

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON AVICENNIA MARINA (FORSK.) VIERH GROWN IN EGYPT

Thesis
Submitted for the Partial Fulfillment of the Degree of
Master of Science in Botany

By

Nabeha Samir Ibraheem Mohamad B.Sc. in Science (Botany-Chemistry)

Ain Shams University
Faculty of Science
Botany Department



Ain Shams University Faculty of Sciece Botany Department

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON AVICENNIA MARINA (FORSK.) VIERH GROWN IN EGYPT

Thesis

Submitted for the Partial Fulfillment of the Degree of Master of Science in Botany

By

Nabeha Samir Ibraheem Mohamad

B.Sc. in Science (Botany-Chemistry)

("

Supervised By

Prof. Dr. Seham M. Ali Moustafa

Professor of Plant Physiology, Botany Department, Faculty of Science, Ain Shams University

Prof. Dr. M. Magdel-Din Hussein

Professor of Chemistry of Microbial and Natural Products National Research Centre

Prof. Dr. Abla Hassan Nassar

Professor of Plant Physiology (Plant Tissue Culture and Biotechnology),

Botany Department, Faculty of Science, Ain Shams University

Ain Shams University
Faculty of Science
Botany Department

Approval Sheet

Title of the Thesis: Physiological and Biochemical Studies on

Avicennia marina (Forsk.) Vierh Grown in Egypt

Degree: Master of science in Botany

Name: Nabeha Samir Ibraheem Mohamad

This Thesis for Master of Science Degree has been approved by:

- Prof. Dr. Mohamed Abdo K. Shaddad

Professor of Plant Physiology, Botany Department, Faculty of Science, Assuit University

- Prof. Dr. Hamdia Mahmoud Abd El- Samad

Professor of Plant Physiology, Botany Department, Faculty of Science, El- Minia University

- Prof. Dr. Seham M. Ali Moustafa

Professor of Plant Physiology, Botany Department, Faculty of Science, Ain Shams University

- Prof. Dr. M. Magdel-Din Hussein

Professor of Chemistry of Microbial and Natural Products, National Research Centre

Date of examination: / /Y··V

| This thesis has not been previously submitted for any degree at |
|---|
| this or at any other university. |
| |
| |
| Signed |
| Nabeha Samir Ibraheem |
| |
| |
| |
| |
| |
| |
| |
| |

AKNOWLEDGEMENT

Firstly and Finally Thanks for Allah

Sincerely, I express my deepest thanks, grateful appreciation, and certainly a conventional word of acknowledgement cannot pay for the valuable guidance I have received from Prof. Dr. Mohamed Hussein Darwesh Professor Magdel-Din Chemistry of Natural and Microbial Products, National Research Centre, Prof. Dr. Seham M. Ali Moustafa, Professor of Plant Physiology, Department of Botany, Faculty of Science, Ain Shams University, and Prof. Dr. Abla Hassan Nassar. Professor of Physiology (Plant Tissue Culture Plant and Biotechnology), Department of Botany, Faculty of Science, Ain Shams University, and for their support, understanding and continuous encouragement.

I also grateful for Prof. Dr. Azza M. Saber El-Shafey, Head of Botany Department, Faculty of Science, Ain Shams University.

I also offer my thanks for all my colleagues at the National Research Centre.

ABSTRACT

Samples of Avicennia marina (Forsk.) Vierh were collected from a location at Safaga Mangrove Stand, 'V' km south Safaga, Red Sea shore, Egypt at different seasons; i.e., summer, autumn, winter, and spring. Natural products were analyzed in leaves, lateral branches, bark and seeds for possible use in certain drug production. The season and the plant organ were detected, where highest amounts of respective plant constituents were attained. In this instance, polysaccharides comprised the major constituents of Avicennia marina organs and could be isolated into two fractions: PI including material of relatively high molecular weight and PII with lower molecular weight. In general, the PI yield in different plant organs at different seasons was higher than that of corresponding PII product. Mostly, the PI products were characterized by relatively higher anticoagulant fibrinolytic and activities corresponding PII products. Seven of the components in the PI fraction and three in the PII product exhibited fibrinolytic activities higher than that of the preparation. standard Hemoclar Sulfating the polysaccharide fractions of Avicennia marina was also found promising, with respect to enhancing their biological activity. Assessing possible growth regulating activities of the polysaccharide fractions of *Avicennia marina* plant organs at different seasons was carried out, using callus tissue initiated from *Helianthus annus* seedlings. The most interesting result was the rooting- inductive effect of the PI products originated from the autumn leaves and the winter bark.

