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RESEARCH ARTICLE

The impact of mushroom on elusive medical biological activities

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Abstract

This study aimed to investigate the possible biological activity of aqueous, methanolic and sulfated extracts of tow fungi (Mushrooms) the white mushroom (Agaricus bisporus) and the oyster mushroom (Pleurotus florida) at 85 °C under acidic, neutral and alkaline conditions. Fibrinolytic, anticoagulation, antioxidant, antitumor and antimicrobial activities were investigated. The Biochemical studies of the chosen two fungi showed the presence of varying amounts and different forms of total ash, protein, lipid, and total carbohydrates. However, Paper chromatography examination showed that the output of the acid hydrolysis to the plants varying from monosaccharaides. Anticoagulation and fibrinolytic properties of the crude extracts as well as of their sulfated derivatives, at 2 mg/ml of extract were tested and show that the acidic extract, sulfated acidic extract, sulfated neutral and sulfated alkaline extract of A. bisporus and P. florida amazingly exhibited fibrinolytic activities equivalent to double of the same amount of standard "Hemoclar". On the other hand, results also showed a promising anticoagulant activities of the sulphated extracts at different concentrations compared with the corresponding native extracts. Also, their antimicrobial activity of the aqueous extracts against both tested Gram positive and negative bacteria, yeast and fungal strains was determined. The chemical modification of the aqueous sulfation can increase significantly its anticoagulation and fibrinolytic activities. And also their extracts show antioxidant activity reached up to 70% comparative by standard. However, the antitumor activity is high for both the native and the sulfated aqueous extracts.

INTRODUCTION

For a long time mushrooms have been playing an important role in several aspects of the human activity. Edible mushrooms, for example, are used extensively in cooking and make part of low calorie diets. Mythology is extensively garnished by mushrooms, typically associated with gnomes, fairies and other fairytale personages. The psychedelic and consciousness expansion properties of some species have pushed mushrooms to become part of some religions. Even toxic mushrooms have found a place of relevance, because of the uniqueness of their compounds that evolved naturally as a protection against consumption **(Bala et al., 2011).**

As listings of mushrooms including their Agaricus bisporus, known as table mushroom, cultivated mushroom or button mushroom, is an edible basidiomycete fungus which naturally occurs in grasslands, fields and meadows. It has spread much more widely and is one of the most widely cultivated mushrooms in the world (**María Elena Valverde**

et al., 2015). The original wild form bears a brownish cap and dark brown gills but more familiar is the current variant with a white form, having white cap, stalk and flesh and brown gills **(Loganathan K. Jagadish, et al., 2009).**

Extracts from fruiting bodies and the mycelia of various mushrooms have been reported for antimicrobial activity against wide range of infectious bacteria **(Hirasawa et al., 1999).**

Also, wild and cultivated mushrooms contain a huge diversity of biomolecules with nutritional **(Kalac P., 2009**) and/or medicinal properties **(Borchers et al., 2004 and Poucheret , et al., 2006)**.

However, due to these properties, they have been recognized as functional foods, and as a source for the development of medicines and nutraceuticals. Fruiting bodies, mycelia and spores accumulate a variety of bioactive metabolites with immunomodulatory, cardiovascular **Yeh M.Y. et al., (2014)** and liver protective, anti fibrotic, antiinflammatory, anti-diabetic, anti-viral, antioxidant, antitumor, and antimicrobial properties **(Borchers A et al., 2004, Gonçalves O. et al., 2011 and J. Lee et al., 2013)**

Furthermore, extracts from fruiting bodies and the mycelia of various mushrooms have been reported for antimicrobial activity against wide range of infectious bacteria **(Hirasawa et al., 1999; Dulger et al., 2002).**

Listings of mushrooms including their Agaricus bisporus, known as table mushroom, cultivated mushroom or button mushroom, is an edible basidiomycete fungus which naturally occurs in grasslands, fields and meadows. It has spread much more widely and is one of the most widely cultivated mushrooms in the world. The original wild form bears a brownish cap and dark brown gills but more familiar is the current variant with a white form, having white cap, stalk and flesh and brown gills **(Loganathan K. Jagadish et al., 2009).**

During the past 40 years, numerous mushroom derived polysaccharides and polysaccharide–protein complexes are being used as one of the major sources of therapeutic agents for immunomodulatory and anti-tumor properties (**Wasser et al., 2002 and Ikekawa , 2001)**

Although modes of actions of these compounds are not clear, nevertheless these are suggested to enhance cellular components of the immune system **(Chihara G., 1992).**

Interestingly, Stimulation of host immune defense system by bioactive polymers from medicinal mushrooms has significant effects on maturation, differentiation and proliferation of many kinds of immune cells in the host and thus they can act as anti-tumor compounds (**Wasser et al., 2002)**

On the other hand, the presence of antioxidant (**Cheung LM et al., 2003) and (Wong JY, and Chye FY., 2009**) and anti-inflammatory **(Jose N., 2004 and Khohno K, et al., 2008)** compounds in mushrooms might be clinically relevant in the management of heart and circulation health complications. There are some mushroom components involved in cardiovascular diseases prevention or treatment (proteins, lipids, vitamins, fibers, phenolic compounds and minerals). However, the implicated mechanisms are not yet completely elucidated. (**Miyazawa N, et al., 2008**)

Furthermore, in previous studies various biological activities such as antioxidant, antibacterial, antifungal **(Turkog˘lu et al., 2007)**, immunomodulatory, antiviral (**Moradali et al., 2007**), antitumor (**Tong et al., 2009; Zhang et al., 2007**), anti-inflammatory **(Komura et al., 2010; Regina et al., 2008)**, cytotoxic (**Zhang et al., 2007**), antiaromatase (**Chen et al., 2006**) and anticholesterole (**Jeong et al., 2010**) activities of these compounds and/or complexes were investigated.

