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STUDIES ON PRELIMINARY PHYTOCHEMICAL, ANTIOXIDANT AND ANTIINFLAMMATORY ACTIVITIES OF PLEUROTUS FLORIDA BY IN VITRO METHOD

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Abstract

The aim of the present work was to study preliminary phytochemical, antioxidant and anti-inflammatory activities of the edible mushroom Pleurotus Florida by invitro method. Powder microscopic characters was studied. The physical evaluation included determination of total ash, determination of acid insoluble ash, determination of water soluble ash, determination of moisture content (loss on drying). The chemical evaluation included qualitative evaluation like test for polysaccharide, test for mucilage, test for amino acid, test for proteins, test for saponins, test for alkaloids, test for tannins, test for flavanoids and quantitative evaluation like thin layer chromatography, column chromatography , IR analysis. The antioxidant activity was carried out by Ferric Reducing Antioxidant Power (FRAP) Assay Method. Invitro anti inflammatory activity was studied using Human RBC Membrane (HRBC) Stabilisation Method and Protein Denaturation Bioassay Method. The value of antioxidant activity of mushroom ranged between 57.79-73.8%.It was found that the methanolic extracts of Pleurotus Florida are effective in inhibiting heat induced hemolysis of HRBC at different concentration. In Protein Denaturation Bioassay Method, the in vitro anti inflammatory effect of mushroom extract was evaluated against denaturation of egg albumin. In conclusion edible mushrooms can be excellent source of Micronutrients, Antioxidant components and Anti inflammatory components. So it could be recommended for formulating Antioxidant, Anti inflammatory dietary supplement.

Key Words: Antioxidant activity, Anti-inflammatory activity**,** Pleurotus Florida.

Introduction

Pharmacognosy is the study of drugs of natural origin. The term comes from two Greek words: "pharmakon" meaning drug or medicine, and "gnosis" meaning knowledge. The American Society of Pharmacognosy defines

pharmacognosy as "the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources".

Plant preparations are said to be medicinal or herbal when they are used to promote health beyond basic nutrition. The part of pharmacognosy focusing on use of crude extract or semi pure mixtures originating from nature namely phytotherapy. Such substance extends beyond the category of crude drugs and their derivatives and includes a variety of commercial products. The aim of the present study involves determination of antioxidant and anti-inflammatory activity of edible mushroom Pleurotus florida.

Mushrooms are important constituents of minor forest produce, on the most abundant biomolecule of this biosphere, that is, cellulose. Presently mushrooms are regarded as a macro-fungus with a distinctive fruiting body which can be either epigeous or hypogeous and large enough to be seen with the naked eyes and to be picked by hand (Chang and Miles, 1992). Only fruiting body of the mushroom can be seen whereas the rest of the mushroom remains underground as mycelium. Mushrooms offer tremendous applications as they can be used as food and medicines besides their key ecological roles. They represent as one of the world's greatest untapped resources of nutrition and palatable food of the future. Mushrooms have been found effective against cancer, cholesterol reduction, stress, insomnia, asthma, allergies and diabetes (Bahl, 1983). Due to high amount of proteins, they can be used to bridge the protein malnutrition gap. Mushroom used as nutrient supplements to enhance immunity in the form of tablets. Due to low starch content and low cholesterol, they suit diabetic and heart patients. One third of the iron in the mushrooms is in available form. Their polysaccharide content is used as anticancer drug. Even, they have been used to combat HIV effectively (Nanba, 1993; King, 1993). Biologically active compounds from the mushrooms possess antifungal, antibacterial, antioxidant and antiviral properties, and have been used as insecticides and nematicides as well. Thus keeping in view the tremendous applications of mushrooms, the present study reviews different aspects of mushrooms towards human health benefits[1]. The standardization of plant drug was done by authentication, organoleptic charecters, morphological, microscopical, physical, chemical and pharmacological evaluation methods according to WHO guidelines.further IR analysis, antioxidant and anti-inflammatory activity was performed

Materials and Methods

Materials: The mushroom was purchased from Thiruvalla. The antioxidant activity was carried out using phosphate buffer, potassium hexocyanoferrate, trichloroacetic acid, ferric chloride and ascorbic acid was taken as control. The

invitro anti-inflammatory activity was studied using alsever solution, phosphate buffer, hyposaline, standard drug hydrocortisone and control. In protein denaturation bioassay method standard drug dimethyl formamide, phosphate buffer, albumin solution was used.

Methods

1. Microscopic Characters

Microscopical study of the organized crude drug is an important parameter for the evaluation.

Powder Microscopy

Dried powder of mushroom was taken in a test tube and mixed with potassium hydroxide. Heated for few minutes. Then the solution was allowed to cool. Water was added into the test tube and allows to settle down. This procedure was reapeted until a clear supernatant solution is obtained. Decant the supernatant solution and mixture was taken on a watch glass. Added concentrated sulphuric acid and phluroglucinol and mixed well. Then it was taken on a glass slide and adds glycerine. Observed under the microscope and identify various powder characters[10].

The mushroom extract contained the following powder characters.

- Endosperm (fig 1)
- Mucilage (fig 2)
- Calcium crystals (fig 3)

2. Physical Evaluation

1. Determination of total ash

Incinerated about 2 to 3 gm accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450 degree until free from carbon, cooled and weighed. If a carbon free ash was not obtained in this way, exhausted the charred mass with hot water, collected the residue on an ashless filter paper, incinerated the residue and the filter paper, added the filterate evaporate to dryness, and ignited at a temperature not exceeding 450 degree. Calculated the percentage of ash with reference to the air dried drug[9].

