrooms showed significant antioxidant properties measured on the basis of EC50 values. Each species showed different antioxidant activities with highly effective and less effective EC50 values. Better antioxidant properties of Ramaria botrytis and Clavaria fragilis are due to presence of higher phenolic compounds, ß-carotene, lycopene, ascorbic acids, anthocyanidins, and tocopherol amounts in them which break the free radical chain by donating an electron to stabilize and terminate radical chain reactions or by showing pro-oxidant by maintaining the transition metal ions, Fe2⁺ and Cu2⁺ in their reduced forms. High reducing power of some species might be due to the presence of higher amounts of reducers in them. Role of antioxidants present in coral mushrooms can demonstrate their protective properties via two main types of antioxidants, namely, primary (chain breaking, free radical scavengers) and secondary or preventive. Secondary antioxidants are the consequence of deactivation of metals, inhibition or breakdown of lipid hydroperoxides, regeneration of primary antioxidants34,35.
Table 4. Amino acid (%) profiling of coral mushroom species collected from Northwestern Himalayas. Values are expressed as mean ± SE and different letters represent the significant difference in each column with p ≤ 0.05 according to Tukey’s test. ND = not detected.

Table 5. Bioactive profiling of coral mushroom species collected from Northwestern Himalayas. Values are expressed as mean ± SE and different letters represent the significant difference in each column with p ≤ 0.05 according to Tukey’s test. ND = not detected.

The substances present in these mushrooms exhibit antioxidant activity by acting as inducers or promoting cell signals, leading to changes in gene expression, which result in the activation of enzymes that eliminate reactive oxygen species.

All the species showed antimicrobial activities. The results of inhibition zones produced against a large number of pathogenic bacteria are listed in Table 6. The minimum inhibition concentration against all the pathogenic bacteria was determined. The MIC values for E. coli showed a range of 18-80 mg/mL for all the species. MIC values for P. aeruginosa showed 80, 100, 60, 80, 100 mg/mL for R. botrytis, R. rubripersiansis, R. flavescens, R. aurea, R. stricta, R. flavescens, R. aurea, and R. stricta, respectively. The screening of antibacterial activities of the extracts is in conformity with the previous studies on other medicinal and edible species.

The results obtained for broad spectrum and higher concentrations are similar as obtained for other widely used medicinal species of mushrooms. Extracts from all the species showed activity against both gram-positive and gram-negative strains. The sensitivity of gram-positive bacteria to the mushroom extracts is in conformity with the previous studies on other medicinal and edible species due to the membrane composition of the bacterial stains. The susceptibility of gram-positive and gram-negative bacteria to the mushroom extract might be due to the presence of low molecular weight or high molecular weight compounds.
The detector and injector temperature were 250 °C and 300 °C respectively. N2 and H2 were used.

The column temperature was programmed from 70 to 200 °C with 2 minutes hold at 70 °C, 6.5 rise/min and 5 minutes.

The total weight - (moisture content) was used in the acid-alkali method (1.25% each). Total carbohydrates percentage was calculated by the difference as the percentage of water.

The column with a cross-linked 5% phenyl-methyl polysiloxane (15 m × 0.53 mm i.d.) was used. The oven temperature was held at 200 °C. The detector and injector temperature were 250 °C and 300 °C respectively. N2 and H2 were used as carrier and fuel gases, respectively.

Values are expressed as mean ± SE and different letters represent the significant difference in each column with p ≤ 0.05 according to Tukey’s test.