However, in recent years, multiple drugs resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists for searching new antimicrobial substances from various sources which are the good sources of novel antimicrobial chemotherapeutic agents **(Karaman et al., 2003)**

Accordingly, researchers showed antimicrobial activity of several mushrooms (**Gezer et al., 2006; Mercan et al., 2006 Turkoglu, et al., 2007).** Thus Mushrooms are one of the largest untapped sources of powerful and new pharmaceutical products (**Wasser SP et al., 2002)**

Materials and Methods

1- Materials:

1.1: Mushroom: Tow mushrooms were used in this study –

a) the white mushroom , (**Agaricus bisporus**) collected from (JUNCAO – Technology – China)
b) the oyster mushroom (**Pleurotus florida**) kindly collected from (Agriculture Research Center "ARC" - Biological Agriculture Department - Central lab for Agriculture climate)

1.2: Microorganisms and media:

Eight well identified test microorganisms including (Aspergillus niger, Aspergillus Flavus, Penicillium chrysogenum, Escherichia coli, Klebsiella pneumonia, Bacillus subtilis, Staphylococcus aureus and Candida Albicans) ,that used in this study were kindly provided from the National Research Centre, Dokki, Cairo, Egypt.

1.2.1: Preparation of standard bacterial suspensions: according to (**Miles and Misra, 1938**).

1.2.2: Preparation of standard fungal suspensions: The fungal cultures used were maintained on potato dextrose agar. **(Brantner et al., 1994)**

1.3: Chemicals and reagents:

(Barium chloride-Tween 20) reagent: This was prepared according to (**Larsen et. al., 1986**)

Lowery reagents: **Lowry A**: 2% sodium carbonate in 0.IN sodium hydroxide solution.

Lowry B: **(**1% cupric sulphate solution) – (2% sodium potassium tartrate solution).

Folin-Ciocalteu's Phenol Reagent: One volume of Folin- Ciocalteu's phenol reagent was diluted with two volumes of water before use to determine soluble protein.

Standard antibiotic discs: Streptomycin (10µg/disc) and erythromycin (15µg/disc) were purchased from Bioanalyse company, Ltd., Ankara, Turkey and Griseofulvin (20 mg/ml) from local pharmacy (125 mg/tablet).

Heparin: (Heparin sodium) this was purchased from SIGMA chemicals Co., U.S.A.

Hemoclar: (Pentosan sulfuric polyester) it was the commercial product prepared by Clin- Midy Paris and supplied by the Nile Co. pharmaceuticals. Cairo, Egypt.

Plasma: Human plasma was purchased from The Holding Company for Biological Products and Vaccines (EGYVAC-VACSERA), El-batal Ahmed Abdel Aziz Street, Dokki, Egypt.

Calcium chloride solution 2 % (w/v) & **Saline solution 0.9 % (w/v)** and **Other Chemicals:** All chemicals were analytical grade – products purchased from Sigma, Mark and BDH companies.

2: Methods:

2.1: Biochemical analysis of the mushroom

2.1.1: Determination of moisture content and ash : was attained according to **(A. O. A. C., 1970**).

2.1.2: Determination of total lipids: according to the Method of **(A. O. A. C. 1970).**

2.1.3: Analysis of the polymeric carbohydrates:

2.1.3.1: Acid hydrolysis: according to the modified method of (**Fischer and Dorfel 1955**).

2.1.3.2: Qualitative examination of the hydrolysis products: This was performed by chromatography of the hydrolysates on thin-layer chromatography (TLC) according to **(Adachi, 1965) and** detection of spots was achieved by spraying with an aniline-phthalate reagent (**Partridge, 1949**).

2.1.3.3: Quantitative Determination of the Hydrolysis Products: by the method of (**Wilson 1959**).

2.1.5: Determination of total carbohydrates: was determined, after hydrolysis according to **(Haug and Larsen, 1962)**. The color density was measured at 490 according to (**Dubois et al., 1956**).

2.1.6: Determination of total nitrogen and crude protein: was determined according to the adopting the usual micro-Kjeldahl method of **(A. O. A. C. 1970**).

2.2: Preparation of organic, methanolic and aqueous extracts:

2.2.1: Grinding of the selected plant materials.

2.2.2: Extraction with Methanol: according to **(Menaga D, 2012)**

2.2.3: Aqueous extraction of defatted plant material: with HCl (pH 3), Extraction with hot water (pH 6.6) and Extraction with (1N) NaOH by using the method of (**Jindal and Mukherjee 1970).**

2.3: Analysis of aqueous extracts: This was done using the same methods previously adopted for analysis of the mushroom sample except the protein assay which performed according to the method of (**Lowry et al., 1951**) .

2.4: Sulphation of aqueous extracts: achieved adopting the method of (**Hussein, 1994**)

2.4.1: Determination of Sulfate Ester Groups: was done in two following steps:

2.4.1.1: Cleavage of the Sulfate Ester Groups: by using the method of (**Larsen et. al., 1986**)

2.4.1.2: Turbidimetric Assay of the Liberated Sulfate: Sulfate content of the aforementioned hydrolyzate was determined adopting the turbidimetric procedure of (**Garrido, 1964)** with some modifications.

2.5: Biological activities of aqueous extracts:

2.5.1:-Anti-coagulation activity: by using the method of (**U.S.A., Pharmacopoeia 1960**).

2.5.2: Evaluation of Fibrinolytic activity: according to (**U. S. A., Pharmacopeia, 1960**).

2.5.3: Antimicrobial activity in vitro:

2.5.3.1: Testing for antibacterial activity: agar diffusion method was adopted according to (**Kavanagh, 1972**).

2.5.3.2: Anti-fungal activity: The cup-plate agar diffusion method was adopted according to **(Brantner**, **et. al., 1994)**.

