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STUDIES ON THE PHYTOCHEMICAL, ANTIDIABETIC, ANTI-INFLAMMATORY AND ANTIOXIDANT PROPERTIES OF VARIOUS EXTRACTS OF *AGARICUS BISPORUS*

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Abstract:

The Agaricus bisporus fungi posses all types of phytochemicals which are essential for the normal functioning of human body. The fungus is rich in protein and fibre content as being analysed in the concerned study. The concerned fungus is very important as being antidiabetic, anti-inflammatory and antioxidant in nature. The various extracts of the fungus was analysed for the antidiabetic, anti-inflammatory and antioxidant property determination. Among the various extracts, the water extract of the concerned fungus had been found to be much more effective as per its antidiabetic; anti-inflammatory and antioxidant power is taken into consideration. The petroleum extracts had been found less effective as an antidiabetic, anti-inflammatory, and antioxidant agent. The methanol extracts show all the concerned activities in between water and petroleum extracts of the concerned fungi.

Keywords: *Agaricus bisporus, Antioxidant, antidiabetic, anti-inflammatory, Phytochemicals,*

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INTRODUCTION:

A new concept of "functional foods" was introduced since decades as a factor in the analysis of foods after nutrients (1). Detailed information about food bio-actives is required in order to obtain appropriate functional food products. Therefore US, European Union and Asian countries like Japan, South Korea etc have drafted and revised various regulatory guidelines on functional foods and their health claims (2). Mushrooms come under the most prominent foods being consumed by the humans, so had been collected and cultivated for hundreds of years in Asian countries, like China and Japan (3). Mushrooms are neither plants nor animals and have been placed in a kingdom, called Myceteae (5). The word mushroom did mean different thing to different people and countries. In broad sense "mushrooms are macro fungi having distinctive fruiting body, being large enough to be seen with naked eye and to be picked by hand" (6). Thus, Edible mushrooms have been used by humans for food from centuries and have been appreciated because of the flavor as well as medicinal and tonic attributes (7). However, the complete awareness of mushrooms as a healthy food and as an important source of biological active substances with medicinal value has only recently emerged (8). Mushrooms are considered to be healthy food because they possess low in calories and fat but rich in proteins and dietary fibers. The mushroom protein contains all type of essential amino acids required by humans, also mushrooms are a relatively good source of the mineral nutrients like phosphorus, iron and vitamins, including thiamine, riboflavin, ascorbic acid, ergo sterol, and niacin (9).

MATERIALS AND METHODS:

Study area and fungi collection

The concerned fungus was collected from the local market of Dehradun. The fungi parts were segregated shade dried and powdered in mixture. The powder was used for experiment.

Extraction

50 gms of the powder were weighed separately and accurately and then extracted in a Soxhlet Apparatus using thimble in order to get the best extract. Various solvents were used depending upon their polarity index with increasing polarity (Petroleum ether, Methanol and Water).

Extraction A:

The sample was extracted with a particular solvent (petroleum ether) in a Soxhlet apparatus for a required period, till no extract was coming out of the sample, as being examined by taking a small amount of the extracted solvent from the main chamber of the Soxhlet apparatus over the watch glass for the appearance of precipitate. After the Extraction with diethyl ether, the extract solution was subjected to filtration to remove the residue from extract. The filtrate was them then collected and evaporated to remove the volatile solvent to its 1/4 volume on water bath at a suitable temperature. The whole Filtrate was then made in solid form (powdered) after being kept in an oven. The residue was collected, and subjected to further extraction process.

Extraction B:

The residue was then extracted with ethanol in a same manner as mentioned above, in extraction A.

Extraction C

The residue from extract B was subjected to water extraction by decoction technique. In this technique the extract was dissolved in 500 ml of water. The whole solution was heated over water bath to remove all the water from the extract. Finally additional 500 ml of water was added to the extract, the extracted solution was finally evaporated to remove nearly 250ml of water. The solution was then subjected to filtration. The filtrate was then evaporated to remove nearly 1/4th of its volume. Finally the extract was dried in an oven at a temperature range 30-50^{0}C.

Chemical Tests:

Test for Alkaloids:

Solvent free extract 50mg was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloid reagents as follows:

(a) Wagner's test: To a few ml of filtrate, few drops of Wagner reagent were added by the side of the test tube. A reddish-brown precipitate confirmed the test.

Wagner's reagent: Iodine (1.27g) and potassium iodide (2g) was dissolved in 5ml of water and make up 100ml with distilled water.

(b) Hager's test: To a few ml of filtrate, 1 or 2ml of Hager's reagent were added. A prominent yellow precipitate indicated the test as positive.

Hager's reagent: Saturated aqueous solution of picric acid.

Test for Cardiac Glycosides:

Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for Flavonoid

a)Alkaline reagent test:

An aqueous solution of extract was treated with 10% ammonia hydroxide solution. Yellow fluorescence indicates the presence of Flavanoids.

Test for Tannins:
About 2.5 g of the plant extract was dissolved in 5 ml of distilled water, filtered and ferric chloride reagent added to the filtrate. A blue-black, green, or blue-green precipitate was taken as evidence for the presence of tannins**.**

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 Terpenoids:
Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Anti-inflammatory Activity of *Agaricus bisporus (button mushroom)* extracts
Inflammation is a complex physiological process mediated by a variety of signaling molecules produced by leukocytes, macrophages, and mast cells. Inflammation is a tissue response to injury characterized by increased blood flow to the tissue causing increased temperature, redness, swelling, and pain. Macrophages play an important role in inflammatory disease through the release of inflammatory mediators such as nitric oxide (NO), prostaglandin (PG) E2, and pro-inflammatory cytokines.

Preparation of Human Red Blood Cells (HRBC) suspension
Fresh whole human blood was collected and mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05% citric acid and 0.42 % sodium chloride in water). The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline **(10), (11)**

Heat Induced Hemolysis
The principle involved here is stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10 % v/v] with various concentrations of plant extracts and standard drug diclofenac sodium of various concentrations (50, 100, 200, 300, 400, 500 µg) and control (distilled water instead of hyposaline to produce 100 % hemolysis) were incubated at 37ºC for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 **nm (12).**
The percentage of hemolysis of HRBC membrane can be calculated as follows:

% Hemolysis = (Absorbance of Test Sample / Absorbance of Control) x 100

The percentage of HRBC membrane stabilization can be calculated as follows:

% Protection = 100 – [(Absorbance by Test sample / Absorbance by Control) X 100]

Antioxidant Activity
DPPH Method
DPPH Scavenging activity was measured by the Spectrophotometric method. A stock solution of DPPH (1.5 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml of methanol. Decrease in the absorbance in presence of sample extract at different concentration (10-125 µg/ml) was noted after 15 min. IC_{50} was calculated from % inhibition.

