

Extraction, Purification and evaluation
of some antitumor compounds from
Pleurotus ostreatus

By

Lena Ahmed Saleh Al-Faqeeh

**A Thesis Submitted
to
Faculty of Science**

**In Partial Fulfillment of the
Requirements for
the Degree of
Master of Science
(Microbiology)**

**Botany Department
Faculty of Science
Cairo University**

2010

ABSTRACT

Student Name: Lena Ahmed Saleh Al-Faqeeh

Title of the thesis: Extraction, Purification and evaluation of some antitumor compounds from *Pleurotus ostreatus*

Degree: Master of Science (Microbiology)

A factorial design L18 ($2^1 \times 1^1 \times 3^6$) was used to construct 18 media with different composition and condition, in addition to modified medium containing all the optimum factors for maximizing the proteoglycan production by the edible mushroom *Pleurotus ostreatus*. Media 16, 18 and modified were selected for mycelial proteoglycan study, whereas fruiting bodies grown on rice straw were chosen for fruiting bodies proteoglycan study. Complete purification scheme using ion exchange on DEAE- cellulose and gel filtration chromatography on sephadex G-100 revealed the presence of three peaks in fruiting bodies proteoglycan (FF1, FF2 and FF3) and one main fraction in mycelial proteoglycan (MF1). The FTIR revealed spectra of β -glucosidic bond, OH, C-O and C-O-C stretching bonds and spectra peaks of amide I, II and III. The HPLC analysis of the monosaccharides composition of *Pleurotus ostreatus* proteoglycan indicated the presence of arabinose, fructose, mannose, glucose and galactose in varying ratios among pure fractions. The amino acids analysis of proteoglycans showed the presence of 18 amino acids with leucin being of highest quantity. *In vitro* anticancer assay of the proteoglycans of *P.ostreatus* revealed its potent anticancer activity against cervical carcinoma (HELA), breast carcinoma (MCF7) and larynx carcinoma (HEP2) and to less extent on liver carcinoma (HEPG2) cell line while colon carcinoma (HCT116) was resistant. The crude extracts were more efficient than the pure fractions. *In vivo* assays using head and neck cancer bearing mice indicating that the pure fractions were more necrotic to cancer cells than the crude extracts. For rapid prediction of cancer advancement and healing in head and neck bearing mice, three biological markers were assayed which are plasma catalase, Red blood cells glutathione peroxidase, plasma lipid peroxidase and three

elements which are Zinc, Copper and Selenium. Measurements of these markers in head and neck bearing mice treated with *P.ostreatus* proteoglycans predicted a sign of cancer inhibition or healing.

Keywords: *Pleurotus ostreatus*, cancer disease, anticancer activity, proteoglycan compounds, cancer markers.

Supervisors:

Signature:

1- **Prof.Dr. Tahany M. A. Abdel Rahman**

2- **Dr. Tarek A. A. Moussa**

3- **Prof.Dr. Nahed Zakaria Heikel**

Prof. Dr. Efat Shabana

Chairman of Botany Department
Faculty of Science- Cairo University

APPROVAL SHEET FOR SUBMISSION

**Extraction, Purification and evaluation
of some antitumor compounds from
*Pleurotus ostreatus***

By

Lena Ahmed Saleh Al-Faqeeh

**This thesis has been approved for submission by the
supervisors:**

1- Prof. Dr. Tahany M. A. Abdel Rahman Cairo University

Signature:

2- Dr. Tarek A. A. Moussa Cairo University

Signature:

3- Prof.Dr. Nahed Zakaria Heikel Cairo University

Signature:

Prof. Dr. Efat Shabana

**Chairman of Botany Department
Faculty of Science- Cairo University**

ACKNOWLEDGEMENTS

For the one who guided me, instructed me, support me, learn me, took my hand and picked me up (thank you **God**)

I would like to express my deepest appreciation to **Prof. Dr. Tahany M. A. Abdel Rahman**, Professor of Microbiology, Botany Department, Faculty of Science, Cairo University for supervision, valuable discussion, supporting, understanding and kindness.