2. Determination of acid insoluble ash

To the crucible containing total ash added 25ml of dilute hydrochloric acid. Collected the insoluble matter on an ashless filter paper and washed with hot water until the filterate is neutral. Transfered the filter paper containing the insoluble matter to the original crucible. Dried on a hot plate and ignited to constant weight. Allowed the residue to cool in a suitable dessicator for 30min and weighed without delay. Calculated the content of acid insoluble ash with reference to the air dried drug.

3. Determination of water soluble ash

Boiled the ash for 5 min with 25ml of water, collected insoluble matter in a crucible or on an ashless filtere paper, washed with hot water and ignited for 15 min at a temperature not exceeding 450 degree, subtracted the weight of the insoluble matter from the weight of the ash, the difference in weight represents the water soluble ash. Calculated the percentage of water soluble ash with reference to the air dried drug.

4. Determination of moisture content (Loss on drying)

Weighed about 1gm of the powdered drug into a weighed flat and thin porcelain dish. Dried in the oven at 100 degree celsius and cooled it. It was continued until a constant weight was obtained and loss of weight was thus determined.

3. Chemical Evaluation

1. Qualitative Evaluation

By phytochemical screening the presence of polysaccharide, mucilage, amino acid, proteins, saponins, tannins, flavanoids, alkaloids was confirmed in the mushroom extract.(table 1)

2. Quantitative Evaluation

A. Extraction Method

For the present study, all the procured, selected and dried species of edible mushrooms was cleaned to remove any residual compost/soil and subsequently air dried in the oven at 50^{o}C for 48hours. The entire dried mushroom was ground to fine powder and stored in air tight dessicator at room temperature for further analysis. Then dried mushroom powder was homogenized in 70% ethanol. The homogenate was stirred on a magnetic stirrer for 2h at 4^{o}C. The mixture was centrifuged at 10,000rpm for 20 minutes. The supernatant was concentrated by using the vacuum evaporator[13].

B. Thin layer chromatography

Thin layer chromatography (TLC) is a chromatographic technique used to separate mixture of components into individual components by using stationary phase and mobile phase. Thin layer chromatography is performed on glass plate which is coated with a slurry of silica gel G. This layer of adsorbent is known as stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as mobile phase) is drawn up the plate via capillary action. Different analytes ascend the TLC plate at different rates, and separation is achieved.(figure 4,5,6) (table 2).

C. Column Chromatography

In column chromatography, the stationary phase a solid adsorbent (silica gel G) was placed in a vertical glass column and the mobile phase a liquid was added to the top.

1. Preparation of column

The column was packed by simple dry pack method. To fill the column pours the gel into the column by pouring using a beaker. After filling tap the column on bench top to pack the silica gel. When properly packed the silica gel fills the column to just below the indent. This leaved a space of 10-30cm on the top of the adsorbent for the addition of solvent. Clamped the filled column securely to a ring stand using three pronged clamp[5].(figure 7)

2. Pre-eluate the column

Pre eluted the column using a non polar solvent like hexane. Added hexane to the top of the silica gel. The solvent flows slowly down the column. Allowed the top level to drop by gravity[6].

3. Load sample on to the silica gel column

In the wet method the sample to be purified (separated in to components) was dissolved in a small amount of solvent. The solution was loaded on to the column. The sample was allowed to sink in to the column. Once it was in the column, fresh eluting solvent was added to the top and elution begins to process.(figure 8)

4. Elute the column

Added solvent to column without disturbing column through the side of the tube. Allowed the solvent to the very top of the silica, do not allow silica go dry. Added fresh solvent as necessary. The sample moved through the column. Coloured sample was collected. The beaker was changed as soon as the colored compound begins to elute[14].(figure 9)

5. Elute the column with second elution solvent

Since separating one or more compound, a number of eluting solvents was used. The solvents used are chloroform, ethyl acetate, ethanol, water etc in different ratios

- Hexane-100ml
- Chloroform-50ml+ethyl acetate-50ml
- Ethylacetate-50ml+ethanol 50ml
- Ethanol 50ml+water 50ml

6. Analyze the fractions

Performed IR to analyze elutes. One of the compositions of the each fraction was known, the fraction containing same compound was combined.

D. IR Analysis

The infrared spectrum of a sample was recorded by passing a beam of infrared light through the sample. When the frequency of the IR was the same as the vibrational frequency of a bond, absorption occurs .Examination of the transmitted light reveals how much energy was absorbed at each frequency (wavelength).This can be achieved by scanning the wavelength range using a monochromator. Alternatively the whole wavelength range was measured at once using a fourier transform instrument and then a transmittance or absorbance spectrum is generated using a dedicated procedure[8]. Analysis of the position shape and intensity of peaks in this spectrum reveals details about the molecular structure of the sample[4]. This technique worked almost exclusively on samples with covalent bonds .Simple spectra was obtained from samples with few IR active bonds and high levels of purity. More complex molecular structures lead to more absorption bands and more complex spectra. The technique was used for the characterization of very complex mixtures[7]. Spectra issues with infrared fluorescence are rare. The eluate of mushroom obtained from the column chromatography was subjected to IR spectroscopy and the graphs were obtained which were interpreted for various organic (figure 10,11,12,13) (table 3,4,5,6)

Pharmacological Evaluation

1. Antioxidant Activity

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's). Mushrooms are rich sources of proteins, vitamins and minerals. They are also having antioxidant activity. Antioxidants are chemical compounds which protect cells from damage by free radicals. The main characteristic of an antioxidant is its ability to trap free radicals[2]. (figure-14). For the present study, Antioxidant activity of edible mushroom *Pleurotus florida* was collected. These samples of mushrooms were identified by botanist. Antioxidant activity of these mushrooms was determined by Ferric Reducing Antioxidant Power Assay (FRAP) method, using 80% ethanolic extract of Pleurotus *florida.* To observe the antioxidant activity of these mushrooms, all the procured, selected and dried species of edible mushrooms were cleaned to remove any residual compost/soil and subsequently air dried in the oven at 50^{o}C for 3h. The entire dried mushroom was ground to fine powder and stored in air tight dessicator at room temperature for further analysis. Then 10 grams dried mushroom

powder was homogenized in 70% ethanol. The homogenate was stirred on a magnetic stirrer for 2h at 4^{o}C. The mixture was centrifuged at 10,000rpm for 20 minutes. The supernatant was concentrated by using the vacuum evaporator. The ascorbic acid was taken as control[3].