2.5.3.2: Anti-viral activity : by two steps**:**

- **cytotoxicity assay (TC^{50}):** of the extracts were tested by method (**Mossman, 1983**)
- **Plaque reduction assay:** was carried out according to the method of (**Hayden et al., 1980**)

2.5.4: Antioxidant test by DPPH Radical Scavenging Assay according to the method of **(Gamez EJ, et al., 1998) and (Liu L, et al., 2009).**

2.5.5: Antitumor activity by using SRB Cell survival assay: Cell survival will be determined using SulphoRhodamine-B (SRB) method as previously described by (**Skehan et al., 1990)**

Results and Discussion

1. Biochemical composition evaluation of investigated mushroom :

1.1. Chemical composition of the investigated mushroom:

Table (1): Biocemical analysis of Ash, lipid, total carbohydrates and their monosaccharide constituents of investigated mushroom expressed as (mg/100mg)

Mushroom	Ash	Lipid	Protein	Total Carbohydrates	Monosaccharide Composition (w/w %)					
					U.A	Gal	Glu	Man	Ara	Xyl
A. bisporus	9.23	2.6	45.35	37.51	0.8	18.5	57.8	7.9	9.8	5.2
P. florida	6.66	3.1	25.92	39.25	0.6	19.9	52.7	9.4	11.6	5.8

U.A (Uronic acid), Gal (Galactose), Glu (Glucose), Man (Mannose), Ara (Arabinose) and Xyl (xylose)

The results recorded in Table (1) and illustrated graphically in Figures (1) showed that the ash content highly in A. bisporus (6.66%) and low in P.florida (9.23%) of its dry weight, the highest was in the rhizome of Agaricus bisporus (9.23%), while the lowest was in the Pleurotus florida (6.66%). However determination of lipid contents of the investigated mushroom score in (3.1) in P. florida higher than A. bisporus in (2.6) .However the obtained results are in consistent with that obtained by, **(Nuhu Alam et al, 2008) and (Barros, et al, 2007).**

As part of our continuing studies to achieve some information about the composition of carbohydrate part, the results of these chromatographic investigations Table (1) and Figure (2) revealed the presence of galactose, glucose, mannose, xylose and arabinose as structural unites the polymeric seed carbohydrates. Any way this may due to the mushroom mode of nutrition and as a mechanism of compound turn over.

Figure (1): Ash, Lipid, protein and total carbohydrates in investigated mushroom

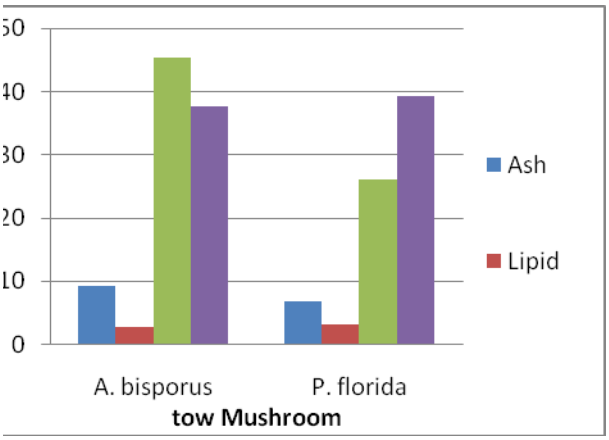
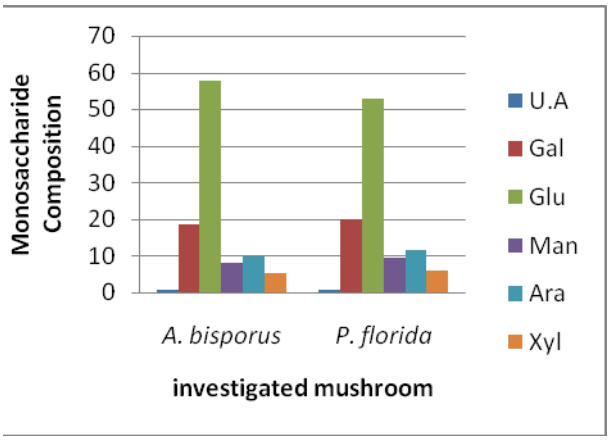


Figure (2): Monosaccharide Composition of investigated mushroom.



The chemical analysis of aqueous and methanolic extracts of mushroom under study as was follows: The extraction of the investigated mushroom was done with water under different PH conditions and methanol. The efficiency of extraction processes, for isolating aqueous extracts, was investigated and chemical characteristics of the extracts have been determined. This was carried out by sulfation of the each aqueous extracts and the chemical analysis of the resulted sulfated products indicated the presence of sharp increases in their sulfate contents which aimed improve biological activities as compared to those of corresponding origins. With a view to the immediate goal of production of extracts that might be promising for drug production. However the obtained results are in agreement with that obtained by **(Ajith and, Janardhanan, 2003) .**

1.2. The biochemical composition of aqueous and methanolic extracts of white mushroom Agaricus bisporus.

Table (2): Yield, sulphate content, protein, total carbohydrates and monosaccharide constituents in aqueous and methanolic extracts of white mushroom "Agaricus bisporus" (mg/100mg)

Extract	Yield	Sulfate content	Protein	Total Carbohydrates	Monosaccharide Composition (w/w %)					
					U.A	Gal	Glu	Man	Ara.	Xyl
A-1	12.3	3.4	36.36	38.52	0.7	16.6	52.9	9.8	13.6	6.1
A-2	65.5	2.3	40.25	42.63	1.2	17.9	56.3	11.1	11.3	2.2
A-3	16.8	2.4	37.65	39.68	0.8	19.7	55.6	8.9	12.9	2.1
A-4	42.4	2.2	52.55	35.95	1.1	18.3	51.8	10.1	16.5	2.2

A-1 (Acidic), A-2 (Neutral), A-3 (Alkaline), A-4 (Methanolic) extract of Agaricus bisporus, U.A (Uronic acid), Gal (Galactose), Glu (Glucose), Man (Mannose), Ara (Arabinose) and Xyl (xylose)

