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Morphology, Qualitative Phytochemical Analysis and Antimicrobial Activities of *Ramaria botrytis* from Davangere, Karnataka, India

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ABSTRACT

This study focuses on the identification and characterization of *Ramaria botrytis*, collected from near canteen of Davangere University, Karnataka, India in July and August 2024. The fungus exhibits distinctive morphological features, including acanthohyphae, which play a crucial role in its ecological interactions and nutrient absorption. Qualitative phytochemical analysis and antimicrobial activities of the ethyl acetate and chloroform extracts of *R. botrytis* are also studied.

Key words: *Ramaria botrytis*, Morphology, Qualitative phytochemical analysis, Antimicrobial activities.

INTRODUCTION

Ramaria botrytis (Pers.) Ricken is an edible species of coral fungus in the Gomphaceae family. The species was first named as *Clavaria botrytis* in 1797 by Persoon. It is often referred to as clustered coral, pink-tipped coral mushroom, or cauliflower coral. Its sturdy fruit body, which resembles certain coastal coral, can reach heights of 20 cm and diameters of up to 15 cm. Dense branches that are bulging at the extremities and divide into many tiny branchlets grow from its sturdy, enormous base (Ali *et al.,* 2024). The branches start out white but eventually turn buff or tan, with pink to crimson tips. It is white flesh, thick. The globular spores, which have continuous patterns, are around 13.8 by 4.7 µm in size. They leave behind a yellowish tint. This is widely distributed species Australia, Asia, North America, North Africa, and central and eastern Europe. The fungus, which is mycorrhizal in nature, grows on broadleaf trees and bears fruit on the ground in wooded areas (Martin *et al.*, 2020). Several coral fungal species that apparently resemble *R. botrytis* can frequently be identified by comparing their habitats or by characteristics such as color or branching shape. However, in certain cases, microscopy is needed to make a definitive distinction between them. Young specimens of *Ramaria botrytis* have a mild, fruity taste, and the fruit bodies are edible **(**Sowmya and Ramalingappa 2024; Tijani *et al.,* 2024**)**. In the present paper, the morphology of *Ramaria botrytis*, qualitative phytochemical analysis and antimicrobial activities of the ethyl acetate and chloroform extracts are presented.

MATERIALS AND METHODS

In month of July and August 2024, specimens of *Ramaria botrytis* were collected from near canteen of Davangere University, Davangere, Karnataka, India. Samples were kept in sterilized polythene bags. Then the samples were transferred to sterile polythene zip lock bags and the samples were carried to the Department of microbiology laboratory of Davangere University, to carry out the further experiments (Ghobad-Nejhad *et al.,* 2024). The collected samples were processed by following the standard techniques (Bhanja *et al.,* 2020). Slides for microscopic examination were prepared by taking freshly collected materials and mounted in clear lacto-phenol cotton blue stain (Ghosh *et al.,* 2020). The microphotographs of samples were taken by using camera with micro lens and the detailed morphological observations was carried out at different magnifications (10X, 40X and 100X). *Ramaria botrytis* was identified by the presence of distinctive branching structure, along with tip of the branches can be reddish, which is one of the characteristics that help in its identification.

Material examined: India, Karnataka, near the university canteen, 14.393795°N 75.963203°E, on mushy leaves of almonds, on 25th July 2024.

Qualitative phytochemical analysis of *Ramaria botrytis*

To determine whether the mushroom sample contained bioactive chemicals, qualitative phytochemical analysis was conducted (Raaman,

2006; Killedar *et al.,* 2024; Abu-Tahon *et al*., 2024).

Test for Alkaloid: *Mayer's test:* After adding a few drops of Mayer's reagent (potassium mercuric iodide solution) to 1 ml of solution, the mixture was checked for turbidity or precipitation. A cream-colored precipitation indicates the presence of alkaloid.

Keller-Killiani test (Test for cardiac glycosides): One ml of the test solution was taken, followed by adding 1 ml of glacial acetic acid and one or two drops of ferric chloride solution. Then, without shaking the test tube, 0.5 ml of concentrated sulfuric acid was gradually inserted along the sides. The presence of deoxysugar, a feature of cardenolides, is indicated by a reddish-brown ring at the interface of 2 liquids.

Tannins and Phenolic compounds test (Lead test): After adding 1 to 3 drops of ferric chloride to 1 ml of test solution, the mixture was examined for blue or green hues.

Saponins test (Foam test): The presence of saponins is indicated by the development of a stable, persistent froth after 10 min of vigorous shaking of the test solution in a test tube.

Terpenoids and Phytosterols test (Salkowski test): An equal amount of chloroform was added to 0.5 ml of the test solution, and then sulfuric acid was poured along the test tube's sides without shaking. The presence of phytosterols is indicated by the chloroform layer turning reddish-brown.