Ferric Reducing Antioxidant Power (FRAP) Assay Method (table 7)

Mushroom Pleurotus florida extract of various concentration was mixed with 0.75ml of phosphate buffer (0.2M, P^{H} 6.6) and 0.75ml of potassium hexacyanoferrate ($K_3Fe(CN)_6$) (1%w/v) followed by incubating at 50^{0}c in a water bath for 20min. the reaction was stopped by adding 0.75ml of trichloroaceticacid (TCA)solution (10%) and then centrifuged at 3000 r/min for 10 min. 1.5ml of supernatant was mixed with 1.5ml of distilled water and 0.1ml of ferric chloride solution (0.1%w/v) for 10min. The absorbance was measured spectrophotometrically at 700nm as the reducing power. The percent inhibition over radical shown by different mushroom extract at different concentration was calculated by using the formula[15]:

Percent inhibition = $100*A_0-A_{b/}A_0$

A_0 – Absorbance of the reaction mixture

A_b - Absorbance of the blank

The principle of FRAP (ferric reducing antioxidant power) method:

The FRAP (ferric reducing antioxidant power) method relies on the reduction by the antioxidants, of the complex ferric ion-TPTZ (2, 4, 6-tri (2-pyridyl)- 1,3,5-triazine). The binding of Fe2+ to the ligand creates a very intense green color. The absorbance can be measured to test the amount of iron reduced and can be correlated with the amount of antioxidants. Ascorbic acid was used as references.(Figure 15,16) (table 8). It was revealed from the results that all the concentrations of the mushroom extract and Ascorbic acid (control) showed antioxidant activity. However, the maximum antioxidant activity was observed at highest concentration and lowest activity at lower concentrations. The antioxidant activity of mushroom extracts might be due to phenolics and other secondary metabolites accumulated by mushroom. . It is also suggested that mushroom rich in antioxidant activity have been shown to play an important role in prevention of cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's). They are highly nutritive as they contain good quality proteins, vitamins and minerals. They are low calorie food with very little fat and are highly suitable for obese persons. With no starch and very low sugars, they are the delight of the diabetics. In adequate quantities and low in sugars they can serve as medicinal foods for diabetes.

2. Invitro Antiinflammatory Activity

Inflammation is the reaction of living tissues to injury, infection or irritation. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis etc. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane1. HRBC or erythrocyte membrane is analogous to the lysosomal membrane2 and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of human red blood cell membrane (HRBC) by hypo tonicity induced membrane lysis can be taken as an in vitro measure of anti inflammatory activity of the drugs or mushroom extracts.

Method I

The Human RBC Membrane Stabilisation Method

Preparation of HRBC suspension

The human RBC membrane stabilisation method was used for the study. Blood was collected from healthy human volunteer who has not taken any NSAID for 2 weeks prior to the experiment mix with equal volume of alsever solution (anticoagulant) (2%dextrose ,0.8%sodium citrate,0.5%citric acid,0.42%sodium chloride)and centrifuge at 3000 rpm. The packed cells were washed with isosaline and 10%v/v suspension was made.

Heat Induced Haemolysis

The principle involved was stabilisation of human RBC membrane by hypotonicity induced membrane lysis. The assay mixture contain 1ml phosphate buffer,(ph 7.4,0.15 molar)2ml hyposaline (0.36%),0.5 ml hrbc suspension(10%v/v) with 3ml,6ml,9ml,12ml,15ml plant extracts and standard drug hydrocortisone and control (distiled water instead of hyposaline to produce 100% haemolysis) were incubated at 37 degree celsius for 30 min and centrifuge respectively.The haemoglobin content in the suspension was estimated using spectrophotometer at 560nm.The percentage haemolysis of hrbc membrane can be calculated as follows: (table 9)

% Hemolysis = (Optical density of Test sample / Optical density of Control) X 100

It was assumed that the haemolysis produced by the control is100%.

The percentage of HRBC membrane stabilisation can be calculated as:

% Protection = 100 – [(Optical density of Test sample / Optical density of Control) X 100]

The inhibition of hypo tonicity induced Human RBC membrane i.e., stabilization of HRBC membrane was taken as a measure of the anti inflammatory activity. The percentage of membrane stabilization for methanolic extracts of *pleurotus florida* and hydrocortisone were done. Methanolic extracts of *pleurotus florida are* effective in inhibiting the heat induced hemolysis of HRBC at different concentrations (3, 6, 9, 15 ml) as shown in table. It showed the maximum inhibition 61.3580% at 15 ml. with the increasing concentration the membrane hemolysis is decreased. And membrane stabilization / protection were increased. Hence anti inflammatory activity of the extracts was concentration dependent[12].