Protocol for DPPH Free Radical Scavenging Activity
Preparation of stock solution of the sample:-
100 mg of extract was dissolved in 100 ml of methanol to get 1000 µg/ml solution.
1. Dilution of test solution: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml solution of test were prepared from stock solution.
2. Preparation of DPPH solution: 15 mg of DPPH was dissolved in 10 ml of methanol. The resulting solution was covered with aluminum foil to protect from light.
3.Estimation of DPPH scavenging activity: 75 µl of DPPH solution was taken and the final volume was adjusted to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 75 µl of DPPH and 100 µl of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Absorbance at zero time was taken in UV-Visible at 517 nm for each concentration. Final decrease in absorbance of DPPH with sample of different concentration was measured after 15 minute at 517 nm.

Percentage inhibitions of DPPH radical by test compound were determined by the following formula.

% Reduction = Control absorbance – Test absorbance/ Control absorbance x 100
Calculation of IC50 value using graphical method.

***In-vitro* inhibition of extracts by alpha-amylase, alpha-glucosidase enzymes**
Inhibition of alpha amylase Enzyme
Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitory activity was based on the starch iodine method that was originally developed by **(13).** In alpha amylase inhibition method 1ml substrate- potato starch (1% w/v), different concentrations of (Acarbose std drug /Plant extracts), 1ml of alpha amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2 pH) was added. NOTE- Potato starch solution, alpha amylase solution and drug solution was prepared in acetate buffer. The above mixture was incubated for 1 hr. Then 0.1 ml Iodine-iodide indicator (635mg Iodine and 1gm potassium iodide in 250 ml distilled water) was added in the mixture. Absorbance was taken at 565 nm in UV-Visible spectroscopy. % inhibition was calculated and all the tests were performed in triplicate.

Inhibition of alpha-glucosidase Enzyme
The inhibitory activity was determined by incubating a solution of starch substrate (2% w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37°C. The reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1U/ml) to it followed by incubation for 40 min at 35°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540nm (14).
Calculation of 50% Inhibitory Concentration (IC_{50})
The concentration of the plant extracts required to scavenge 50% of the radicals (IC_{50}) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (% I) was calculated by

% I = (Ac-As)/Ac X 100 (15)

Where ***Ac*** is the absorbance of the control and ***As*** is the absorbance of the sample.

Procedure for the estimation of Amino-acids by ninhydrin method

Introduction: - The amino acids are colourless ionic compounds the forms the basic building blocks of proteins. Apart from being bound as proteins, amino acids also exist in the free form in many tissues and are known as free amino acid. They are mostly water soluble in nature. Very often in plants during disease conditions the free amino acid composition exhibits a change hence, the measurement of the total free amino acids gives the physiological and health states of the plants.

Principle: - Ninhydrin, a powerful oxidizing agent, decarboxylates the alpha amino acids and yields an intensity coloured bluish purple product which is calorimetrically measured at 570nm.

Reagents Required
1. Ninhydrin:- dissolve 0.8gm stannous chloride ($SnCl_2.2H_2O$) in 500ml of 0.2M citrate buffer(pH 5.0) add this solution to 20gm of ninhydrin in 500ml of methyl cellosolve (2methoxy ethanol).
2. 0.2M Citrate Buffer pH5.0 Solution A: 0.2M citric acid. Solution B: 0.2M sodium citrate mix 20.5ml of solution A with 29.5ml of solution B and check pH.
3. Dilute Solvent: mix equal volume of water and n-propanol and use.
4. Standard: dissolve 50mg leucine in 50ml of distilled water in a volumetric flask. Take 10ml of this stock standard and dilute to 100ml in another flask for working standard solution.

Procedure
1.Pipette out 0.2, 0.4, 0.6, 0.8, 1.0 ml of standard amino acid solution to the resp. labeled test tubes.
2.Add distilled water in all the test tubes and make up the volume to 1ml.
3.Add 1ml of distilled water to the test tube to make up the volume to 1ml.
4.Now add 1ml of ninhydrin reagent to all the test tube including the blank test tube and unknown test tube.
5.Mix the contents of the test tubes by shaking the tubes.
6.Then cover all the test tubes with papers.
7.Place all the test tubes in boiling water bath for 15minutes.
8.Cool the test tubes in cold water and add 5ml of diluents solvents to each test tube and mix well.
9.Now record the absorbance at 570nm of each solution using a colorimeter.
Then plot the standard curve by taking concentration along X axis and absorbance at 570nm along Y-axis.

Estimation of Crude Fibre

Crude fibre consists largely of cellulose and lignin (97%) plus some mineral matter. It represents only 60–80% of the cellulose and 4–6% of the lignin. The crude fibre content is commonly used as a measure of the nutritive value of poultry and livestock feeds and also in the analysis of various foods and food products to detect adulteration, quality and quantity.

Principle

During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of the native cellulose and considerable degradation of lignin occur. The residue obtained after final filtration is weighed, incinerated, cooled and weighed again. The loss in weight gives the crude fibre content.

Materials

Sulphuric acid solution (0.255 ± 0.005 N): 1.25 g concentrated sulphuric acid diluted to
100 mL (concentration must be checked by titration).
Sodium hydroxide solution (0.313 ± 0.005 N): 1.25 g sodium hydroxide in 100 mL distilled water (concentration must be checked by titration with standard acid).

Procedure

1.Extract 2 g of ground material with ether or petroleum ether to remove fat (Initial boiling temperature 35–38°C and final temperature 52°C). If fat content is below 1% extraction may be omitted.
2.After extraction with ether boil 2 g of dried material with 200 mL of sulphuric acid for 30 min with bumping chips.
3.Filter through muslin and wash with boiling water until washings are no longer acidic.
4.Boil with 200 mL of sodium hydroxide solution for 30 min.
5.Filter through muslin cloth again and wash with 25 mL of boiling 1.25% H_2SO_4, three 50 mL portions of water and 25 mL alcohol.
6.Remove the residue and transfer to ashing dish (preweighed dish W1).
7.Dry the residue for 2 h at 130 ± 2°C. Cool the dish in desiccators and weigh (W2).
8.Ignite for 30 min at 600 ± 15°C.
9.Cool in a desiccator and reweigh (W3).

