

EVALUATING PHYSIOLOGICAL AND BIOCHEMICAL
RESPONSES OF *AMARANTHUS CRUENTUS* TOWARDS
POLYCYCLIC AROMATIC HYDROCARBONS INDUCED
OXIDATIVE STRESS

THESIS

SUBMITTED
FOR
AWARD OF DEGREE OF

MASTER OF PHARMACY IN PHARMACOGNOSY

IN THE SCHOOL OF STUDIES OF NATURAL RESOURCES
SUBMITTED TO
GURU GHASIDAS VISHWAVIDYALAYA
BILASPUR (C.G.)

(A Central University established by the Central Universities Act 2009 No. 25 of 2009)



SESSION 2020-2022

SUPERVISED BY
Dr. Vivekananda Mandal
(M.Pharm, Ph.D.)
Assistant Professor

SUBMITTED BY
Pragya Gupta
M. Pharm. IV semester
Enroll No: GGM/20/06330
Roll No: 20706310

DEPARTMENT OF PHARMACY,
GURU GHASIDAS VISHWAVIDYALAYA BILASPUR (C.G.)

(A Central University established by the Central Universities Act 2009 No. 25 of 2009)

**EVALUATING PHYSIOLOGICAL AND BIOCHEMICAL
RESPONSES OF *AMARANTHUS CRUENTUS* TOWARDS
POLYCYCLIC AROMATIC HYDROCARBONS INDUCED
OXIDATIVE STRESS**

THESIS

**SUBMITTED
FOR
AWARD OF DEGREE OF**

MASTER OF PHARMACY IN PHARMACOGNOSY

IN THE SCHOOL OF STUDIES OF NATURAL RESOURCES

SUBMITTED TO

GURU GHASIDAS VISHWAVIDYALAYA

BILASPUR (C.G.)

(A Central University established by the Central Universities Act 2009 No. 25 of 2009)



SESSION 2020-2022

SUPERVISED BY

**Dr. Vivekananda Mandal
(M.Pharm. Ph.D.)
Assistant Professor**

SUBMITTED BY

**Pragya Gupta
M. Pharm. IV semester
Enroll No: GGV/20/06330
Roll No: 20706010**

DEPARTMENT OF PHARMACY,

GURU GHASIDAS VISHWAVIDYALAYA, BILASPUR (C.G.)

(A Central University Established by the Central University Act-2009 No. 25 of 2009)



**DEPARTMENT OF PHARMACY,
GURU GHASIDAS VISHWAVIDYALAYA, BILASPUR (C.G.)**

(A Central University Established by the Central University Act-2009 No. 25 of 2009)

Tel. No. 07752-260027; Fax No. 07752-260148

FORWARDING CERTIFICATE

This is to certify that **Miss Pragya Gupta** D/o Mr. Tapeswar Gupta is a student of M. Pharm. IV semester in Department of Pharmacy (GGV) has submitted her dissertation entitled “**Evaluating Physiological and Biochemical Responses of *Amaranthus cruentus* towards Polycyclic Aromatic Hydrocarbons Induced Oxidative Stress**” for the fulfillment of the requirement for the Degree of **Master of Pharmacy (Pharmacognosy)** at **Department of Pharmacy, Guru Ghasidas Vishwavidyalaya Bilaspur (C.G.)**. She has completed her dissertation work under the supervision of **Dr. Vivekananda Mandal** (Assistant professor).

I hereby forward her dissertation report in M. Pharm.(Pharmacognosy) during the academic session 2020-2022.

FORWARDED BY

Dr. Bharti Ahirwar

Associate Professor and HOD

Department of Pharmacy,

Guru Ghasidas Vishwavidyalaya Bilaspur (C.G.)

Date: 19/11/2022

Place: GGV, Bilaspur.

Head

**Department of Pharmacy
Guru Ghasidas Vishwavidyalaya
(A Central University)
Bilaspur (C.G.)**



**DEPARTMENT OF PHARMACY,
GURU GHASIDAS VISHWAVIDYALAYA, BILASPUR (C.G.)**

(A Central University Established by the Central University Act-2009 No. 25 of
2009)

Tel. No. 07752-260027; Fax No. 07752-260148

CERTIFICATE

This is to certify that **Miss Pragya Gupta** D/o Mr. Tapeswar Gupta student of M. Pharm. IV semester, Department of Pharmacy, Guru Ghasidas Vishwavidyalaya Bilaspur (C.G.) has submitted her dissertation report entitled "**Evaluating Physiological and Biochemical Responses of *Amaranthus cruentus* towards Polycyclic Aromatic Hydrocarbons Induced Oxidative Stress**" for the fulfillment of the requirement for the Degree of **Master of Pharmacy (Pharmacognosy)** at Department of Pharmacy, Guru Ghasidas Vishwavidyalaya Bilaspur (C.G.). She has completed her dissertation report under my supervision.

I hereby forward her project report for the award of degree of M. Pharm. (Pharmacognosy) during the academic session 2020-2022. I wish her every success in future life.

Supervised By

Dr. Vivekananda Mandal

Assistant Professor

Department of Pharmacy,

Ghasidas Vishwavidyalaya Bilaspur (C.G.)

