INTEGRATED COMPARATIVE STUDY OF PHYTOCHEMICALS, ANTIOXIDANT PROPERTIES, AND ANTIBACTERIAL ACTIVITIES OF *PLEUROTUS SAJOR-CAJU*

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Abstract: *Pleurotus sajor – caju* is one of the most popular mushroom belong to the genus Pleurotus and family Pleurotaceae. There is an immense potential for mushroom in India, where a very large population are vegetarian in habit. The cultivation of oyster mushrooms, which have strong nutritional characteristics, is best suited for North East India. It is an appropriate fungus that can produce protein from different agricultural wastes. The objective of our study was to determine the phytochemical components, antioxidant and antibacterial properties of the methanolic and aqueous extracts of *P. sajor-caju.* The antioxidant activity was measured using DPPH and H2O2 techniques. The highest DPPH free radical scavenging activity and H_2O_2 scavenging activity was shown at 500µg/ml for both extracts. The antibacterial activity was tested against pathogenic bacteria *Escherichia coli*,, *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumoniae.* The antibacterial activity against *E. coli* produced the best results in methanolic extract. This study depicts the medicinal properties of the species which can be explored further.

Key words: Oyster mushroom, *Pleurotus sajor –caju*, Phytochemical, Antioxidant, DPPH, H_2O_2, Antibacterial.

INTRODUCTION:

India is a huge geographical area with high potential and a rich culture of medicinal herbs and spices, with over 2000 species. Therefore, only a very small number of traditional medicines—Ayurvedic, Unani, and Siddha—have been chemically and pharmacologically examined for possible therapeutic benefits [5].

Few species of edible mushrooms are as highly valued in terms of nutrition, taste and its therapeutic activity [11]. *Pleurotus sajor-caju*, also referred to as the oyster mushroom which is belongs the Pleurotus species, is the third-largest type of fungus grown for commercial purposes worldwide [3]. Mushrooms are considered to be among the most popular foods and are known to contain a variety of biopharmaceutical chemicals, because they are higher in protein (40–49%) than many other vegetables. *P. sajor caju* mushrooms contain bioactive substances that are used to treat a wide range of illnesses in people and are also crucial to the healing process such as anticancer, antibacterial, antidiabetic, antidiuretic, and anti-inflammatory properties [4].

According to Egra et al., 2019 was reported that oyster mushrooms are contain 19–35 percent protein, which is made up of 9 amino acids; 1,7–2,2% fat, of which 72% is unsaturated fatty acid; carbohydrates; vitamins B (thiamine, riboflavin, and niacin); D and C; minerals (K, P, Na, Ca, Mg, Zinc, Fe, Mn, Co, and Pb); and very low levels of metal-microelements [6]. According to Brains et al. (2016), *P. sajor caju* contains phytoconstituents and has strong antibacterial qualities against both gram positive and gram negative bacteria [2]. The identification of phytoconstituents, antioxidants, and antibacterial activity will help to increase knowledge *of P. sajor caju's* therapeutic potential and benefit the food and pharmaceutical industries.

MATERIAL AND METHODS:

SAMPLE COLLECTION:

The *P. sajor caju* was collected from Sarihajan market, Karbi Anglong District of Assam during the month of May 2023.

Sample preparation: Fresh, healthy, mature mushrooms were harvested, cleaned, weighed, chopped into small pieces, and then dried at 45^0C in a hot air oven. The dried mushroom was ground into a powder, and extraction process was carried out using maceration technique by Singh et al., 2008[13].

PHYTOCHEMICAL SCREENING:

Qualitative phytochemical analysis of *P. Sajor –Caju* was carried out as follows using standard procedure by Gul et al., 2018 with little modifications [7].

TEST FOR ALKALOIDS:

Mayer's test: 2ml of methanolic extract were treated with 2ml of 1% HCL and few drops of mayer's reagent.

TEST FOR CARBOHYDRATES:

Fehling's test: Equal volume of Fehling A and Fehling B were added with methanolic extract and boiled.

TEST FOR SAPONIN:

2ml of methanolic extract were added in 5 ml of distilled water and mixed it for 2 to 3 minutes. The form formation indicates the presence of saponin.

TEST FOR TERPINOIDS:

Salkowski test : 2ml of methanolic extract were dissolve in 2ml of chloroform and evaporated to dry and added 2 ml of concentrated H_2SO_4 heated. Formation of grayish colour indicates the presence of terpenoids.

TEST FOR PROTEIN:

Ninhydrine test : 2ml of methanolic extract were boil with 2ml of 0.2 % of ninhydrin. Formation of violet colour indicates the presence of protein.

