Antioxidant Assessment of *Pleurotus sajor-caju* Extracts Obtained through Hot Extraction Process

5% wet weight basis spawn grain was mixed with the substrate and lled into polythene bags. e mouth of the bag was tied with rubber band and 12 holes of about 1cm diameter were made; two at each corner of the base, four each on the broader area and one each on the narrow, rectangular side to drain out extra water and for proper aeration [11].

Mushroom sample collection

Fruiting bodies were collected and air dried. Dried materials were crushed and ground into ne powder with mortar and pestle and it was kept under refrigeration at 4°C for further analysis.

Preparation of extracts

Powdered sample was sequentially extracted with hexane (HX), ethyl acetate (EA), Methanol (MeOH) and water (AQ) in Soxhlet apparatus for 8 h [12]. Solvent was removed completely under reduced pressure and dried extracts were preserved at –20°C. e dried residues were reconstituted in DMSO for determination of biochemical activities.

Biochemical screening

Qualitative biochemical analysis was carried out for identi cation of avonoids, terpenoids, saponins, anthraquinone, tannin and phlobatannins in *P. sajor-caju* extracts using standard procedures [13].

Determination total avonoid content

Aluminum chloride colorimetric method [14] with minor modi cation was used for determination of avonoid content in extract fractions. Small amount (0.2 ml) of extract in pure DMSO (2 mg/ml) was separately mixed with 1.8 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. Tubes were incubated at room temperature for 30 min and then absorbance of the reaction mixture was measured at 415 nm. Calibration curve was prepared using quercetin as standard compound. e amount of avonoids in the test samples was expressed as µg quercetin equivalent/mg sample (µg QE/mg). Experiments were performed in triplicate and the results were expressed as mean ± SD.

Determination of total phenolics

Total phenolic content in extract fractions was determined according to the protocol [15] with some modi cations [16]. Modi cation included dissolution of extracts in DMSO instead of water. 0.2 ml of sample (2 mg/ml in DMSO) was diluted to 3 ml with water. Small amount (0.5 ml) of two-fold-diluted FCR was added and the contents were mixed. A er 3 min, 2 ml of 20% sodium carbonate solution was added and the tubes were placed in boiling water bath for one min followed by cooling. e absorbance was measured at 650 nm. e concentration of phenols in the extract samples was expressed as µg propyl gallate equivalents/mg sample (µg PGE/mg). e estimation was performed in triplicate, and the results were expressed as mean ± SD.

DPPH radical scavenging activity

e free radical scavenging activity of the extract fractions was measured *in vitro* by DPPH assay [15] with minor modi cation [16]. DMSO was used as solvent for dissolving extracts instead of methanol. ree milliliters of 0.1 mM DPPH solution prepared in methanol was added to 1 ml of the test extracts (40-100 µg/ml) dissolved in DMSO. e content was mixed and allowed to stand at room temperature for 30 min in dark. e reduction of DPPH free radical was measured by recording the absorbance at 517 nm. e percentage scavenging activities (% Inhibition) at di erent concentrations of the extracts were calculated using the following formula.

$$(\%)\ I = ((Ac - As)/Ac) \times 100$$

Where I is inhibition, *A*c and *A*s are the absorbance values of the control and the sample respectively. ree replicates were made for each sample and results were expressed as mean ± SD.

Reducing power assay

e reducing power of test extracts was determined by the protocol [17]. One ml aliquots of extracts (200-1000 µg/ml) prepared in DMSO was taken in test tubes. To each test tube 2.5 ml of phosphate bu er (0.2 M, pH 6.6) and 2.5 ml of 1% potassium hexacyanoferrate ($K_3Fe(CN)_6$) were added and contents were mixed. Tubes were incubated at 50°C in a water bath for 20 min. e reaction was stopped by adding 2.5 ml of 10% TCA and then centrifuged at 4000 g for 10 min. One ml of the supernatant was mixed with 1 ml of distilled water and 0.5 ml of $FeCl_3$ solution (0.1%, w/v) and kept at 25°C for 2 min. e absorbance was measured at 700 nm. Higher absorbance indicated higher reducing capability of the sample. All the tests were run in triplicate and results were reported as mean ± SD.

Metal ion chelating activity

e chelation of ferrous ions by the *P. sajor caju* extracts was estimated by the method of Dinis et al. [18] as modi ed by us. Modi cation included dissolution of extracts in DMSO instead of methanol. Brie y, the extract samples (200 µl) of di erent concentrations were added to a solution of 2 mM/L ferrous sulphate (0.05 ml). e reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixture was shaken vigorously and le standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. Metal ion chelating ability (percentage inhibition of ferrozine-Fe^{2+} complex formation) was calculated using the formula.

$$\text{Metal ion chelating ability } (\%) = ((Ac - As)/Ac) \times 100$$

Where *A*c is the absorbance of control and *A*s is absorbance in the presence of the sample. e results were expressed as mean ± SD of three replicates.

Biochemicals	Methanol Extract	Aqueous Extract
Tannins	-	-
Flavonoids	+	+
Terpenoids	+	-
Anthraquinones	+	+
Phlobatannins	-	-
Saponins	+	+

Table 1: Biochemical screening of *P. sajor-caju* extracts.