Method II

Protein Denaturation Bioassay Method

The standard drug (ibuprofen) mushroom extract were dissolved in minimum quantity of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2M, p^H 7.4). 5ml, 10ml, 15ml mushroom extract and standard drug ibuprofen was mixed with albumin solution in phosphate buffer and incubated at 27^0c in incubator for 15 minutes. Denaturation was induced by keeping the reaction mixture at 60^0c in water bath for 10 minutes. After cooling the turbidity was measured at 660 nm (UV- visible spectrophotometer). Percentage of inhibition of denaturation was calculated from control. Where no drug was added each experiment was done in triplicate and average is taken. The ibuprofen was used as standard drug. The percentage inhibition of denaturation was calculated by using following formula.(table 10)

$$\% \text{ INHIBITION} = 100*(A_o - A_b)/A_o$$

In the present investigation, the in vitro anti-inflammatory effect of mushroom extract was evaluated against denaturation of egg albumin. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation. Ibuprofen was used as standard drug.

Results

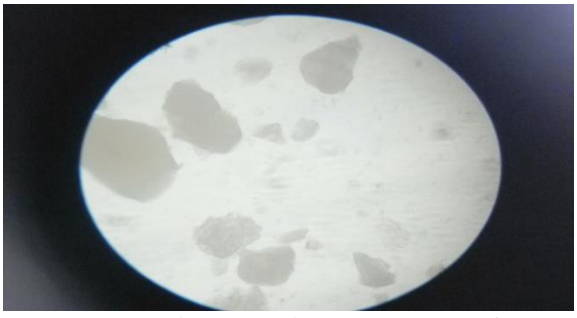

Figure-1: Powder microscopic characters of Endosperm.

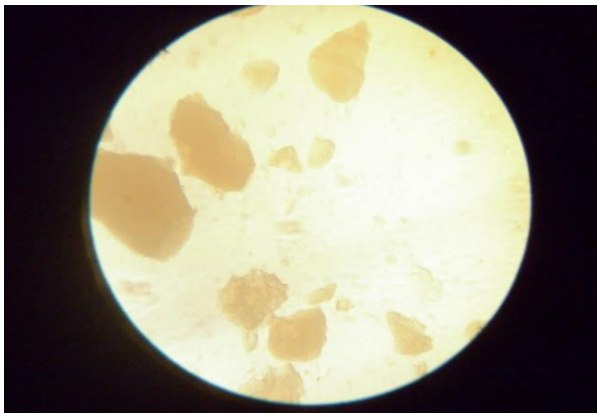

Figure-2: Powder microscopic characters of Mucilage.

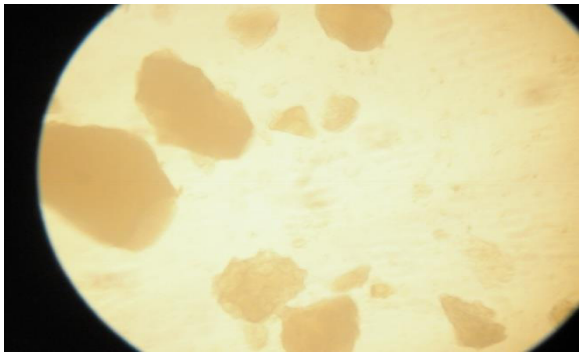

Figure 3: Powder microscopic characters of Calcium Crystals.

Figure-4: Thin layer chromatography of phenol.

Figure-5: Thin layer chromatography of flavanoids.

Figure 6: Thin layer chromatography of alkaloids.

Figure 7: Preparation of column.

Figure 8: Application of sample.

Figure 9: Collection of Eluate.

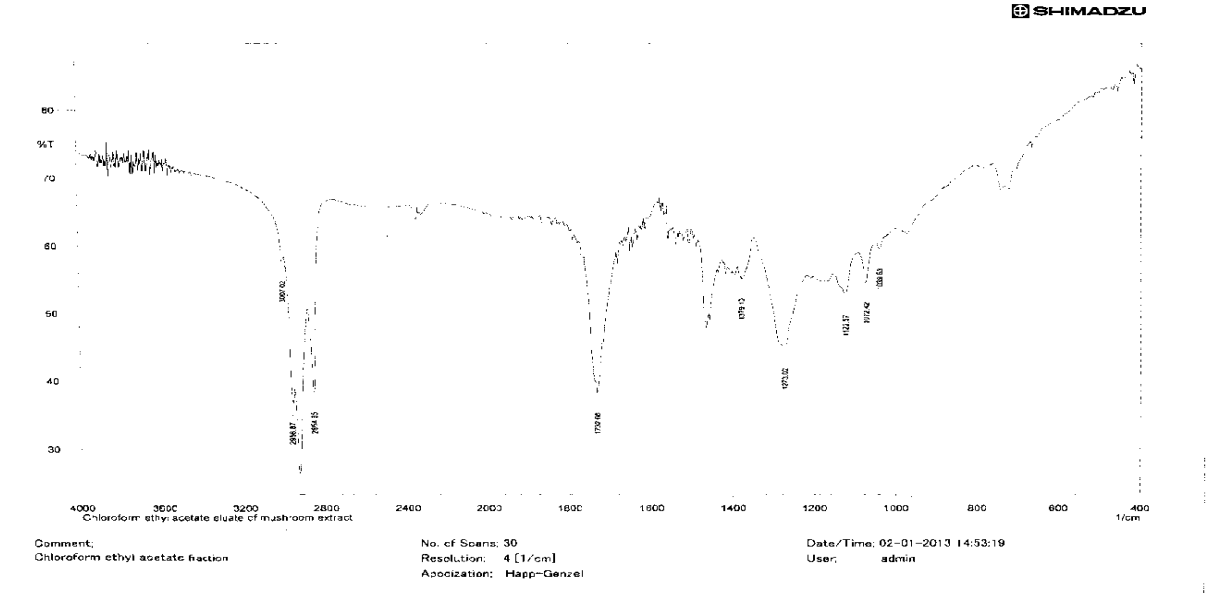

Figure 10: IR Spectrum of Chloroform: Ethyl acetate fraction.