### Table 6. EC50 values (mg/mL) exhibiting antioxidant activities of coral mushrooms from Northwestern Himalayas.

| Species          | DPPH radicals scavenging activity | ABTS radicals scavenging activity | Scavenging ability on superoxide anion radicals | Ferric reducing antioxidant power | Iron Chelating effect |
|------------------|----------------------------------|----------------------------------|-------------------------------------------------|----------------------------------|-----------------------|
| R. batratus      | 0.68 ± 0.0*                      | 0.39 ± 0.0*                      | 0.86 ± 0.0*                                     | 0.92 ± 0.0*                      | 1.12 ± 0.0*           |
| R. rubripersaenens | 0.76 ± 0.0*                      | 0.43 ± 0.0*                      | 1.12 ± 0.1*                                     | 1.15 ± 0.0*                      | 1.22 ± 0.0*           |
| R. flavus        | 0.88 ± 0.0*                      | 0.49 ± 0.0*                      | 1.35 ± 0.0*                                     | 1.18 ± 0.0*                      | 1.34 ± 0.0*           |
| R. flavescens    | 1.52 ± 0.0*                      | 0.52 ± 0.0*                      | 1.48 ± 0.0*                                     | 1.22 ± 0.0*                      | 1.38 ± 0.0*           |
| R. aurea         | 1.58 ± 0.0*                      | 0.59 ± 0.0*                      | 1.55 ± 0.0*                                     | 1.28 ± 0.0*                      | 1.44 ± 0.0*           |
| R. stricta       | 1.72 ± 0.0*                      | 0.92 ± 0.0*                      | 1.58 ± 0.3*                                     | 1.32 ± 0.0*                      | 1.58 ± 0.0*           |
| Clavaria fragilis| 0.69 ± 0.0*                      | 0.40 ± 0.0*                      | 0.88 ± 0.0*                                     | 0.93 ± 0.0*                      | 1.14 ± 0.0*           |
| C. coralloides   | 0.75 ± 0.0*                      | 0.41 ± 0.0*                      | 1.11 ± 0.0*                                     | 1.13 ± 0.0*                      | 1.21 ± 0.0*           |
| C. purpurea      | 0.86 ± 0.0*                      | 0.47 ± 0.0*                      | 1.30 ± 0.0*                                     | 1.16 ± 0.0*                      | 1.32 ± 0.0*           |
| C. vermicularis  | 1.48 ± 0.0*                      | 0.50 ± 0.0*                      | 1.44 ± 0.0*                                     | 1.20 ± 0.0*                      | 1.34 ± 0.0*           |
| C. amena         | 1.52 ± 0.0*                      | 0.56 ± 0.0*                      | 1.50 ± 0.0*                                     | 1.26 ± 0.0*                      | 1.48 ± 0.0*           |
| C. rosea         | 1.68 ± 0.0*                      | 0.89 ± 0.0*                      | 1.53 ± 0.0*                                     | 1.30 ± 0.0*                      | 1.48 ± 0.0*           |
| Ascorbic acid    | 0.11 ± 0.0*                      | 0.29 ± 0.0*                      | 0.09 ± 0.0*                                     | 0.07 ± 0.0*                      | —                     |
| EDTA             | —                                | —                                | —                                               | —                                | 0.27 ± 0.0*           |

### Materials and Methods

#### Collection and Processing of Samples.
All the samples were collected during the frequent surveys to the different regions of Northwestern Himalayas. Informations about the culinary status were collected from native inhabitants. The species were taxonomically identified at Mushroom Research Centre, CSK Himachal Pradesh Agriculture University. Twelve species belonging to two genera Ramaria and Clavaria were subjected to detailed studies on biochemical profiling and their biological activities. The samples were vacuum dried and preserved in air-tight cellophane bags, with a small amount of 1-4-paradichlorobenzene in porous packets to keep them free of insects, for further analysis. Samples are deposited and available at Mushroom Research Centre (No. R501–R512).

#### Chemical Profiling.
For chemical profiling of nutrients, samples were powdered and analyzed for protein, fat, carbohydrates, ash, and crude fibres. Crude protein contents were estimated using the Kjeldahl method by calculating total nitrogen (N) and protein contents were expressed by N × 4.3844. Crude fat was estimated using a Soxhlet apparatus by extraction of powered samples with petroleum ether. Ash content was calculated by incineration in silica dishes at 525 ± 20 °C containing 5–10 g/sample. Fibre contents were estimated on de-fatted samples using the acid-alkali method (1.25% each). Total carbohydrates percentage was calculated by the difference as the total weight - (moisture content + protein content + crude fat + ash content + crude fibres). Minerals were analyzed using Atomic Absorption Spectrophotometer (Perkin Elmer Analyst A 400, Waltham, MA, USA).

Preliminary tests were performed to check the presence of toxic metals. For this, diluted HCl (2%) and copper foil (1 × 1/2 cm strips) pretreated with concentrated HNO3 were taken. After that powered samples were acidified with 10–20 mL of 5 diluted HCl (2%) until colour changed from fairy pink to lilium. After this strips of copper foil were added and boiled for 30 min with addition of water from time to time to replace the losses by evaporation. The heavy metals got deposited on the copper foil and color was noted after 30 min. The color and results were interpreted for the presence of heavy metals.