Calculation

% crude fiber in ground sample = Loss in weight on ignition $\frac{(W_2 - W_1) - (W_3 - W_1)}{\text{Weight of the sample}}$

Observations and Results

A) Qualitative Analysis

Table: 1 Phytochemical Tests for *Agaricus bisporus*

S. No	Phytochemical tests	Petroleum ether	Methanol	Water
1	Alkaloid Test (1)	-ve	-ve	+ve
2.	Alkaloid test (2)	-ve	-ve	+ve
3.	Flavonoids Test	-ve	+ve	+ve
4.	Tannins Test	+ve	-ve	+ve
5.	Saponin Test	-ve	+ve	+ve
6.	Phenol Test	-ve	+ve	+ve
7.	Carbohydrates	-ve	-ve	-ve
8	Phytobatinnin	-ve	+ve	-ve

Protein content in various extracts of *Agaricus bisporus*

Protein content in petroleum ether extract of *Agaricus bisporus* = 0.345 **µg/ml**
Protein content in methanol extract of *Agaricus bisporus* = 0.451 **µg/ml**
Protein content in water ether extract of *Agaricus bisporus* = 0.545 **µg/ml**

Crude Fibre content in various extracts of *Agaricus bisporus*

% of Fibre content in Petroleum Ether extract = 5.9%
% of Fibre content in Methanol extract = 6.2%

Antioxidant Power of *Agaricus bisporus*

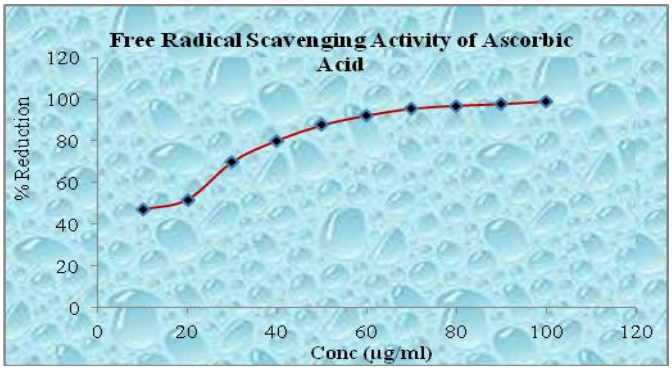
An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thioles,

ascorbic acid or polyphenols. Although oxidation reactions are crucial for life, they can also be

damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases.

Table 2: DPPH Free Radical Scavenging Activity of Ascorbic Acid

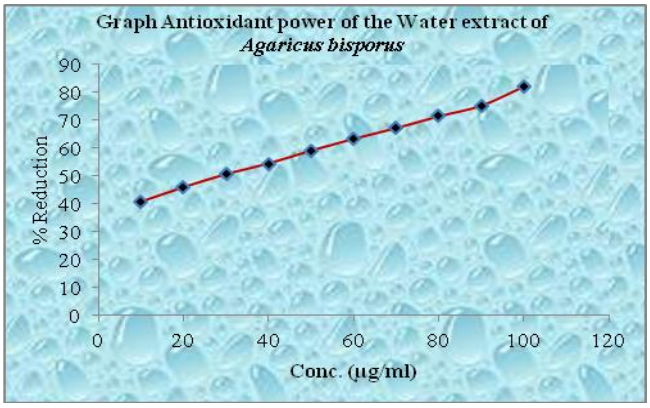
S. No.	Conc. (µg/ml)	Absorb of Ascorbic acid	% Red	IC_{50} Value
1.	10	0.292	46.93	17
2.	20	0.269	51.81	
3.	30	0.244	80.01	
4.	40	0.226	80.2	
5.	50	0.202	87.55	
6.	60	0.181	92.24	
7.	70	0.162	95.51	
8.	80	0.141	96.93	
9.	90	0.122	97.75	
10.	100	0.088	98.97	



Graph.1: showing DPPH Free Radical Scavenging Activity of Ascorbic Acid

Table 3: Showing the antioxidant power of the Water extract of *Agaricus bisporus*

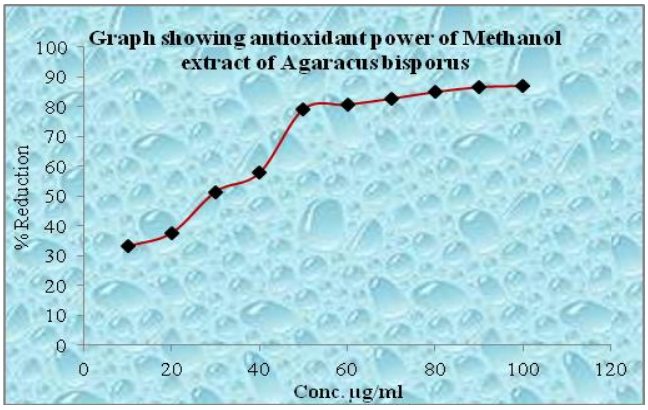
S. No.	Conc. (µg/ml)	Absorb. of Pet. ether Extract	% Reduction	IC_{50} (µg/ml)
1.	10	0.260	40.63	26
2.	20	0.236	45.90	
3.	30	0.098	50.60	
4.	40	0.098	54.45	
5.	50	0.061	59.10	
6.	60	0.038	63.33	
7.	70	0.022	67.21	
8.	80	0.015	71.45	
9.	90	0.011	75.30	
10.	100	0.005	82.16	



Graph 2: showing the antioxidant power of the Water extract of *Agaricus bisporus*

Table 4: Showing the antioxidant power of the Methanol extract of *Agaricus bisporus*

