

## **Integrative Analysis of *Astraeus asiaticus*: DNA Barcoding, Nutritional Composition, and Medicinal Properties of a Wild Edible Mushroom commonly found in Jharkhand State.**

**Neha Rai<sup>1\*</sup>, Dr. Navita Gupta<sup>2</sup>**

<sup>1</sup>Research Scholar, Department of Zoology, BBMKU, Dhanbad.

Email:- nrail7940@gmail.com

<sup>2</sup>Associate Professor, Department of Zoology, BBMKU, Dhanbad.

Email: -navitagupta\_bksc@yahoo.con.in

### **Abstract**

Rugra (commonly identified as *Astraeus asiaticus*) is a wild edible mushroom that holds significant nutritional and medicinal importance especially in the tribal and rural regions of Jharkhand, India. These macro fungi are sources of energy and are rich in essential proteins, carbohydrates, dietary fibers, and micronutrients like potassium, phosphorus, and iron. Due to the high nutritional value, Rugra serves as an important dietary supplement for local populations. This macro fungus also contains various bioactive compounds such as alkaloids, Flavonoids, polysaccharides, phenolics, and triterpenoids that exhibit various medicinal and potent antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties. These macro fungi have been traditionally harvested during the monsoon season almost all parts of Jharkhand state and have been an integral part of Jharkhand's indigenous cuisine, especially as local delicacies in curries and stews. Rugra are valued not only for its taste but also for its health benefits. Beyond its culinary appeal, these macro fungi have a unique cultural identity and knowledge system in various indigenous tribal communities such as the Munda, Oraon, and Santhal. These communities recognize its importance as therapeutic value in folk medicine. The collection, consumption, and trade of Rugra mushroom support the local livelihoods that make it a vital component for ecological sustainability and socio-economic resilience in the region. This underexplored macro fungus exemplifies its importance in the biodiversity, culture, tradition, nutrition, and health, especially in Jharkhand, that further plan for the scientific exploration and conservation of wild variety.

**Keywords:** *Astraeus asiaticus*, Rugra, nutritional and pharmacological potential, Jharkhand.

### **Introduction**

*Astraeus asiaticus* are one of the edible macro fungi that are popular as delicacy in Jharkhand which are valuable resource of different nutrition and bioactive compounds. *Astraeus asiaticus* belongs to genus *Astraeus* within the family *Diplocystaceae* under division *Basidiomycota* of Kingdom Fungi. These mushrooms are also commonly called as "false

earthstars" due to their superficial resemblance to true earthstars "*Geastrum* species" (Phosri *et al.*, 2007).

*Astraeus* are saprophytic and ectomycorrhizal fungus, forms symbiotic associations with trees and typically appear during the monsoon or post-monsoon season. They are found in sandy loam or lateritic soils with good drainage. These macro fungi generally have ectomycorrhizal collaboration (mycorrhizal symbioses) with rhizosphere root trees that belongs to *Fagaceae*, *Dipterocarpaceae*, *Pinaceae*, *Betulaceae*, *Ericaceae* families such as *Shorea robusta* (Sal Tree), *Madhuca indica*, *Terminalia belerica*, *Petrocarpus marsupium* (Wilson *et al.*, 2012; Harley *et al.*, 1997). Three major species of *Astraeus* that are found in India are *A. odoratus*, *A. hygrometricus* and *A. asiaticus* (Hembrom *et al.*, 2014; Karun and Sridhar, 2014; Phosri *et al.*, 2012) that are predominately distributed in Uttar Pradesh, Himachal Pradesh, Uttarakhand, Punjab, Madhya Pradesh, Chhattisgarh, Jharkhand, Odisha, West Bengal, Karnataka, and Kerala (Pavithra *et al.*, 2015; Topno & Srivastava, 2021).

Jharkhand, known for its rich biodiversity and forest wealth, harbors numerous wild edible fungi, among which *Astraeus asiaticus* (commonly known as *Rugra* or *Rugda*) holds significant ethnobiological importance. Regarded as a seasonal delicacy among the indigenous tribal communities, *Rugra* is not only a traditional food source but also an underexplored reservoir of nutritional and pharmacological compounds. With the increasing global interest in natural and functional foods, there is a renewed focus on wild mushrooms like *Rugra* that bridge the gap between traditional dietary practices and modern nutraceutical innovations. This study aims to bridge the knowledge gap by systematically analysing the nutritional and medicinal properties of *Astraeus asiaticus*, assess its proximate composition, including macronutrients and micronutrients, identify and quantify its bioactive compounds; and to evaluate its antioxidant, antimicrobial, and anti-inflammatory activities through *in vitro*. The findings of this study could provide a scientific basis for promoting *Astraeus asiaticus* as a functional food and a source of novel therapeutic agents.

## Materials and Methodology

### Collection of *Astraeus asiaticus* samples

Samples of *Astraeus asiaticus* (Phosri M.P. Martín & Watling) (*Rugra*) was collected under sterile conditions and proper handling procedures in the month of July/August from local market in Dhanbad, Ranchi, Bokaro steel city and Jamshedpur of Jharkhand state during the monsoon season.

**Identification of Mushroom sample (*Astraeus asiaticus*)**

Most used DNA markers for fungal identification are the 18S ribosomal RNA (18S rRNA) gene (Banos *et al.*, 2018) and the internal transcribed spacer (ITS) region (Cheng *et al.*, 2014; Das *et al.*, 2013). The Genomic DNA was extracted using a CTAB-based protocol optimized for fungal samples. The extracted DNA was further purified to ensure extraction of high purity DNA by elimination of RNA contamination (Bellemare *et al.*, 2018; Fredricks *et al.*, 2005). The purity and concentration of the DNA were assessed using a Nanodrop spectrophotometer, where A260/A280 and A260/A280 ratios were checked. High-quality DNA exhibited ratios close to 1.8 respectively (García-Alegría *et al.*, 2020). The Polymerase chain reaction (PCR) amplification was carried out using universal primers targeting the conserved 18S rRNA region (e.g., NS1/NS4 primers) and ITS region (e.g., ITS1/ITS4 primers). The PCR products were resolved on a 1.5% agarose gel stained with ethidium bromide and visualized under UV light. Successful amplification yielded single, distinct bands corresponding to the expected sizes of the 18S rRNA (~1,800 bp) and ITS (~500-700 bp) regions. The PCR products were then purified using a commercial PCR purification kit (e.g., Qiagen PCR Purification Kit) to remove residual primers, nucleotides, and enzymes. The purified products were sequenced bidirectionally using the Sanger sequencing method on an automated sequencer. The resulting sequences were assembled and edited using software like MEGA or BioEdit to ensure high-quality reads (Altschul *et al.*, 1997; Barry 2013; Pavlopoulos *et al.*, 2010). The obtained sequences were trimmed for quality and aligned using bioinformatics tools such as MEGA or BLAST against the GenBank database to identify homologous sequences. The sequences were compared against reference databases using the BLAST algorithm (Zhang *et al.*, 2018). Species-level identification was confirmed based on sequence similarity thresholds of  $\geq 97\%$  for ITS and  $\geq 95\%$  for 18S rRNA (Kõljalg *et al.*, 2013). Phylogenetic analyses were conducted to validate the taxonomic placement of *A. asiaticus*. Neighbor-joining and maximum likelihood methods were used to construct phylogenetic trees, providing insights into evolutionary relationships with closely related taxa.

**Processing of mushroom (*Astraeus asiaticus*) sample**

The fresh fruiting bodies that were collected under sterile conditions were cleaned thoroughly by distilled water and sterile brushes to remove debris and soil particles. Immediate post-harvest processing is one of the crucial that ensures prevention of enzymatic degradation and microbial contamination (Boa, 2004). The cleaned *Rugra* samples were then subjected to air-drying for 5-7 days in a humidity-controlled environment at room temperature (Heleno *et al.*,

2015). The dried mushrooms were pulverized using a sterile grinder to produce a fine powder, then sieved for uniform particle size, and stored in airtight containers under low humidity and temperature conditions to preserve phytochemical stability (Kalac, 2016).

### **Proximate Composition and nutritional Analysis**

The proximate composition, including moisture, crude protein, crude fat, ash, and carbohydrate content, was determined following standard AOAC methods (AOAC, 2019). Moisture content was estimated by oven-drying the sample at 105°C until a constant weight was achieved. Crude protein content was measured using the Kjeldahl method, where the total nitrogen content was multiplied by a factor of 6.25. Crude fat was extracted using a Soxhlet apparatus with petroleum ether as the solvent. Ash content was determined by incinerating the sample in a muffle furnace at 550°C for 4 hours. The mineral composition, including calcium (Ca), potassium (K), magnesium (Mg), iron (Fe), and zinc (Zn), was determined using an atomic absorption spectrophotometer (AAS). Dried mushroom powder (1gm) was subjected to acid digestion with a mixture of nitric acid and perchloric acid (3:1) before analysis (Barros *et al.*, 2008). Standards for each mineral were used to calibrate the spectrophotometer. The vitamin content, particularly ascorbic acid (vitamin C) and B-complex vitamins was analysed using high-performance liquid chromatography (HPLC). (Heleno *et al.*, 2010). All experiments were conducted in triplicate, and results were expressed as mean  $\pm$  standard deviation (SD). Statistical analyses, including one-way ANOVA, were performed using SPSS software (version 26.0) to determine significant differences ( $p < 0.05$ ) among nutritional components.

### **Extract preparation**

Approximately 10 g of dried sample powder of *Astraeus asiaticus* was soaked in 100 mL of each solvent (Water, Hydro-alcohol, Ethyl alcohol, Ethyl acetate, Acetone) for 48 hours at room temperature, with intermittent shaking. The extracts were filtered using Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at temperatures not exceeding 40°C to prevent the degradation of heat-sensitive compounds. The concentrated extracts were stored at 4°C in airtight containers for further analysis (Abubakar & Haque, 2020)

### **Qualitative analysis of phytochemicals**

Phytochemical analysis is an essential step in understanding the bioactive compounds present in *Astraeus asiaticus* (Rugra), an edible mushroom widely consumed in Jharkhand for its nutritional and medicinal benefits. Qualitative screening defines the presence or absence of

different phytochemicals such as Alkaloids, Flavonoids, Phenols, Terpenes, Coumarins, etc. (Dubale *et al.*, 2023; Nortjie *et al.*, 2022).

### **Quantitative estimation of Flavonoids and Phenolics**

The Flavonoids and phenolic compounds are one of the most prominent classes of bioactive secondary metabolites in plants that have been widely recognized for their potent antimicrobial and antioxidant activities. Some naturally occurring polyphenolic molecules act as natural defence agents in plants and can offer significant therapeutic potential in humans. These prominent bioactive compounds exert their effects by disrupting microbial cell walls and membranes, inhibiting nucleic acid synthesis, and inactivating microbial enzymes, making them effective against a wide spectrum of Gram-positive and Gram-negative bacteria, as well as fungi (Lobiuc *et al.*, 2023; Shamsudin *et al.*, 2022). The antioxidant activity of these bioactive molecules is attributed to their strong capacity to scavenge reactive oxygen species (ROS), chelate metal ions, and inhibit the oxidative enzymes, that protects the cells from oxidative stress-induced damage. The presence of hydroxyl groups in their structures enables them to donate hydrogen atoms or electrons, neutralizing free radicals effectively. Due to these properties, flavonoids and phenolics are extensively explored in the development of natural alternatives to synthetic antibiotics and preservatives, and in managing oxidative stress-related diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions (Mutha *et al.*, 2021; Muflihah *et al.*, 2021). The Aluminium chloride ( $\text{AlCl}_3$ ) colorimetric method is a widely used technique for the quantitative estimation of total flavonoid content in plant extracts (Chandra *et al.*, 2014; Ramos *et al.*, 2017; Sultana *et al.*, 2024). The formation of a flavonoid–aluminium complex, particularly with flavones and flavonols, yields a stable yellow coloration measurable at an absorbance of around 415 nm using a UV-Visible spectrophotometer. A standard calibration curve is prepared using known concentrations of quercetin (or rutin), and the total flavonoid content is expressed in terms of milligrams of quercetin equivalent per gram of dry weight of the sample (mg QE/g dry weight).

