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Analysis of Antioxidant activity, Total Phenol, Total Flavonoid and screening of Phytocomponents in *Pleurotus platypus* and *Pleurotus eous*

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ABSTRACT

Qualitative and quantitative Phytocomponents test were screened in Pleurotus eous and Pleurotus platypus, according to the results tannin, saponin, flavonoids, and Anthroquinones are presented in both the Pleurotus sp. But terpenoids was present in Pleurotus platypus. While Phlobatannins, Steroids, and Cardiac glycosides were absent in both Pleurotus species. Antioxidant activity was determined by DPPH method and the percentage of Pleurotus platypus was 95.2 % in paddy straw substrate. In banana substrates Pleurotus eous shows 87.4 %. Total Phenolic compound was higher 770mg/100gm in Pleurotus platypus which was cultivated in Black pod when compared to other substrates and Pleurotus eous shows highest 695mg/100gm amount in corn straw. The total flavonoid compound was higher in Pleurotus platypus which was cultivated in Teak leaves 473mg/100gm when compared to other substrates and Pleurotus eous shows highest amount 397mg/100gm in Paddy straw.

KeyWords: Antioxidant activity, Total Phenol, Total Flavonoid and screening of phytocomponents.

INTRODUCTION

oyster mushrooms have shown that they serve as repositories of B-vitamins such as niacin, flavin and pyridoxine [1] organic acids such as ascorbate, shikimate, malate and fumarate; carbohydrates such as the _-glucans; monoterpenoid and diterpenoid lipids; proteins such as hydrophobins and trace elements such as selenium [2,3]. These substances have been found

through several *in vitro* and *in vivo* studies to be responsible for the antimicrobial, antioxidant, and antitumor, antihypertensive and antiaging potentials of edible mushrooms The recognition of *Garnodema lucidium* also called reshi as an antioxidant mushroom is due to its phenolic, terpenoids and polysaccharide polypeptide contents. These bioactive compounds mediate biological activities including stimulation of interleukin-12 production, nitric oxide syntheses activation, free radical scavenging and iron chelating properties [4, 5].

In recent decades, the essential oils and various extracts of plants have been of great interest as they have been the sources of natural products. In order to prolong the storage stability of foods, synthetic antioxidants are used for industrial processing. But according to toxicologists and nutritionists, the side effects of some synthetic antioxidants used in food processing such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have already been documented. The antioxidant activity by nitric oxide scavenging assay and also the biocidal activities against various bacteria like Gram negative E.coli, Gram positive S.aureus, M.luteus and B.licheniformis of all the synthesized ligands and complexes.[6].

The many pharmacological effects of phenolic compounds and flavonoids are linked to their ability to act as strong antioxidants and free radical scavengers, to chelate metals, and to interact with enzymes, adenosine receptors, and biomembranes [7]. It was reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds [8]. Phenolic compounds, especially phenolic acids and flavonoids, are ubiquitously present in vegetables, fruits, seeds, tea, wines and juices; thus they are an integral part of the human diet. Recently, they have received much attention since many epidemiological studies suggest that consumption of polyphenol-rich foods and beverages is associated with a reduced risk of cardiovascular diseases, stroke and certain forms of cancer.

EXPERIMENTAL SECTION

Sample Preparation

Aqueous extract of *Pleurotus eous and Pleurotus platypus* samples were used to carry out the Qualitative and Quantitative analysis using standard procedures to identify the phyto constituents as described by [9, 10, 13&14].

Phytochemical Screening

Test for Tannins

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for browrish green or a blue-black colouration.

Test for Phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatinins.

Test for Saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a

stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Flavonoids
5ml of the diluted ammonia solution was added to a portion of aqueous filterate of plant extract followed by the addition of concentrated sulphuric acid formation of yellow color.

Test for Steroids
Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H2S04. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for Terpenoids
Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for Cardiac glycosides
2 ml of glacial acetic acid containing one drop of ferric chloride solution was add to 5ml of the aloe vera extract. This was sunder layer with 1ml of concentrated sulphuric acid. Formation of a brown ring at the interface indicates the presence of cardiac glycosides.

Test for Anthroquinones
0.5gm of the extract was boiling with 10ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette out into another test tube & 1ml of diluted ammonia was added. The resulting solution was observed for colour change.

Quantitative Analysis
Quantitative Analysis of *Pleurotus eous and Pleurotus platypus was* carried out by described method as Alkaloid [11, 12 & 13].

Antioxidant Activity – DPPH Method
Pleurotus eous and *Pleurotus platypus* was smashed were extracted with 250ml of methanol using the soxhlet extractor for 72 hrs at a temperature not exceeding the boiling point of the solvent. From the extracted sample antioxidant activities was carried out by [15] and FRAP Method [16].

Estimation of Total Phenol
Standard phenol solution:
100mg of Gallic acid is dissolved in little amount of distilled water and the volume is made up to 100ml with distilled water. Different concentration of the standard was obtained by appropriate dilution with distilled water. The concentration of the solution will be 100micro gram/ml.