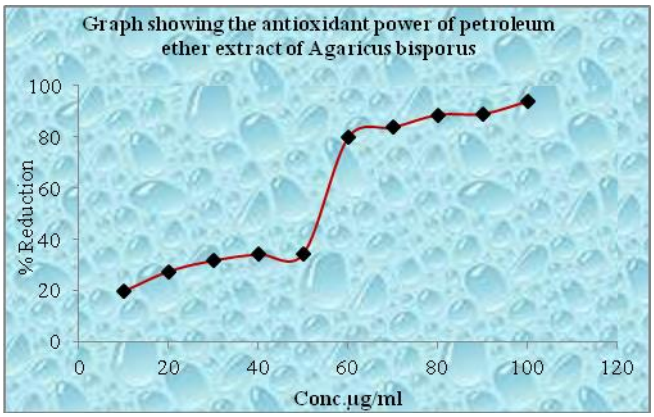
S. No.	Conc. (µg/ml)	Absorb of Methanol Extract	% Red	IC_{50} (µg/ml
1.	10	0.228	33.06	29
2.	20	0.206	37.55	
3.	30	0.138	51.42	
4.	40	0.107	57.75	
5.	50	0.102	79.18	
6.	60	0.095	80.61	
7.	70	0.085	82.65	
8.	80	0.074	84.89	
9.	90	0.066	86.53	
10.	100	0.064	86.93	



Graph 3: showing the antioxidant power of the methanol extract of *Agaricus bisporus*

Table: 5. showing the antioxidant power of the petroleum ether extract of *Agaricus bisporus*

S. No.	Conc. (µg/ml)	Absorb. of water extract	% Red.	IC_{50} (µg/ml)
1	10	0.394	19.59	53
2	20	0.356	27.34	
3	30	0.335	31.63	
4	40	0.323	34.08	
5	50	0.322	34.28	
6	60	0.099	79.79	
7	70	0.08	83.67	
8	80	0.056	88.57	
9	90	0.054	88.97	
10	100	0.03	93.87	



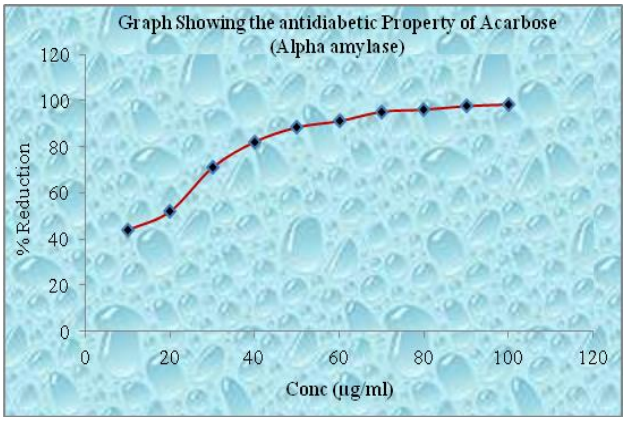
Graph 4: Showing the antioxidant power of the petroleum ether extract of *Agaricus bisporus*

Antidiabetic property of Various Extracts of *Agaricus bisporus* by inhibition of Alpha Amylase

The alpha-amylase enzyme inhibition by various plant extracts and their comparison with the standard **(ACAROSE)** have been analysed by spectrophotometric method. All the extracts of fungi show alpha-glucosidase inhibition and the results are tabulated in **(Tables).** The graph was plotted between the percentage inhibition and the extract concentrations and is shown in **(Figs).** The **IC_{50}** value of all the plant extracts have been calculated and are shown in **(Tables).** The highest alpha-glucosidase enzyme inhibition was shown by **(water)** extract having the **IC_{50}** value of **(22).** The plant extract as per their inhibition potential follows the order as **(water extract > methanol extract >Petroleum ether extract).**

Table: 6. Inhibition of alpha amylase enzymes by Acarbose Standard

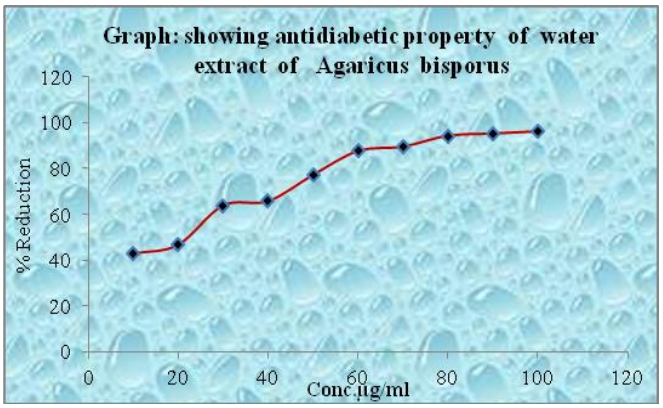
S. No.	Conc. (µg/ml)	Absorbance of Acarbose	% Red.	IC_{50} (µg/ml)
1.	10	0.291	44.13	17
2.	20	0.267	52.21	
3.	30	0.242	71.11	
4.	40	0.227	82.21	
5.	50	0.204	88.51	
6	60	0.185	91.21	
7.	70	0.163	95.23	
8.	80	0.146	96.14	
9.	90	0.123	97.71	
10.	100	0.086	98.37	



Graph 5: showing the antidiabetic property of the Acarbose (Alpha amylase)

Table 7: Showing the Antidiabetic property of the water extract of *Agaricus bisporus*

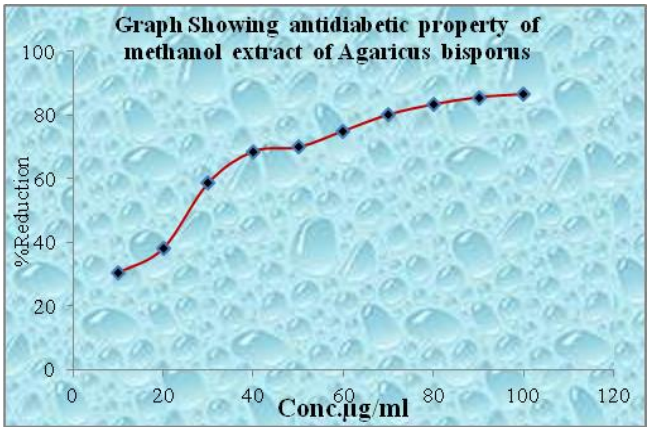
S. No.	Conc. (µg/ml)	Absorb of pet. Ether extract	% Red	IC_{50} (µg/ml
1.	10	0.060	42.85	22
2.	20	0.056	46.66	
3.	30	0.038	63.80	
4.	40	0.036	65.71	
5.	50	0.024	77.14	
6	60	0.013	87.61	
7.	70	0.011	89.52	
8.	80	0.006	94.28	
9.	90	0.005	95.23	
10.	100	0.004	96.19	