Flavonoids test:

Lead acetate test: One ml of test solution was taken, 1 ml of 10% lead acetate solution was added, and the appearance of yellow colored precipitate signifies the presence of flavonoids.

Ferric Chloride test: One ml of test solution was taken, ferric chloride solution was added drop by drop. The formation of greenish black color indicates the presence of flavonoids.

Test for Proteins

Biuret test: One ml of biuret reagent was added to 1 ml of test solution, shake well and warm it on water bath. Appearance of red or violet color indicates presence of proteins.

Test for Fixed oils and Fatty acids

Spot test: After adding a drop of extract to the filter paper, it was seen that the existence of fixed oils and fats is indicated by oil staining on the filter paper.

Antibacterial activity of *Ramaria botrytis* (Agar well diffusion method)

The antimicrobial activities of the organic extracts (ethyl acetate and chloroform) of the edible mushroom *Ramaria botrytis* on 5 bacterial strains: *Salmonella* sp., *Staphylococcus* sp., *Klebsiella* sp., *Neisseria* sp. and *Streptococcus* sp. were tested. Initially Mueller- Hinton agar plates were prepared. Each microbial isolates were swabbed on media plates. Wells were formed using a cork borer, and then 1 ml of mushroom extract was added. As a control, distilled water has been used (Killedar and Ramalingappa, 2024). With the proper extract and test organism, all of the preloaded plates were incubated for 24 h at 36°C. After the incubation time, the zone of inhibition (mm) was determined (Sakemi *et al*., 2022).

RESULT AND DISCUSSION

The fresh mushroom samples were collected from Davangere University campus as shown in the **Figure 1A, B, C.** It is identified as *Ramaria botrytis* (Pers.) Ricken. It initially with short stout stem, divides and redivides to form a cauliflower-like structure and whitish, later on become pinkish at the tip, surrounded by whitish margin, and divided into several small branchlets, 5–6 mm in length. Spores transparent and ellipsoid to sub-fusiform **(Figure 1E,F)**, 11-20 x 5-6 µm, with ornamentation aggregating into spinlike structures and also into faint long striations as they get old, its white to creamy- white colour when young turn to yellowish white in the final stage. The branch tips are whitish red when initial stages, fading to pallid buff over the time. Acanthohyphae are characterized by their branched, septate hyphae that often have pointed or thorn like projections (acanths). This gives them a distinctive appearance compared to other types of hyphae **(**Bisht *et al.,* 2024**)**. It plays a role in ability of the fungus to anchor itself to substrates and may assist in nutrient absorption. This projection can also help in the interaction with surrounding environment, including other fungi and plant roots. Acanthohyphae can be observed under a microscopic (**Figure 1D**), their branched structure and pointed projections are evident. This morphological characteristic is crucial for mycologists when distinguishing *Ramaria* species

from other similar fungi. *R. botrytis* has several notable applications, particularly in the fields of health and nutrition. *R. botrytis* is considered its culinary uses, it is included in various health food combinations due to its nutritional benefits and pleasant flavor (Hawksworth *et al.*, 2024). Recent studies have shown that the extract from *Ramaria botrytis* can favorably affect the development and proliferation of HeLa cells in tissue culture, suggesting possible uses in biomedical research.