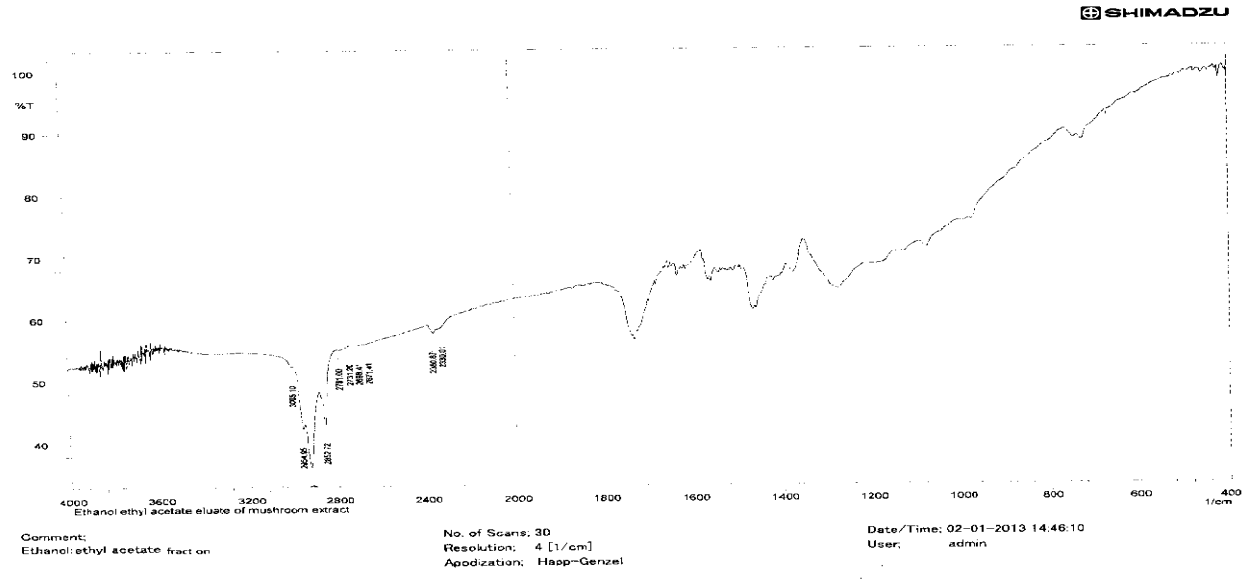

Figure 11: IR spectrum of ethanol: ethyl acetate fraction.

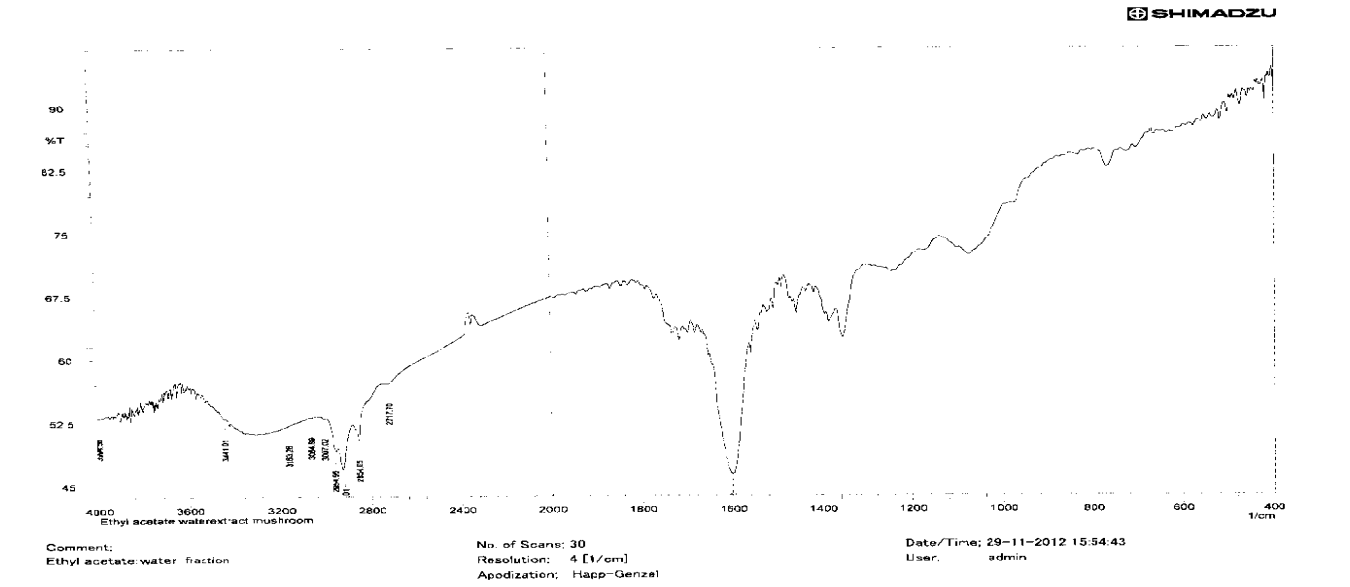

Figure 12: IR Spectrum of ethyl acetate: water fraction.