Contents

| Subjects | Page |
|--|------|
| Introduction | |
| Aim of work | |
| Materials and Methods | |
| Materials | |
| Mangrove plants | |
| Chemicals | |
| Methods | |
| Chemical analysis of the mangrove plant | |
| samples | |
| Determination of moisture content | |
| Determination of ash | |
| Determination of total lipids | |
| Determination of low-molecular weight | |
| carbohydrates | |
| Determination of total nitrogen and crude | |
| protein | |
| Determination of cellulose and hemicellulose | |
| Determination of lignin | |
| Determination of pectin | |
| Isolation of different forms of alkali-extractable | |
| polysaccharides from the mangrove plant | |
| Analysis of the alkali-extractable | |
| polysaccharides | |
| Determination of total carbohydrates | |
| Qualitative examination and quantitative | |
| determination of the sugar moieties comprising | |
| the isolated alkali-extractable polysaccharides | |
| Determination of soluble protein | |
| Biological activities of the isolated | |
| polysaccharides Anti-googylation activity | |
| Anti-coagulation activity | |
| Fibrinolytic activity | |

| Subjects | Page |
|--|------|
| Fractional precipitation of polysaccharides with | |
| ethanol | |
| Sulfation of the isolated polysaccharides | |
| Cleavage of the sulfate ester group | |
| Turbidimetric assay of the liberated sulfate | |
| Reaction with toluidine blue | |
| Plant tissue culture techniques | |
| Seed sterilization, germination and preparation | |
| of explants for callus induction | |
| Formation, preparation of culture medium and | |
| inoculation of explants | |
| Treatment of callus with polysaccharides | |
| Determination of mono and disaccharides in | |
| callus tissues | |
| Results | |
| Discussion | |
| English summary | |
| References | |
| الموجز باللغة العربية | I |
| | I |

List of Tables

| | List of Tables | | | | |
|---------|--|------------|--|--|--|
| No. | Table | Page | | | |
| I. Meta | I. Metabolic constituents of Avicennia marina as affected by | | | | |
| seasona | l variation | | | | |
| | Biochemical constituents of Avicennia marina | | | | |
| | leaves as influenced by seasonal variation. | | | | |
| | Biochemical constituents of Avicennia marina | | | | |
| | branches as influenced by seasonal variation. | | | | |
| | Biochemical constituents of Avicennia marina | | | | |
| | bark as influenced by seasonal variation. | | | | |
| | Biochemical constituents of Avicennia marina | | | | |
| | seeds as influenced by seasonal variation. | | | | |
| II. Iso | lation and characterization of alkali-ex | ktractable | | | |
| polysac | charides | | | | |
| | Yeild and analytical characteristics of alkali- | | | | |
| | extractable polysaccharides isolated from | | | | |
| | Avicennia marina leaves. | | | | |
| | Yeild and analytical characteristics of alkali- | | | | |
| | extractable polysaccharides isolated from | | | | |
| | Avicennia marina branches. | | | | |
| | Yeild and analytical characteristics of alkali- | | | | |
| | extractable polysaccharides isolated from | | | | |
| | Avicennia marina bark. | | | | |
| | Yeild and analytical characteristics of alkali- | | | | |
| | extractable polysaccharides isolated from | | | | |
| | Avicennia marina seeds. | | | | |
| | Monosaccharide constituents of alkali- | | | | |
| | extractable polysaccharides isolated from | | | | |
| | Avicennia marina leaves. | | | | |
| | Monosaccharide constituents of alkali- | | | | |
| | extractable polysaccharides isolated from | | | | |
| | Avicennia marina branches. | | | | |
| | Monosaccharide constituents of alkali- | | | | |
| | extractable polysaccharides isolated from | | | | |
| | Avicennia marina bark. | | | | |
| | Monosaccharide constituents of alkali- | | | | |
| | extractable polysaccharides isolated from | | | | |
| | Avicennia marina seeds. | | | | |
| | | | | | |
| | | | | | |