The Folin–Ciocalteu (F/C) method has been widely used for the quantitative estimation of total phenolic content (TPC) in plant extracts. The principle is based on the reduction of the Folin–Ciocalteu reagent, a mixture of phosphomolybdic and phosphotungstic acids, by the phenolic compounds under alkaline conditions, that results in the formation of a blue-colored complex. This complex has maximum absorbance at 760–765 nm, The absorbance is proportionate to the concentration of phenolics present in the sample. A calibration curve is prepared using gallic acid as the standard, and the results are expressed as milligrams of

gallic acid equivalents of the sample (mg GA/g dry weight) (Ainsworth & Gillespie, 2007; Singleton *et al.*, 1999). The quantitative estimation of Flavonoids and Phenolics of Rugra (*Astraeus asiaticus*) was evaluated and expressed as mean  $\pm$  standard deviation (SD) across multiple replicates of each solvent extracts of all collected sample from different geographical locations.

### **Pharmacological analysis**

The pharmacological potential of *Astraeus asiaticus* (Rugra), an edible mushroom commonly found in Jharkhand, was evaluated through antimicrobial and antioxidant assays. The study focused on the efficacy of solvent extracts (acetone, ethyl alcohol, ethyl acetate, and aqueous) against microbial pathogens and oxidative stress, aiming to explore the bioactive properties of macro fungus.

### **Anti-microbial activity**

The antimicrobial activity study for different solvent extracts of *Astraeus asiaticus* was evaluated using the agar well diffusion method. This method is employed technique for assessing the inhibitory potential of natural compounds against microbial pathogens (Balouiri *et al.*, 2016). The test organisms such as gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and fungal strains (*Candida albicans* and *Aspergillus niger*), were obtained from standard culture collections, grown on their respective nutrient agar (bacteria) or potato dextrose agar (fungi) under appropriate incubation conditions. The solvent extracts were prepared in various solvents (e.g., acetone, ethyl alcohol, and aqueous, hydroalcohol) and concentrated using a rotary evaporator. For the assay, sterile Petri dishes were prepared with 20 mL of molten agar, and wells (8 mm diameter) were punched into the solidified medium. Each well was filled with 50 $\mu$ L of extract (concentration: 100 mg/mL) and allowed to diffuse at 4°C for 1 hour. Positive controls (standard antibiotics such as ampicillin for bacteria and fluconazole for fungi) and negative controls (solvent without extract) were included. The plates were incubated at 37°C for bacterial strains and 28°C for fungal strains for 24-48 hours. The antimicrobial activity was assessed by measuring the diameter of the zone of inhibition (ZOI) in millimeters (in mm) around each well using a digital caliper. Triplicates were performed for each extract against each microorganism to ensure accuracy and reproducibility.

### **Antioxidant assay**

Antioxidant properties of plant extracts refer to their ability to neutralize free radicals, reduce oxidative stress, and prevent cellular damage caused by reactive oxygen species (ROS). The antioxidant activity of the extracts was evaluated using the DPPH (2,2-diphenyl-1-

picrylhydrazyl) radical scavenging assay and the FRAP (Ferric Reducing Antioxidant Power) assay.

#### **DPPH (2,2-diphenyl-1-picrylhydrazyl) radical assay** (Yamauchi *et al.*, 2024)

This method is a widely used method to evaluate the free radical scavenging ability of plant extracts and other antioxidant compounds. This method relies on the reduction of the stable DPPH radical, which is characterized by its deep violet colour with an absorption peak at 517 nm. When antioxidants donate an electron or hydrogen atom to the DPPH radical, it becomes a stable diamagnetic molecule, leading to a color change from violet to yellow. The extent of decolorization is proportional to the antioxidant capacity of the sample. The absorbance is then measured spectrophotometrically, and the percentage inhibition of DPPH radicals is calculated.

#### **FRAP assay** (Benzie & Strain, 1999; Benzie & Szeto, 1999):

The FRAP assay is a simple and reliable method for assessing the reducing power of antioxidants in plant extracts and other samples. This assay is based on the reduction of the ferric-tripyridyltriazine ( $\text{Fe}^{3+}$ -TPTZ) complex to its ferrous ( $\text{Fe}^{2+}$ ) form by electron-donating antioxidants under acidic conditions, resulting in a blue-colored product with a strong absorption peak at 593 nm. High FRAP values indicate greater reducing power and, consequently, stronger antioxidant potential.

#### **Minimal Inhibitory concentration (MIC)**

The Minimal Inhibitory Concentration (MIC) determination by the tube dilution method is a widely accepted quantitative technique for evaluating the antimicrobial efficacy of solvent extracts of *Astraeus asiaticus* (Rugra). In this method, serial two-fold dilutions of various solvent extracts (such as aqueous, ethanol, acetone, ethyl acetate, and hydro-alcoholic) are prepared in sterile nutrient broth media, typically ranging from 1500  $\mu\text{g/mL}$  to 1  $\mu\text{g/mL}$ . Each dilution is inoculated with a standardized microbial suspension (bacterial or fungal), and the tubes are incubated under optimal growth conditions—typically 37 °C for bacteria and 28–30 °C for fungi. After 18–72 hours, the tubes are examined visually for turbidity or growth, with the MIC defined as the lowest extract concentration that completely inhibits visible microbial growth. Controls include sterility (extract only), growth (organism only), solvent control (DMSO), and positive antibiotic controls. This method enables a reliable assessment of the antimicrobial potency of Rugra extracts and allows comparison across different solvents based on their inhibitory concentrations. Each solvent extract of Rugra was dissolved in DMSO to make a stock solution of 20 mg/ml (Andrews 2001).

### ***Invitro* anti-inflammatory assay**

The in-vitro anti-inflammatory assay using the albumin denaturation method commonly used technique to evaluate the anti-inflammatory potential of natural extracts, phytochemicals, and bioactive compounds. The principle is based on the ability of anti-inflammatory agents that inhibit the heat-induced denaturation of proteins that mimics the denaturation of tissue proteins during inflammation. The denatured proteins lose their tertiary and secondary structures that leads to the increase in turbidity that can be quantified spectrophotometrically by recording the absorbance at 660 nm. (Sakat *et al.*, 2010). The reaction mixture contains 1% bovine serum albumin (BSA), Tris-HCl buffer (pH 6.8), and various concentrations of the test sample were incubated at room temperature followed by controlled heating at 55°C to induce denaturation. After cooling, the absorbance was measured, and the percentage inhibition of protein denaturation was calculated in comparison to a control. This percentage correlates with the sample's anti-inflammatory activity, and results can be compared against standard drugs such as diclofenac sodium or aspirin. This method provides a reproducible and cost-effective approach for preliminary anti-inflammatory screening of medicinal plants and fungal extracts.

### ***Invitro* anti-diabetic assay**

The *Invitro* anti-diabetic activity for different plant extracts were accessed by the  $\alpha$ -amylase inhibition assay. This assay simulates the delay of digestion of carbohydrate and glucose absorption in the gastrointestinal tract. The principle relies on the ability of a test sample to inhibit the activity of the  $\alpha$ -amylase enzyme that hydrolyses starch into maltose and glucose. By inhibiting this enzyme, the postprandial rise in blood glucose can be reduced which mimics the mechanism of antidiabetic drugs like acarbose. The procedure includes the reaction mixture that contains  $\alpha$ -amylase enzyme solution and the test sample which are pre-incubated at 37°C for 10 minutes, followed by the addition of a starch solution as a substrate. After 30 mins of incubation, the reaction is stopped using 3,5-dinitrosalicylic acid (DNSA) reagent and the mixture is then boiled for 5 minutes to develop a colored complex. The absorbance is measured at 540 nm using a spectrophotometer. The percentage inhibition of  $\alpha$ -amylase is calculated compared to a control without the extract. (Kazeem *et al.*, 2013).

## **Results and Discussion**

### **Collection of samples**

The *Astraeus asiaticus* sample were collected across various locations such as Dhanbad, Ranchi, Bokaro steel city and Jamshedpur of Jharkhand state during the monsoon season (Figure 1). The healthy disease-free mushroom sample of *Astraeus asiaticus* were then

sorted, washed properly to remove visible dirt and contaminants, surface sterilized and are allowed to dry in well-ventilated room to remove excess water. The sample were coded as (F1: Dhanbad, F2: Ranchi, F3: Bokaro, F4: Jamshedpur).



Dhanbad (F1)

Ranchi (F2)

Bokaro (F3)

Jamshedpur (F4)

Figure 1: *Astraeus asiaticus* (Rugra) sample collected from different locations in Jharkhand.

### Identification of sample by DNA barcode (ITS sequencing)

Genomic DNA was isolated from the fruiting body of *Astraeus asiaticus* by cetyltrimethylammonium bromide (CTAB) method, purified and solubilized in TE (Tris-EDTA) buffer. The DNA was further purified by Phenol:Chloroform:iso-amyl alcohol method. Then PCR amplification of 18s rRNA and ITS region was done by using universal primers ITS1/ITS4 respectively. The PCR amplified product was checked using 1.2% agarose gel prepared in 0.5 TBE assay buffer containing 0.5 $\mu$ g/ml ethidium bromide. The PCR products in the gel can be visualized by using UV Trans-illuminator (Figure 2).

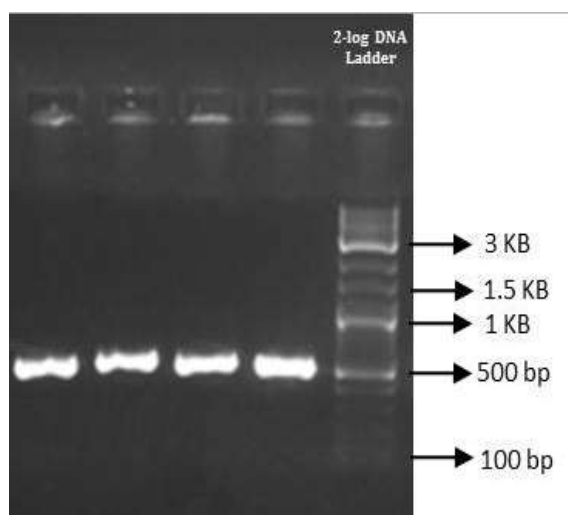


Figure 2: Agarose gel electrophoresis of PCR amplified product (ITS)

Lane 1-Isolate F1; lane 2-Isolate F2; lane 3- Isolate F3; Lane 4: Isolate F4; Lane 5: 2log DNA ladder. (Approx size: ~500bp)

The PCR product was further purified by ExoSAP treatment that further removes unwanted primers and dNTPs. The sequencing of each PCR product was carried out using Thermal cycler Big Dye Terminator sequencing kit for the purified PCR product and analysed using

Sequence scanner software (Applied Biosystem). The sequences for ITS regions of Sample F1, F2, F3 and F4 are:

**Sample F1 (Dhanbad)**

>SR3897-1-ITS

GGAAGGATCAGAGGGAATTCTGAGGCGGGAGAAGCGCAAGTGGGTTCTAGCAATTCGGGAGTGCAGTC  
GCTGGCCATTCGGGGCATGTGCACGTCTTCTGGAGTGTCTGCCTTTCGGACCTCCGAACCCTCTCCTA  
TACCTCCCAAACACACCTGTGTGCACCTGTTGTAGGCCTTGTCTATTAGGCAGACCTATGTATTACTT  
TCATAAACATCGCATGTATAAAAAGAATGTTTGAACAACGATATATATGAATAAATATAACTTTTCAGCA  
ATGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAATTGCGATAAGTAATGTGAATTGCAGAT  
TTTCCGTGAATCATCGAATCTTTGAACGCACCTTGCCTCCTTGGTATTCCGAGGAGCATGCCTGTTT  
GAGTGTTCATCGAAATCTCAAAAATCCTAGCTTTGCCTTGTTCGGAGCTTGGTTTTTGGACTTTGGGAGTT  
TGCGGGCGACCCCTTTGCCTTGGGATGTTCGGCTCTCCTTAAATGTATTAGCAGTGGGTGCAAGTCCTT  
TGCATGGCACGGCCTGTTTCGACGTCTAGTGTATCGTTCGGGCTGGAAGTGCTCGGAATCGACGTGTC  
TCATGCTTCCCAACTTTTACATGCGCCAAGTTTCAGTCTAGGCTACTCAAGCGTGGTGTCTTTTAAAC  
TCTTAAGGCATTACATCAGTGACGACTCGTTCCGC

**Sample F2 (Ranchi)**

>SR3897-2-ITS

TAGAATTCTACTGAGGCGGGAGAGCGCAAGGGGGTTCTAGCATTTCGGAGTGCTGTTCGCTGGCCTTTC  
GGGGCATGTGCACGTCTTCGGAGTGTCTGCCTTTCGGACCTCCGAACCCTCTCCTATAACCTCCCAAACA  
CACCTGTGTGCACGTATTGTATATACCTTGTCTATTAGGCAGACCTATGTATTACTTTTCATAAACATC  
GCATGTATAAAAAGAATGTTTGAACAACGATATATATGAATAAATATAACTTTTCAGCAATGGATCTCTT  
GGCTCTCGCATCGATGAAGAACGCAGCGAATTGCGATAAGTAATGTGAATTGCAGATTTTCCGTGAAT  
CATCGAATCTTTGAACGCACCTTGCCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGGAGTGTTCATCG  
AAATCTCAAAAATCCTAGCTTTGCCTTCTACGGAGCTTGGTTTTTGGACTTTGGGAGTTTGGGGCGAC  
CCCTTTGCTTTGGGATGTTCGGCTCTCCTTAAATGTATTAGCAGTGGGTGCAAGTCCTTTGCATGGCAC  
GGCTGTTTCGACGTCTAGTGTATCGTTCGGGCTGGAAGTGCTTGGATTGACGTGTCTCATGCT  
TCCAACTTTTACATGCGCCAAGTTTAGTCTAGGCTACTCTAGCGTGTGTCTTTTTCTCTAAGGC

**Sample F3 (Bokaro Steel City)**

>SR3897-3-ITS

CGGATTCTGAGGGCGGGAAGCGCAGGGGGTTCTAGCATTTCGGAGTGCTGTTCGCTGGCCTTTCGGGGC  
ATGTGCACGTCTTCGGAGTGTCTGCCTTTCGGACCTCCGAACCCTCTCCTATAACCTCCCAAACACACCT  
GTGTGCACCTGTTGTAGGCCTTGTCTATTAGGCAGACCTATGTATTACTTTTCATAAACATCGCATGTA  
TAAAAGAATGTTTGAACAACGATATATATGAATAAATATAACTTTTCAGCAATGGATCTCTTGGCTCTC  
GCATCGATGAAGAACGCAGCGAATTGCGATAAGTAATGTGAATTGCAGATTTTCCGTGAATCATCGAA  
TCTTTGAACGCACCTTGCCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGGAGTGTTCATCGAAATCTC  
AAAATCCTAGCTTTGCCTTGTTCGGAGCTTGGTTTTTGGACTTTGGGAGTTTGGGGCGACCCCTTTGC  
TTTGGGATGTTCGGCTCTCCTTAAATGTATTAGCAGTGGGTGCAAGTCCTTTGCATGGCACGGCCTGTT  
CGACGTCTAGTGTATCGTTCGGGCTGGAAGTGCTTGGATTGACGTGTCTCATGCTTCCAACTTTTAC  
ATGCGCCAAAGTTTAGTCTAGGCTACTCTAGCGTGTGTCTTTTTCTCTAAGGCTTGACCTCAAATCAG  
GTAGAAACTACCCGCTGAACCTAAAGCATATCAAT

**Sample F4 (Jamshedpur)**

>SR3897-4-ITS

GAATTCTGGAGGGGGGGGAGCGCAGGGGGTTCTAGCATTTCGGAGTGCTGTTCGCTGGCCTTTCGGGG  
CATGTGCACGTCTTCGGAGTGTCTGCCTTTCGGACCTCCGAACCCTCTCCTATAACCTCCCAAACACACC  
TGTGTGCACCTGTTGTAGGCCTTGTCTATCAGGCAGACCTATGTATTACTTTTCATAAACATCGCATGT  
ATAAAAAGAATGTTTGAACAACGATATATATGAATAAATATAACTTTTCAGCAATGGATCTCTTGGCTCT  
CGCATCGATGAACAACGCACCGAATTGCGATAAGTAATGTGAATTGCAGATTTTCCGTGAATCATCGA  
ATCTTTGAACGCACCTTGCCTCCTTGGTATTCCGAGGAGCATGCCTGTATGAGTGTTCATCGAAATCT  
CAAAAATCCTAGCTTTGCCTAGTTCGGAGCTTGGTTTTTGGACTTTGGGAGTTTGGGGCGACCCCTTTG

CTCTGGGAGGTCGGCTCTCCTTAAATGTATTAGCAAAGGGTGCAAGTCCTTTGCATGGCACGGCCTGT  
 TCGACGTCATAGTGATCGTCGCGGGCAGGAAAATGCCTGGATGGACGTGTCTCATGCTTCCAACCTTTT  
 ACATGCGCCAAGTTTAGTCTAGGCTACTCTAGCAGTGTGTCCCATTCTCTACAGGCTGACCTCAAATC  
 AGTAGAATT

The sequences of ITS region obtained for all the samples were then compared with NCBI GeneBank databases by BLAST tool of NCBI (Figure 3). Similar sequences obtained were then analysed by constructing phylogeny tree by MEGA 11 software to validate the taxonomic placement of *A. asiaticus* (Figure 4). MEGA 11 (Molecular Evolutionary Genetics Analysis version 11) a comprehensive bioinformatics software that are widely used for species determination and evolutionary tree (phylogenetic) analysis. This allows users to align DNA or protein sequences, compute evolutionary distances, and construct phylogenetic trees using various methods such as Neighbor-Joining, Maximum Likelihood, and Maximum Parsimony method. MEGA 11 helps in identifying species relationships and inferring evolutionary lineages by analysing the sequence divergence and similarity. (Tamura *et al.*, 2021).

Description	Score	E value	Accession
<b>Astraeus asiaticus [basidiomycete fungi]</b>			
<a href="#">Next</a> <a href="#">Previous</a> <a href="#">First</a>			
<a href="#">Astraeus asiaticus voucher Rug-11a-WB internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i</a>	1146	0.0	<a href="#">OP964740</a>
<a href="#">Astraeus asiaticus voucher CUH/FN/JH/SP/05 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene</a>	1142	0.0	<a href="#">MZ749749</a>
<a href="#">Astraeus asiaticus voucher Rug-14b-JH internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and in</a>	1136	0.0	<a href="#">OP965022</a>
<a href="#">Astraeus asiaticus isolate RUG-a small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S</a>	1136	0.0	<a href="#">MN257431</a>
<a href="#">Astraeus asiaticus voucher Rug-12a-JH internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and in</a>	1136	0.0	<a href="#">OP964930</a>
<a href="#">Astraeus asiaticus isolate Arora02-121 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S r</a>	1136	0.0	<a href="#">EU718089</a>
<a href="#">Astraeus asiaticus strain N4b internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal tran</a>	1136	0.0	<a href="#">QQ253541</a>
<a href="#">Astraeus asiaticus isolate RMUAA28 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complet</a>	1131	0.0	<a href="#">MW403858</a>
<a href="#">Astraeus asiaticus 18S rRNA gene, 5.8S rRNA gene, 28S rRNA gene, ITS1 and ITS2, strain ASTRAE-64</a>	1131	0.0	<a href="#">AJ629400</a>
<a href="#">Astraeus asiaticus 18S rRNA gene, 5.8S rRNA gene, 28S rRNA gene, ITS1 and ITS2, strain ASTRAE-65</a>	1131	0.0	<a href="#">AJ629401</a>
<a href="#">Astraeus asiaticus strain N3a internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete seque</a>	1131	0.0	<a href="#">QQ253540</a>

**Taxonomic Identification of Isolate F1 (SR3897-1-ITS) by BLAST algorithm.**

Description	Score	E value	Accession
<b>Astraeus asiaticus [basidiomycete fungi]</b>			
<a href="#">Next</a> <a href="#">Previous</a> <a href="#">First</a>			
<a href="#">Astraeus asiaticus isolate RUG-a small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S</a>	1157	0.0	<a href="#">MN257431</a>
<a href="#">Astraeus asiaticus strain N4b internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal tran</a>	1157	0.0	<a href="#">QQ253541</a>
<a href="#">Astraeus asiaticus voucher Rug-14b-JH internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and in</a>	1157	0.0	<a href="#">OP965022</a>
<a href="#">Astraeus asiaticus voucher Rug-12a-JH internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and in</a>	1157	0.0	<a href="#">OP964930</a>
<a href="#">Astraeus asiaticus voucher Rug-11a-WB internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i</a>	1157	0.0	<a href="#">OP964740</a>
<a href="#">Astraeus asiaticus voucher CUH/FN/JH/SP/05 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene</a>	1151	0.0	<a href="#">MZ749749</a>
<a href="#">Astraeus asiaticus isolate RMUAA28 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complet</a>	1151	0.0	<a href="#">MW403858</a>
<a href="#">Astraeus asiaticus 18S rRNA gene, 5.8S rRNA gene, 28S rRNA gene, ITS1 and ITS2, strain ASTRAE-64</a>	1151	0.0	<a href="#">AJ629400</a>
<a href="#">Astraeus asiaticus 18S rRNA gene, 5.8S rRNA gene, 28S rRNA gene, ITS1 and ITS2, strain ASTRAE-65</a>	1151	0.0	<a href="#">AJ629401</a>
<a href="#">Astraeus asiaticus 18S rRNA gene, 5.8S rRNA gene, 28S rRNA gene, ITS1 and ITS2, strain ASTRAE-54</a>	1151	0.0	<a href="#">AJ629397</a>
<a href="#">Astraeus asiaticus voucher Rug-11b-WB internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i</a>	1149	0.0	<a href="#">OP964705</a>
<a href="#">Astraeus asiaticus voucher Rug-14a-JH internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, comp</a>	1147	0.0	<a href="#">OP965021</a>
<a href="#">Astraeus asiaticus isolate Arora02-121 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S r</a>	1147	0.0	<a href="#">EU718089</a>
<a href="#">Astraeus asiaticus strain N3a internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete seque</a>	1146	0.0	<a href="#">QQ253540</a>
<a href="#">Astraeus asiaticus 18S rRNA gene, 5.8S rRNA gene, 28S rRNA gene, ITS1 and ITS2, strain ASTRAE-8</a>	1146	0.0	<a href="#">AJ629382</a>
<a href="#">Astraeus asiaticus voucher Rug-12b-JH internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and in</a>	1144	0.0	<a href="#">OP964931</a>

Taxonomic Identification of Isolate F2 (SR3897-2-ITS) by BLAST algorithm.

