Antimicrobial and Antioxidant and Antituberculosis Effect of Sponge Collospongia Auris

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**Abstract:** Few investigations have been conducted on endemic species of Collospongia auris, despite the fact that marine sponges are well known for their cytotoxic, antimicrobial, and antifungal properties. The impact of methanolic extracts of sponge C. aureus is used to illustrate the area's potential for the discovery of marine biomolecules with antibacterial, antioxidant, and antitubercular properties. Three bacterial strains were assessed for their antitubercular and antioxidant properties: Proteus mirablis, Klebsilla pneumoniae, and Escherichia coli. The disc diffusion technique was used to assess antimicrobial activity by figuring out the lowest bactericidal and inhibitory concentrations. Strong antioxidant properties and a notable antibacterial impact against every tested bacterial species were demonstrated by the C. aureus extract. The anti-mycobacterial activity of C. aureus is only mild.

**Keywords:** Novel drug development, Antioxidant activity; Marine sponges; Reactive free radical, *Mycobacterium*, Health and wellbeing.

# Introduction

Investigating new pharmaceuticals is essential to overcoming the shortcomings of the ones that are currently in use [(Aparna et al., 2021; Ganapathy & Professor and Head of 2021)](https://paperpile.com/c/qEdP1b/Y8YtR+vOmnu). It is well known that harmful bacteria have become resistant to new antibiotic generations, and that some of them may be able to withstand every antibiotic currently in use and cause acute infections that call for more costly and toxic medications [(Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/qEdP1b/HfSoC). Similar to bacteria, viruses undergo mutations throughout time and become resistant to antiviral medications [(Sanjuán & Domingo-Calap, 2016)](https://paperpile.com/c/qEdP1b/m1TIo). One of the most deadly illnesses in the world today is cancer [(Poornima et al., 2021)](https://paperpile.com/c/qEdP1b/oocsx).In 2018, there were an estimated 10 million cancer-related fatalities out of 18 million cancer cases. By 2030, there will likely be 26.4 million cancer cases annually worldwide, with 17 million deaths [(Bray et al., 2018)](https://paperpile.com/c/qEdP1b/JdBlh); [(Davies-Bolorunduro et al., 2019)](https://paperpile.com/c/qEdP1b/20Gz1). Numerous diseases, including cancer, Alzheimer's disease, cardiovascular disease, early aging, ischemia, liver damage, arteriosclerosis, inflammation, skin damage, diabetes mellitus, and arthritis, are linked to oxidative stress [(Pandiyan et al., 2022)](https://paperpile.com/c/qEdP1b/oYa7t). The most widely utilized synthetic antioxidants today, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylated hydroquinone (TBHQ), are hazardous and may have harmful and cancerous side effects [(Nieva-Echevarría et al., 2015)](https://paperpile.com/c/qEdP1b/jeSfL) [(Chandra et al., 2020)](https://paperpile.com/c/qEdP1b/YVUXC). Therefore, the hunt for bioactive substances may provide new substances that address all of these problems with chemotherapy [(Ganapathy 2021)](https://paperpile.com/c/qEdP1b/UTODB).Natural products derived from a variety of sources, such as microorganisms, animals, and plants, have been used to cure a wide range of human and animal illnesses. They also hold promise as a source of more powerful medications to replace the shortcomings of traditional medications [(Law et al., 2020; Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/qEdP1b/zGIPi+oYa7t+8gjAu). The constraints of acquiring pharmaceuticals by field-harvesting vast quantities of macroorganisms would be overcome by using microorganisms, which are more suited for the large-scale generation of bioactive chemicals [(Chokkattu et al., 2022; Molinski et al., 2009; Ramamurthy et al., 2022)](https://paperpile.com/c/qEdP1b/bgHSL+vNNsY+fKkjB). As of right now, marine microorganisms are thought to be a comparatively underappreciated source for the identification of bioactive chemicals. They may also yield unique bioactive compounds with respect to their structure and bioactivity [(Adnan et al., 2018)](https://paperpile.com/c/qEdP1b/rM04T). Actinomycetes are among the marine microorganisms that create a great deal of bioactive chemicals with a variety of biological activities, including antiviral, antioxidant, immunosuppressive, anticancer, and enzyme inhibitory qualities [(Hu et al., 2018)](https://paperpile.com/c/qEdP1b/vj99m).Because of the variety of their primary and secondary chemical components and metabolites, marine sponges have been regarded as a very fertile ground for the identification of bioactive natural chemical substances for the past few decades [(Jain & Verma, 2022; Marya et al., 2022)](https://paperpile.com/c/qEdP1b/pGVOl+zdsZP).It has been demonstrated that marine sponges generate a vast array of bioactive compounds with potential for therapeutic use, including antibiotics, anticancer, antiviral, anti-inflammatory, and immunosuppressive compounds [(Sreevarun et al., 2023; Wadhwani et al., 2022)](https://paperpile.com/c/qEdP1b/5vMNH+AMYbt). Up to 2012, more than 15,000 marine products had been identified and examined during the previous 20 years. The best producers of a wide variety of natural ingredients have been highlighted. There were 1152 natural products in total in 2019 (the most recent year for which we have data). With 269 new compounds reported, the number of novel natural chemical components from the phylum Porifera (sponges) has somewhat increased compared to prior years. This is mostly because multiple studies have reported numerous new metabolites from individual collections. Through its methanol extract, the sponge Collospongia auris's antibacterial and antioxidant properties are the main focus of this investigation.