For monosaccharides composition, samples were extracted with 70% aqueous methanol (2.5 mL and 1.5 mL). After this, the extracts were centrifuged at 4000 rpm (4 °C) for 10 min. Supernatants were collected and volumes were made up to 5 mL with 70% methanol. The extract was passed through Millipore filter (0.45 m) and injected to the HPLC.

#### Bioactive Profiling.
**Fatty Acid Composition.** The samples were dissolved in 1 mL of solution (sodium hydroxide pellets (45 g) in 300 mL of 50% methanol and vortexed for 1 min. The solution was left for 5 minutes at 100 °C, vortexed again for 1 minute, and left at 100 °C in a water bath for 25 min. Methylation was done by adding 2 mL of solution (6N hydrochloride in methanol) and vortexing for 1 min followed by heating (80 °C) in a water bath. For extraction of fatty acids, 1.25 mL of solution (25 mL methyl ter-butyl ether added to hexane) was added, and the solution was shaken for 10 min. Upper layer was removed and 3 mL of solution (10% sodium hydroxide in water while stirring) was added. Finally, the top phase (2/3) was removed and transferred into a gas chromatography vial and injected. An Agilent 7890A gas chromatograph equipped with a flame ionization detector (FID) and fused silica column (25 m × 0.200um × 0.33 μm, Specol, Sigma) was used in split mode of 40:1. The column temperature was programmed from 70 to 200 °C with 2 minutes hold at 70 °C, 6.5 rise/min and 5 minutes hold at 200 °C. The detector and injector temperature were 250 °C and 300 °C respectively. N2 and H2 were used as carrier and fuel gases, respectively.

molecular weight compounds as peptides and proteins in the mushrooms that allow the opening of the pores for ions transport and inhibit bacterial growth[24,25]. Difference in the antibacterial activities for different species is due to the production of different antimicrobial compounds by different species.
Species & Con. (%) & Escherichia coli & Klebsiella pneumoniae & Vibrio cholerae & Pseudomonas aeruginosa & Vibrio alginolyticus & Streptococcus pneumonia & Positive Control (Tetracycline) & Negative Control EDTA

**R. botrytis** & 25 & 6.2 ± 0.1 & — & — & 2.2 ± 0.1 & — & — & 10.91 ± 1.0
 & 50 & 8.2 ± 0.3 & — & 3.2 ± 0.2 & — & 2.1 ± 0.2 & — & 13.32 ± 1.1
 & 75 & 10.2 ± 0.4 & 4.2 ± 0.5 & 4.3 ± 0.2 & 2.2 ± 0.2 & 3.3 ± 0.2 & — & 17.23 ± 1.8
 & 100 & 11.1 ± 1.9 & 5.3 ± 0.6 & 5.1 ± 0.3 & 3.3 ± 0.6 & 4.1 ± 0.3 & — & 18.92 ± 2.1

**R. rubripersica** & 25 & 5.8 ± 0.2 & — & 1.2 ± 0.0 & — & — & — & 10.24 ± 1.3
 & 50 & 7.8 ± 0.3 & — & 2.7 ± 0.1 & — & 1.2 ± 0.1 & — & 13.19 ± 1.4
 & 75 & 9.5 ± 0.4 & 1.2 ± 0.5 & 3.4 ± 0.2 & 1.8 ± 0.1 & 2.5 ± 0.6 & — & 16.21 ± 1.4
 & 100 & 10.4 ± 1.2 & 3.3 ± 0.6 & 4.1 ± 1.0 & 2.3 ± 0.4 & — & 4.5 ± 0.8 & 18.61 ± 2.3

**R. flava.** & 25 & 5.4 ± 0.2 & — & 1.0 ± 0.0 & — & — & — & 10.11 ± 2.5
 & 50 & 7.2 ± 0.3 & — & 2.5 ± 0.3 & — & — & — & 13.54 ± 1.6
 & 75 & 9.8 ± 1.4 & — & 3.2 ± 0.2 & — & 2.3 ± 0.1 & — & 17.55 ± 1.7
 & 100 & 10.1 ± 1.2 & 2.3 ± 0.1 & 3.4 ± 0.8 & 1.9 ± 0.2 & 3.4 ± 0.5 & — & 18.37 ± 2.4