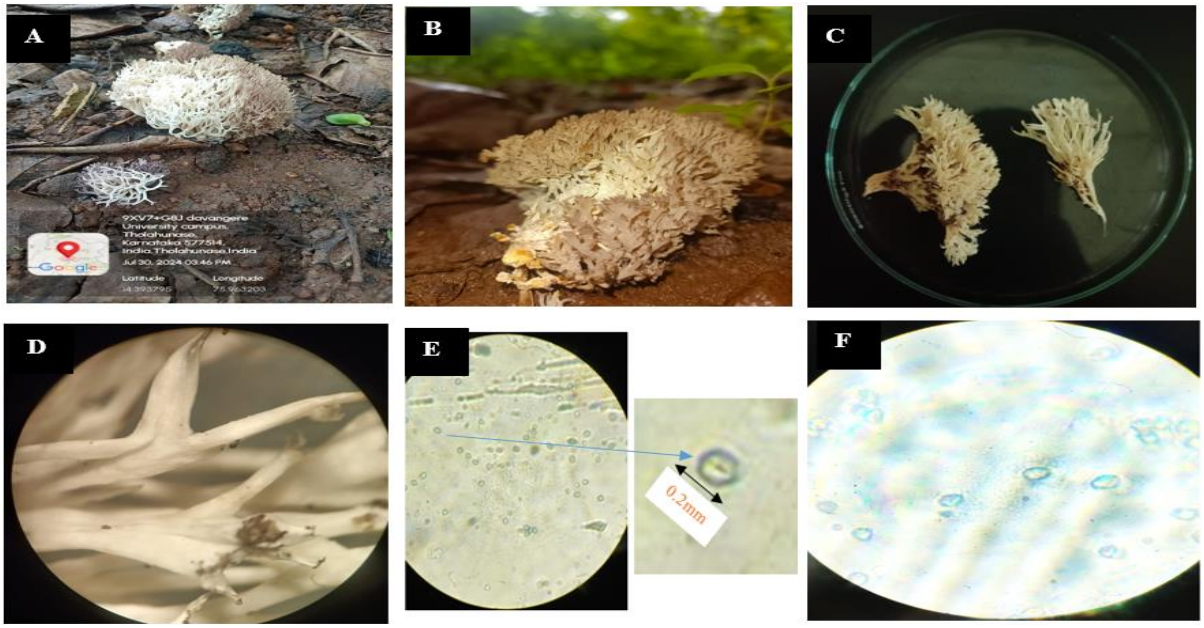

Figure 1: *Ramaria botrytis*. **A,** *R. botrytis* in natural habitat; **B**, *R. botrytis* with cluster coral branches; **C**, *R. botrytis* with root length of 1.7 mm; **D,** Stereo binocular microscopic view of *R. botrytis* with illumination (40X); **E,** Spores 10X; **F**, Spores 100X.

Phytochemical analysis of ethyl acetate and chloroform extracts of the mushroom showed the presence of phytoconstituents such as terpenoids, saponins, steroids and glycosides. The presence of deoxysugar, a feature of cardenolides, is shown by the reddish-brown ring that forms at the interface of two liquids, it indicates the positive results for cardiac glycosides. The extract of *R. botrytis* did not turn blue or green color after addition of two drops of ferric chloride, it indicated the negative results for tannins. The test for saponins showed positive result by formation of stable foam. Terpenoids test showed positive result by formation of reddish-brown color ring which indicated the presence of phytosterols. The extract of *Ramaria botrytis* did not show greenish black colour in the lower chloroform layer which indicated the absence of flavonoids. In lead acetate test, the appearance of yellow color precipitate formed indicated the presence of flavonoids. The appearance of red or violet colour indicated the presence of proteins. In fatty acids or fixed oil test, oil stains on filter paper revealed the presence of fixed oils **(Table 1).**

Table1: Phytochemical analysis of *Ramaria botrytis.*

Bioactive compound	Test	Chloroform Extract	Ethyl acetate Extract
Alkaloids	Mayer's test	Positive	Positive
Cardiac Glycosides	Keller Killiani test	Negative	Positive
Tannins	Lead test	Negative	Negative
Saponins	Foam test	Positive	Negative
Terpenoids	Salkowski test	Positive	Positive
Flavonoids	Lead acetate test	Positive	Positive
	Ferric chloride test	Negative	Negative
Proteins	Biuret test	Positive	Negative
Fatty acids	Spot test	Positive	Positive

Different qualitative tests were conducted to identify classes of phytochemicals, such as phenolic compounds and flavonoids, known for antioxidant and antimicrobial properties. Alkaloids contribute to antimicrobial, anti-cancer, and anti-inflammatory effects. Terpenoids known for anti-inflammatory and antimicrobial activities. Polysaccharides often associated with immunomodulatory effects. Steroids may have antibacterial and antifungal effects. Toth *et al*. (2013) reported identification and quantification of bioactive compounds present in mushrooms by phytochemical analysis. They suggested that the bioactive compounds are responsible for various therapeutic effects, including antioxidant, anti-inflammatory, and antimicrobial properties. For extraction processes various solvents (example: methanol, ethyl acetate, chloroform, acetone, or water) were used to extract the bioactive compounds from the mushroom tissue and the choice of solvent can affect the efficiency of the extraction, as different compounds have varying solubilities (Courtney *et al*., 2012).

The mushroom extracts showed antibacterial activity by forming a zone around the wells against selected bacterial strains as shown in **Figure 2 and Figure 3.** *Streptococcus* species showed maximum inhibition against *Ramaria botrytis* extract (both in chloroform and ethyl acetate) followed by *Staphylococcus* sp., *Neisseria* sp., *Salmonella* sp.