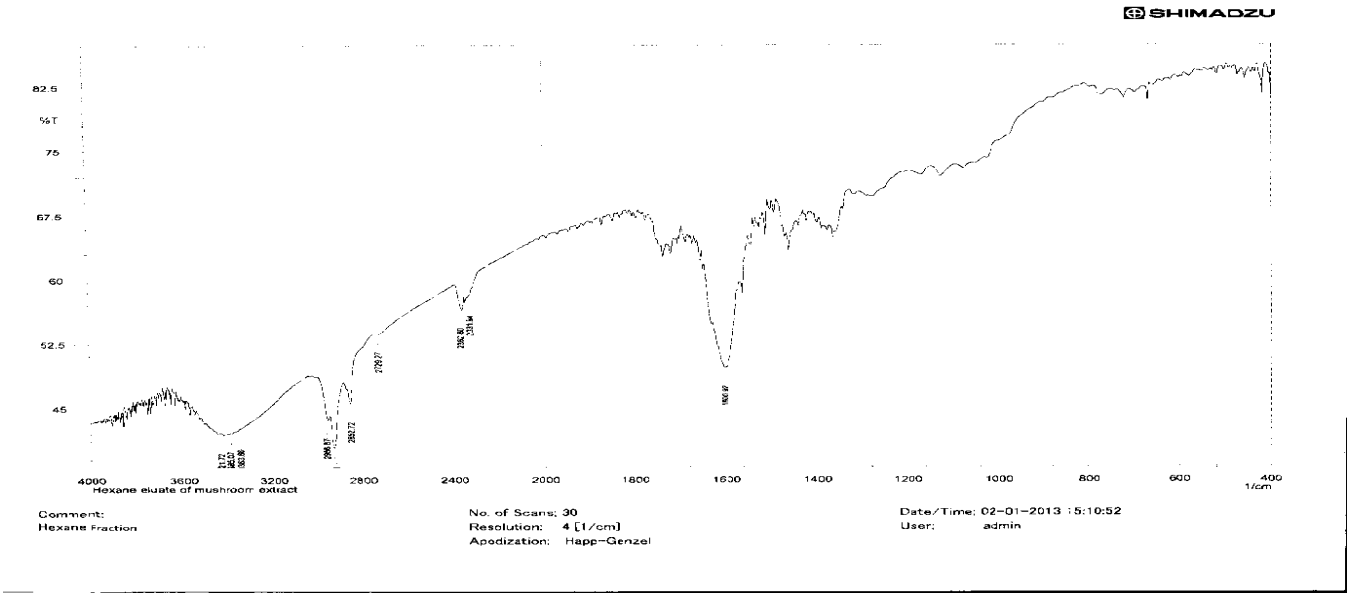

Figure 13: IR spectrum of hexane fraction.

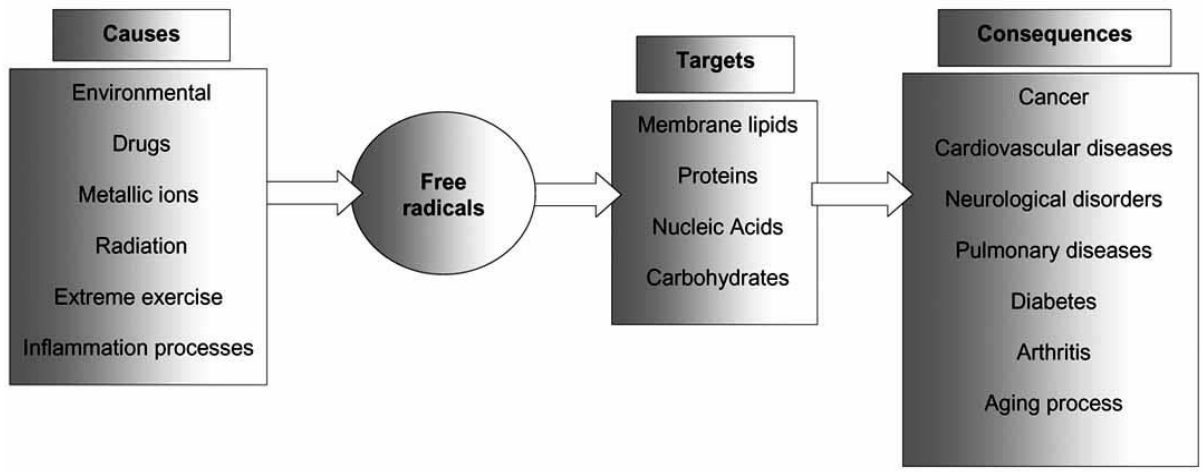

Figure 14: Anti oxidant activity.

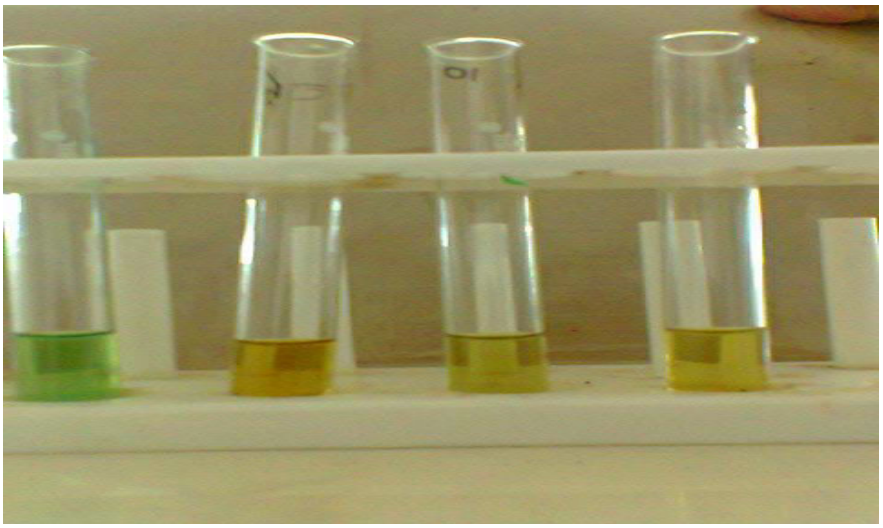

Figure 15: Ferric Reducing Antioxidant Power Assay Method (FRAP).