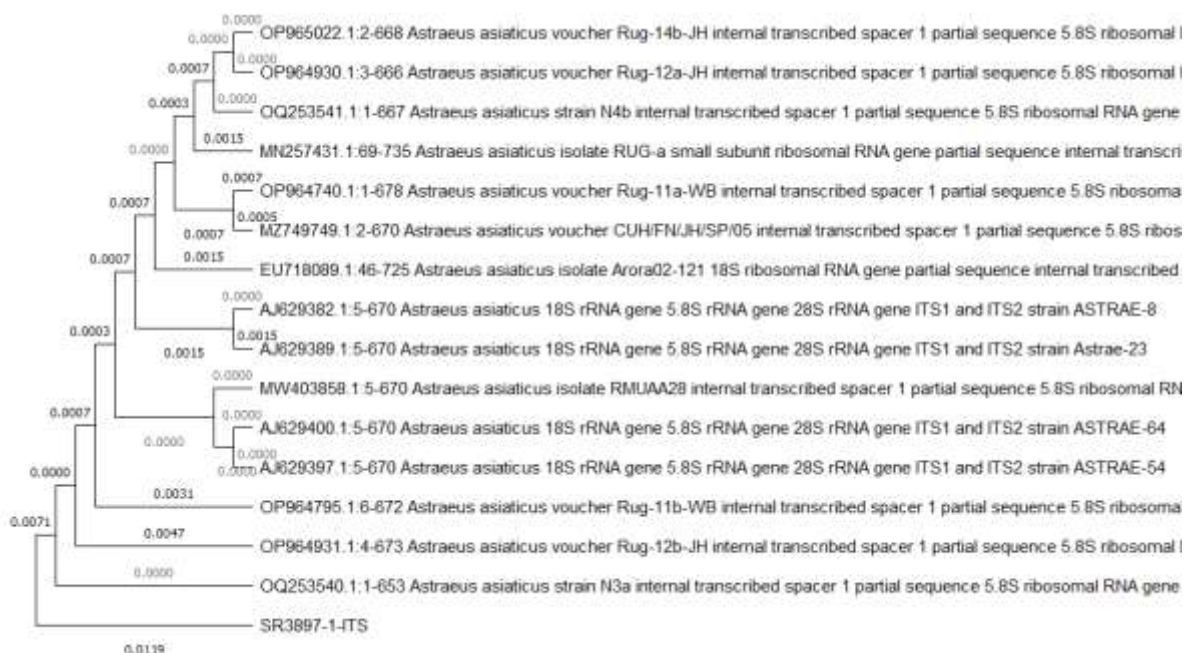
Description	Score	E value	Accession
<b>Astraeus asiaticus [basidiomycete fungi]</b>			
▼ Next   ▲ Previous   ◀ First			
<a href="#">Astraeus asiaticus voucher Rug-14b-JH internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene and in</a>	1275	0.0	<a href="#">OP965022</a>
<a href="#">Astraeus asiaticus voucher Rug-12a-JH internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene and in</a>	1275	0.0	<a href="#">OP964930</a>
<a href="#">Astraeus asiaticus voucher Rug-11a-WB internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene and ir</a>	1267	0.0	<a href="#">OP964740</a>
<a href="#">Astraeus asiaticus voucher Rug-11b-WB internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene and ir</a>	1264	0.0	<a href="#">OP964795</a>
<a href="#">Astraeus asiaticus isolate Arora02-121 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S rri</a>	1264	0.0	<a href="#">EU718089</a>
<a href="#">Astraeus asiaticus voucher Rug-12b-JH internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene and in</a>	1260	0.0	<a href="#">OP964931</a>
<a href="#">Astraeus asiaticus strain N4b internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene and internal tran</a>	1260	0.0	<a href="#">OQ253541</a>
<a href="#">Astraeus asiaticus genes for ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, strain Aswan29</a>	1247	0.0	<a href="#">AB507404</a>
<a href="#">Astraeus asiaticus isolate RUG-a small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8:</a>	1236	0.0	<a href="#">MN257431</a>
<a href="#">Astraeus asiaticus isolate RMUAA28 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene, complete</a>	1225	0.0	<a href="#">MW403858</a>
<a href="#">Astraeus asiaticus 18S rRNA gene, 5.8S rRNA gene, 28S rRNA gene, ITS1 and ITS2, strain ASTRAE-64</a>	1225	0.0	<a href="#">AJ629400</a>
<a href="#">Astraeus asiaticus 18S rRNA gene, 5.8S rRNA gene, 28S rRNA gene, ITS1 and ITS2, strain ASTRAE-85</a>	1225	0.0	<a href="#">AJ629401</a>

Taxonomic Identification of Isolate F3 (SR3897-3-ITS) by BLAST algorithm.

Description	Score	E value	Accession
<b>Astraeus asiaticus [basidiomycete fungi]</b>			
▼ Next   ▲ Previous   ◀ First			
<a href="#">Astraeus asiaticus isolate RUG-a small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8:</a>	1120	0.0	<a href="#">MN257431</a>
<a href="#">Astraeus asiaticus strain N4b internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene and internal tran</a>	1120	0.0	<a href="#">OQ253541</a>
<a href="#">Astraeus asiaticus voucher Rug-14b-JH internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene and in</a>	1120	0.0	<a href="#">OP965022</a>
<a href="#">Astraeus asiaticus voucher Rug-12a-JH internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene and in</a>	1120	0.0	<a href="#">OP964930</a>
<a href="#">Astraeus asiaticus voucher Rug-11a-WB internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene and ir</a>	1120	0.0	<a href="#">OP964740</a>
<a href="#">Astraeus asiaticus isolate RMUAA28 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene, complete</a>	1112	0.0	<a href="#">MW403858</a>
<a href="#">Astraeus asiaticus 18S rRNA gene, 5.8S rRNA gene, 28S rRNA gene, ITS1 and ITS2, strain ASTRAE-64</a>	1112	0.0	<a href="#">AJ629400</a>
<a href="#">Astraeus asiaticus 18S rRNA gene, 5.8S rRNA gene, 28S rRNA gene, ITS1 and ITS2, strain ASTRAE-65</a>	1112	0.0	<a href="#">AJ629401</a>
<a href="#">Astraeus asiaticus 18S rRNA gene, 5.8S rRNA gene, 28S rRNA gene, ITS1 and ITS2, strain ASTRAE-54</a>	1112	0.0	<a href="#">AJ629397</a>
<a href="#">Astraeus asiaticus voucher Rug-14a-JH internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene, comp</a>	1110	0.0	<a href="#">OP965021</a>
<a href="#">Astraeus asiaticus voucher Rug-11b-WB internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene and ir</a>	1109	0.0	<a href="#">OP964795</a>
<a href="#">Astraeus asiaticus isolate Arora02-121 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S rri</a>	1109	0.0	<a href="#">EU718089</a>

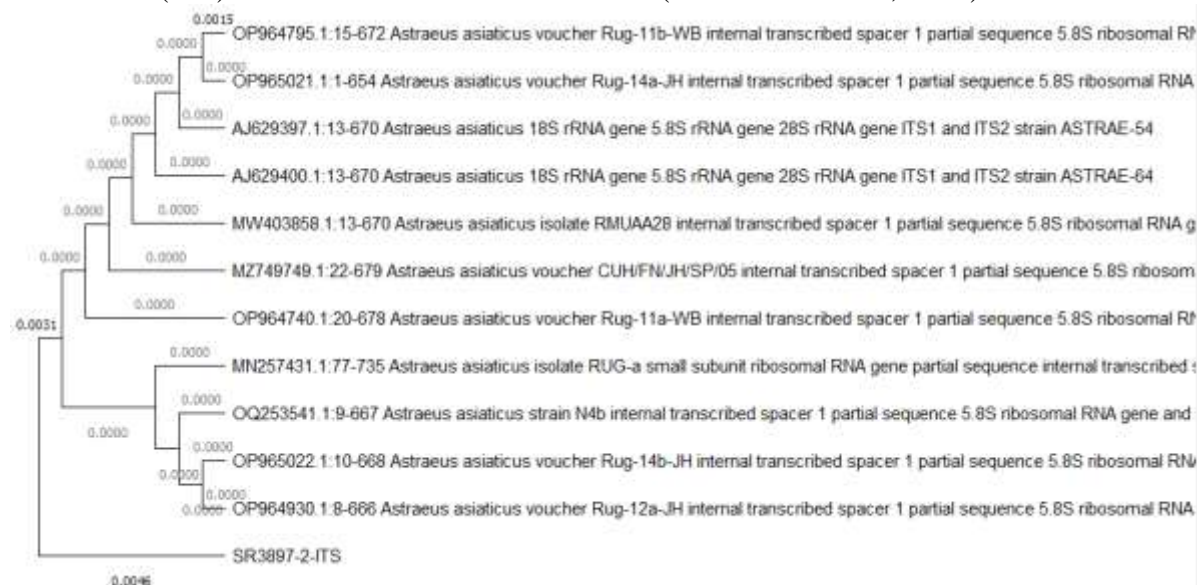
Taxonomic Identification of Isolate F4 (SR3897-4-ITS) by BLAST algorithm.

Figure 3: Taxonomic identification of Isolate F1, F2, F3 and F4 by BLAST algorithm.

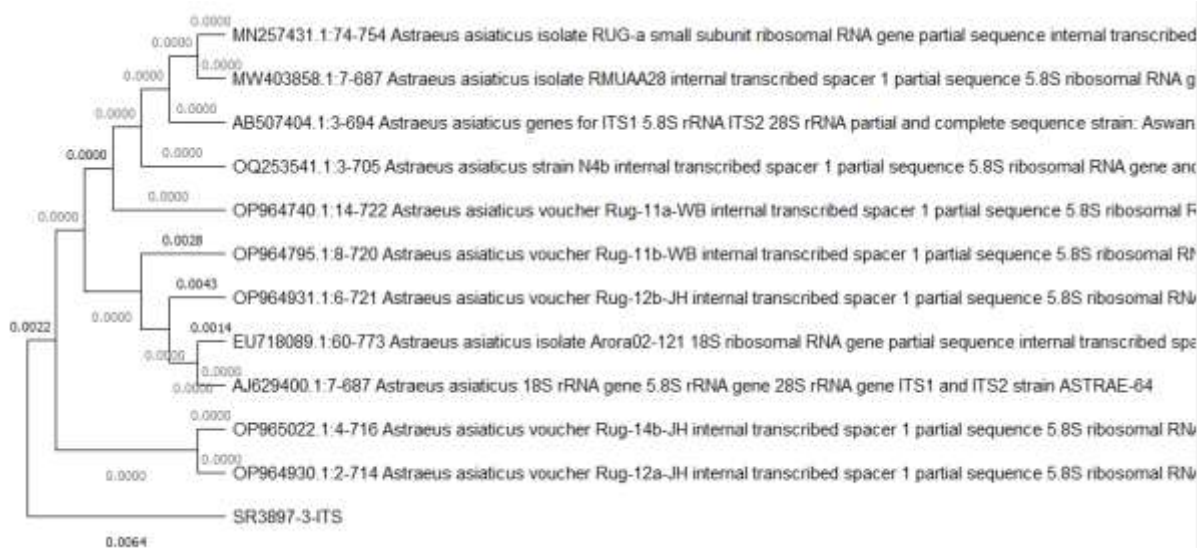


The evolutionary history for F1 (SR3897-1-ITS) was inferred by using the Maximum

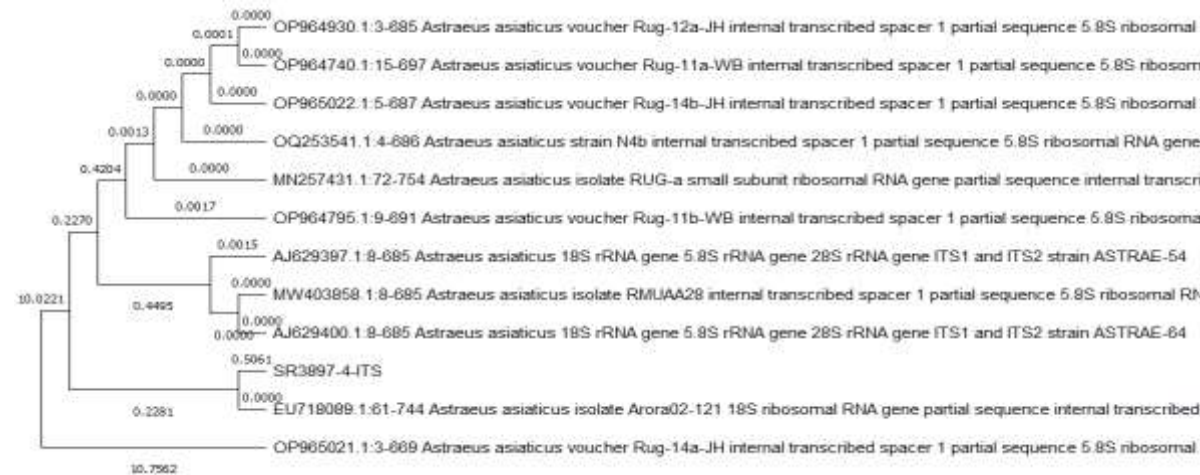
Likelihood (ML) method and Jukes-Cantor model (Jukes and Cantor, 1969).