**R. flavescens** & 25 & 5.2 ± 0.8 & — & — & — & — & — & 10.76 ± 1.2
 & 50 & 6.9 ± 0.5 & — & 2.2 ± 0.1 & — & — & — & 12.32 ± 2.1
 & 75 & 9.2 ± 1.3 & — & 2.8 ± 0.4 & — & 1.6 ± 0.2 & — & 16.21 ± 1.3
 & 100 & 10.2 ± 2.2 & 1.1 ± 0.0 & 3.2 ± 0.7 & 1.4 ± 0.1 & 2.5 ± 0.8 & — & 17.85 ± 2.1

**R. aurea** & 25 & 4.8 ± 0.5 & — & — & — & — & — & 10.27 ± 1.2
 & 50 & 6.4 ± 1.3 & — & — & — & — & — & 13.38 ± 1.4
 & 75 & 8.7 ± 1.4 & — & — & — & — & — & 16.20 ± 2.1
 & 100 & 9.9 ± 1.2 & — & — & — & 2.5 ± 0.2 & 2.4 ± 0.2 & — & 17.82 ± 1.8

**R. stricta** & 25 & 4.4 ± 0.5 & — & — & — & — & — & 10.21 ± 1.2
 & 50 & 6.2 ± 0.3 & — & — & — & — & — & 13.22 ± 1.3
 & 75 & 8.2 ± 0.4 & — & — & — & — & — & 17.58 ± 1.3
 & 100 & 9.1 ± 0.2 & — & — & — & — & — & 18.26 ± 1.8

**Clavaria fragilis** & 25 & 6.1 ± 1.2 & — & — & 2.3 ± 0.0 & — & — & 10.91 ± 1.5
 & 50 & 7.8 ± 1.3 & — & 3.1 ± 0.4 & 3.1 ± 0.3 & 1.3 ± 0.1 & 1.1 ± 0.0 & 13.24 ± 1.6
 & 75 & 9.8 ± 1.4 & — & 4.1 ± 0.3 & 4.9 ± 0.2 & 2.3 ± 0.2 & 2.2 ± 0.0 & 15.23 ± 1.8
 & 100 & 10.6 ± 1.2 & — & 6.8 ± 1.3 & 3.2 ± 0.0 & 3.4 ± 0.0 & — & 17.92 ± 2.2

**C. coralloides** & 25 & 4.5 ± 0.8 & — & — & 1.3 ± 0.0 & — & — & 10.20 ± 1.3
 & 50 & 5.8 ± 0.7 & — & 2.8 ± 0.1 & — & — & — & 13.24 ± 1.5
 & 75 & 7.4 ± 0.4 & — & 3.5 ± 0.2 & 1.9 ± 0.0 & 2.6 ± 0.4 & — & 17.20 ± 1.3
 & 100 & 8.7 ± 1.5 & — & 3.9 ± 1.3 & 2.7 ± 0.3 & 4.6 ± 0.3 & — & 18.22 ± 2.2

**C. purpurea** & 25 & 5.5 ± 0.2 & — & — & 1.2 ± 0.0 & — & — & 10.75 ± 1.2
 & 50 & 7.4 ± 0.7 & — & 2.7 ± 0.3 & — & 0.9 ± 0.0 & — & 13.28 ± 1.4
 & 75 & 8.9 ± 1.3 & — & 3.4 ± 0.1 & 1.1 ± 0.0 & 2.5 ± 0.0 & — & 16.20 ± 1.2
 & 100 & 9.5 ± 1.0 & — & 3.8 ± 0.1 & 2.1 ± 0.5 & 3.6 ± 0.8 & — & 18.52 ± 2.2

**C. vermicularis** & 25 & 5.5 ± 0.4 & — & — & — & — & — & 10.91 ± 1.5
 & 50 & 7.3 ± 0.6 & — & 2.4 ± 0.1 & — & — & — & 13.32 ± 1.1
 & 75 & 8.5 ± 1.6 & — & 2.0 ± 0.4 & — & 1.7 ± 0.2 & — & 16.21 ± 1.2
 & 100 & 9.9 ± 1.9 & — & 3.5 ± 0.7 & 1.5 ± 0.0 & 2.3 ± 0.2 & — & 17.90 ± 2.2