Graph 6: showing the antidiabetic property of the water extract of *Agaricus bisporus*

Table 8: showing the antidiabetic property of the methanol extract of *Agaricus bisporus*

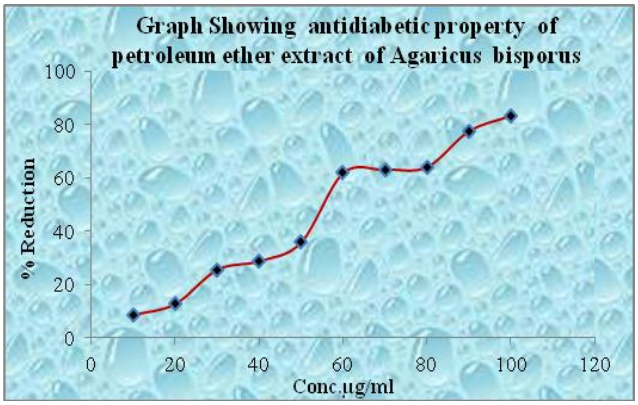
S. No	Conc. (µg/ml)	Absorb. of methanol Extract	% Red	IC_{50} (µg/ml
1.	10	0.217	30.44	26
2.	20	0.193	38.14	
3.	30	0.129	58.65	
4.	40	0.098	68.58	
5.	50	0.094	69.87	
6	60	0.078	75.00	
7.	70	0.062	80.12	
8.	80	0.052	83.33	
9.	90	0.045	85.57	
10.	100	0.042	86.53	



Graph 7: showing the antidiabetic property of the Methanol extract of *Agaricus bisporus*

Table 9: Showing the antidiabetic property of the petroleum ether extract of *Agaricus bisporus*

S. No.	Conc. (µg/ml)	Absorbance of water Extract	% Red	IC_{50} (µg/ml)
1.	10	0.311	8.65	55.5
2.	20	0.272	12.82	
3.	30	0.232	25.64	
4.	40	0.222	28.84	
5.	50	0.200	35.89	
6.	60	0.119	61.85	
7.	70	0.115	63.14	
8.	80	0.112	64.10	
9.	90	0.070	77.56	
10.	100	0.052	83.33	



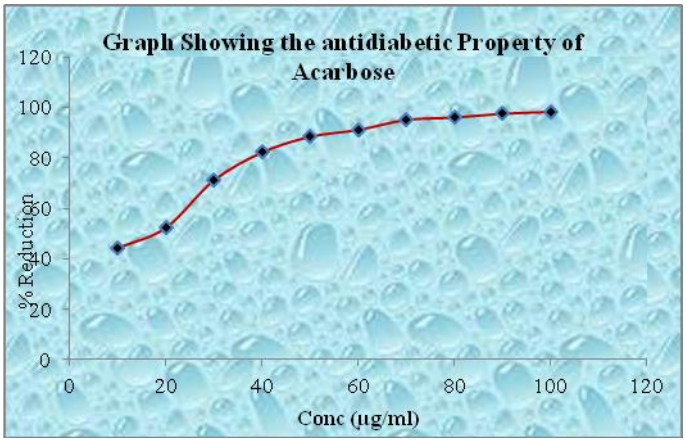
Graph 8: Showing the antidiabetic property of the petroleum ether extract of *Agaricus bisporus*

Antidiabetic Activity by inhibition of Alpha glucosidase enzyme

The alpha-glucosidase enzyme inhibition by various plant extracts and their comparison with the standard **(ACAROSE)** have been analysed by spectrophotometric method. All the plant extracts **(Petroleum ether, Methanol and Water)** show alpha-glucosidase inhibition and the results are tabulated in **(Tables).** The graph was plotted between the percentage inhibition and the extract concentrations and is shown in **(Figs).** The **IC_{50}** value of all the plant extracts have been calculated and are shown in **(Tables).** The highest alpha-glucosidase enzyme inhibition was shown by **water** extract having the **IC_{50}** value of **(25).** The plant extract as per their inhibition potential follows the order as **(water extract > methanol extract > petroleum ether).**

Table 10: Inhibition of alpha glucosidase enzymes by Acarbose Standard

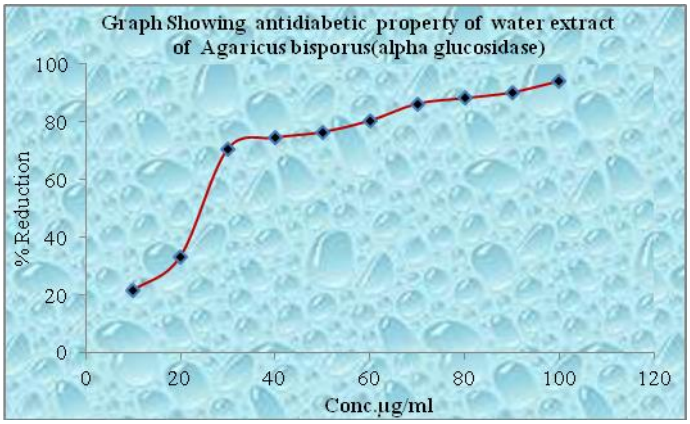
S. No.	Conc. (µg/ml)	Absorbance of Acarbose	% Red.	IC_{50} (µg/ml
1.	10	0.291	44.13	
2.	20	0.267	52.21	
3.	30	0.242	71.11	
4.	40	0.227	82.21	
5.	50	0.204	88.51	18
6	60	0.185	91.21	
7.	70	0.163	95.23	
8.	80	0.146	96.14	
9.	90	0.123	97.71	
10.	100	0.086	98.37	



Graph 9: showing the antidiabetic property of the Acarbose

Table 11: Showing the antidiabetic property of the water extract of *Agaricus bisporus*