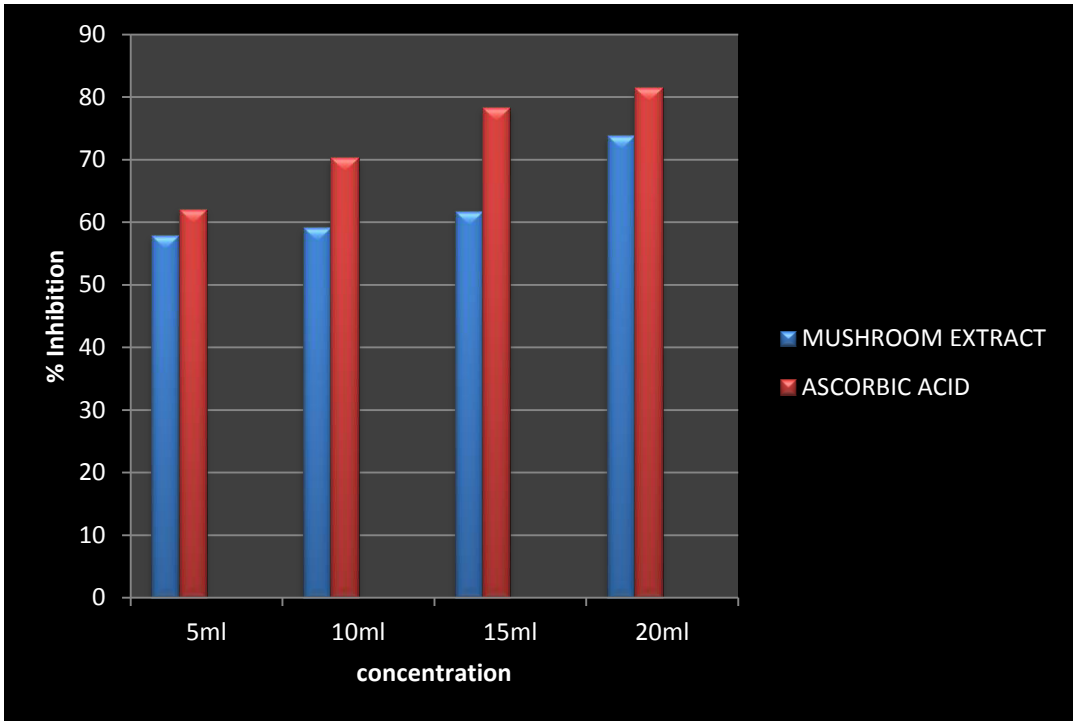

Figure 16: % INHIBITION V/S CONCENTRATION GRAPHS.

Table-1: Qualitative Analysis.

SERIAL NO.	CHEMICAL TEST	OBSERVATION
1	**Test for polysaccharide**	
	a) Molisch test	+
	b) Fehlings test	-
	c) Benedicts test	-
2	**Test for mucilage**	
	a) Aqueous extract+KOH solution	+
	b) Aqueos extract+ruthenium red	+
3	**Test for amino acid**	
	a) Millons test	+
	b) Ninhydrin test	+
4	**Test for proteins**	
	a) Biuret test	+
	b) Millons test	+

	c) Xanthoprotein test	+
5	**Test for saponins**	
	a) Sample is treated with water and shaken well	+
	b) Haemolytic test	+
6	**Test for alkaloids**	
	a) Mayers test	+
	b) Wagners test	+
	c) Hagers test	+
	d) Dragendroffs test	+
7	**Test for tanins**	
	a) To the solution add 5%ferric chloride	+
	b) To the solution add lead acetate	+
	c) To the solution add bromine water	+
	d) To the solution add potassium permanganate	+
	e) To the acetic acid	+
8	**Test for flavanoids**	
	a) Sample is treated with lead acetate	+
	b) Sample solution is treated with sodium hydroxide and dilute hydrochloric acid	+

Table-2: Thin layer chromatography.

SL NO	STATIONARY PHASE	MOBILE PHASE	R_f VALUE	ACTIVE CONSTITUENTS
1	Silica gel G	Ethyl acetate : formic acid : acetic acid: water (100:11:11:27)	Spot 1 = 0.69	Flavanol[11] Quercetin
2	Silica gel G	methanol: ammonium hydroxide (200:3)	Spot 1=0.1829 Spot 2=0.4152	Atropine Thebaine
3	Silica gel G	aceticacid: chloroform (1:9)	Spot 1=0.55	Parahydroxy benzoic acid

Table-3: Interpretation of hexane fraction.

MUSHROOM EXTRACT IR SPECTROSCOPICAL VALUES	INTERPRETATION (HEXANE FRACTION)
2956.87	ALKANES
2852.72	ALKANES
2729.27	ALDEHYDE
2362.80	ALKYNES
2331.94	ALKYNES
1600.92	PHENYL

Table-4: Interpretation of Chloroform and Ethyl acetate fraction.

MUSHROOM EXTRACT IR SPECTROSCOPICAL VALUES	INTERPRETATION (CHLOROFORM AND ETHYLACETATE FRACTION)
3007.02	AROMATIC RING
2956.87	ALKANES/HYDROXYL GROUP OF ALCHOHOL, PHENOL,HYDROGEN BONDED ACID
2854.65	ALKANES/HYDROXYL GROUP OF ALCHOHOL, PHENOL,HYDROGEN BONDED ACID
1732.08	KETONES/ALDEHYDES/ACID/ESTER
1379.70	NITRO GROUP
1273.02	ETHER/CARBOXYLIC ACID/ALCHOHOL/ESTER
1272.22`	ETHER/CARBOXYLIC ACID/ALCHOHOL/ESTER
1072.12	ETHER/CARBOXYLIC ACID/ALCHOHOL/ESTER
1039.63	ETHER/CARBOXYLIC ACID/ALCHOHOL/ESTER

Table-5: Interpretation of Ethanol and Ethyl acetate fraction.