**C. amoena** & 25 & 4.9 ± 0.8 & — & — & — & — & — & 10.21 ± 1.2
 & 50 & 6.6 ± 1.2 & — & — & — & — & — & 13.26 ± 1.4
 & 75 & 8.9 ± 1.9 & — & 1.2 ± 0.0 & — & — & — & 17.15 ± 1.3
 & 100 & 9.7 ± 1.8 & — & 1.8 ± 0.1 & 2.5 ± 0.2 & 1.1 ± 0.0 & 2.4 ± 0.2 & — & 18.24 ± 2.2

**C. rosea** & 25 & 4.4 ± 0.5 & — & — & — & — & — & 10.84 ± 1.2
 & 50 & 6.2 ± 0.3 & — & — & — & — & — & 13.24 ± 1.3
 & 75 & 8.2 ± 0.4 & — & 1.3 ± 0.0 & — & 1.3 ± 0.0 & — & 17.43 ± 1.3
 & 100 & 9.1 ± 0.2 & — & 2.2 ± 0.0 & 1.1 ± 0.0 & 2.5 ± 0.2 & — & 18.91 ± 2.3

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**Table 7.** Inhibition zone (mm) shown by coral mushroom species of Northwestern Himalayas against human pathogenic bacterial strains. Values are expressed as mean ± SE and different letters represent the significant difference in each column with p ≤ 0.05 according to Tukey's test.
| Species             | Cons. (%) | Escherichia coli | Klebsiella pneumonia | Vibrio cholerae | Pseudomonas aeruginosa | Vibrio alginolyticus | Streptococcus pneumonia |
|---------------------|-----------|------------------|----------------------|-----------------|------------------------|----------------------|------------------------|
| R. botrytis         | 20        | +++              | +++                  | +++             | +++                    | +++                  | +++                    |
|                     | 40        | ++               | ++                   | ++              | +                      | ++                   | +                      |
|                     | 60        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 80        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 100       | +                | +                    | +               | +                      | +                    | +                      |
| R. rubripersanensis | 20        | +++              | +++                  | +++             | +++                    | +++                  | +++                    |
|                     | 40        | ++               | ++                   | ++              | +                      | ++                   | +                      |
|                     | 60        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 80        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 100       | +                | +                    | +               | +                      | +                    | +                      |
| R. flavus           | 20        | +++              | +++                  | +++             | +++                    | +++                  | +++                    |
|                     | 40        | ++               | ++                   | ++              | +                      | ++                   | +                      |
|                     | 60        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 80        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 100       | +                | +                    | +               | +                      | +                    | +                      |
| R. flavescens       | 20        | +++              | +++                  | +++             | +++                    | +++                  | +++                    |
|                     | 40        | ++               | ++                   | ++              | +                      | ++                   | +                      |
|                     | 60        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 80        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 100       | +                | +                    | +               | +                      | +                    | +                      |
| R. aurea            | 20        | +++              | +++                  | +++             | +++                    | +++                  | +++                    |
|                     | 40        | ++               | ++                   | ++              | +                      | ++                   | +                      |
|                     | 60        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 80        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 100       | +                | +                    | +               | +                      | +                    | +                      |
| R. stricta          | 20        | +++              | +++                  | +++             | +++                    | +++                  | +++                    |
|                     | 40        | ++               | ++                   | ++              | +                      | ++                   | +                      |
|                     | 60        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 80        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 100       | +                | +                    | +               | +                      | +                    | +                      |
| Clavaria fragilis   | 20        | +++              | +++                  | +++             | +++                    | +++                  | +++                    |
|                     | 40        | ++               | ++                   | ++              | +                      | ++                   | +                      |
|                     | 60        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 80        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 100       | +                | +                    | +               | +                      | +                    | +                      |
| C. coralloides      | 20        | ++               | ++                   | ++              | ++                     | ++                   | ++                     |
|                     | 40        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 60        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 80        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 100       | +                | +                    | +               | +                      | +                    | +                      |
| C. purpurea         | 20        | ++               | ++                   | ++              | ++                     | ++                   | ++                     |
|                     | 40        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 60        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 80        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 100       | +                | +                    | +               | +                      | +                    | +                      |
| C. vermicularis     | 20        | ++               | ++                   | ++              | ++                     | ++                   | ++                     |
|                     | 40        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 60        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 80        | +                | +                    | +               | +                      | +                    | +                      |
|                     | 100       | +                | +                    | +               | +                      | +                    | +                      |

Continued
kept in the dark for 90 min after that absorbance was read at 725 nm. Gallic acid was used to calculate the standard
(3 mL) was added to the mixture, and the volume was adjusted with distilled water to 10 mL. The reaction was
(1 mL) were mixed with Folin and Ciocalteu’s phenol reagent. Now, 1 mL of saturated sodium carbonate solution
samples were again vortexed with hexane (4 mL). After this, 2 mL of saturated NaCl aqueous solution was added,
were made by authentic standards.