# Materials and methods

## Sample collection and extraction

Collospongia auris sponge samples (Figure.1) were gathered from the coastal regions of the Rameshwaram Gulf Biosphere Reserve (Lat’ 9.2876° N, Long’ N: 79.3129° E). Samples of collected sponge were thoroughly cleaned with fresh water before being extracted using methanol (weight/volume). After drying, the methanol extract was ground into a powder. Biological screening was then conducted using the dried extract.



**Figure.1:** Sponge samples of *Collospongia auris*

## Antimicrobial screening

Proteus mirabilis (ATCC 3524), Escherichia coli (ATCC 9593), and Klebsiella pneumonia (ATCC 1474) are examples of human urinary pathogenic bacteria that were employed for antimicrobial screening. Using the agar well diffusion method, the antibacterial activity of the Collospongia auris methanol extract was ascertained. A sterile swab was used to apply an inoculum of 106 CFU/ml of each bacterial culture to be tested on nutrient agar plates for bacteria. Following this, 8 mm-diameter wells were punched into the agar medium, filled with 100 μl (25 mg/ml) of sponge extract, and left to diffuse for two hours at room temperature. After that, the plates were incubated for 24 hours at 37° in the upright position. Standard antibiotics such as Ampicillin (10 μg/mL) for bacteria were used as positive controls, whereas wells with the same volume of methanol were used as negative controls. For every extract, three duplicates were tested against every test organism.

## Antioxidant assay

## DPPH Assay

1.0 mg of sponge extract and 0.25 mL of 0.5 mM DPPH in ethanol were combined. At room temperature, the absorbance was measured at 517 nm after 20 minutes of standing. When the absorbance at 517 nm decreased with the addition of the sample in comparison to the control, the DPPH radical scavenging activity (%) was computed [(Kedare & Singh, 2011)](https://paperpile.com/c/qEdP1b/ZAkqL).

## Nitric oxide scavenging activity

Through the interaction of oxygen molecules, nitric oxide and sodium nitroprusside react to produce nitric ions. Using the Griess reagent, this reaction was measured. The nitric oxide scavenging assay was carried out using a slightly modified version [(Sreejayan & Rao, 1997)](https://paperpile.com/c/qEdP1b/6Gsbu). After adding 1.0 mg of the sponge extract to 3 ml of phosphate-buffered saline containing 10 mM sodium nitroprusside, the mixture was incubated for 150 minutes at 25 °C. Every 30 minutes, 0.5 ml of an aliquot of the incubated sample was taken out and replaced with 0.5 ml of Griess reagent. The measurement of color absorption took place at 546 nm. The medicine used as a comparison was quercetin.

## Ferrous ion chelating activity

The ferrous ion chelating action of sponge extract was investigated using existing approach [(Dinis et al., 1994)](https://paperpile.com/c/qEdP1b/paz68). A 0.05 ml solution of 2 mM FeCl2 was mixed with 1 mg of sponge extract. After adding 0.2 ml of 5 mM ferrozine to start the reaction, the mixture was agitated briskly and allowed to stand at room temperature for ten minutes. After some time, the solution's absorbance at 562 nm was measured using spectrophotometry. A control run with just ferrozine and Fecl2 was carried out. Every analysis and test was performed three times and averaged. The following formula provided the percentage of inhibition of the development of the ferrozine-Fe2+ complex:

% Inhibition = [(A0 – A1) / A0] x 100

Where A0 was the absorbance of the control and A1 was the absorbance in the presence of the seahorse sample and standard.