MUSHROOM EXTRACT IR SPECTROSCOPICAL VALUES	INTERPRETATION (ETHANOL AND ETHYL ACETATE FRACTION)
3005.10	AROMATIC RING
2954.95	ALKANES
2852.72	ALKANES
2791.00	ALDEHYDE
2731.20	ALDEHYDE
2698.41	ALDEHYDE
2671.41	ALDEHYDE

Table-6: Interpretation of Ethyl acetate and Water fraction.

MUSHROOM EXTRACT IR SPECTROSCOPICAL VALUES	INTERPRETATION (ETHYL ACETATE AND WATER FRACTION)
3441.01	AMINES
3163.26	CARBOXYLIC ACID
3064.89	ALKENES/AROMATIC RING
3007.02	ALKENES/AROMATIC RING
2954.95	ALKANES
2854.65	ALKANES
2717	ALDEHYDE
1600	PHENYL

Table-7: Ferric Reducing Antioxidant Power Assay Method.

SL.NO	MUSHROOM EXTRACT(mg/ml)	%INHIBITION	ASCORBIC ACID(mg/ml)	%INHIBITION
1	5	57.79	5	62.1
2	10	59.14	10	70.4
3	15	61.8	15	78.3
4	20	73.8	20	81.5

Table 8: The principle of FRAP (ferric reducing antioxidant power) method.

Antioxidant capacity assay	Principle of the method	End-product determination
Ferric Reducing Antioxidant Power Assay (FRAP)	Antioxidant reaction with a Fe(III) complex	Colorimetry

Table-9: Calculation of heat induced haemolysis.

Sl NO:	Sample	Absorbance(nm)	% haemolysis	% protection
1	Mushroom extract:3ml	0.1067	45.67	54.33
2	Mushroom extract:6ml	0.0702	30.06	69.94
3	Mushroom extract:9ml	0.0519	22.23	77.77
4	Mushroom extract:12ml	0.0377	16.15	83.85
5	Mushroom extract:15ml	0.0347	14.88	85.12
6	Standard drug: hydrocortisone	0.0148	6.35	93.65
7	Control-Distilled water	0.2338	0	100

Table-10: Percentage inhibition of protein denaturation bioassay method.

Sl no:	Sample	% inhibition
1	Ibuprofen (std drug)	98.50
2	Mushroom extract: 5ml	72.00
3	Mushroom extract: 10 ml	79.84
4	Mushroom extract: 15 ml	85.35

Discussion

The in vitro antioxidant and anti-inflammatory of edible mushroom Pleurotus florida was studied. The investigation had opened a possibility of use of these mushroom in drug development for human consumption possibly for the treatment of various inflammatory conditions and various disease associated with oxidative stress such as cardiovascular disease, diabetes, cancer etc. Mushroom Pleurotus florida belonging to Class Basidiomycetes and Family Agaricaceae is popularly known as 'dhingri' in India. Microscopical study of the organized crude drug is an important parameter for the evaluation. Microscopical characters of the mushroom powder was studied and contains the following powder characters such as endosperm, Mucilage, Calcium crystals. Various physical parameters like total cash value, acid insoluble ash, water soluble ash, loss on drying was performed. Total ash valve of the sample was found to be 1.66%w/w. Acid insoluble ash of the sample was found to be 0.51%w/w. water soluble ash of the sample was found to be 0.34%w/w. By phytochemical screening the presence of polysaccharide, mucilage, amino acid, proteins, saponins, tannins, flavanoids, and alkaloids was confirmed in the mushroom extract. Thin layer chromatography (TLC) is a chromatographic technique used to separate mixture of components into individual components by using stationary phase and mobile phase. Thin layer chromatography was performed and contained:flavanol, quercetin, atropine, thebaine, parahydroxybenzoic acid. In column chromatography, the stationary phase a solid adsorbent (silica gel 60-120 mesh) was placed in a vertical glass column and the mobile phase a liquid was added to the top. Performed IR to analyze of fraction of the column. Chemical evaluation like Qualitative Evaluation and Quantitative Evaluation such as thin layer chromatography, Column chromatography, IR Analysis was performed. The total antioxidant activity of edible mushroom Pleurotus florida was evaluated by FRAP method, the results being expressed as % of inhibition. The values ranged between 57.79-73.8%. Antioxidant act as major defense against radical mediated toxicity by protecting damage caused by free radicals. The highest antioxidant activity was observed at highest concentration of mushroom extract. Recently mushroom plays an important role by providing antioxidant activity in the treatment and prevention of various free radical induced pathological condition. Anti-inflammatory activity was studied by two methods such as The Human RBC Membrane Stabilization Method; Protein Denaturation Bioassay Method. It was also positive correlation with concentration of the mushroom extract.

Our present in vitro studies on Pleurotus florida extracts demonstrated the depression of inflammation. Due to the presence of active principles such as flavonoids and polyphenols may responsible for this activity. Hence, Pleurotus florida can be used as a potent anti inflammatory agent.

Conclusion

The invitro antioxidant and anti inflammatory activity of edible mushroom pleurotus florida was studied. The investigation has opened a possibility of use of these mushroom in drug development for human consumption possibily for the treatment of various inflammatory conditions and various disease associated with oxidative stress such as cardiovascular disease, diabetes, cancer etc

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