Ascorbic acid contents were quantified by colorimetric assays. For quantification, standard ascorbic
ples were mixed with butylated hydroxytoluene (BHT) in hexane (10 mg/mL; 100 μL) and IS solution in hexane
(0.372 g/mL; 250 μL). Now, samples (300 mg) were vortexed for 1 min with methanol (4 mL). The samples were again vortexed with hexane (4 mL). After this, 2 mL of saturated NaCl aqueous solution was added, and the mixture was vortexed (1 min), followed by centrifugation at 4000 g for 5 min and the upper layer was separated. The samples were again re-extracted twice with hexane. The extracts were then vacuum-dried and re-dissolved in hexane (1 mL), followed by dehydration with anhydrous sodium sulphate, then filtered and trans-
ferred into a dark injection vial, and analyzed by HPLC (Waters India Pvt. Ltd.). Chromatographic comparisons were made by authentic standards.

Tocopherol Composition. Tocopherols were estimated using HPLC following standard methods. Firstly, samples were mixed with butylated hydroxytoluene (BHT) in hexane (10 mg/mL; 100 μL) and IS solution in hexane (0.1 mg/mL; 250 μL). Now, samples (300 mg) were vortexed for 1 min with methanol (4 mL). The samples were again vortexed with hexane (4 mL). After this, 2 mL of saturated NaCl aqueous solution was added, and the mixture was vortexed (1 min), followed by centrifugation at 4000 g for 5 min and the upper layer was separated. The samples were again re-extracted twice with hexane. The extracts were then vacuum-dried and re-dissolved in hexane (1 mL), followed by dehydration with anhydrous sodium sulphate, then filtered and trans-
ferred into a dark injection vial, and analyzed by HPLC (Waters India Pvt. Ltd.). Chromatographic comparisons were made by authentic standards.

Table 8. MIC of studied coral mushroom species against tested pathogenic bacterial strains. *MIC concentration: − No growth; + Cloudy solution (slight growth); ++ Turbid solution (strong growth); +++ highly turbid solution (dense growth). Control readings = Nil for all the concentrations.

| Time (h) | Flow | A % | B % |
|----------|------|-----|-----|
| 1        | 1.00 | 100 | 0   |
| 10       | 1.00 | 100 | 0   |
| 20       | 1.00 | 100 | 0   |

Profiling of Other Bioactive Compounds. β-carotene and lycopene were estimated from powdered samples. Samples (~5 g) were extracted with 100 mL of methanol at 25°C (150 rpm) for 24 hours and filtered through Whatman no. 2 filter paper. The residues were again re-extracted with 2 additional 100 mL portions of methanol. These extracts were evaporated to dryness at 42°C, then redissolved in methanol at a concentration of 50 mg/mL, and stored at 4°C. The dried methanolic extracts (100 mg) were shaken vigorously with acetone/hexane mixture (4:6) for 1 minute and filtered. The absorbance of the filtrate was measured at 453, 505, and 663 nm. β-carotene and lycopene content were estimated using the following equation: Lycopene (mg/100 mL) = (0.0458 \times A_{663}) + (0.372 \times A_{505}) - (0.0806 \times A_{453}) and β-carotene (mg/100 mL) = (0.216 \times A_{663}) - (0.304 \times A_{505}) + (0.452 \times A_{453}).