TEST FOR PHENOL AND TANNINS:

2ml of methanolic extract was treated with 2ml of 2% FeCl3 solution. A black colour indicates the presence of phenol and tannins.

TEST FOR FLAVONOIDS:

Alkaline reagent test: Methanolic extract was treated with 10% Al(OH_3). Formation of yellow colour indicates presence of flavonoids.

TEST FOR GLYCOSIDE:

Borntrager test: 2ml of methanolic extract was treated with 3ml CaCl3 and 10% ammonia solution. Formation of pink colour indicates the presence of glycosides.

IN-VITRO ANTIOXIDANT SCAVENGING ACTIVITY:

The antioxidant activity of methanolic extract of *P. sajor caju* was determined by in-vitro free radical scavenging assays using DPPH free radical followed by Abe et al., 1998 [1]. The stock solution of each 1ml extract, as well as standard was prepared in 1ml of methanol to achieved the 1mg/ml concentration. Dilution was done in the concentration of 100, 200, 300, 400 and 500 µg/ml. Diluted solution (0.5ml of each sample solution) was mixed with methanol and 2.5 ml of 0.1mM DPPH solution. After 30 minutes of incubation in dark, the O.D of the sample was recorded at 517 nm using UV-VIS spectrophotometer. The IC_{50} value was calculated as mean value± Standard deviation. Ascorbic acid is used as standard. The experiment was done in triplicates.

The formula of scavenging activity percentage of methanolic extract:

$$\text{Scavenging \%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

ESTIMATION OF H_2O_2 SCAVENGING ACTIVITY:

The H_2O_2 scavenging activity of *P. sajor caju's* methanolic and aqueous extract was assessed using the Ruch et al., 1989 technique [12]. 3.4 mL of 0.1M phosphate buffer (pH 7.4) was used to dissolve various extract concentrations (from 100 µg to 500

µg/ml), which were then combined with 600 µL of 43 mM H_2O_2. After 10 minutes, the absorbance of the reaction mixture was measured at 230 nm and recorded. The following formula was used to determine the H_2O_2 scavenging activity:

Scavenging %= Absorbance of control- Absorbance of sample × 100

Absorbance of control

TOTAL YIELD OF EXTRACT:

The fully matured *P.sajor caju* (1kg) was collected for analysis. Sample was washed and removed all dirt. After remove of the excess water it was dried and grinded to power form. Extract preparation was done by maceration method using solvent of methanol and water.

DETERMINATION OF ANTIBACTERIAL ACTIVITY:

The methanolic extract of *P. sajor caju* was used for antimicrobial activity by following Agar Well Diffusion Assay using nutrient agar media as described in Ibekwe et al, 2001[8]. The standard solutions of Streptomycin of the concentration of 1mg/ml will be used. The different dilutions of methanolic extract in concentration 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml was determined and compared with the standard. The antibacterial activity was tested against pathogenic gram-negative bacteria *E.coli and Staphylococcus aureus* and gram positive bacteria such as *Bacillus subtilis* and *Klebsiella pneumoniae.*

RESULTS AND DISCUSSION:

The qualitative phytochemical analysis results showed the presence of important phytoconstituents like protiens, carbohydrates, phenols, flavonoids, glycosides, steroids, alkaloids and saponin in methanolic extract of *P. sajor caju.* The aqueous extract showed the presence of protiens, carbohydrates, phenols, flavonoids, alkaloids, glycosides and saponin. The results have been tabulated in **Table 1**.

TABLE 1: RESULTS OF PHYTOCHEMICAL SCREENING OF BOTH EXTRACT.

Phytoconstituents	Aqueous extract	Methanolic extract
Alkaloids	+	+
Protiens	+	+
Carbohydrates	+	+
Phenols	+	+
Flavonoids	+	+
Glycosides	+	+
Steroids	-	+
Terpenoids	-	-
Saponin	+	+

TOTAL ANTI-OXIDANT ACTIVITY:

DPPH ASSAY:

Total anti-oxidant activity was determined in 100, 200, 300, 400 and 500µgm/ml concentration of methanolic and aqueous extract of *P. sajor caju*. The highest anti-oxidant activity was found at 500µg/ml with a percentage inhibition of 94.56 and 93.06 in methanolic and aqueous extract respectively. Whereas 100µg/ml concentration showed scavenging activity (%) of 59 in methanolic extract and 55 in aqueous extract respectively. The results indicated that increase in the concentration of extract, percentage of scavenging activity increased in both extract which has mentioned below the **Table 2** and **Table 3**