The evolutionary history for F2 (SR3897-2-ITS) was inferred by using the Maximum Likelihood (ML) method and Jukes-Cantor model (Jukes and Cantor, 1969).



The evolutionary history for F3 (SR3897-3-ITS) was inferred by using the Maximum Likelihood (ML) method and Jukes-Cantor model (Jukes and Cantor, 1969).



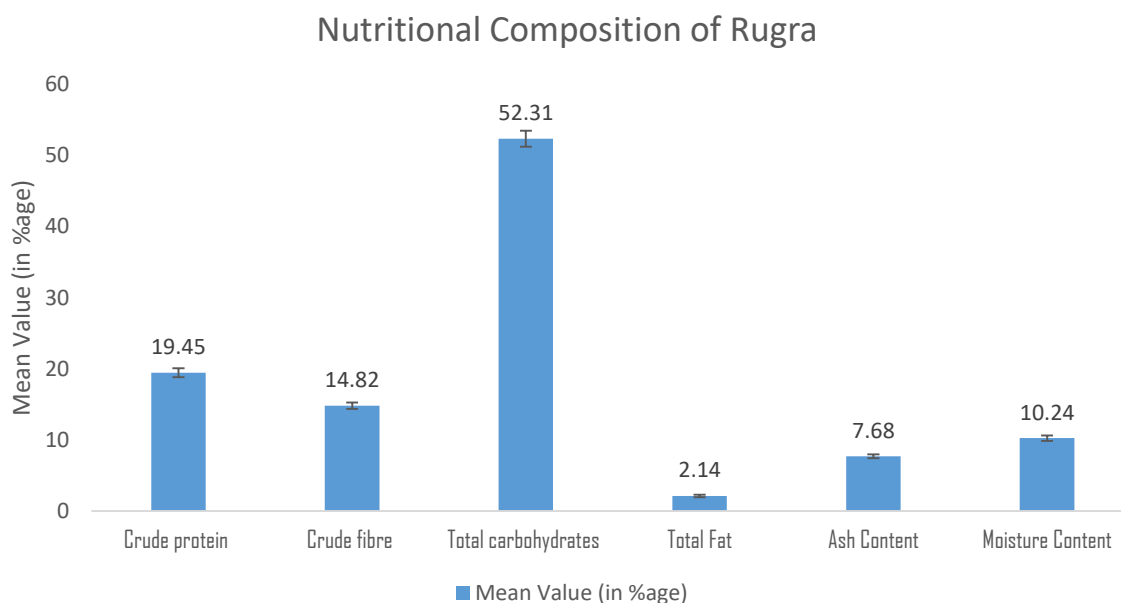
The evolutionary history for F4 (SR3897-4-ITS) was inferred by using the Maximum

Likelihood method and Hasegawa-Kishino-Yano model (Hasegawa *et al.*, 1985).

Figure 4: Computational analysis for determination of evolutionary relationship by MEGA 11 software (Tamura *et al.*, 2021).

### Nutritional assessment of Rugra

The nutritional composition of Rugra (*Astraeus asiaticus*) was evaluated and expressed as mean  $\pm$  standard deviation (SD) across multiple replicates. Major components analysed included crude protein, crude fiber, total carbohydrates, ash, moisture, and fat content. The mean values ( $\pm$  SD) were as follows: Crude protein ( $19.45 \pm 0.62\%$ ), Crude fibre ( $14.82 \pm 0.45\%$ ), Total carbohydrates ( $52.31 \pm 1.12\%$ ), Ash content ( $7.68 \pm 0.27\%$ ), Moisture content ( $10.24 \pm 0.39\%$ ), and Total fat ( $2.14 \pm 0.18\%$ ) (Graph: 1). To assess the statistical significance of differences among nutritional parameters, one-way analysis of variance (ANOVA) was conducted using SPSS software (version 26.0). The results indicated statistically significant differences among the nutritional components ( $p < 0.05$ ), confirming the compositional diversity within the mushroom matrix. These findings underscore Rugra's potential as a nutritionally rich wild edible mushroom traditionally consumed in Jharkhand and other tribal regions of India.



Graph 1: Nutritional composition of *Rugra (Astraeus asiaticus)* across multiple replicates.

The moisture content of fresh Rugra was found to be ~85–90% that is one of the major characteristics of wild mushrooms which upon drying, the fruiting bodies yield concentrated amounts of dry matter that are rich in proteins, carbohydrates, and dietary fibres. The crude protein content in edible macro fungus varies between 18–25% of the dry weight, that are

comparable to legumes and are significantly higher than most staple cereals (Manzi *et al.*, 2004). This high protein content is particularly valuable for the rural and tribal populations in Jharkhand, where protein malnutrition remains a concern. The carbohydrates form the bulk of the mushroom's dry mass, that constitute approximately 50–60% which includes simple sugars and complex polysaccharides like  $\beta$ -glucans, are known to have prebiotic and immunomodulatory properties (Heleno *et al.*, 2015). The dietary fibre content, comprising mainly chitin and non-starch polysaccharides, plays a vital role in digestive health and may contribute to glycemic control and lipid metabolism. Rugra has a low lipid content (2–4%), mostly composed of unsaturated fatty acids such as linoleic acid that constitute beneficial omega-6 fatty acid (Barros *et al.*, 2008). The profile of low-fat, high-protein, and fiber-rich makes Rugra a low-calorie, nutrient-dense food that is suitable for lifestyle-related disease management, including obesity and cardiovascular disorders. The elemental analysis reveals that the macro fungus are rich source of essential minerals such as potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), zinc (Zn), and iron (Fe), especially Potassium that supports blood pressure regulation and cardiovascular health. Presence of bioavailable form of iron and zinc can effectively address the deficiencies in Jharkhand's anemic and undernourished populations (Kalač, 2009).

### Processing of sample

The healthy disease-free mushroom samples was washed properly, surface sterilized and dried in well-ventilated room for 7-15 days to reduce moisture content. The dried sample was then milled at controlled temperature and sieved properly to get uniform particle size (Figure 5). The reduced sample was kept in airtight container for further analysis.



Figure 5: Processed *Astraeus asiaticus* (Rugra) sample

### Extract preparation

For preparation of solvent extract, 10 gm of mushroom powder was soaked in 100 mL of each solvent (Water, Hydro-alcohol, Ethyl alcohol, Ethyl acetate, Acetone) for 48 hours at

room temperature, with intermittent shaking. After 48 hours, sample was filtered, and filtrate was collected (Figure 6).

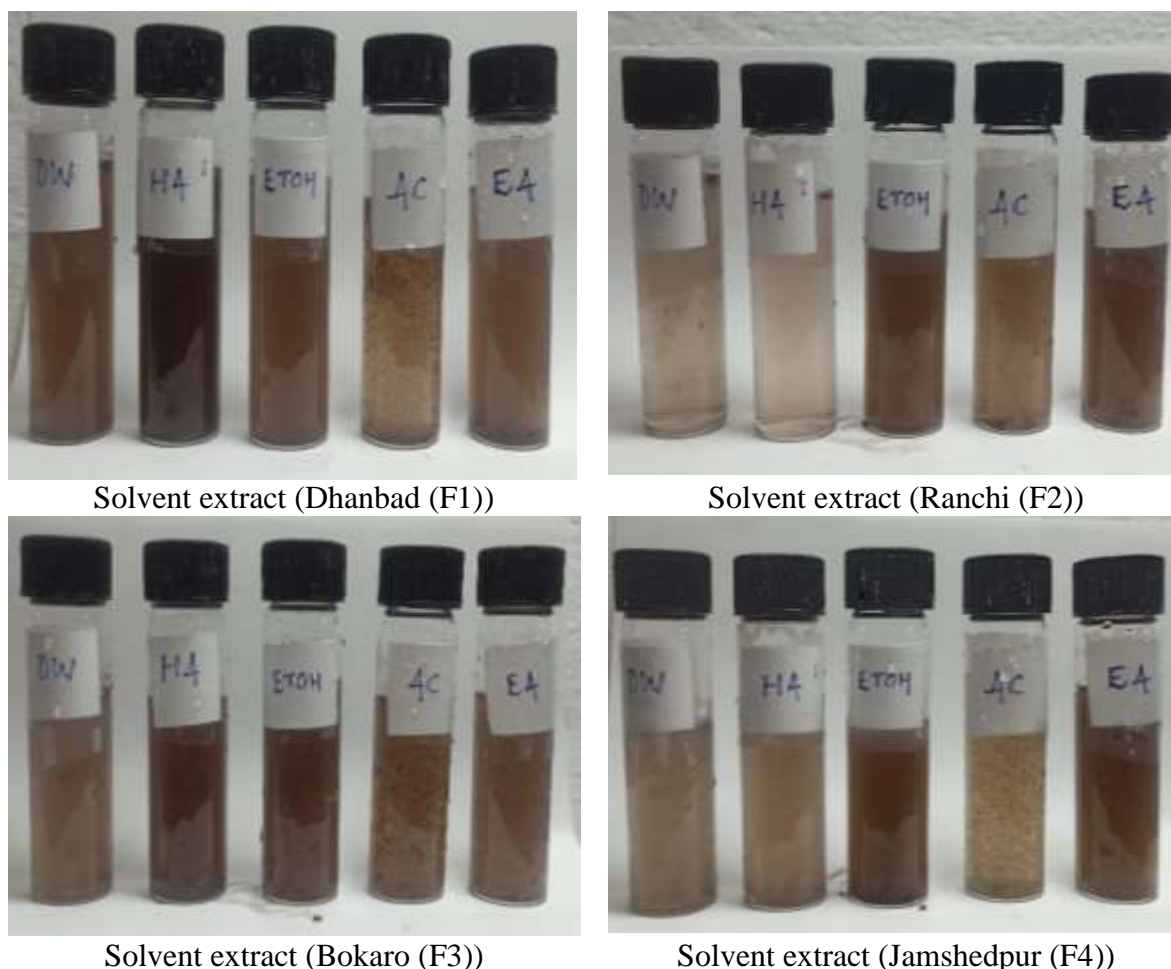


Figure 6: Solvent extracts (Filtrate) of sample  
**Qualitative analysis**

Phytochemical profiling of *Astraeus asiaticus* collected from four locations in Jharkhand (F1: Dhanbad, F2: Ranchi, F3: Bokaro, F4: Jamshedpur) was conducted using various solvent extracts (Water, Hydro-alcohol, Ethyl alcohol, Ethyl acetate, Acetone) to explore its pharmacological potential. Observations revealed a distinct distribution of secondary metabolites, indicating solvent-specific and location-specific variations.