0.5ml of freshly prepared sample was taken in the test tubes. 8ml of distilled water was added to all the tubes. 0.5ml of Folin Ciocalteau reagent added to all the tubes. All the tubes were kept in B.O.D for incubation at 40ċ for 10 minutes. Then, 1ml of sodium carbonate solution was added

to all the tubes. Then, the tubes were kept in the dark for incubation for one hour. The colour so developed was read spectrophotometrically at765 nm [17].

Estimation of Total Flavonoids

Standard phenol solution:

100mg of tannic acid is dissolved in little amount of distilled water and the volume is made up to 100ml with distilled water. Different concentration of the standard was obtained by appropriate dilution with distilled water. The concentration of the solution will be 100micro gram/ml [17].

Procedure:

0.5ml of aqueous extract of sample is diluted with 3.5ml of distilled water at zero time. 0.3ml of 5% Sodium Nitrate was added to the tubes. After 5 minutes, 0.3ml of 10% Aluminium chloride was added to all the tubes. At 6^{th} minute, 2ml of 1M Sodium Hydroxide was added to mixture. Immediately, the contents of the reaction mixture were diluted with 2.4ml of distilled water and mixed thoroughly and read at 510nm.Gallic acid was used as the standard compound

RESULTS

Table: 1. Screening of Phytocomponents in *Pleurotus eous* & *Pleurotus platypus*

S.No	Parameter	*Pleurotus eous*	*Pleurotus platypus*
1.	Tannin	Presence	Presence
2.	Phlobatannins	Absence	Absence
3.	Saponin	Presence	Presence
4.	Flavonoids	Presence	Presence
5.	Steroids	Absence	Absence
6.	Terpenoids	Absence	Presence
7.	Cardiac glycosides	Absence	Absence
8.	Anthroquinones	Presence	Presence

Estimation of Antioxidant activity

Pleurotus platypus and *Pleurotus eous* was cultivated in different substrates and harvested. Fresh fruit bodies of above species were used to estimate the amount of antioxidant activity by DPPH method. According to the results antioxidant activity was higher 95.2mg/100gm in *Pleurotus platypus* which was cultivated in paddy straw when compared to other substrates and *Pleurotus eous* 87.4mg/100gm. Results were shown in table: 2

Table: 2 Antioxidant activity of *Pleurotus platypus* in different substrates

S. No	Test for Antioxidant activity	DPPH Method (Inhibition %)				
		S1	**S2**	**S3**	**S4**	**S5**
1.	***Pleurotus platypus***	95.2	93.6	92.7	91.4	93.7
2.	***Pleurotus eous***	86.7	87.2	85.5	86.8	87.4

Pleurotus platypus and *Pleurotus eous* was cultivated in different substrates and harvested. Fresh fruit bodies of above species were used to estimate the amount of total phenol. According to the results Phenolic compound was higher 770mg/100gm in *Pleurotus platypus* which was cultivated in Black pod when compared to other substrates and *Pleurotus eous* shows highest 695mg/100gm amount in corn straw. Results were shown in table: 3

Table: 3Total Phenols activity of *Pleurotus platypus* in different substrates

Sl. No.	Name of the Sample	Gallic acid equivalent (mg/100g)				
		Paddy Straw	corn straw	Black Pod	Teak Leaves	Banana Leaves
1.	*P. platypus*	738	755	770	725	735
2.	*P. eous*	678	695	680	635	655

Pleurotus platypus and *Pleurotus eous* was cultivated in different substrates and harvested. Fresh fruit bodies of above species were used to estimate the amount of total phenol. According to the results total flavonoid compound was higher in *Pleurotus platypus* which was cultivated in Teak leaves 473mg/100gm when compared to other substrates and *Pleurotus eous* shows highest amount 397mg/100gm in Paddy straw. Results were shown in table: 4

Table: 4 Total Phenols activity of *Pleurotus platypus* in different substrates

Sl. No.	Name of the Sample	Tannic acid equivalent (mg/100g)				
		Paddy Straw	corn straw	Black Pod	Teak Leaves	Banana Leaves
1.	*P. platypus*	458	446	465	473	453
2.	*P. eous*	397	383	375	390	375

CONCLUSION

The many pharmacological effects of phenolic compounds and flavonoids are linked to their ability to act as strong antioxidants and freeradical scavengers, to chelate metals, and to interact with enzymes, adenosine receptors, and biomembranes. Qualitative and quantitative Phytocomponents test were screened in *Pleurotus eous* and Pleurotus platypus, according to the results Tannin, Saponin, Flavonoid, and Anthroquinones are presented in both the *Pleurotus sp*. But Terpenoids was present in Pleurotus platypus. While Phlobatannins, Steroids, and Cardiac glycosides were absent in both Pleurotus species. Antioxidant activity was determined by DPPH method and the percentage of *Pleurotus platypus* was 95.2 **%** in paddy straw substrate. In banana substrates *Pleurotus eous* shows 87.4 %**.** According to the results Phenolic compound was higher 770mg/100gm in *Pleurotus platypus* which was cultivated in Black pod when compared to other substrates and *Pleurotus eous* shows highest 695mg/100gm amount in corn straw. The total flavonoid compound was higher in *Pleurotus platypus* which was cultivated in Teak leaves 473mg/100gm when compared to other substrates and *Pleurotus eous* shows highest amount 397mg/100gm in Paddy straw.

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