Table 2: DPPH FREE RADICAL SCAVENGING ACTIVITY; % INHIBITION ± SE OF METHANOLIC EXTRACT OF *P.SAJOR CAJU*

Sl. No	Concentration (µg/ml)	% of Inhibition	
		Sample	Standard
1.	100	59±0.32	94.65±0.35
2.	200	76±0.94	94.87±0.43
3.	300	88.01±1.10	95.34±0.76
4.	400	92.03±1.22	97.12±0.55
5.	500	94.56±1.50	98.23±0.65

Table 3: DPPH FREE RADICAL SCAVENGING ACTIVITY; % INHIBITION ± SE OF AQUEOUS EXTRACT OF *P.SAJOR CAJU*

Sl. No	Concentration (µg/ml)	% of Inhibition	
		Sample	Standard
1.	100	55±0.12	94.65±0.13
2.	200	66±0.44	94.87±0.33
3.	300	78.11±0.53	95.34±0.65
4.	400	88.04±1.01	97.12±0.47
5.	500	93.06±1.20	97.20±0.78

ESTIMATION OF H_2O_2 SCAVENGING ACTIVITY:

Total anti-oxidant activity was determined in 100, 200, 300, 400 and 500µgm/ml concentration of methanolic and aqueous extract of *P. sajor caju*. The highest anti-oxidant activity was found in 500µg/ml with a scavenging activity (%) of 96.66 and 95.26 in methanolic and aqueous extract respectively. Whereas 100µg/ml concentration showed scavenging activity (%) of 69 in methanolic extract and 65 in aqueous extract respectively. The results evaluated that increase in the concentration of extract, percentage of scavenging activity increased in both extract which has mentioned below the **Table 4** and **Table 5.**

Table 4: H_2O_2 SCAVENGING ACTIVITY; % INHIBITION ± SE OF METHANOLIC EXTRACT OF *P.SAJOR CAJU*

Sl. No	Concentration (µg/ml)	% of Inhibition	
		Sample	Standard
1.	100	69±0.12	93.05±0.05
2.	200	79±0.99	94.07±0.33
3.	300	85.05±1.10	96.14±0.46
4.	400	93.13±1.20	97.02±0.50
5.	500	96.66±1.60	98.13±0.55

Table 5: H_2O_2 SCAVENGING ACTIVITY; % INHIBITION ± SE OF AQUEOUS EXTRACT OF *P.SAJOR CAJU*

Sl. No	Concentration (µg/ml)	% of Inhibition	
		Sample	Standard
1.	100	65±0.14	93.15±0.08
2.	200	75±0.08	94.27±0.30
3.	300	82.25±0.67	95.04±0.06
4.	400	92.03±0.08	96.06±0.10
5.	500	95.26±1.03	97.03±0.04

TOTAL EXTRACT YIELD:

Total yield of extract was found to be 5.5 gm/kg in methanolic extract and 4.3 gm/kg in aqueous extract of dried *P. sajor caju (***Table 6** and **Figure 1).**

TABLE 6: TOTAL YIELD OF METHANOLIC AND AQUEOUS EXTRACT OF DRIED *P. SAJOR CAJU*

Sl. No	Methanolic extract	Aqueous extract
1.	5.5±1.2	4.3±1.3

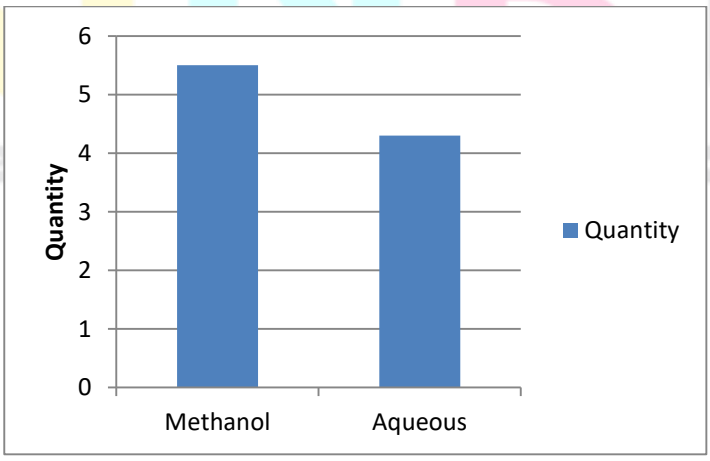

FIG 1: TOTAL YIELD OF *P. SAJOR CAJU* (GM/KG)

ANTI-BACTERIAL ACTIVITY:

Both the gram positive and gram negative bacteria were significantly inhibited by the antibacterial activity of the both extract of *P. sajor caju*. The highest zone of inhibition was observed both methanolic and aqueous extract results showed 15mm in methanolic extract and 13mm in aqueous extract at the concentration of 100µg/ml. The Agar well diffusion method were compared to the standard antibiotic *Streptomycin* at a concentration of 1 mg/ml, whose zone of inhibition is recorded and displayed below the **Figure 2 and Figure 3.**