Data obtained from Table (2) and Figure (3) showed a high variation in the yield of Agaricus bisporus, the highest value in aqueous extract was recorded in neutral of (65.8 %) and lowest in acidic (12.3%). From these results, the highest sulfate content was recorded in acidic aqueous extract (3.4%) and the lowest in methanolic extract (2.2%). The highest soluble-protein values in methanolic aqueous extract were recorded (52.55%) and the lowest in acidic aqueous extract (36.36%). Whereas we can be also note that the highest total carbohydrate in aqueous extract recorded in neutral (42.63%) and lowest in methanolic (35.95%). However the obtained results are in consistent with that obtained by **(Wang X. et al., 2014) and (Beluhan and ,Ranogajec, 2011)**

Figure (3): Yield, total carbohydrates, protein and sulphate content in aqueous and methanolic extracts of Agaricus bisporus

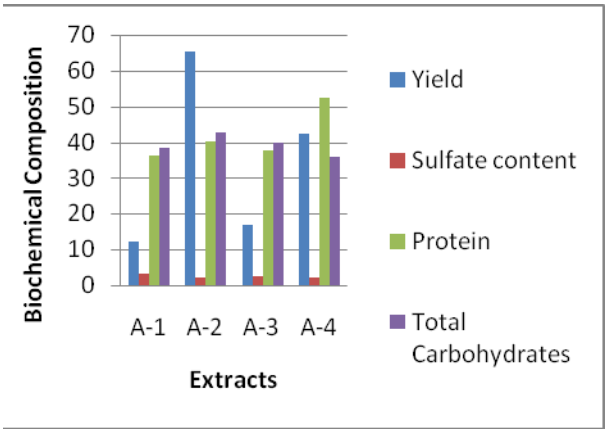
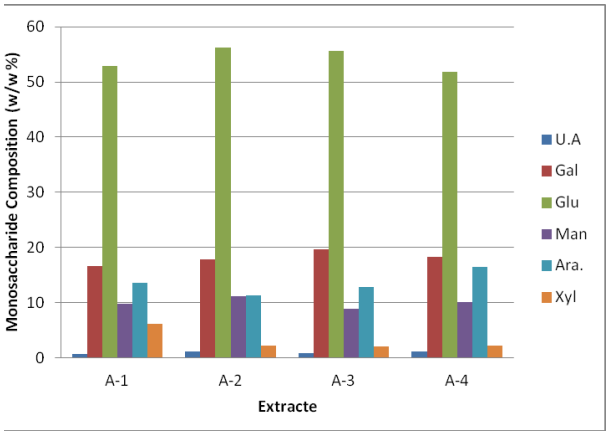


Figure (4): Monosaccharide constituents of acid hydrolysis of Agaricus bisporus in crude aqueous and methanolic extracts.



As seen in the Table (2) and Figure (4) chromatographic analysis of the acid hydrolysates of various aqueous extracts revealed the presence of galactose, glucose, mannose, arabinose, xylose and glucuronic acid in variations values. However the obtained results are in consistent with that obtained by **(Wasser, 2002)**

Table (3): Yield, sulphate content, protein, total carbohydrates and monosaccharide constituents in aqueous and methanolic chemically modified (sulfated) extracts of white mushroom "Agaricus bisporus" (mg/100mg)

Extract	Yield	Sulfate content	Protein	Total Carbohydrates	Monosaccharide Composition (w/w %)					
					U.A	Gal	Glu	Man	Ara.	Xyl
SA-1	68.2	25.2	40.46	42.55	0.9	15.9	53.2	8.8	14.1	7,1
SA-2	61.3	35.8	43.75	47.35	0.6	17.5	55.1	9.3	12.2	5.3
SA-3	55.8	39.9	41.35	44.72	0.7	18.6	52.8	9.5	13.5	4.9
SA-4	52.4	23.6	45.35	38.51	0.8	16.9	54.2	10.4	11.6	6.1

SA-1 (Sulphated acidic), SA-2 (Sulphated neutral), SA-3 (Sulphated alkaline) and SA-4(Sulphated methanolic) extract of Agaricus bisporus, U.A (Uronic acid), Gal (Galactose), Glu (Glucose), Man (Mannose), Ara (Arabinose) and Xyl (xylose)

Data obtained from Table (3) and Figure (5) showed a high variation in the yield of Agaricus bisporus aqueous extract, the highest value in sulfated extract the acidic extract was record highest value of (68.2 %) and lowest of (52.4 %) in methanolic extract. From these results, the highest sulfate content was recorded in sulfated extract the highest value recorded in alkaline of (39.9%) and lowest of (23.6%) in methanolic extract. The highest soluble-protein values in sulfated extract highest value was obtained in methanolic extract of (45.35%) and lowest of (40.46%) in acidic extract. Whereas we can be also note that the highest total carbohydrate in sulfated extract highest value of (47.35%) in neutral extract and lowest value of (38.51%) in methanolic extract.

Figure (5): Yield, total carbohydrates, protein and sulphate content in aqueous and methanolic sulfated extracts of Agaricus bisporus

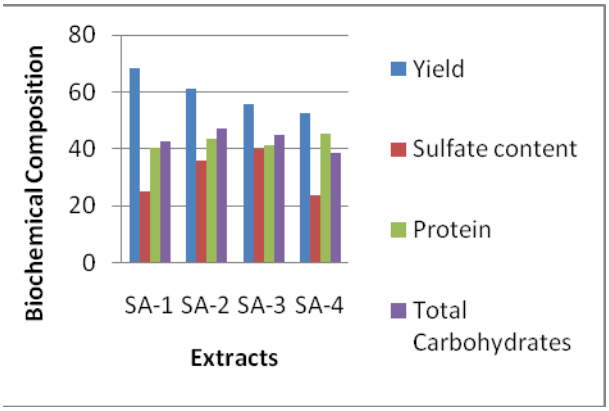
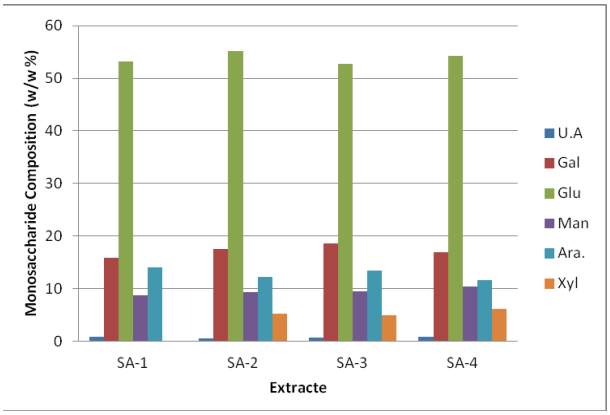