Alkaloids and saponins were consistently detected across all hydro-alcohol (HA) extracts, suggesting their ubiquitous presence in *A. asiaticus*. Acetone (AC) extracts exhibited the most diverse profiles, containing alkaloids, glycosides, saponins, tannins, and, in some cases, flavonoids, phenols, quinones, and resins, highlighting their utility in extracting bioactive compounds. Ethyl alcohol (EtOH) and ethyl acetate (EA) extracts predominantly contained alkaloids, glycosides, saponins, Flavonoids, phenols and tannins, while aqueous (DW) extracts showed notable levels of proteins, carbohydrates, and terpenoids in samples F2

(Ranchi) and F4 (Jamshedpur). Phenols, flavonoids, and terpenoids were most prominent in F3 (Bokaro), particularly in the Ethyl acetate, Distilled water, Acetone and Ethyl alcohol extracts, suggesting antioxidant and antimicrobial potential. The presence of tannins and glycosides in most extracts underscores their astringent and cardioprotective properties, respectively.

Pharmacologically, alkaloids detected across all samples are associated with antimicrobial and anti-inflammatory activities, while saponins contribute to immunomodulatory and cholesterol-lowering effects. The flavonoids and phenols in F3 and F4 extracts indicate strong antioxidant and free radical scavenging properties. The presence of sterols and terpenoids, particularly in AC and HA extracts of F3 and F4, suggests anti-inflammatory and anticancer potential. Glycosides and tannins, found in multiple extracts, support cardioprotective and wound-healing applications. Quinones and resins, detected in F2 extracts, are known for their antimicrobial and anticancer properties. The study determines the potential of *Astraeus asiaticus* as a reservoir of bioactive compounds with diverse pharmacological potentials. The solvent-dependent extraction of these metabolites emphasizes the need for targeted extraction methods to maximize their therapeutic applications.

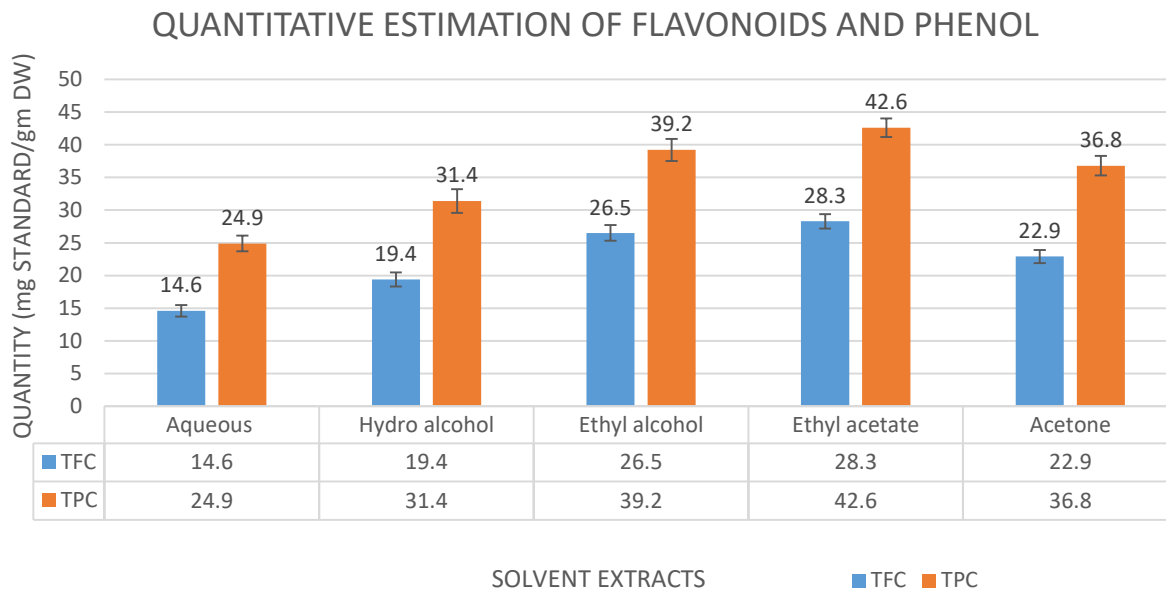
### Quantitative estimation of Flavonoids and Phenolics

The quantitative estimation of Flavonoids and phenolics in different solvent extracts were calculated from the respective standard curve (Quercetin standard curve and Gallic standard curve). The total phenolic content (TPC) and total flavonoid content (TFC) of *Astraeus asiaticus* (Rugra) were quantitatively determined in five different solvent extracts: acetone, ethanol, ethyl acetate, aqueous, and hydroalcoholic. The Folin–Ciocalteu method was used for TPC estimation (expressed in mg gallic acid equivalent [GAE]/g dry extract), and the aluminum chloride colorimetric method was used for TFC (expressed in mg quercetin equivalent [QE]/g dry extract). The values represent mean  $\pm$  SD. The studies revealed that ethyl acetate and ethanol extracts contain the highest concentration of phenolics and flavonoids, while aqueous extracts contained the least (Table 1; Graph 2)

Table 1: Quantitative analysis of Total Flavonoid content and Total Phenolics content in different extracts of Rugra (*Astraeus asiaticus*).

Sl. No.	Extract	Total Flavonoid content (TFC) (mgQE/gm dry weight $\pm$ 0.5)	Total Phenolic Content (TPC) (mgGA/gm dry weight $\pm$ 0.5)
1	Aqueous	14.6 $\pm$ 0.9	24.9 $\pm$ 1.2
2	Hydro-alcohol	19.4 $\pm$ 1.1	31.4 $\pm$ 1.8

<b>3</b>	<b>Ethyl alcohol</b>	$26.5 \pm 1.2$	$39.2 \pm 1.7$
<b>4</b>	<b>Ethyl acetate</b>	$28.3 \pm 1.1$	$42.6 \pm 1.4$
<b>5</b>	<b>Acetone</b>	$22.9 \pm 1.0$	$36.8 \pm 1.5$



Graph 2: Quantitative analysis of Total Flavonoid content and Total Phenolics content in different extracts of Rugra (*Astraeus asiaticus*).

The higher phenolic and flavonoid content in the ethyl acetate and ethanol extracts of *Astraeus asiaticus* (Rugra) can be correlated with the antioxidant and other bioactive properties. The presence of these polyphenolic compounds can be co-related with their free-radical scavenging, anti-inflammatory, antimicrobial, and anti-diabetic properties (Ferreira et al., 2009; Giri & Biswas, 2021). Dasgupta *et al.*, (2015) and Acharya *et al.*, (2022) have reported that the *Astraeus hygrometricus*, where observed significantly high phenolic and flavonoid content in methanolic and ethyl acetate extracts. The semi-polar nature of ethyl acetate and ethanol helps in efficient extraction of diverse metabolites that includes flavonoids, tannins, and phenolic acids, as compared to aqueous solvents that mainly extract hydrophilic constituents. Furthermore, several studies were also done on edible mushrooms such as *Ganoderma lucidum*, *Pleurotus ostreatus*, and *Lentinula edodes* that demonstrates the role of phenolics and flavonoids as major contributors to bioactivity (Barros *et al.*, 2007; Ferreira *et al.*, 2009).

### Invitro anti-microbial assay

The in vitro antimicrobial activity for solvent extracts of *Astraeus asiaticus* was evaluated against selected pathogenic microorganisms that includes gram-positive bacteria

(*Staphylococcus aureus*, *Bacillus subtilis*), gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and fungal strains (*Candida albicans*, *Aspergillus niger*). The solvent extracts that were tested for anti-microbial activity included acetone, ethanol, aqueous, and hydroalcoholic fractions, The ethanolic and acetone extracts exhibited the highest antibacterial activity, especially against *Staphylococcus aureus* and *Escherichia coli*, as indicated by larger zones of inhibition (ranging from 15–22 mm), which were comparable to or slightly lower than standard antibiotics (e.g., ciprofloxacin) used as positive control. The aqueous extract showed moderate to low activity, while the hydroalcoholic extract demonstrated broad-spectrum activity, inhibiting both gram-positive and gram-negative strains effectively. In case of antifungal assays, the acetone and hydroalcoholic extracts of *Rugra* demonstrated notable inhibition against *Candida albicans* and *Aspergillus niger*, with the zone of inhibition ranging from 12–18 mm. The aqueous extract had shown the least activity against the target pathogenic fungus. These observations revealed the presence of polar and semi-polar bioactive compounds such as flavonoids, phenolics, terpenoids, and polysaccharides that may contribute to the antimicrobial effects. Studies have reported antimicrobial potential in *Astraeus spp.* that support the hypothesis of role of mushroom and its constituent biometabolites against pathogenic bacteria and fungus (Kumar *et al.*, 2017; Latha & Babu, 2020; Sundari & Rajalakshmi, 2022).

#### **Minimal inhibitory concentration (MIC)**

The Minimal Inhibitory Concentration (MIC) of different solvent extracts of *Astraeus asiaticus* (*Rugra*) was determined using the tube dilution method against selected pathogenic microorganisms, including gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and fungi (*Candida albicans*, *Aspergillus niger*). The acetone and ethanolic extracts exhibited the lowest MIC values, ranging from 125–250 µg/mL against pathogenic bacterial strains and 250–500 µg/mL for activity against pathogenic fungal strains that indicate strong antimicrobial potential. The hydroalcoholic extract also demonstrated broad-spectrum efficiency against pathogens such as *B.subtilis* and *P. aeruginosa*, with MIC values ranging from 250–500 µg/mL. The aqueous extract had shown higher MIC values (500–1000 µg/mL) in contrast to the other extracts suggesting its lower potency against pathogenic micro-organisms. The MIC values observed against *S. aureus* and *E. coli* were notably lower in the acetone extract, that signifies the role of these organic solvents in efficiently extracting phenolic and flavonoid compounds responsible for antimicrobial activity (Latha & Babu, 2020; Sundari & Rajalakshmi, 2022). The higher MIC values (500–1000 µg/mL) against *Candida albicans*

and *Aspergillus niger* for acetone and ethanolic extracts suggest fungal resistance that may be slightly higher than bacterial pathogens under the tested conditions.

The MIC data strongly support the hypothesis that the solvent extracts of *Astraeus asiaticus* contains potential phytoconstituents that have anti-microbial potential, particularly the acetone and ethanol extract. These solvents are known to dissolve a wide range of bioactive secondary metabolites such as terpenoids, alkaloids, and phenolic compounds, which contribute to microbial growth inhibition (Jonathan & Fasidi, 2003). Kumar *et al.*, 2017, found the extracts of *Astraeus hygrometricus* had shown significant antimicrobial effects, with MIC values ranging between 125–250 µg/mL against different pathogenic bacterial strains. The study also suggests the improvement in extraction efficiency of hydroalcoholic extract due to its polar and non-polar property and broadens antimicrobial activity. Macro fungi like *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Lentinula edodes* have reported to exhibit MIC values within similar ranges against multidrug-resistant pathogens, supporting the broader relevance of these results (Kalaivani *et al.*, 2014; Alves *et al.*, 2012). These findings affirm the importance of macro fungus in ethnomedicinal use in indigenous communities and underscore its potential as a natural antimicrobial agent, especially in the context of increasing antibiotic resistance in different pathogens.