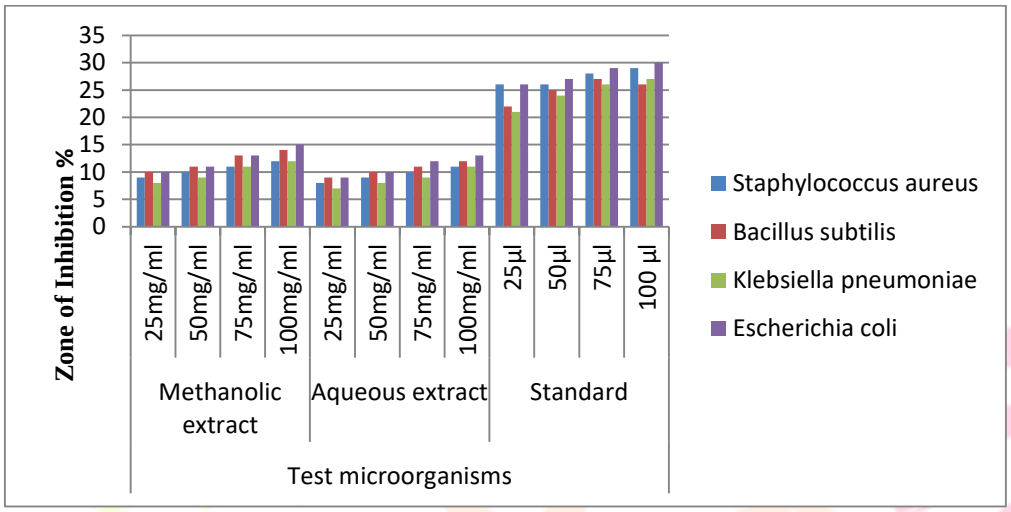

FIG 2: GRAPH FOR ANTIBACTERIAL ACTIVITIES OF METHANOLIC AND AQUEOUS EXTRACT OF *P. SAJOR-CAJU* ALONG WITH STANDARD.

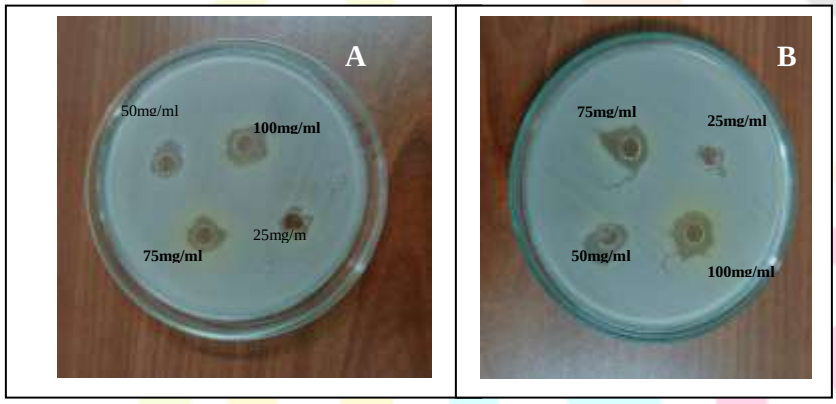

FIG 3: A: ZONE OF INHIBITION OF METHANOLIC EXTRACT AGAINST E.COLI, B: ZONE OF INHIBITION OF AQUEOUS EXTRACT OF *P. SAJOR – CAJU* AGAINST *E.COLI.*

The antibacterial activity of oyster mushrooms was found to be strongest in ethanolic extract against *Staphylococcus aureus* and highest in aqueous extract against *Escherichia coli*, according to a previous publication by Ikon et al. 2018 [9]. In the present study, the antibacterial activity of oyster mushroom against *Escherichia coli* was showed significant inhibition in both extracts.

Thillaimaharani et al. 2013 [14] reported that methanolic extract exhibited the lowest antibacterial activity against to *Staphylococcus aureus*, whereas ethanolic extract demonstrated the best antioxidant activity. In the present study, methanolic extract demonstrated the strongest antioxidant activity in compared to aqueous extract, in both the DPPH and H_2O_2 assays. Also methanolic extract shown highest antibacterial activity against *Escherichia coli*.

CONCLUSION:

The oyster mushroom *Pleurotus sajor caju* showed strong antibacterial and antioxidant qualities in both methanolic and aqueous extracts. Comparatively in this study methanolic extract showed best result than aqueous extract. Therefore, oyster mushrooms may be considered as therapeutic food that has its own natural antibacterial and antioxidant properties. This study would encourage the oyster mushroom cultivation and consumption which can also raise the economic standards in the rural areas.

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