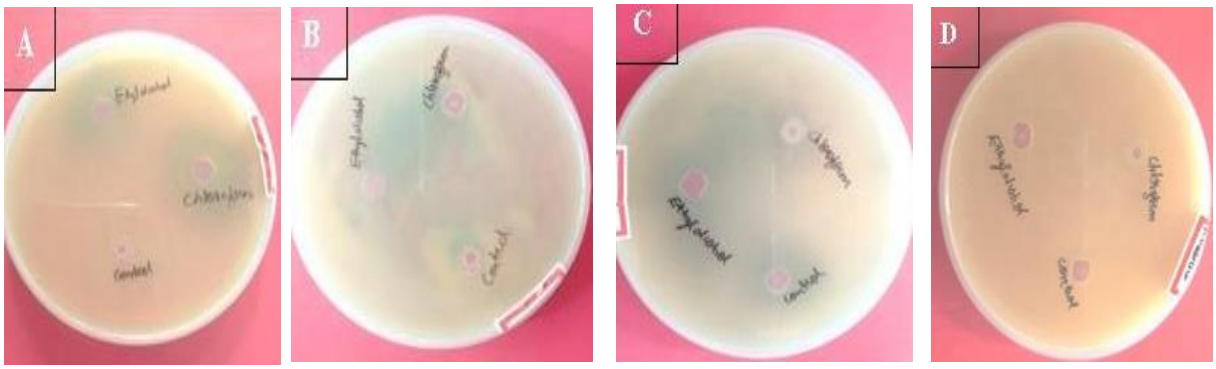

Figure 2: Antibacterial activity of *Ramaria botrytis*. **A.** *R. botrytis* extract against *Salmonella* sp.; **B**. *R. botrytis* extract against *Staphylococcus* sp.: **C**. *R. botrytis* extract against *Streptococcus* sp.; **D.** *R. botrytis* extract against *Klebsiella* sp.

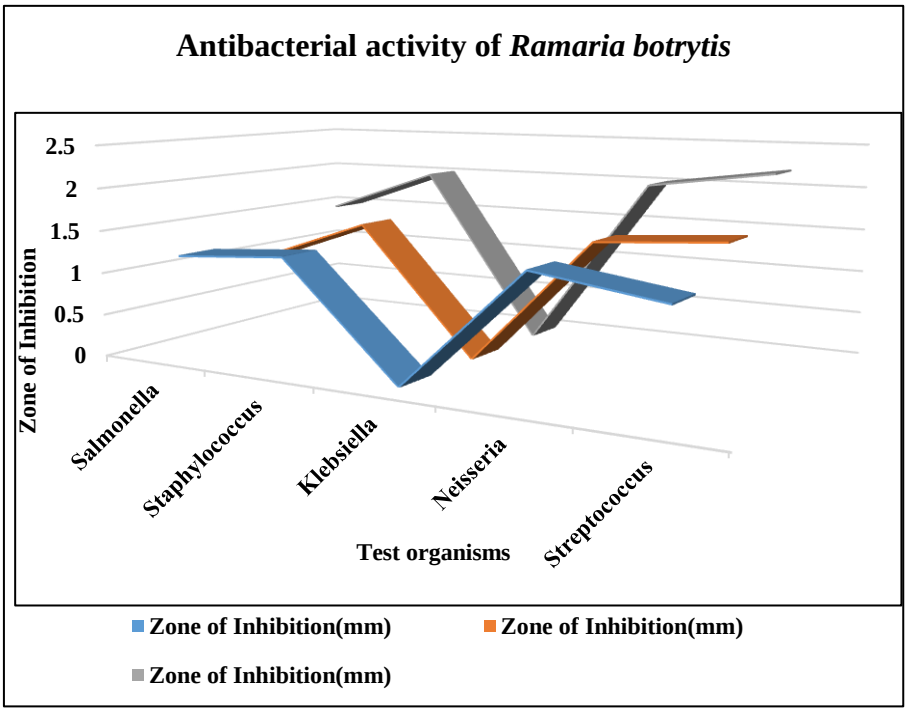

Figure 3: Antibacterial activity of *Ramaria botrytis* against bacterial species in mm

Younis *et al*. (2015) observed that water extract of *Pleurotus ostreatus* showed antimicrobial activity, most sensitive test bacteria were *Staphylococcus aureus* followed by *Escherichia coli* and they identified an antimicrobial compound 3-(2-aminopheny1thio)-3-hydroxypropanoic acid from *Pleurotus ostreatus.* Jankov *et al*. (2024) investigated the antibacterial activity of *Agaricus bisporus* extracts against *Escherichia coli* and *Staphylococcus aureus* using different concentrations. The methanolic extract demonstrated the most potent effect, suggesting the need for further research to isolate and characterize the antibacterial properties of this mushroom for practical disease control measures.

Menaga *et al*. (2024) explored the use of horse gram (*Macrotyloma uniflorum*) on *Pleurotus florida* mushrooms and its antimicrobial activity against human pathogens. The researchers found that horse gram supplementation and straw size reduction improved mushroom yields. The study also revealed the presence of bioactive compounds in *Pleurotus florida* extracts, indicating its potential antimicrobial activity. The findings suggest that these factors could be used as alternative medical treatments to address antibiotic resistance.

CONCLUSION

The study highlights the morphology of *Ramaria botrytis*, its potential health benefits and its antibacterial activity, suggesting potential for natural antimicrobial agents. The research also provides ecological and habitat insights. Morphological observations further enhance the scientific documentation of *Ramaria botrytis*.

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