Phenolic compounds were quantified using Folin and Ciocalteu’s phenol reagent. Briefly, powdered samples (1 mL) were mixed with Folin and Ciocalteu’s phenol reagent. Now, 1 mL of saturated sodium carbonate solution (3 mL) was added to the mixture, and the volume was adjusted with distilled water to 10 mL. The reaction was kept in the dark for 90 min after that absorbance was read at 725 nm. Gallic acid was used to calculate the standard curve (0.01–0.4 mM; R² = 0.9999) and the results were expressed as milligrams of gallic acid equivalents per gram of extract. Ascorbic acid contents were quantified by colorimetric assays. For quantification, standard ascorbic acid solution (5 mL L-ascorbic acid in 3% phosphoric acid) was added to 5 mL of phosphoric acid. A microburette was filled with dye, and the samples were titrated with the dye solution to a pink color, which persisted for 15 seconds. The dye factor (milligrams of ascorbic acid per milliliter of dye using formula: 0.5/titrate) was determined. Sample (10 g) was grounded in metaphosphoric acid, and the volume was increased up to 100 mL. It was titrated...
after filtration until a pink color appeared\(^1\). The amount of ascorbic acid was calculated with the use of the following equation: \(\text{mg of ascorbic acid per} 100 \text{ g or mL} = \text{titrate} \times \text{dye factor} \times \text{vol. made aliquot of extract} \times \text{wt. of sample} \times 100\). Anthocyanidins were quantified by using standard protocol with minor modifications\(^3\). Briefly, samples (0.7 g) were mixed with the solvent (mixture of 85 : 15 (v/v) of ethyl alcohol and hydrochloric acid 1.5 M) followed by ultrasoundication for 15 min and filtration through Whatman filter paper no. 1. Standard solution was prepared with cyaniding chloride with a concentration of 5–15 \(\mu\text{g/mL}\). The absorption was measured at 546 nm. The total quantity of anthocyanidins expressed in g of cyaniding chloride/100 g extract.

Bioactivities. Antioxidant Activities. DPPH scavenging activity was measured with adding DPPH (200 \(\mu\text{L}\)) solution at different concentrations (2–10 mg/mL) to 0.05 mL of the samples dissolved in ethanol. An equal amount of ethanol was added to the control. Ascorbic acid was used as the control\(^\text{50}\). The absorbance was read after 20 min at 517 nm and the inhibition was calculated using the formula DPPH scavenging effect \((\%) = \frac{A_0 - A_\text{sample}}{A_0} \times 100\), where \(A_0\) represented the absorbance of the control and \(A_\text{sample}\) represented the absorbance in the presence of the sample.

ABTS radical scavenging activity was measured following standard protocol\(^\text{51}\). Samples were added to 4 mL of diluted ABTS\(^+\) solution (prepared by adding 7 mM of the ABTS stock solution to 2.45 mM potassium persulfate, kept in the dark, at room temperature, for 12–16 h before use). The solution was then diluted with 5 mM saline (pH 7.4) phosphate-buffered and absorbance was measured at 730 nm after 30 min. The ABTS radical scavenging activity was calculated as \(\%\text{Scavenging Effect} = \frac{A_\text{control} - A_\text{sample}}{A_\text{control}} \times 100\) for the control without the polysaccharide sample and \(A_\text{control}\) is the absorbance of the blank and \(A_\text{sample}\) is the absorbance in the presence of the extract\(^\text{52}\).

The scavenging activities of superoxide anion radicals were measured following standard method\(^\text{34}\). Samples and Tris-HCl buffer (50.0 mM, pH 8.2, 3 mL) were incubated in a water bath at 25°C for 20 min and after this pyrogallic acid (5.0 mM, 0.4 mL) was added. HCl solution (8.0 M, 0.1 mL) was added to terminate the reaction after 4 min. The absorbance of the mixture was measured at 320 nm. The scavenging activity was calculated using the following formula: scavenging ability \((\%) = \frac{(1 - \frac{A_\text{sample}}{A_\text{control}})}{A_\text{control}} \times 100\). Where \(A_\text{control}\) is the absorbance of control without the polysaccharide sample and \(A_\text{sample}\) is the absorbance in the presence of the polysaccharide sample.