Figure (6): Monosaccharide constituents of acid hydrolysis of Agaricus bisporus in sulfated aqueous and methanolic extracts



On the other hand in sulfated extract as seen in the Table (3) and Figure (6) Chromatographic analysis of the acid hydrolysates of various aqueous extracts revealed the presence of galactose, glucose, mannose, arabinose, xylose and glucuronic acid in variations values .

1.3. Biochemical composition aqueous and methanolic extracts of oyster mushroom "Pleurotus florida".

Data obtained from Table (4) and Figure (7) showed a wide range of variation in the yield of oyster mushroom Pleurotus florida aqueous extract in the highest value was recorded in neutral extract of (58.4%) and lowest in alkaline extract (12.6%). The data also indicated that the highest sulfate content was recorded in acidic aqueous extract (1.8%) and lowest in methanolic of (1.1%).

Table (4): Yield, sulphate content, protein, total carbohydrates and monosaccharide constituents of aqueous and methanolic extracts of Oyster mushroom "Pleurotus florida" (mg/100mg)

Extract	Yield	Sulfate content	Protein	Total Carbohydrates	Monosaccharide Composition (w/w %)					
					U.A	Gal	Glu	Man	Ara.	Xyl
P-1	15.2	1.8	32.5	40.2	0.8	21.4	51.9	11.2	9.8	4.9
P-2	58.4	1.5	34.6	45.5	0.7	19.8	52.7	20.7	4.6	1.5
P-3	12.6	1.7	65.9	42.6	1.1	25.1	49.8	13.9	6.7	3.4
P-4	36.8	1.1	36.7	38.9	1.0	19.7	50.1	11.7	10.9	6.6

P-1 (Acidic), P-2 (Neutral), P-3 (Alkaline), P-4 (Methanolic) extract of Pleurotus florida, U.A (Uronic acid), Gal. (Galactose), Glu. (Glucose), Man. (Mannose), Arab. (Arabinose) and Xyl. (Xylose

Noteworthy, the highest soluble-protein values were recorded of (65.9%) in alkaline aqueous extract and lowest in acidic aqueous extract of (32.5%). And also the highest value of total carbohydrate contents of (45.5%) in neutral extract and lowest in methanolic extract (38.9%).

Figure (7): Yield, total carbohydrates, protein and sulphat content of P. florida in crude aqueous and methanolic extracts

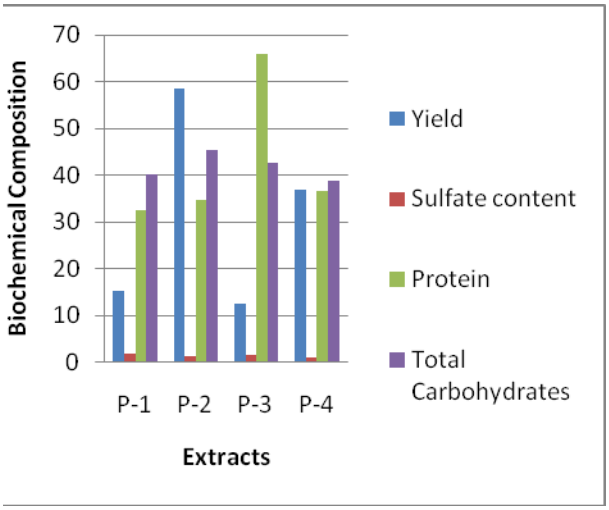
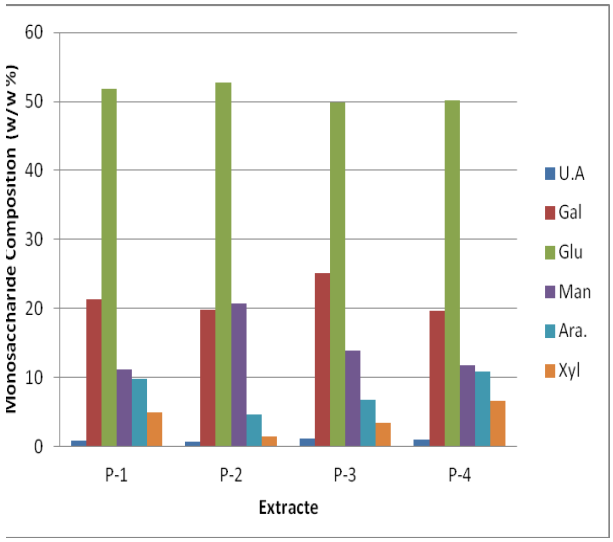


Figure (8): Monosaccharide constituents of acid hydrolysis of P. florida in aqueous and methanolic extracts



As seen in the Table (4) and Figure (8) chromatographic analysis of the acid hydrolysates of various aqueous extracts of P. florida revealed the presence of galactose, glucose, mannose, arabinose, xylose and glucuronic acid. Furthermore, quantitative determination of the aforementioned monosaccharide components indicated that the majors are glucose and galactose while the other sugars are the minors **(Yun-Tao Liu, et al., 2012).**