Also, I would like to express my deepest grateful to **Dr. Tarek A. A. Moussa**, Associate Professor of Microbiology, Botany Department, Faculty of Science, Cairo University for supervision, scientific advice through the work, continuous guidance and encouragement and understanding.

Also, I would like to express my appreciation to **Prof. Dr. Nahed Z. Heikel**, Professor of Microbiology, Botany Department, Faculty of Science, Cairo University for understanding and kindness

Also, I would like to thank **German Academic Exchange Service** that gave me the chance for preparation of master's degree and for their support throughout the period of scholarship.

Dedication

To the three pillars of my life: my parents, my brothers and sisters for their supporting, understanding and love.

List of Appreviation

EAT	:	Ehrlich ascites tumor
PBS	:	Phosphate buffer saline.
FF1	:	Fruiting bodies fraction 1.
FF2	:	Fruiting bodies fraction 2.
FF3	:	Fruiting bodies fraction 3.
MF1	:	Mycelial fraction 1.
HELA	:	Cervical carcinoma cell line.
MCF7	:	Breast carcinoma cell line.
HEP2	:	Larynx carcinoma cell line.
HCT116	:	Colon carcinoma cell line.
HEPG2	:	Liver carcinoma cell line.
PPI	:	Proteoglycan Production Index
C/P	:	Carbohydrate/Protein.
P/C	:	Protein/Carbohydrate.
H and E	:	Hematoxylin and Eosine stain

X

List of Tables

- Table 1.** Cultural factors levels
- Table 2.** L18 ($2^1 \times 1^1 \times 3^6$) orthogonal array of the designed experimental media
- Table 3.** Effect of medium compositions on the mycelial growth and proteoglycan production in *Pleurotus ostreatus*
- Table 4.** Differentiated effect of the selected media (16 and 18) in comparison with modified medium on growth and proteoglycan production in *Pleurotus ostreatus*
- Table 5.** Effect of substrate types on fruiting bodies production in *Pleurotus ostreatus* and their proteoglycan content
- Table 6.** Purification scheme of proteoglycan compound extracted from *Pleurotus ostreatus* fruiting bodies and mycelium
- Table 7.** HPLC analysis of monosaccharides in the purified fractions from mycelium and fruiting bodies of *Pleurotus ostreatus*.
- Table 8.** Amino acids composition of crude proteoglycan complex extracted from *Pleurotus ostreatus* and its peak fractions
- Table 9.** Effect of proteoglycan complex extracted from fruiting bodies and mycelium of *P. ostreatus* on the head and neck cancer biomasses of different mice groups
- Table 10.** Plasma catalase activity in male and female mice injected with head and neck cancer and treated with proteoglycans complex extracted from fruiting bodies and mycelium of *Pleurotus ostreatus*.
- Table 11.** Plasma lipid peroxidase activity in male and female mice injected with head and neck cancer and treated with proteoglycans complex extracted from fruiting bodies and mycelium of *Pleurotus ostreatus*
- Table 12.** Red blood cells Glutathione peroxidase activity in male and female mice injected with head and neck cancer and treated with proteoglycans complex extracted from fruiting body and mycelium of *Pleurotus ostreatus*

Table 13. Serum selenium in male and female mice injected with head and neck cancer and treated with proteoglycans complex extracted from fruiting bodies and mycelium of *Pleurotus ostreatus*

Table 14. Serum zinc in male and female mice injected with head and neck cancer and treated with proteoglycans complex extracted from fruiting bodies and mycelium of *Pleurotus ostreatus*

Table 15. Serum copper in male and female mice injected with head and neck cancer and treated with proteoglycans complex extracted from fruiting bodies and mycelium of *Pleurotus ostreatus*.

Table 16. Illustrate the IC_{50} ($\mu\text{g ml}^{-1}$) recorded in the *in vitro* assays

Table 17. Expresses the different degrees of cancer cells, inflammatory cells infiltration, necrobiosis and necrosis in head and neck cancer bearing mice.