**Invitro anti-inflammatory assay:** The in-vitro anti-inflammatory potential of *Astraeus asiaticus* (Rugra) was assessed using the albumin denaturation assay. The percentage inhibition of protein denaturation increased in a concentration-dependent manner across all solvent extracts. Ethyl acetate and ethanolic extracts had shown highest inhibition of protein denaturation, with maximum inhibition of 85.4% and 82.1% respectively at 500 µg/mL, which was comparable with percentage inhibition of standard (diclofenac sodium) having value of 92.3%. The acetone and hydroalcoholic extracts also had substantial anti-inflammatory activity with inhibition ranging from 60.2% to 76.8%, The aqueous extract had shown least activity with maximum of 51.6% at 500 µg/mL. The above study had indicated that *Astraeus asiaticus* possess significant anti-inflammatory properties, especially when the extracts (ethyl acetate and ethanol) have semi-polar characteristics. The albumin denaturation assay that mimics inflammation-induced protein denaturation in tissue cells, the inhibition of denaturation of proteins suggests the presence of phytoconstituents that stabilizes the protein structures and helps in prevention of inflammatory responses (Mizushima & Kobayashi, 1968). Earlier studies on wild edible mushrooms, especially *Astraeus hygrometricus*, demonstrated strong anti-inflammatory activity attributed to phenolics, flavonoids, and terpenoids (Dasgupta *et al.*, 2015; Acharya *et al.*, 2022). Studies also showed that the ethyl

acetate and ethanol extracts, contained the polyphenolic compounds that were potent inhibitors of inflammatory mediators such as prostaglandins and cytokines (Alves *et al.*, 2012). Other edible macro fungus such as *Ganoderma lucidum* and *Pleurotus ostreatus* have shown similar bioactivities in comparable assays, suggesting the therapeutic potential of edible mushrooms as anti-inflammatory agents (Ferreira *et al.*, 2010). The relatively lower activity in aqueous extracts may be due to the limited solubility of non-polar metabolites that supports the solvent-dependence of bio efficacy. The growing interest for a safe and natural anti-inflammatory agent, *A. asiaticus* can hold itself as potential and valuable candidate for sourcing of bioactive compounds for nutraceutical or pharmaceutical applications, especially in inflammation-related conditions.

### **In vitro antioxidant assay**

The antioxidant potential of various solvent extracts (acetone, ethanol, ethyl acetate, aqueous, and hydroalcoholic) of *Astraeus asiaticus* (Rugra) was evaluated using two standard in-vitro assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and FRAP (Ferric Reducing Antioxidant Power) assay. The DPPH assay reflects the ability of the extract to neutralize free radicals by hydrogen donation, while the FRAP assay indicates the reducing power of the extract, correlating with its electron-donating capacity. In DPPH Assay, all the solvent extracts demonstrated dose-dependent radical scavenging activity. Ethyl acetate and Ethanol extracts exhibited the strongest activity, with  $IC_{50}$  values of 45.3  $\mu\text{g/mL}$  and 51.7  $\mu\text{g/mL}$ , respectively, that were comparable to the standard antioxidant ascorbic acid ( $IC_{50} = 32.5 \mu\text{g/mL}$ ). The acetone extract showed moderate activity ( $IC_{50} = 62.8 \mu\text{g/mL}$ ), followed by the hydroalcoholic ( $IC_{50} = 75.2 \mu\text{g/mL}$ ) and aqueous extracts ( $IC_{50} = 90.5 \mu\text{g/mL}$ ).

In the FRAP assay, antioxidant power was expressed in terms of  $\mu\text{mol Fe}^{2+}$  equivalents per gram of extract. The ethyl acetate extracts again showed the highest ferric-reducing ability (1024.7  $\mu\text{mol Fe}^{2+}/\text{g}$ ), followed by ethanol (950.6  $\mu\text{mol Fe}^{2+}/\text{g}$ ), acetone (825.3  $\mu\text{mol Fe}^{2+}/\text{g}$ ), hydro alcohol (701.8  $\mu\text{mol Fe}^{2+}/\text{g}$ ), and aqueous extract (545.1  $\mu\text{mol Fe}^{2+}/\text{g}$ ). The study demonstrates that *Astraeus asiaticus* possesses significant antioxidant properties, with solvent polarity playing a crucial role in the extraction of antioxidant compounds. The Ethyl acetate and ethanol, being moderately polar, are capable for extracting diverse range of antioxidant phytochemicals such as phenolics, flavonoids, ascorbic acid, and terpenoids that contribute to radical scavenging and reducing capabilities. The hydroalcoholic extract, though moderate in activity, offers a broader extraction spectrum and still provides considerable antioxidant effects. Earlier studies on macro fungi reported that ethyl acetate and ethanolic extracts of *Astraeus hygrometricus* exhibited high antioxidant capacity due to the presence of total

phenolics and flavonoids (Acharya *et al.*, 2015; Giri & Biswas, 2021). Similarly, Ferreira *et al.* 2009 observed potent antioxidant activity in wild mushrooms, and their role as natural sources for dietary antioxidants.

### **Invitro anti-diabetic assay**

The anti-diabetic activity of different solvent extracts (acetone, ethanol, ethyl acetate, aqueous, and hydroalcoholic) of *Astraeus asiaticus* (Rugra) was assessed using the  $\alpha$ -amylase inhibition assay, a well-established in-vitro model to simulate carbohydrate metabolism inhibition and postprandial blood glucose control. All solvent extract exhibited dose-dependent inhibitory effects on  $\alpha$ -amylase activity. extracts, the ethyl acetate and ethanol extracts showed the most potent inhibitory activity, with  $IC_{50}$  values of 54.6  $\mu$ g/mL and 61.2  $\mu$ g/mL, respectively. These values are moderately comparable to the standard reference drug acarbose ( $IC_{50} = 38.4 \mu$ g/mL). The acetone, hydroalcoholic and aqueous extracts exhibited relatively lower activity. *Astraeus asiaticus* possess significant in-vitro  $\alpha$ -amylase inhibitory activity especially ethyl acetate and ethanolic fractions, that proves that those fractions were capable of modulating carbohydrate digestion and glucose absorption. Previous observations indicate the importance of phenolic and flavonoid compounds in mushrooms which can inhibit carbohydrate-hydrolysing enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase (Apostolidis *et al.*, 2007; Patel & Goyal, 2011). Comparable anti-diabetic activities have been reported in related species such as *Astraeus hygrometricus* and *Ganoderma lucidum*. Dasgupta *et al.*, (2015) studied that the ethanolic extracts of *A. hygrometricus* exhibited potential for  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition due to presence of triterpenoids and flavonoids. Furthermore, Giri and Biswas (2021) found that the mushroom extract that are rich in flavonoids can competitively inhibit  $\alpha$ -amylase in a concentration-dependent manner. These findings can support the traditional use of Rugra in indigenous diets and folk medicine in Jharkhand, not only for nutrition but also for managing metabolic disorders such as diabetes.

### **Conclusion**

The study of *Astraeus asiaticus* offers a promising avenue for advancing our understanding of the nutritional and medicinal potential of wild edible mushrooms. By integrating traditional knowledge with modern scientific approaches, this research seeks to unlock the full potential of this underutilized natural resource. The nutritional assessment of Rugra underscores its role as a valuable dietary component with both macro- and micronutrient benefits. Its high protein, low fat, and rich mineral profile, coupled with bioactive compounds, position it as a functional food with potential applications in dietary supplementation, health promotion, and food-based interventions. Further exploration of its

cultivation, preservation, and integration into formal food systems could enhance its accessibility and impact, particularly in nutritionally vulnerable regions like Jharkhand. If its pharmacological properties are harnessed and documented further, Rugra could be processed into capsules, powders, or nutraceutical formulations, providing year-round income for rural communities and boosting local bioeconomy.

## Reference

- Acharya, K., Bera, P., & Ghosh, S. (2022). Anti-inflammatory and immunomodulatory activity of bioactive components from edible mushrooms: A mechanistic insight. *Frontiers in Pharmacology*, 13, 860071. <https://doi.org/10.3389/fphar.2022.860071>
- Acharya, K., Ghosh, S., & Mitra, P. (2015). Antioxidant and anti-inflammatory activity of *Astraeus hygrometricus* in vitro. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(9), 408–411.
- Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature Protocols*, 2(4), 875–877. <https://doi.org/10.1038/nprot.2007.102>.
- Andrews J. M. (2001). Determination of minimum inhibitory concentrations. *The Journal of antimicrobial chemotherapy*, 48 Suppl 1, 5–16. [https://doi.org/10.1093/jac/48.suppl\\_1.5](https://doi.org/10.1093/jac/48.suppl_1.5)
- Apostolidis, E., Kwon, Y. I., & Shetty, K. (2007). Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. *Innovative Food Science & Emerging Technologies*, 8(1), 46–54. <https://doi.org/10.1016/j.ifset.2006.06.001>
- Altschul SF Madden TL Schäffer AA Zhang J Zhang Z Miller W Lipman DJ, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, *Nucleic Acid Res.*, 1997, vol. 25 (pg. 3389-3402).
- Alves, M. J., Ferreira, I. C. F. R., Dias, J., Teixeira, V., Martins, A., & Pintado, M. (2012). A review on antimicrobial and anti-inflammatory activity of mushroom (Basidiomycetes) extracts and isolated compounds. *Phytotherapy Research*, 26(10), 1421–1438. <https://doi.org/10.1002/ptr.4610>.
- AOAC. (2019). Official Methods of Analysis of AOAC International (21st ed.). Rockville, MD: AOAC International.
- Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, 6(2), 71–79. <https://doi.org/10.1016/j.jpha.2015.11.005>
- Banos, S., Lentendu, G., Kopf, A., Wubet, T., Glöckner, F. O., & Reich, M. (2018). A comprehensive fungi-specific 18S rRNA gene sequence primer toolkit suited for diverse research issues and sequencing platforms. *BMC microbiology*, 18(1), 190. <https://doi.org/10.1186/s12866-018-1331-4>
- Barry G. Hall, Building Phylogenetic Trees from Molecular Data with MEGA, *Molecular Biology and Evolution*, Volume 30, Issue 5, May 2013, Pages 1229–1235, <https://doi.org/10.1093/molbev/mst012>.
- Barros, L., Ferreira, M. J., Queirós, B., Ferreira, I. C. F. R., & Baptista, P. (2007). Total phenols, ascorbic acid,  $\beta$ -carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry*, 103(2), 413–419. <https://doi.org/10.1016/j.foodchem.2006.07.038>
- Barros, L., Baptista, P., Correia, D. M., Morais, J. S., & Ferreira, I. C. F. R. (2008). Effects of conservation treatment and cooking on the chemical composition and antioxidant activity of Portuguese wild edible mushrooms. *Journal of Agricultural and Food Chemistry*, 55(12), 4781–4788. <https://doi.org/10.1021/jf8003114>