## Reducing power assay

Sponge extracts' capacity to reduce was measured using the existing methods [(Bhalodia et al., 2013)](https://paperpile.com/c/qEdP1b/TAZLN). One milligram of sponge extract was combined with one milliliter of distilled water, phosphate buffer [2.5 milliliters, 0.2M (pH 6.6)], and 2.5 milliliters of potassium ferricyanide [K3 Fe (CN) 6] (1%). The reaction mixture was incubated for 20 minutes at 50 degrees Celsius. Ten percent trichloroacetic acid (2.5 ml) was added to the reaction mixture, and it was centrifuged at 1000g for 10 minutes. The absorbance was measured at 700 nm in a spectrophotometer after the top layer of solution (2.5 ml) was combined with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%).

## Anti-tuberculosis assay

The REMA was utilized to investigate the antimycobacterial activity of 80% methanolic crude extracts of C. aureus against Mycobacterium TB (H37Rv strain, SIT777 strain) (Folkvardsen et al., 2013). As a measure of cellular viability or growth inhibition, resazurin was used to conduct the susceptibility test in 96 microplates (wells). To achieve final sample concentrations ranging from 0.78 μg/mL to 100 μg/mL, working solutions of the examined extracts were diluted in MiddleBrook 7H9 broth supplemented with OADC instead. The extracts or drug-free medium containing strain suspensions served as the negative control, while rifampicin, dissolved in DMSO, served as the positive control medication. Both the drug/extract-free control wells and all testing wells received 100 microliters of MiddleBrook 7H9 broth and the test inoculum.

The first well of each row was then filled with 100 microliters of working extract solutions, from which two-fold dilution series were created using the micro plate column. Every concentration of the extract was tested twice. After that, each microplate was parafilm-sealed and incubated for five to seven days at 37°C in a standard environment. Following the incubation period, each well received 25 μL of resazurin 0.02% w/v, which was then incubated for 24 hours at 37°C to produce color. The lowest drug/extract concentration that stopped the Resazurin reagent from becoming pink instead of blue was known as the visual MIC. Pink hue in the well was evaluated as growth occurring, while blue color was regarded as no mycobacterial growth(Rafi et al., 2024).

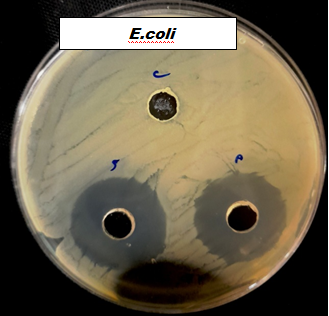
## Statistical analysis

The experiments were done in triplicate assay to obtain standard error mean ± values. One-Way ANOVA was performed to validate the p value of significance where p>0.5 was considered significant. SPSS package was used for One way ANOVA.

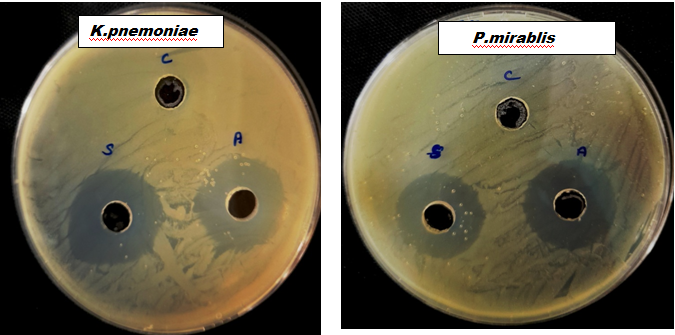
# Results

## Antimicrobial effect

Collospongia auris extract showed a significant antimicrobial effect against urinary pathogens. The extract showed a 4.5 mm zone of inhibition against K.pneumoniae bacteria and a 5.1 mm zone of inhibition against P.mirabilis and 7.0 mm zone of inhibition against E.coli bacteria. Collospongia auris extract does not show any antibacterial effect against any fungal agents (Tuluwengjiang et al., 2024)



(a)