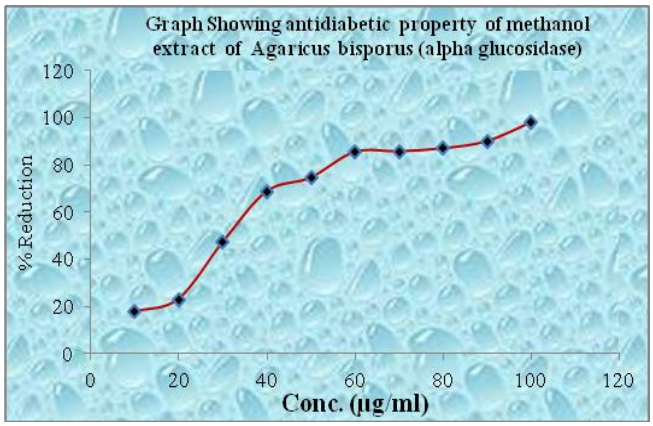
S. No.	Conc. (µg/ml)	Absorbance of water extract	% Red	IC_{50} (µg/ml)
1.	10	0.040	21.56	
2.	20	0.034	33.33	
3.	30	0.015	70.58	
4.	40	0.013	74.50	
5.	50	0.012	76.47	25
6	60	0.010	80.39	
7.	70	0.007	86.27	
8.	80	0.006	88.23	
9.	90	0.005	90.19	
10.	100	0.003	94.11	



Graph 10: showing the antidiabetic property of the water extract of *Agaricus bisporus*

Table 12: Showing the antidiabetic property of the methanol extract of *Agaricus bisporus*

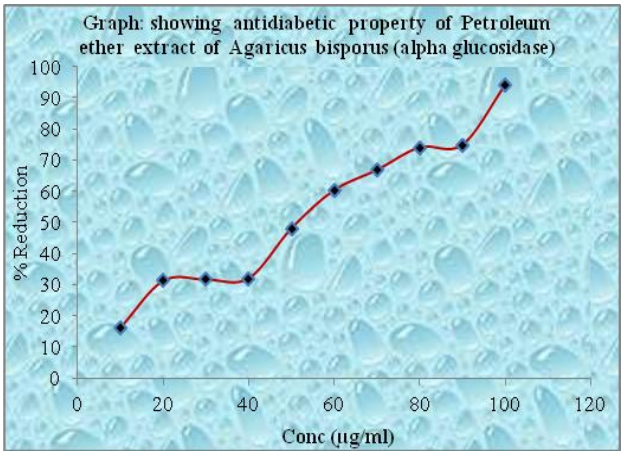
S. No	Conc. (µg/ml)	Absorbance of methanol	% Red	IC_{50} (µg/ml)
1.	10	0.701	17.62	30
2.	20	0.655	23.03	
3.	30	0.045	47.12	
4.	40	0.266	68.74	
5.	50	0.216	74.61	
6	60	0.123	85.54	
7.	70	0.121	85.78	
8.	80	0.011	87.07	
9.	90	0.084	90.12	
10.	100	0.016	98.11	



Graph 11: Showing the antidiabetic property of the methanol extract of *Agaricus bisporus*

Table: 13. Showing the antidiabetic property of the Petroleum ether extract of *Agaricus bisporus*

S. No.	Conc. (µg/ml)	Absorb. of water Extract	% Red.	IC_{50} (µg/ml)
1.	10	0.714	16.09	51
2.	20	0.594	31.19	
3.	30	0.582	31.60	
4.	40	0.579	31.96	
5.	50	0.442	48.06	
6	60	0.338	60.28	
7.	70	0.281	66.78	
8.	80	0.221	74.03	
9.	90	0.214	74.85	
10.	100	0.051	94.00	



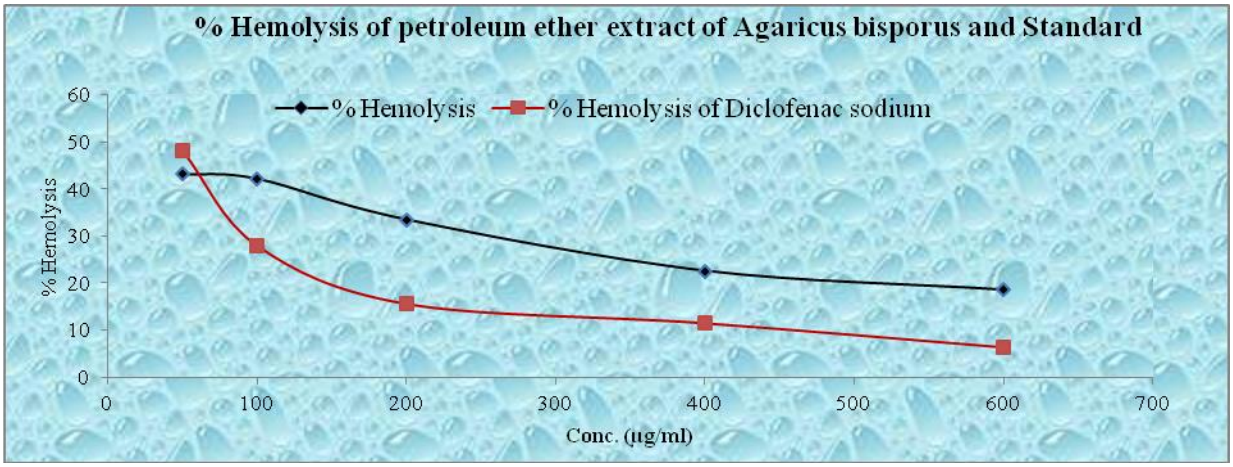
Graph: 12. Showing the antidiabetic property of the petroleum ether extract of *Agaricus bisporus*

Anti-inflammatory

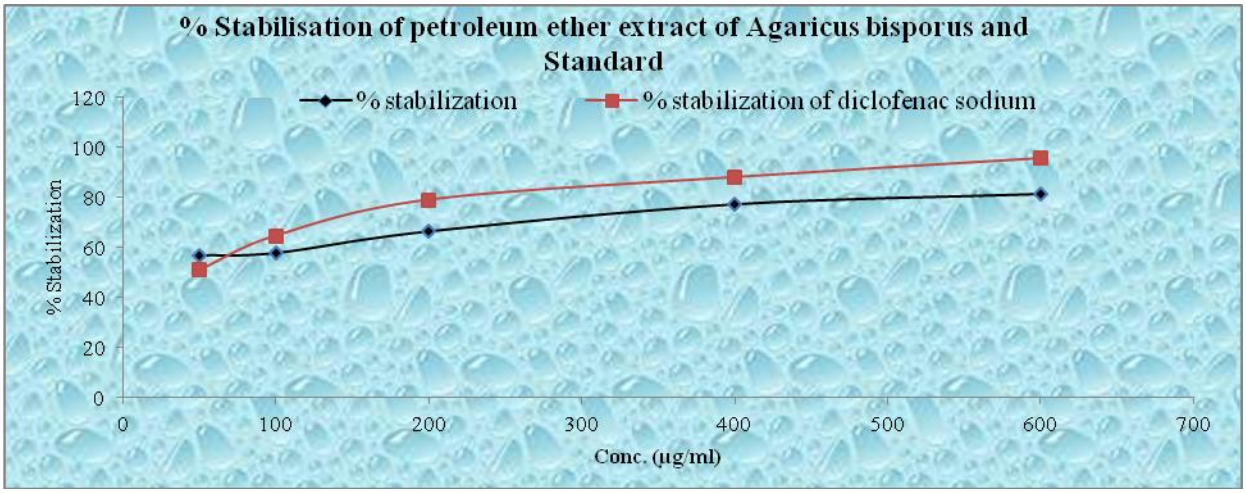
Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers. In the case of oxidative stress, reactive oxygen species are generated. Hence, antioxidants that can scavenge these reactive oxygen species can be beneficial in the treatment of inflammatory disorders. Synthetic antioxidants have restricted use because of their toxicity and DNA damaging properties. Whereas natural products obtained from plant extracts prove to be a better alternative against such modern medicines that have numerous side effects.