| No. | Table | Page | | | |
|-----|---|------|--|--|--|
| | III. Anticoagulation and fibrinolytic activity of the isolated polysaccharides | | | | |
| | Biological activities of alkali-extractable polysaccharides isolated from <i>Avicennia marina</i> leaves. | | | | |
| | Biological activities of alkali-extractable polysaccharides isolated from <i>Avicennia marina</i> branches. | | | | |
| | Biological activities of alkali-extractable polysaccharides isolated from <i>Avicennia marina</i> bark. | | | | |
| | Biological activities of alkali-extractable polysaccharides isolated from <i>Avicennia marina</i> seeds. | | | | |
| | Chemical and biological characteristics of various polysaccharides fractions isolated from autumn leaves of <i>Avicennia marina</i> | | | | |

List of Figures

| List of Figures | | | |
|-----------------|--|------|--|
| No. | Figure | Page | |
| | Biochemical constituents of <i>Avicennia marina</i> leaves as influenced by seasonal variation. | | |
| | Biochemical constituents of <i>Avicennia marina</i> branches as influenced by seasonal variation. | | |
| | Biochemical constituents of <i>Avicennia marina</i> bark as influenced by seasonal variation. | | |
| | Biochemical constituents of <i>Avicennia marina</i> seeds as influenced by seasonal variation. | | |
| | Yeild and analytical characteristics of alkali- extractable polysaccharides isolated from <i>Avicennia marina</i> leaves. | | |
| | Yeild and analytical characteristics of alkali- extractable polysaccharides isolated from <i>Avicennia marina</i> branches. | | |
| | Yeild and analytical characteristics of alkali- extractable polysaccharides isolated from <i>Avicennia marina</i> bark. | | |
| | Yeild and analytical characteristics of alkali- extractable polysaccharides isolated from <i>Avicennia marina</i> seeds. | | |
| | A chromatogram showing the monosaccharide constituents of alkali-extractable polysaccharides isolated from <i>Avicennia marina</i> leaves. | | |
| | A chromatogram showing the monosaccharide constituents of alkali-extractable polysaccharides isolated from <i>Avicennia marina</i> branches. | | |
| | A chromatogram showing the monosaccharide constituents of alkali-extractable polysaccharides isolated from <i>Avicennia marina</i> bark. | | |

| No. | Figure | Page |
|-----|--|------|
| | A chromatogram showing the monosaccharide constituents of alkali-extractable polysaccharides isolated from | |
| | Avicennia marina seeds. | |
| | Biological activities of alkali-extractable polysaccharides isolated from <i>Avicennia marina</i> leaves. | |
| | Biological activities of alkali-extractable polysaccharides isolated from <i>Avicennia marina</i> branches. | |
| | Biological activities of alkali-extractable polysaccharides isolated from <i>Avicennia marina</i> bark. | |
| | Biological activities of alkali-extractable polysaccharides isolated from <i>Avicennia marina</i> seeds. | |
| | Sunflower hypocotyls cultivated on MS medium | |
| | Friable callus produced from sunflower hypocotyls cultivated on MS medium supplemented with or without <i>Avicennia marina</i> polysaccharides | |
| | The effect of different polysaccharides extracted from <i>Avicennia marina</i> on the percentage of glucose in <i>Helianthus annus</i> L. callus tissues | |
| | The effect of different polysaccharides extracted from <i>Avicennia marina</i> on the percentage of sucrose in <i>Helianthus annus</i> L. callus tissues | |