List of figure

- Fig. (1).** Typical elution profile for the behaviour of *P. ostreatus*. Mycelial proteoglycan on DEAE- cellulose using Phosphate buffer saline pH (7.4).
- Fig. (2).** Typical elution profile for the behaviour of *P. ostreatus* mycelial proteoglycan on Sephadex G-100 using Phosphate buffer saline pH (7.4).
- Fig. (3).** Typical elution profile for the behaviour of *P.ostreatus* fruiting bodies proteoglycan on DEAE- cellulose using Phosphate buffer saline pH (7.4).
- Fig. (4).** Typical elution profile for the behaviour of *P.ostreatus* fruiting bodies proteoglycan on Sephadex G-100 using Phosphate buffer saline pH (7.4).
- Fig. (5).** FTIR spectra of crude proteoglycan complex and its fraction FF1, FF2 and FF3 extracted from fruiting bodies of *P.ostreatus*
- Fig. (6).** FTIR spectra of crude proteoglycan complex and its fraction MF1 extracted from mycelial of *P.ostreatus*.
- Fig. (7).** HPLC chromatography of monosaccharides in purified fractions from mycelium and fruiting bodies of *Pleurotus ostreatus*.
- Fig. (8).** *In vitro* anticancer activity of crude proteoglycan complex extracted from *P.ostreatus* mycelium grown on PDA (basal medium).
- Fig. (9).** *In vitro* anticancer activity of crude proteoglycan complex extracted from *P. ostreatus* mycelium grown on (medium 16).
- Fig. (10).** *In vitro* anticancer activity of crude proteoglycan complex extracted from *P. ostreatus* mycelium grown on (medium 18).
- Fig. (11).** *In vitro* anticancer activity of crude proteoglycan complex extracted from *P. ostreatus* mycelium grown on (modified medium).
- Fig. (12).** *In vitro* anticancer activity of proteoglycan fraction MF1 extracted from *P. ostreatus* mycelium grown on (modified medium) as compared to crude proteoglycan complex

- Fig. (13).** *In vitro* anticancer activity of crude proteoglycan complex extracted from *P. ostreatus* fruiting bodies.
- Fig. (14).** *In vitro* anticancer activity of proteoglycan fraction FF1 extracted from *P. ostreatus* fruiting bodies as compared to crude proteoglycan complex.
- Fig. (15).** *In vitro* anticancer activity of proteoglycan fraction FF2 extracted from *P. ostreatus* fruiting bodies as compared to crude proteoglycan complex.
- Fig. (16).** *In vitro* anticancer activity of proteoglycan fraction FF3 extracted from *P. ostreatus* fruiting bodies as compared to crude proteoglycan complex.
- Fig. (17).** Plasma catalase activity in male and female mice injected with head and neck cancer and treated with proteoglycans complex extracted from fruiting bodies and mycelium of *Pleurotus ostreatus*.
- Fig. (18).** Plasma lipid peroxidase activity in male and female mice injected with head and neck cancer and treated with proteoglycans complex extracted from fruiting bodies and mycelium of *Pleurotus ostreatus*
- Fig. (19).** Red blood cells Glutathione peroxidase activity in male and female mice injected with head and neck cancer and treated with proteoglycans complex extracted from fruiting body and mycelium of *Pleurotus ostreatus*
- Fig. (20).** Serum selenium in male and female mice injected with head and neck cancer and treated with proteoglycans complex extracted from fruiting bodies and mycelium of *Pleurotus ostreatus*
- Fig. (21).** Serum zinc in male and female mice injected with head and neck cancer and treated with proteoglycans complex extracted from fruiting bodies and mycelium of *Pleurotus ostreatus*
- Fig. (22).** Serum copper in male and female mice injected with head and neck cancer and treated with proteoglycans complex extracted from fruiting bodies and mycelium of *Pleurotus ostreatus*