Table 14: Report of anti-inflammatory effect of petroleum ether extract of Agaricus bisporus and Standard on HRBC membrane hemolysis and membrane stabilization

Conc. of Standard/ Plant Extract (µl/ml)	% Hemolysis	% stabilization	% Hemolysis of Diclofenac sodium	% stabilization of diclofenac sodium
50	56.82	43.18	47.97	50.9
100	57.84	42.16	27.89	64.6
200	66.52	33.48	15.63	78.97
400	77.45	22.55	11.54	87.99
600	81.59	18.49	6.44	95.52



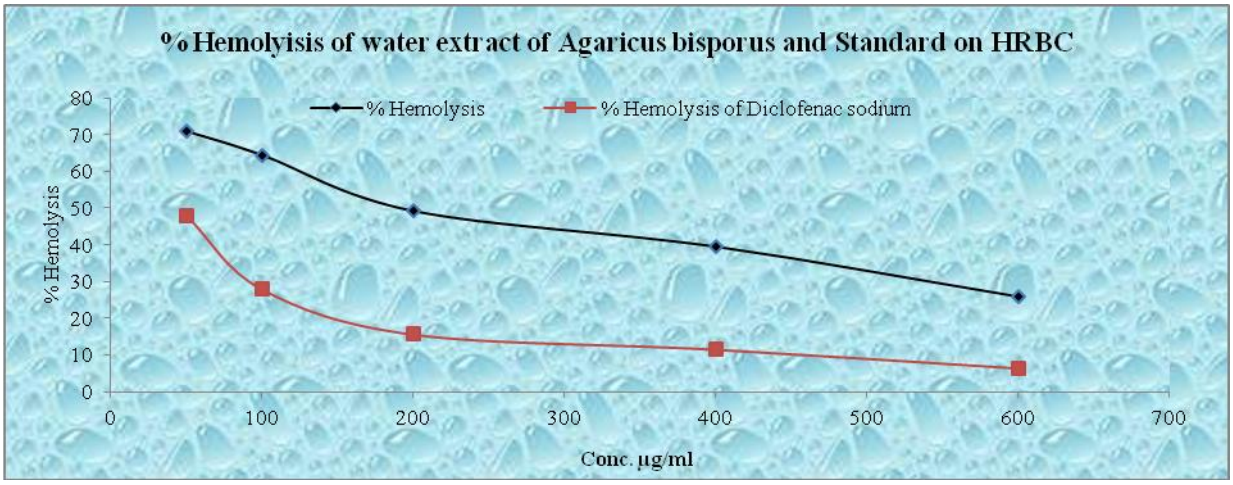
Graph 13: showing % hemolysis of petroleum ether extract of Agaricus bisporus and Standard on HRBC membrane hemolysis and membrane stabilization



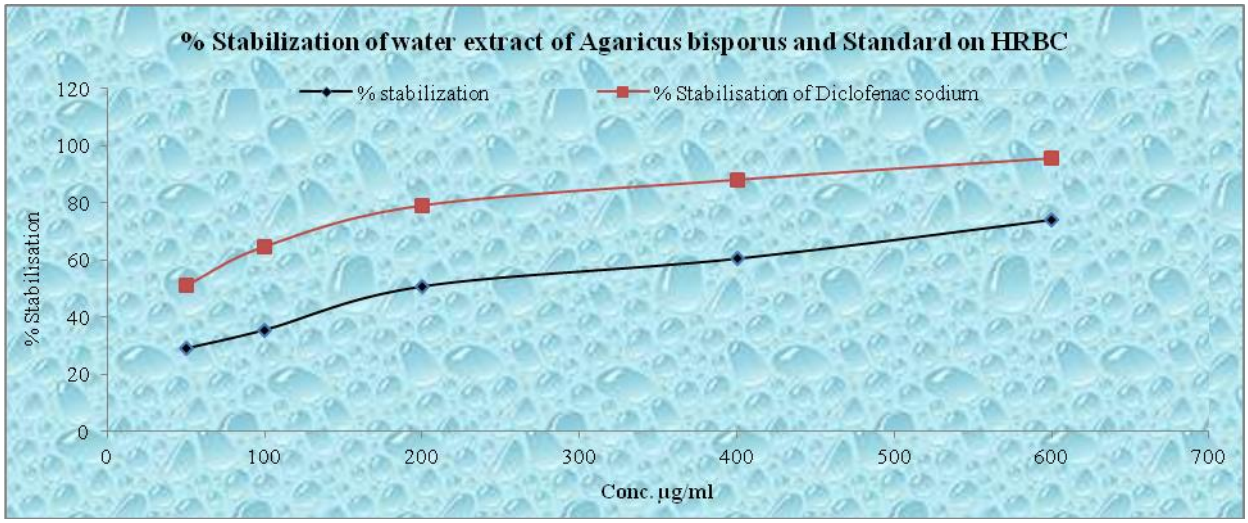
Graph 14: showing % Stabilisation of petroleum ether extract of Agaricus bisporus and Standard on HRBC membrane hemolysis and membrane stabilization

Table 15: Showing anti-inflammatory of water extract of Agaricus bisporus and Standard on HRBC membrane hemolysis and membrane stabilization