- Boa, E. (2004). *Wild Edible Fungi: A Global Overview of Their Use and Importance to People*. Food and Agriculture Organization of the United Nations.
- Bellemare, A., John, T., & Marquetteau, S. (2018). Fungal Genomic DNA Extraction Methods for Rapid Genotyping and Genome Sequencing. *Methods in molecular biology (Clifton, N.J.)*, 1775, 11–20. [https://doi.org/10.1007/978-1-4939-7804-5\\_2](https://doi.org/10.1007/978-1-4939-7804-5_2)
- Harley J., Smith S., Read D. (1997). Mycorrhizal symbiosis. 172. Academic Press.
- Benzie, I. F., & Strain, J. J. (1999). Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in enzymology*, 299, 15–27. [https://doi.org/10.1016/s0076-6879\(99\)99005-5](https://doi.org/10.1016/s0076-6879(99)99005-5)
- Benzie, I. F., & Szeto, Y. T. (1999). Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *Journal of agricultural and food chemistry*, 47(2), 633–636. <https://doi.org/10.1021/jf9807768>
- Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., Elsohly, M. A., & Khan, I. A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: a comparative study. *Evidence-based complementary and alternative medicine : eCAM*, 2014, 253875. <https://doi.org/10.1155/2014/253875>.
- Dasgupta, A., Acharya, K., & Mitra, P. (2015). In vitro antidiabetic activity of *Astraeus hygrometricus* (Pers.) Morg. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(9), 408–411.
- Das, S. K., Mandal, A., Datta, A. K., Gupta, S., Paul, R., Saha, A., Sengupta, S., & Dubey, P. K. (2013). Nucleotide sequencing and identification of some wild mushrooms. *TheScientificWorldJournal*, 2013, 403191. <https://doi.org/10.1155/2013/403191>.
- Dasgupta, A., Acharya, K., & Mitra, P. (2015). Antioxidant and anti-inflammatory activity of *Astraeus hygrometricus* (Pers.) Morg. extract in vitro. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(9), 408–411.
- Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., & Suleman, S. (2023). Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. *Journal of experimental pharmacology*, 15, 51–62. <https://doi.org/10.2147/JEP.S379805>.
- Ferreira, I. C. F. R., Barros, L., & Abreu, R. M. V. (2010). Antioxidants in wild mushrooms. *Current Medicinal Chemistry*, 16(12), 1543–1560. <https://doi.org/10.2174/092986710790980985>
- Fredricks, D. N., Smith, C., & Meier, A. (2005). Comparison of six DNA extraction methods for recovery of fungal DNA as assessed by quantitative PCR. *Journal of clinical microbiology*, 43(10), 5122–5128. <https://doi.org/10.1128/JCM.43.10.5122-5128.2005>.
- García-Alegría, A. M., Anduro-Corona, I., Pérez-Martínez, C. J., Guadalupe Corella-Madueño, M. A., Rascón-Durán, M. L., & Astiazaran-Garcia, H. (2020). Quantification of DNA through the NanoDrop Spectrophotometer: Methodological Validation Using Standard Reference Material and Sprague Dawley Rat and Human DNA. *International journal of analytical chemistry*, 2020, 8896738. <https://doi.org/10.1155/2020/8896738>.
- Giri, S., & Biswas, G. (2021). Evaluation of phenolic content and antioxidant potential of wild edible mushrooms from Eastern India. *Journal of Food Biochemistry*, 45(5), e13713. <https://doi.org/10.1111/jfbc.13713>.
- Giri, S., & Biswas, G. (2021). In-vitro antidiabetic activity of selected wild edible mushrooms from Eastern India. *Journal of Ethnopharmacology*, 267, 113532. <https://doi.org/10.1016/j.jep.2020.113532>
- Hasegawa M., Kishino H., and Yano T. (1985). Dating the human-ape split by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22:160-174.

- Hembrom, M.E., Parihar, A., Martín, M.P., *et al.*, 2014. First report of *Astraeus odoratus* from India. *Kavaka* **42**:16-19.
- Heleno, S. A., Barros, L., Sousa, M. J., Martins, A., & Ferreira, I. C. F. R. (2015). Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. *Food Chemistry*, *119*(4), 1443–1450.
- Heleno, S. A., Ferreira, I. C. F. R., Antonio, A. L., Queiroz, M. J. R. P., & Barros, L. (2015). Nutritional value, bioactive compounds, and antioxidant properties of three edible mushrooms from Poland. *Food Bioscience*, *11*, 48–55. <https://doi.org/10.1016/j.fbio.2015.04.008>.
- Jonathan, S. G., & Fasidi, I. O. (2003). Antibacterial activities of two Nigerian edible macrofungi: *Lentinus subnudus* and *Termitomyces striatus*. *Nigerian Journal of Botany*, *16*, 52–57.
- Jukes T.H. and Cantor C.R. (1969). Evolution of protein molecules. In Munro HN, editor, *Mammalian Protein Metabolism*, pp. 21-132, Academic Press, New York.
- Kalač, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, *113*(1), 9–16. <https://doi.org/10.1016/j.foodchem.2008.07.077>
- Kalac, P. (2013). A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. *Journal of the Science of Food and Agriculture*, *93*(2), 209–218.
- Kalaivani, K., Kalaiselvi, M., & Senthilkumar, P. (2014). Evaluation of antibacterial activity of *Pleurotus ostreatus* against human pathogens. *Asian Journal of Pharmaceutical and Clinical Research*, *7*(2), 181–183.
- Karun, N.C., and Sridhar, K.R. 2014. A preliminary study on macrofungal diversity in an arboretum and three plantations of the southwest coast of India. *Current Research in Environmental & Applied Mycology*, **4**(2):173-187; doi: 10.5943/Cream/4/2/5.
- Köljalg, U., Nilsson, R. H., Abarenkov, K., *et al.* (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology*, *22*(21), 5271–5277. <https://doi.org/10.1111/mec.12481>.
- Kumar, V., Bhowmik, D., & Dutta, M. (2017). Evaluation of antimicrobial activity of wild edible mushroom *Astraeus hygrometricus* from Tripura, North-East India. *International Journal of Pharmacy and Pharmaceutical Sciences*, *9*(3), 137–141. <https://doi.org/10.22159/ijpps.2017v9i3.16242>.
- Latha, S., & Babu, A. (2020). Antimicrobial properties of wild edible mushroom *Astraeus hygrometricus* collected from Western Ghats. *Journal of Applied Pharmaceutical Science*, *10*(2), 083–088. <https://doi.org/10.7324/JAPS.2020.102010>
- Lobiuc, A., Pavăl, N. E., Mangalagiu, I. I., Gheorghită, R., Teliban, G. C., Amăriucăi-Mantu, D., & Stoleru, V. (2023). Future Antimicrobials: Natural and Functionalized Phenolics. *Molecules (Basel, Switzerland)*, *28*(3), 1114. <https://doi.org/10.3390/molecules28031114>.
- Manzi, P., Aguzzi, A., & Pizzoferrato, L. (2004). Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry*, *82*(4), 549–552. [https://doi.org/10.1016/S0308-8146\(02\)00516-4](https://doi.org/10.1016/S0308-8146(02)00516-4)
- Muflihah, Y. M., Gollavelli, G., & Ling, Y. C. (2021). Correlation Study of Antioxidant Activity with Phenolic and Flavonoid Compounds in 12 Indonesian Indigenous Herbs. *Antioxidants (Basel, Switzerland)*, *10*(10), 1530. <https://doi.org/10.3390/antiox10101530>
- Mutha, R. E., Tatiya, A. U., & Surana, S. J. (2021). Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. *Future journal of pharmaceutical sciences*, *7*(1), 25. <https://doi.org/10.1186/s43094-020-00161-8>
- Mizushima, Y., & Kobayashi, M. (1968). Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *Journal of Pharmacy and Pharmacology*, *20*(3), 169–173. <https://doi.org/10.1111/j.2042-7158.1968.tb09718.x>

- Nortjie, E., Basitere, M., Moyo, D., & Nyamukamba, P. (2022). Extraction Methods, Quantitative and Qualitative Phytochemical Screening of Medicinal Plants for Antimicrobial Textiles: A Review. *Plants (Basel, Switzerland)*, 11(15), 2011. <https://doi.org/10.3390/plants11152011>.
- Patel, D. K., & Goyal, R. K. (2011). Cardioprotective and antidiabetic effects of dietary flavonoids: A review of evidence from preclinical and clinical studies. *Journal of Advanced Pharmaceutical Technology & Research*, 2(4), 236–243. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3249744>
- Pavithra M., Greeshma A., Karun N., Sridhar K. (2015). Observations on the *Astraeus* spp. of Southwestern India. *Mycosphere*. 6 (4): 421-432.
- Pavlopoulos, G. A., Soldatos, T. G., Barbosa-Silva, A., & Schneider, R. (2010). A reference guide for tree analysis and visualization. *BioData mining*, 3(1), 1. <https://doi.org/10.1186/1756-0381-3-1>
- Phosri C, Martín MP, Sihanonth P, Whalley AJ, Watling R (2007). "Molecular study of the genus *Astraeus*". *Mycological Research*. 111 (3): 275–86. doi:10.1016/j.mycres.2007.01.004. PMID 17360168.
- Phosri, C., Martín, M. P., Watling, R., & Whalley, A. J. S. (2012). The genus *Astraeus*: Hygroscopic earthstars. *Fungal Diversity*, 55(1), 103–115. <https://doi.org/10.1007/s13225-012-0152-3>.
- Ramos, R. T. M., Bezerra, I. C. F., Ferreira, M. R. A., & Soares, L. A. L. (2017). Spectrophotometric Quantification of Flavonoids in Herbal Material, Crude Extract, and Fractions from Leaves of *Eugenia uniflora* Linn. *Pharmacognosy research*, 9(3), 253–260. <https://doi.org/10.4103/pr.pr.143.16>.
- Sakat, S. S., Juvekar, A. R., & Gambhire, M. N. (2010). *In-vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata* Linn. *International Journal of Pharma and Bio Sciences*, 1(2), 1–9.
- Shamsudin, N. F., Ahmed, Q. U., Mahmood, S., Ali Shah, S. A., Khatib, A., Mukhtar, S., Alsharif, M. A., Parveen, H., & Zakaria, Z. A. (2022). Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation. *Molecules (Basel, Switzerland)*, 27(4), 1149. <https://doi.org/10.3390/molecules27041149>.
- Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology*, 299, 152–178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
- Sultana, S., Hossain, M. L., Sostaric, T., Lim, L. Y., Foster, K. J., & Locher, C. (2024). Investigating Flavonoids by HPTLC Analysis Using Aluminium Chloride as Derivatization Reagent. *Molecules (Basel, Switzerland)*, 29(21), 5161. <https://doi.org/10.3390/molecules29215161>.
- Sundari, T., & Rajalakshmi, R. (2022). Investigation of the antimicrobial potential of macrofungi extracts against human pathogens. *Mycosphere*, 13(1), 45–54. <https://doi.org/10.5943/mycosphere/13/1/5>.
- Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* <https://doi.org/10.1093/molbev/msab120>.
- Topno K., Srivastava A. (2021). Identification of puffball found in Chota Nagpur plateau through molecular characterization. *Journal of Pharmacognosy and Phytochemistry*. 10 (4): 404-407.
- Wilson A., Binder M., Hibbett D. (2012). Diversity and evolution of ectomycorrhizal host associations in the Sclerodermatineae (Boletale, Basidiomycota). *New Phytologist*. 194 (4): 1079-1095.
- Wu, Z., Tsumura, Y., Blomquist, G., & Wang, X. R. (2003). 18S rRNA gene variation among common airborne fungi, and development of specific oligonucleotide probes for the detection of fungal isolates. *Applied and environmental microbiology*, 69(9), 5389–5397. <https://doi.org/10.1128/AEM.69.9.5389-5397.2003>.

Xie Q, Lin J, Qin Y, Zhou J, Bu W. Structural diversity of eukaryotic 18S rRNA and its impact on alignment and phylogenetic reconstruction. *Protein Cell*. 2011 Feb;2(2):161-70. doi: 10.1007/s13238-011-1017-2. Epub 2011 Mar 12. PMID: 21400046; PMCID: PMC4875256.

Yamauchi, M., Kitamura, Y., Nagano, H., Kawatsu, J., & Gotoh, H. (2024). DPPH Measurements and Structure-Activity Relationship Studies on the Antioxidant Capacity of Phenols. *Antioxidants (Basel, Switzerland)*, 13(3), 309. <https://doi.org/10.3390/antiox13030309>

Zhang, D., Kan, X., Huss, S. E., Jiang, L., Chen, L. Q., & Hu, Y. (2018). Using Phylogenetic Analysis to Investigate Eukaryotic Gene Origin. *Journal of visualized experiments : JoVE*, (138), 56684. <https://doi.org/10.3791/56684>