Table (5): Yield, sulphate content, protein, total carbohydrates and monosaccharide constituents of chemically modified (sulfated) aqueous and methanolic extracts of Oyster mushroom "Pleurotus florida" (mg/100mg)

Extract	Yield	Sulfate content	Protein	Total Carbohydrates	Monosaccharide Composition (w/w %)					
					U.A	Gal	Glu	Man	Ara.	Xyl
SP-1	14.9	38.6	29.2	42.3	0.7	22.7	50.4	10.6	9.6	6.0
SP-2	9.7	29.9	31.7	46.6	0.8	18.6	51.9	19.8	5.0	3.9
SP-3	58.5	46.7	32.4	43.8	1.0	24.6	48.9	13.7	6.5	5.3
SP-4	11.3	43.8	34.7	60.4	0.9	19.4	49.5	10.9	11.1	8.2

SP-1 (Sulphated acidic), SP-2 (Sulphated neutral), SP-3 (Sulphated alkaline), SP-4 (Sulphated methanolic) extract of Pleurotus florida, U.A (Uronic acid), Gal. (Galactose), Glu. (Glucose), Man. (Mannose), Arab. (Arabinose) and Xyl. (Xylose

Data obtained from Table (5) and Figure (9) showed a wide range of variation in the yield of oyster mushroom Pleurotus florida highest value was recorded of (58.5) in alkaline and lowest in neutral of (9.7%). The data also indicated that the highest sulfate content in sulfated extract the highest value of (46.7%) recorded in alkaline and the lowest value seen in neutral of (29.9%) noteworthy, in sulfated extract soluble-protein obtained the highest value of (34.7%) in methanolic extract and lowest in acidic extract of(29.2%). And also sulfated extract the highest value recorded of (60.4%) in methanolic extract and in acidic recorded lowest value of (42.3%).

Figure (9): Yield, total carbohydrates, protein and sulphat content of P. florida in sulfated aqueous and methanolic extracts

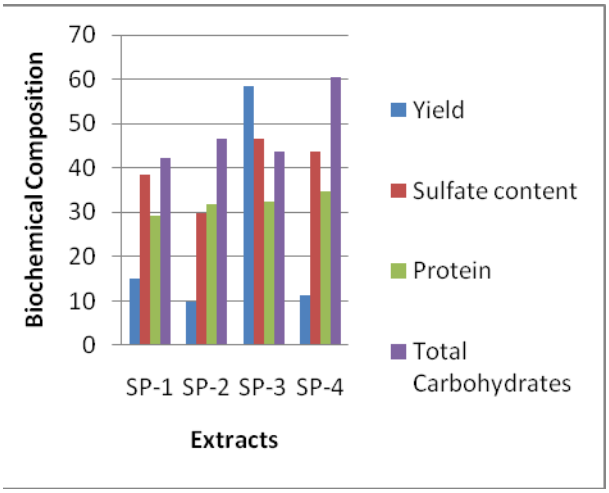
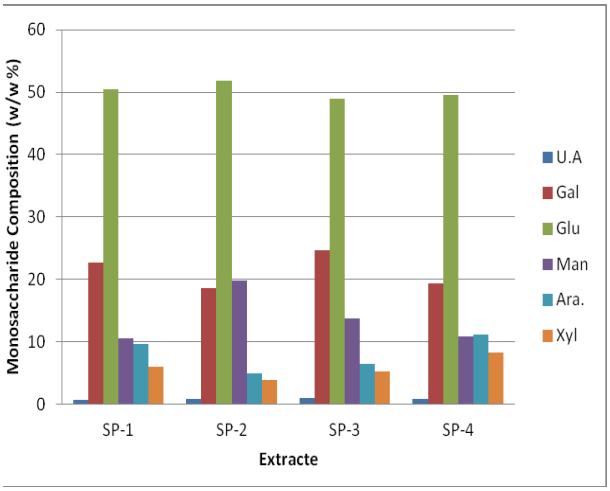


Figure (10): Monosaccharide constituents of acid hydrolysis of P. florida in sulfated aqueous and methanolic extracts.



As seen in the Table (5) and Figure (10) Chromatographic analysis of the acid hydrolysates of various aqueous extracts of P. florida revealed the presence of galactose, glucose, mannose, arabinose, xylose and glucuronic acid. Furthermore, quantitative determination of the aforementioned monosaccharide components indicated that the majors are glucose and galactose while the other sugars are the minors.

2. Biological activities:

A part of the present work has been devoted to evaluate the (anticoagulant, fibrinolytic, antimicrobial, antioxidant, antitumor and antiviral activities) of the aqueous and methanolic extracts from white mushroom "Agaricus bisporus" and oyster mushroom "Pleurotus florida". According to (**María Elena Valverde et al., 2015)**

2.1. Testing aqueous extracts for its anticoagulation activity in vitro:

Table (6): The anticoagulation activity of aqueous extracts and their sulphated aqueous extracts against human plasma (min)

Extracts	Clotting Time (min)							
	Concentrations of extracts (µg /ml)							
	cont.	2000	1000	500	200	100	50	25
A-1	5	90	25	---	---	---	---	---
A-2	5	100	30	---	---	---	---	---
A-3	5	60	15	---	---	---	---	---
A-4	5	30	---	---	---	---	---	---
SA-1	5	> 600	> 600	> 600	> 600	250	45	---
SA-2	5	> 600	> 600	> 600	290	120	50	---
SA-3	5	> 600	> 600	320	140	50	10	---
SA-4	5	60	10	---	---	---	---	---
P-1	5	50	10	---	---	---	---	---
P-2	5	25	---	---	---	---	---	---

P-3	5	60	12	---	---	---	---	---
P-4	5	25	---	---	---	---	---	---
SP-1	5	180	75	25	---	---	---	---
SP-2	5	45	10	---	---	---	---	---
SP-3	5	150	60	12	---	---	---	---
SP-4	5	60	---	---	---	---	---	---