Conc. of Standard/ Plant Extract (µl/ml)	% Hemolysis	% stabilization	% Hemolysis of Diclofenac sodium	% Stabilisation of Diclofenac sodium
50	71.03	28.97	47.97	50.9
100	64.49	35.51	27.89	64.6
200	63.24	50.76	15.63	78.97
400	59.51	60.49	11.54	87.99
600	25.86	74.14	6.44	95.52



Graph 15: Showing % hemolysis of water extract of Agaricus bisporus and Standard on HRBC membrane hemolysis and membrane stabilization



Graph 16: Showing % hemolysis of water extract of Agaricus bisporus and Standard on HRBC membrane hemolysis and membrane stabilization

DISCUSSION:

The human biology is very complex system in nature. Its functional biology is contingent upon the simultaneous production and elimination of free radicals produced during the various biomechanisms operating in the body system, excessing the free radical concentration to the threshold level. The threshold crossing levels of free radicals concentration in the body invites the various types of diseases in the body like diabetes, Parkinsons disease, cancer to name a few. The nature has provided the different types of bio-organics available inherently in the forest wealth and vegetable to combat the overproduction of free radicals in the body system. The various phytochemicals so for analysed showed that the fungi in reference possesses all types of phytochemicals, which are necessary as per there medical importance is taken into consideration.

The antidiabetic data as gathered with the application of alpha-amylase enzyme inhibition method and alpha –glucosidase enzyme inhibition method used Acarbose as standard revealed that the % inhibition gets increased with the increase in the concentration of plant extracts and the reference compound. The IC_{50} value was derived from the plots between the % inhibition and the concentration as the corresponding concentration of control or the extracts in the different solvent at 50% inhibition. Among all the extracts water extract had been found to possesses the highest antidiabetic potential followed by methanol and petroleum ether extracts as per the inhibition of alpha amylase and alpha glycosidase enzyme are taken into consideration.

The diseases which effect Liver at an alarming sequence appear to increase in our society. Chemical medicines are found to be cost effective in overcoming such types of diseases and but are also associated with side effects which are the biggest problems in the present life. ***Agaricus bisporus*** extracts have been evaluated for its antidiabetic properties by in-vitro inhibition of alpha amylase and alpha glucosidase enzymes.

Anti- inflammation refers to the property of a medicine or medicine like product to inhibit the process of inflammation or swelling. Anti-inflammatory drugs make half of the analgesics, reducing pain by reducing inflammation at a particular body part of an organism. Only the petroleum ether extract and water extract have been analysed for the anti-inflammatory of the concerned fungi, and via analyzing it had been concluded that the water posses the highest anti-inflammatory activity as compared to the petroleum ether extracts.

Agaricus bisporus plant extracts were analysed for their anti-inflammatory effect and results obtained were found to possess a high level of effect against inflammation. The anti-inflammatory effect of various extracts of plant, fungi and animals is because of the presence of various secondary phytochemicals like flavonoids, alkaloids etc. the most enhancing capacity against the inflammation was found to be due the histamines. So the fungi could be used as best anti-inflammatory source. The anti-inflammatory effect of all the extracts have been carried out in reference to standard diclofenac sodium (anti-inflammatory drug). The inflammation was done by hypotonicity induced membrane lysis. The stabilisation against membrane induced hypotonicity was found to be concentration dependent, and the stabilisation percentage in all the plant extracts was found less than the reference compound (Diclofenac sodium). Among the concerned extracts the highest stabilisation was found in case of methanol extracts followed by water extracts.

The petroleum ether and methanol extracts of the concerned fungi had also been analysed for the estimation of fibre content, in which it had been found that methanol extract possesses the high fibre content (6.2%) and the petroleum ether possesses less fibre content as (5.9%).

The protein content of the fungi in petroleum ether, methanol and water extract had been found to be 0.345, 0.451, 0.545µg/ml respectively.

CONCLUSION:

Diabetes is a metabolic disorder which can be considered as a major cause of high economic loss which can in turn impede the development of nations. Moreover, uncontrolled diabetes leads to many chronic complications such as blindness, heart failure, and renal failure. In order to prevent this alarming health problem, the development of research into new hypoglycemic and potentially antidiabetic agents is of great interest. The study found that since the antidiabetic evaluation is done using extracts. It may be difficult to find the exact mechanism responsible for the hypoglycemic effect. Studies are needed to identify the active compound responsible for the hypoglycemic effect.

The anti-diabetic problem is a serious problem worldwide. Its control is must, because nearly about 70% of people having an age of 40 or above are being affected by this drastic disease. This disease is all about the abnormal behavior of endocrine system of a particular person. Once the enzymatic system gets disturbed the whole mechanism of the bodily

system whether catabolic or anabolic gets disturbed to a large extent. So for the diabetes is taken into considerations a large number of allopathic medicines have been analyzed synthesized and are still the research bodies all over the world under the process to find out the noble and ultimate medicine for the disease in reference. Nowadays the ultimate medicine against the disease is the application of insulin injection directly into the blood stream of the effected person. But as possess the same nature as other allopathic medicines the direct entry of the insulin into the blood stream of the diseased person leads to many side effects. The first effect is the overall weight of the person. Due to the weight loss other type of diseases gets originated. Secondly there is fluctuation in the blood pressure of an effected person. So finally man finds its way towards nature. Every type of problem finally gets its solution from nature, same goes for diabetes. So nowadays the ultimate cure against diabetes is considered to be by the utilization of Ayurvedic system of medicine. A large number of plants have been discovered to possess the anti-diabetic effect. In the present study different plant extracts have been analyzed and discovered to have a prompt effect against diabetes.

Inflammation is a complex localized response to foreign substances such as bacteria or in some instances to internally produced substances with fever usually presenting as one of its sequel. Inflammation underlies almost all disease conditions and it is fundamentally a protective response, the ultimate goal of which is to get rid of the organism of both the initial cause of cell injury (for example microbes and toxins) and the consequences of such injuries. Various medicinal plants provide relief from symptoms comparable to that obtained from allopathic medicines. The majority of clinically important medicines belong to steroidal or non-steroidal anti-inflammatory drugs. Though these drugs have potent activity, they have a number of severe adverse effects such as gastrointestinal disturbances and body fat redistribution. Hence, there is a need to develop safe and new anti-inflammatory agents with minimum side effects. In this scenario, use of plant derived products to treat inflammation and related condition becomes a viable and valid approach.

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