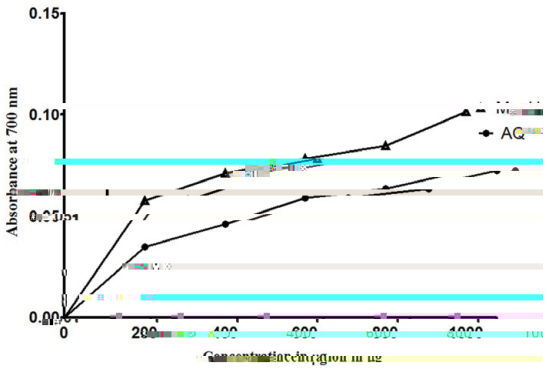

Figure 1: Reducing power assay of *P. sajor-caju* extracts. (Abbreviations: MeOH: Methanolic Extract; AQ: Aqueous Extract.

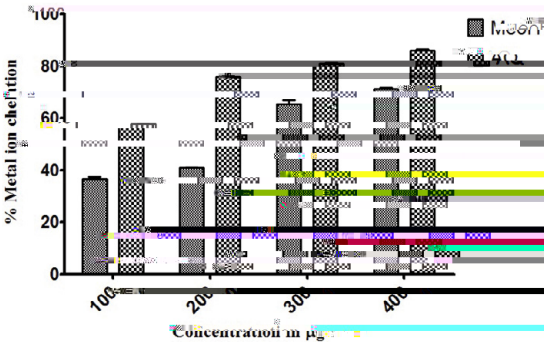

Figure 2: Metal ion chelating activity of *P. sajor-caju* extracts.

aqueous extracts only. Biochemical screening of *P. sajor-caju* extracts exhibited presence of avonoids, terpenoids, reducing sugars, saponins, anthraquinone in MeOH and AQ fractions (Table 1). Tannin and phlobatannins were absent in extracts.

Total phenolic and avonoid contents

Total phenolic contents in AQ and MeOH extracts were 19.78 ± 0.59 µg PGE/mg and 23.59 ± 0.99 µg PGE/mg of samples, respectively. Total avonoids in both the extracts ranged between 23.27-26.44 µg QE/mg of sample.

DPPH assay

P. sajor-caju extracts (AQ and MeOH) showed 2-6% DPPH free radical scavenging potential at test concentrations. Antioxidants react with DPPH free radical and change the color of reaction mixture from violet to yellow. Results demonstrated that both extracts have low capability to donate hydrogen atom.

Reducing Power assay

Reducing power ability of test compounds exhibited the similar pattern as observed in radical scavenging assay (Figure 1). *P. sajor-caju* MeOH extract showed moderate reducing ability (Absorbance 0.06-0.11). AQ extract also showed lower activity (Absorbance 0.03-0.07) in the concentration range 200-1000 µg/ml.

Metal ion chelation

Test extracts displayed concentration dependent metal chelation potential (Figure 2). Aqueous extracts accounted for appreciable metal ion chelating ability (57-86%). Methanolic extracts also showed noticeable chelation activity (36-71%) at test concentrations. Transition metals are responsible for increasing ROS generation in the system. Hence chelation of iron by *P. sajor-caju* extracts shows their ability to diminish ROS production.

Phosphomolybdate assay

e AQ and MeOH extracts derived from *P. sajor-caju* exhibited noticeable AO capacity. e AO capacity of AQ and MeOH extracts was 83.68 ± 0.60 g PGE/mg and 95.89 ± 0.58 g PGE/mg of extract, respectively. Phosphomolybdate assay is used for assessment of total antioxidant capacity of samples.

Discussion

Pleurotus species are promising as medicinal mushrooms and exhibit hematological, antiviral, antitumor, antibacterial, hypocholesterolic, and immunomodulatory activities as well as antioxidant properties. Approximately 40 species of the oyster mushrooms (genus *Pleurotus*) have been reported in the literature. Scienti c studies on macrofungi (mushrooms and entomopathogenic fungi) have reported that extracts derived from fruiting bodies or mycelia possess important medicinal properties [20-22]. In the present work, biochemical analyses of fruiting bodies of *P. sajor-caju* has shown presence of number of bioactive compounds such as avonoids, terpenoids, reducing sugars, saponins, anthraquinone (Table 1). Considerable amount of phenolics and avonoids have been found in AQ and MeOH extracts. ese compounds are known for their medicinal attributes including antioxidant properties [23].

Current work also describes the antioxidant properties of methanolic and aqueous extract of *P. sajor-caju*. Antioxidants counteract the adverse effects of free radicals in the body by scavenging or reducing their formation [5]. DPPH assay is one of the most commonly used methods for screening antioxidant activity of plant extracts [24]. The hot extracts accounted for lower DPPH radical scavenging activity in the experiments which contradict the reports showing higher radical scavenging activity in cold extracts [25]. Lower radical scavenging activity in our study might be due to a chemical alteration in the active compounds present in this mushroom caused by using a higher temperature during hot extraction process. In reducing power assay MeOH and AQ fractions showed moderate activity even at higher concentrations (Figure 1). Dose dependent response in activity pattern was also observed [17]. Lower proton donating ability marks lower scavenging action. Present study exhibited direct correlation between lower radical scavenging actions of hot extracts with lower reducing power.

e transition metal ion, Fe^{2+} possess the ability to move single electrons, by which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals. e main strategy to avoid ROS generation that is associated with redox active metal catalysis involves chelating of the metal ions [26]. Ferrozine can quantitatively form complexes with Fe^{2+}. In the

presence of *P. sajor-caju* extracts formation of red coloured complexes decreased substantially (Figure 2). erefore, measurement of the rate of color reduction helps to estimate the chelating activity of the coexisting chelator present in the samples. Our results have shown

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