(A-1, A-2, A-3, A-4, SA-1, SA-2, SA-3 and SA-4) acidic, neutral, alkaline, methanolic and its sulfated extract of Agaricus bisporus respectively, (P-1, P-2, P-3, P-4, SP-1, SP-2, SP-3 and SP-4) acidic, neutral, alkaline, methanolic and its sulfated extract of Pleurotus florida respectively,

Results in Table (6) are variable in its activity from less activity show in neutral aqueous extract of P. florida (25 min) and highly activity noted in modified aqueous extracts (acidic, neutral and alkaline) of A. bisporus more than (600 min) in the same concentration of extracts in 2000 µg /ml. However the obtained results are in consistent with that obtained by **(Wang et al., 2010)**

Testing of aqueous extracts for its fibrinolytic activity in vitro. 2.2:

As part of our interest in this study was to evaluate various aqueous and methanolic extracts and their corresponding sulphates against fibrinolytic activity compared with standard fibrinolytic, Hemoclar drug (Pentosan sulfuric polyester, product of Clin Midy. Paris). The results in Table (7) showed that the acidic extract, sulfated acidic extract, sulfated neutral and sulfated alkaline extract of A. bisporus, sulfated acidic, sulfated neutral, sulfated alkaline, sulfated methanolic extract of P. florida exhibited fibrinolytic activities equivalent to double of the same amount of standard "Hemoclar" and the acidic extract and neutral extract of P. florida and acidic show fibrinolytic activities about of the same amount of standard "Hemoclar" preparation and (neutral extract of A. bisporus, alkaline, methanolic extract of P. florida not show any fibrinolytic activities. However the obtained results are in consistent with that obtained by other authors, such as, **(Borchers et al., 2004 , Poucheret et al., 2006 and Yeh M.Y. et al., 2014).**

Table (7): Fibrinolytic activities of aqueous extracts and their corresponding chemically modified extracts (2000 µg/ml)

Extracts		**Fibrinolytic activity (2000 µg/ml)**	
		Crud extracts	**Modified extracts**
A. bisporus	Acidic	+8	+9
	Neutral	+1	+7
	Alkaline	+7	+8
	Methanolic	+3	+3
P. florida	Acidic	+6	+8
	Neutral	+6	+7
	Alkaline	+1	+8
	Methanolic	+1	+8
Standard (Heparin)		**+4**	

Lysis of plasma clot using standard Hemoclar ((2000µg/ml): 4(+).,
(+9): Lysis of more than 90 % of plasma clot, (+8): Lysis of more than 80 % of plasma clot
(+7): Lysis of more than 70 % of plasma clot, (+6): Lysis of more than 60 % of plasma clot,
(+5): Lysis of more than 50 % of plasma clot, (+4): Lysis of more than 40 % of plasma clot,
(+3): Lysis of more than 30 % of plasma clot, (+2): Lysis of more than 20 % of plasma clot,
(+1): Lysis of more than 10 % of plasma clot, (---): not Lysis of plasma clot

Antimicrobial activity In vitro. 2.3:

Testing of aqueous extracts for Antibacterial activity: 2.3.1:

Table (8): The Antibacterial activity of Hexane, Chloroform and their aqueous extracts and corresponding chemically modified extracts (mm):

Extracts (20 mg/ml	**Diameter of inhibition zone (mm**		
	Control	**Gram positive**	**Gram Negative**

	solvent	Streptom	B.subtilis	S.aureus	E.coli	K.pneumonia
A-1	--	15	10	12	--	7
A-2	--	18	11	11	6	12
A-3	--	20	--	10	9	8
A-4	--	20	12	12	10	9
SA-1	--	21	10	--	--	8
SA-2	--	19	7	--	9	--
SA-3	--	20	9	8	8	10
SA-4	--	18	9	9	7	--
P-1	--	18	--	10	--	11
P-2	--	19	11	12	11	12
P-3	--	20	--	--	--	--
P-4	--	20	14	9	11	--
SP-1	--	18	--	--	9	--
SP-2	--	19	15	--	8	10
SP-3	--	20	--	--	--	9
SP-4	--	21	--	--	11	--

(A-1, A-2, A-3, A-4, SA-1, SA-2, SA-3 and SA-4) acidic, neutral, alkaline, methanolic and its sulfated extract of Agaricus bisporus respectively, (P-1, P-2, P-3, P-4, SP-1, SP-2, SP-3 and SP-4) acidic, neutral, alkaline, methanolic and its sulfated extract of Pleurotus florida respectively,

Results in Table (8) are variable in its antimicrobial activity the high activity show in methanolic extract of P. florida (14 mm) against B.subtilis and week activity note in modified alkaline aqueous extracts of P. florida activity varied from (zero B.subtilis, S.aureus, E.coli to 9 mm against K.pneumonia) in the same concentration of extracts in 20 mg /ml. However the obtained results are in consistent with that obtained by many authors, **(Hirasawa, et al., 1999; Dulger et al., 2002).**

Testing of aqueous extracts for its Antifungal activity: 2.3.2:

Table (9): The Antifungal activity of aqueous and methanolic extracts and corresponding chemically modified of mushroom extracts (mm).

Extracts (20 mg/ml	Diameter of inhibition zone (mm					
	Control		Organisms			
	solvent	Griseo	C.albicans	A.niger	A.flavus	p.chrysogenum
A-1	--	14	19	16	--	--
A-2	--	16	14	12	--	--
A-3	--	17	--	13	--	--
A-4	--	16	10	--	--	--
SA-1	--	18	9	14	--	12
SA-2	--	15	--	--	--	--
SA-3	--	18	10	9	--	8
SA-4	--	17	12	--	--	--
P-1	--	18	12	17	--	14

P-2	--	17	14	12	--	12
P-3	--	18	--	11	--	--
P-4	--	16	11	--	14	--
SP-1	--	18	--	8	--	--
SP-2	--	17	--	7	--	--
SP-3	--	18	--	--	--	11
SP-4	--	18	9	8	--	--

(A-1, A-2, A-3, A-4, SA-1, SA-2, SA-3 and SA-4) acidic, neutral, alkaline, methanolic and its sulfated extract of Agaricus bisporus respectively, (P-1, P-2, P-3, P-4, SP-1, SP-2, SP-3 and SP-4) acidic, neutral, alkaline, methanolic and its sulfated extract of Pleurotus florida respectively.