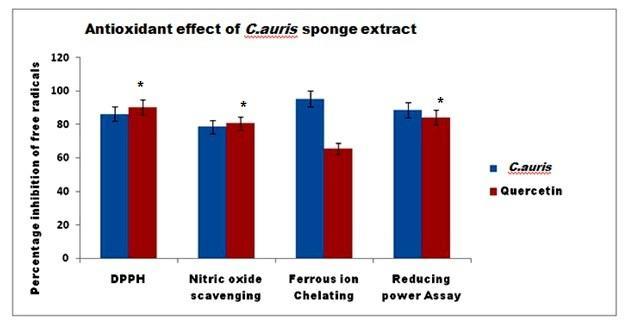


(b) (c)

**igure.2: (a) – (c)**Antibacterial effect of *C.auris* extract.

# Antioxidant effect

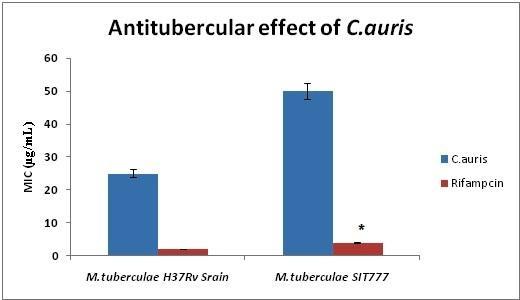
*Collospongia auris* extract exhibited a strong in-vitro antioxidant effect in terms of scavenging free radicals and reducing agents. The extract showed an 86.38% scavenging effect against DPPH radicals alongside a 78.67% scavenging effect against nitric oxide and a 95.39% ferrous ion chelating effect. Almost 88.63% of the reduction percentage of radicals is observed in the reducing power assay. Quercetin was used as a positive control antioxidant standard (Figure.3).



**Figure.3:** Antioxidant effect of *C.auris*

## Anti-tuberculosis effect

*C.auris* extract showed significant antitubercular effect against *M.tuberculae* strains at 25 and 50µg/mL as IC50 value when compared to that of standard Rifampicin drug which showed MIC value at 2 and 4 µg/mL. *C.auris* has moderate anti-tuberculosis effect (Figure.4).



**Figure.4:** Antitubercular effect of *C.auris*

# Discussion

The current investigation demonstrates the sponge C. aureus extract's potent antibacterial activity against tested pathogens, including P. mirablis, K. pneumoniae, and E. coli. The majority of these pathogens are urinary tract pathogens that cause UTIs. Similar to quercetin, a common antioxidant medication, C. aureus extract has a strong antioxidant impact in terms of DPPH scavenging, ferrous ion chelating, reducing power assay, and nitric oxide scavenging ability up to 92%. This is the first method to assess C. aureus extract's antitubercular activity against M. tubercule bacteria. Comparing the C. aureus extract to the common medication Rifampcin, the results show a moderate anti-tubercular impact.With biomass making up as much as 25% of the sponge volume, marine sponges are home to a variety of microbial communities that generate bioactive substances that were previously thought to be the product of the host sponge [(Chokkattu et al., 2023; Solanki et al., 2023)](https://paperpile.com/c/qEdP1b/Ij3D9+n2Zf9).Streptomyces is the most prevalent genus in the phylum Actinomycetes, which are frequently linked to marine sponges [(Abdelmohsen et al., 2014)](https://paperpile.com/c/qEdP1b/eOexc) [(Horn et al., 2015)](https://paperpile.com/c/qEdP1b/5sjNK). Apoptosis, immunity, and cell signaling are just a few of the basic functions in the human body that are impacted by free radicals such reactive oxygen species and reactive nitrogen species. Endogenous antioxidative mechanisms regulate the levels of free radicals because too many of them can harm essential molecules (proteins, lipids, carbohydrates, and DNA) and cause a number of diseases [(Adel et al., 2023; Laghari et al., 2023; Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/qEdP1b/BpN7+WLhx+iJ5Y). These mechanisms include enzymatic substances like glutathione peroxidase, superoxide dismutase, and catalase (CAT) as well as nonenzymatic substances like albumin and bilirubin. However, while under stress, the body's natural defenses against free radicals are weakened, which leads to oxidative stress. Numerous investigations have documented the antioxidant capacity of various Streptomyces species that have been isolated from various maritime environments [(Raghava Rao et al., 2017)](https://paperpile.com/c/qEdP1b/2InMr) [(Kemung et al., 2020; Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/qEdP1b/OUwOV+aILC8).

# Conclusion

*Collospongia auris* extract has significant antimicrobial and antioxidant efficacy than the antituberculosis effect through preliminary experimental results. Further research on this will lead to the isolation of drug candidates for advanced biomedical research.

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