For ferric reducing antioxidant power (FRAP) assay, FRAP reagent was prepared by mixing TPTZ (2.5 mM, 10 mM in 40 mM HCl), 25 mL of 300 mM acetate buffer, and 2.5 mL of FeCl\(_3\) · 6H\(_2\)O. Thereafter, FRAP reagent (1.8 mL) was incubated at 30°C in water bath for 10 min. Then, absorbance was read at 0 min (t0). After this, 100 \(\mu\text{L}\) of sample extract or standard and 100 \(\mu\text{L}\) of distilled water were added to the test tube, mixed, and incubated at 30°C for 30 min. Then, the absorbance was taken at 593 nm (t30). Ferrous sulphate was used as standard\(^\text{55-57}\). FRAP activity was determined against a standard curve of ferrous sulphate and the values were expressed as \(\mu\text{M Fe}^{2+}\) equivalents per gram of extract and calculated using the following equation: FRAP value = Absorbance (sample + FRAP reagent) – Absorbance (sample).

Antibacterial Activities. Extracts of the coral mushrooms powdered samples were prepared to analyze the antibiotic activities. For extract preparation, 5 g of mushrooms were macerated twice with 99.9% ethanol (50 mL) at room temperature for 24 h and filtered. The residues were twice extracted by ultrasonically assisted extraction with 50 mL of ethanol at room temperature for 30 min. The combined extracts were evaporated to dryness under vacuum. Samples were re-dissolved for checking antibacterial effects. Minimal inhibitory concentration (MIC) of the extracts were tested for Escherichia coli, Klebsiella pneumonia, Vibrio cholerae, Pseudomonas aeruginosa, Vibrio alginitolytics and Staphylococcus aureus. The strains were procured from Microbial Type Culture Collection and Gene Bank, CSIR, India. For this, bacterial strains were individually inoculated in the nutrient broth and incubated at 37°C for 24 h. Mueller Hinton agar (MHA) was prepared and autoclaved and poured in petriplates and incubated at 37°C for 24 h. The strains were then inoculated in nutrient broth and incubated at 37°C for 24 h. The diameter (mm) of the growth inhibition halos produced by the extracts was examined. Results were calculated by measuring the zone of inhibition in millimetres (mm)\(^\text{58}\). MIC was determined followed by the turbidimetric method\(^\text{59}\). A stock solution of 100 \(\mu\text{g/mL}\) was prepared and diluted to obtain various ranges of concentrations from 20 to 100 \(\mu\text{g/mL}\). 0.5 mL of each of the dilutions of different concentrations was transferred into sterile test tube containing 2.0 mL of nutrient broth. To the test tubes, 0.5 mL of test organism previously adjusted to a concentration of 10\(^5\) cells/mL was then introduced. A set of test tubes containing broth alone was used as control. All the test tubes and control were then incubated at 37°C for 24 h. After the period of incubation, the tubes were studied for visible signs of growth or turbidity. The lowest concentration coral mushroom extracts that inhibited the growth of bacteria was taken as the minimum inhibitory concentration. All assays were carried out in triplicates and the control test was carried out with the broth alone.
Statistical Analysis. All experiments for chemical and bioactive analysis were performed three times and with three replicates, and the results were expressed as mean ± SD values of 3 observations. The results were analyzed using one-way analysis of variance (ANOVA) and p < 0.05 was considered significant, and SPSS software (SPSS Inc., Chicago, IL, USA, version 16) was used to calculate differences.

Conclusions
Coral mushrooms of Northwestern Himalayas are very important because of their culinary credentials and use from ancient times by native inhabitants. There are no previous reports about the exact chemical composition and bioactivities of these important mushrooms from Northwestern Himalayas. Presently investigated species have been collected from the regions native to northwestern Himalayas and evaluated for the exact composition of bioactive compounds and their in vitro antioxidant and antimicrobial activities. Some species of coral mushrooms are used by local people for culinary purposes. But, their knowledge is restricted to the aged villagers of the regions and hence neglected for the commercial exploitations. All the species were found to be rich in protein, minerals and contained important nutraceuticals such as unsaturated fatty acids, phenolics, carotenoids, ascorbic acid, tocopherols, and anthocyanidins which can be useful for their commercial use for nutritional therapy. All the species are observed free from toxic heavy metals and health promoting constituents like unsaturated fatty acids, essential amino acids, carotenoids, phenolic compounds with advantage of the additive effects of antioxidant compounds. Extracts of these coral mushrooms from Northwestern Himalayas showed broad spectrum of inhibition against human pathogenic bacteria. The extracts can be used as antibacterial constituents against human pathogenic bacterial strains tested presently.

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Author Contributions

S.K.S. carried the work on collection and analysis of samples. N.G. worked on experimental design and data analysis. Both authors participated equally in manuscript preparation.

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