Antifungal activity of aqueous and methanolic extracts their corresponding chemically modified extracts showed significant activity when compared with Griseofulvin as standard antifungal agent Table (9) Highly activity recognized in acidic aqueous extracts of A. bisporus (19 mm) against C. albicans and lowly activity recognized in modified aqueous extracts of P. florida in the same concentration of extracts in 20 mg /ml. Also these results are in accordance with that obtained by **(Turkog˘lu et al., 2007).**

2.3.3: Testing of extracts and its sulfated extracts for its Antiviral activity :

The samples show from very weak to moderate activity against H5N1 influenza virus. Results in Table (10) are variable in its antiviral activity started from zero up to the highest activity show in modified methanolic extract of P. florida , any way these results are in accordance with that obtained by **(Moradali et al., 2007)**

Table (10): Antiviral activity measured using Plaque reduction assay of H5N1 influenza virus

Extracts	Conc.µg/µl	Initial viral count	Viral count (PFU/ml)	% of Inhibition
A-1	50	0.52×10^{10}	0.52×10^{10}	0
	100		0.49×10^{10}	6
A-2	50	0.52×10^{10}	0.49×10^{10}	6
	100		0.35×10^{10}	33
A-3	50	0.52×10^{10}	0.51×10^{10}	2
	100		0.44×10^{10}	15
A-4	50	0.52×10^{10}	0.51×10^{10}	2
	100		0.51×10^{10}	2
SA-1	50	0.91×10^{10}	0.82×10^{10}	10
	100		0.72×10^{10}	21
SA-2	50	0.91×10^{10}	0.72×10^{10}	21
	100		0.72×10^{10}	21
SA-3	50	0.90×10^{10}	0.79×10^{10}	12
	100		0.66×10^{10}	27
SA-4	50	0.90×10^{10}	0.73×10^{10}	19
	100		0.68×10^{10}	24
P-1	50	0.65×10^{10}	0.62×10^{10}	5

	100		0.48 X 10^{10}	26
P-2	50	0.65X 10^{10}	0.65 X 10^{10}	0
	100		0.65 X 10^{10}	0
P-3	50	0.76 X 10^{10}	0.73 X 10^{10}	4
	100		0.73 X 10^{10}	4
P-4	50	0.76 X 10^{10}	0.65 X 10^{10}	14
	100		0.60 X 10^{10}	21
SP-1	50	0.47 X 10^{10}	0.47 X 10^{10}	0
	100		0.28 X 10^{10}	40
SP-2	50	0.47 X 10^{10}	0.47 X 10^{10}	0
	100		0.47X 10^{10}	0
SP-3	50	0.81 X 10^{10}	0.53 X 10^{10}	34.5
	100		0.48 X 10^{10}	41
SP-4	50	0.81 X 10^{10}	0.70 X 10^{10}	13.5
	100		0.37 X 10^{10}	54

Testing of aqueous and methanolic extracts and its sulfated extracts for its Antioxidant activity 2.4:

Antioxidant activities of aqueous and methanolic extracts and its sulfated extracts compared with Rutin and Vitamin C. With regard to scavenging ability on DPPH radicals, Results in table (--) are variable in its activity the highest value show in neutral aqueous extract of A. bisporus (52.82) and the lowest value note in modified alkaline aqueous extracts (31.88) where in the same concentration µg /ml of standard antioxidation agent (Rutin and Vitamin C show that (62.27 and 61.33 respectively. In spite of the obtained results are in consistent with that obtained by many authors**, (Cheung et al., 2003)** and **(Wong and Chye 2009),** nevertheless , the implicated mechanisms are not yet completely elucidated. (**Miyazawa, et al., 2008**) and **J. Lee et al.,(2013)**.

Table(11) Antioxidant activities of aqueous and methanolic extracts and its modified (sulfated) extracts.

Extracts (mg/ml)		**Antioxidant activities**	
		Crude extract	**Modified extract**
A. bisporus	Acidic	46.93	39.34
	Neutral	52.82	48.79
	Alkaline	45.19	47.48
	Methanolic	49.98	40.93
P. florida	Acidic	38.95	35.97
	Neutral	47.56	39.78
	Alkaline	36.78	31.88
	Methanolic	42.95	35.56
Standard	**Rutin**	**62.27**	
	Vitamin C	**61.33**	

2.5: Testing of aqueous and methanolic extracts for its Antitumor test:

Table(12) Testing of extracts and its sulfated extracts for its Antitumor test to HEPG-2 Cells (liver Cancer)

Extracts		IC_{50} (µg/ml)	
		Crude extract	**Modified extract**
A. bisporus	Acidic	0.3032	6.125
	Neutral	4.3571	7.0223
	Alkaline	2.601	4.6828
	Methanolic	22.7938	65.5332
P. florida	Acidic	0.1293	6.664
	Neutral	101.631	9.1037
	Alkaline	3.4366	3.2707
	Methanolic	116.7682	88.0369
Standard (Doxorubicin)		**3.0738**	

IC_{50} meaning (concentration inhibiting 50% of the growth) the results in Table (12) are variable in its antitumor activity the lowest IC_{50} show in acidic aqueous extracts of P. florida (0.1293 µg/ml) and the highest IC_{50} show in Methanolic aqueous extracts of P. florida (116.7682 µg/ml). However the obtained results are in consistent with that obtained by many authors, **(Wasser, et al., 2002), (Ikekawa, 2001), (Tong et al., 2009)** and **(Zhang et al., 2007).**

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