List of photos

- Photo 1: Male control head and neck carcinoma bearing mice treated with PBS**
- Photo 2: Male head and neck carcinoma bearing mice treated with fruiting bodies crude proteoglycan complex**
- Photo 3: Male head and neck carcinoma bearing mice treated with fruiting bodies FF1**
- Photo 4: Male head and neck carcinoma bearing mice treated with fruiting bodies FF2**
- Photo 5: Male head and neck carcinoma bearing mice treated with fruiting bodies FF3**
- Photo 6: Male head and neck carcinoma bearing mice treated with mycelial crude proteoglycan complex**
- Photo 7: Male head and neck carcinoma bearing mice treated with mycelial MF1**
- Photo 8: Female control head and neck carcinoma bearing mice treated with PBS**
- Photo 9: Female head and neck carcinoma bearing mice treated with fruiting bodies crude proteoglycan complex**
- Photo 10: Female head and neck carcinoma bearing mice treated with fruiting bodies FF1**
- Photo 11: Female head and neck carcinoma bearing mice treated with fruiting bodies FF2**
- Photo 12: Female head and neck carcinoma bearing mice treated with fruiting bodies FF3**
- Photo 13: Female head and neck carcinoma bearing mice treated with mycelial crude proteoglycan complex**
- Photo 14: Female head and neck carcinoma bearing mice treated with mycelial MF1**

CONTENTS

	Pages
INTRODUCTION	1
1- REVIEW OF LITRETURE	3
1.1. Nutritive value of Mushrooms	3
1.2. Fruiting bodies production of <i>Pleurotus</i> Species	4
1.3. Antitumor activity of mushrooms	6
1.4. Antitumor activity of <i>Pleurotus ostreatus</i>	12
1.5. Identification and characterization of antitumor compounds from mushrooms	13
2- MATERIALS AND METHODS	18
2.1. Test organism and cultural conditions	18
2.1.1. Optimization of the medium composition and cultural conditions for proteoglycan production	18
2.2. Cultivation of <i>Pleurotus ostreatus</i> fruiting bodies	21
2.3. Extraction of proteoglycan from mycelial mat and fruiting bodies	22
2.4. Biochemical analyses of proteoglycan	22
2.4.1. Carbohydrate content	22
2.4.2. Protein content	22
2.5. <i>In vitro</i> antitumor activity of proteoglycan complex	23
2.6. Purification and characterization of proteoglycan extracted from mycelia and fruiting bodies of <i>P. osteratus</i>	23
2.6.1. Column chromatography using DEAE- cellulose	23
2.6.2. Chromatographic gel filtration by Sephadex G-100 column	23
2.6.3. Fourier Transformed Infra Red (FTIR) analysis	24
2.6.4. High- performance liquid chromatography (HPLC) analysis of monosaccharide	24

2.6.5. Amino acid analysis	24
2.7. <i>In vivo</i> anticancer activity of proteoglycan complex	24
2.7.1. Infection experiment	24
2.7.2. Estimation of tumor markers	25
2.7.2.1. Plasma catalase and Lipid peroxide (Malondialdehyde) activity assay	25
2.7.2.1.1. Determination of Plasma catalase	25
2.7.2.1.2. Determination of Plasma Lipid Peroxidase	27
2.7.2.2. Erythrocyte lysates glutathione peroxidase activity assay	28
2.7.2.3. Serum selenium, zinc and copper estimation	30
2.8. Histopathological assay	31
2.9. Statistical analyses	31
3- EXPERIMENTAL RESULTS	32
3.1. Proteoglycan production by <i>P.ostreatus</i> mycelial mats	32
3.2. Proteoglycan production from <i>P.ostreatus</i> fruiting bodies	37
3.3. Purification of <i>P.ostreatus</i> proteoglycan from mycelial mats	39
3.4. Purification of <i>Pleurotus ostreatus</i> proteoglycan from fruiting bodies	43
3.5. FTIR spectra	46
3.6. Monosaccharides composition in proteoglycan purified fractions	49
3.7. Amino acids composition in crude proteoglycan complex and its pure fractions	49
3.8. <i>In vitro</i> anticancer assays	54
3.9. <i>In vivo</i> anticancer assay	65
3.9.1 Estimation of cancer biomasses in male and female head and neck carcinoma bearing mice.	65
3.10. Histopathological assays	67
3.10.1. Male mice groups	68

3.10.2. Female mice groups	72
3.11. Assay of some enzyme markers activity in Red blood cells and serum of head and neck cancer bearing mice	76
3.12. Assay of some metal markers in mice sera	84
4- DISCUSSION	92
5- SUMMARY	107
6- REFERENCE	110
7- ARABIC SUMMARY	