Date: 09/11/2022

Place: GGU, Bilaspur



**DEPARTMENT OF PHARMACY,
GURU GHASIDAS VISHWAVIDYALAYA, BILASPUR (C.G.)**

(A Central University Established by the Central University Act-2009 No. 25 of
2009)

Tel. No. 07752-260027; Fax No. 07752-260148

DECLARATION

I hereby declare that the dissertation report entitled “**Evaluating Physiological and Biochemical Responses of *Amaranthus cruentus* towards Polycyclic Aromatic Hydrocarbons Induced Oxidative Stress**” was done by me. The entire work was done with the guidance and suggestion received from my supervisor **Dr. Vivekananda Mandal** (Assistant Professor) at Department of Pharmacy, Guru Ghasidas Vishwavidyalaya Bilaspur (C.G.). The same is submitted to Guru Ghasidas Vishwavidyalaya, Bilaspur (C.G.) for the fulfillment of the requirement for the degree of **Master of Pharmacy (Pharmacognosy)**.

Pragya Gupta

Pragya Gupta

M.Pharm. IV Semester

Roll No. 20706010

Enrollment No. GGV/20/06330

Department of Pharmacy

Guru Ghasidas Vishwavidyalaya, Bilaspur (C.G.)

Date: 09/11/2022

Place: GGU, Bilaspur.

ACKNOWLEDGEMENT

I humbly and whole heartedly bow my head before Lord Almighty for blessing me all his grace to successfully complete my studies and research endeavor at Guru Ghasidas University.

It is a great pleasure to acknowledge my deepest thanks and gratitude to everyone who supported me throughout the course of thesis work. I wish to extend my sincere and heartfelt obligation to work all the personages who have helped me in this endeavour. Without their active guidance, help, cooperation and encouragement, I would not have made any progress in my project.

By the grace of Almighty, I take this opportunity to express my deep sense of gratitude to my supervisor **Dr. Vivekananda Mandal**, Assistant Professor, Department of Pharmacognosy, Department of Pharmacy, Guru Ghasidas Vishwavidyalaya, Bilaspur (C.G.) for providing me excellent guidance, motivation, encouragement and care throughout my research work. I am highly thankful to him for critical inputs, thought provoking suggestions during the progress of the work and writing of dissertation.

I am also thankful to **Dr. Bharti Ahirwar** (Head of Department) Pharmacy Guru Ghasidas Vishwavidyalaya, Bilaspur (C.G.) for providing me the required facilities and also their timely words of advice, despite of their busy schedule.

I am thankful to, **Prof. Vinod D. Rangari (Former H.O.D. and Dean)**, **Dr. Arjun Patra**, and **Dr. Neelirose beck** for their constant efforts and support throughout my project work and post-graduation as well.

I would like to thanks all the non-teaching staff of the department for their contribution and assistance.

I would like to thank and express my gratitude to my seniors and friends, **Mr. Kavi Bhushan Chouhan, Mr. Souvik Mukherjee, Dr. Roshni Tandey, Monika Chandrakar, Kajal Lal** who contributed in some or other way for the success of this research work.

I would like to acknowledge the analytical services provided by **Birbal Sahni Institute of Palaeosciences, Lucknow** and **CIL, Central University of Punjab-Bhatinda**.

Now, Last but not the least; my unbound gratitude and affection drive to my beloved parents **Mrs. Tapeswar Gupta, Mrs. Udiyan Gupta** for bringing me up in the best of ways, for rendering me the best of education, for nurturing in me the best of ideals. I am very happy to express my special appreciation and thanks to my brother **Mr. Deepak Gupta** and sister **Miss. Sadhna Gupta** for their limitless love, affection and support.

Sincerely thanks to all.

Date: 09/11/2022

Place: GGU, Bilaspur.

Pragya Gupta
PRAGYA GUPTA

TABLE OF CONTENTS

	Page No
List of Tables	i
List of Figures	ii - iv
1. INTRODUCTION	1-5
2. AIM AND OBJECTIVES	6
3. REVIEW OF LITERATURE	7-39
3.1. An Overview of A Selected Plant	7-12
3.2. Polyaromatic Hydrocarbons (PAHs)	12-18
3.3. Polyaromatic Hydrocarbons (PAHs) Research on Plants	18-34
3.4. Research Status	35
3.4.1. International	35-38
3.4.2. National	38-39
4. MATERIAL AND METHODS	45-65
4.1. Standards and reagents	40
4.2. Apparatus	40-43
4.3. Description of study area	43-45
4.4. Description of control area	43-44
4.5. Description of sample area	44-45
4.6. Collection of plant sample	45
4.7. Plant sample authentication	45
4.8. Processing of plant sample	46
4.9. Estimation of chlorophyll content	46
4.10. Histochemical analysis	47-50
4.10.1. In-vivo localization of reactive oxygen species	47-48

4.10.1.1. In vivo Localization of super oxide anion radical (O_2^-)	47
4.10.1.2. Localization of hydrogen peroxide radical (H_2O_2)	47-48
4.10.1.3. Localization of Malondialdehyde (MDA)	48
4.10.1.4. Visualization of cell death	48
4.10.2. Localization of cell permeability changes	48-49
4.10.3. Localization of phenolic principles	49
4.10.4. In situ-localization of viable plant cells	49
4.10.5. Field emission scanning electron microscope (SEM)	50
4.11. Determination of SOD enzyme activity	50-51
4.12. Nutraceutical profiling	51-60
4.12.1. Protein Extraction	51-53
4.12.2. Determination of carbohydrate content	53-55
4.12.3. Determination of total phenolic content (TPC)	55-57
4.12.4. Total flavonoid content (TFC)	57-58
4.12.5. Anti-oxidant Activity	59-60
4.12.6. LC-MS Analysis	60
4.13. PAH Extraction and enrichment	61-65
4.13.1. Gas Spectrometry Flame Ionization Detector (GC-FID)	65
4.14. Statistical analysis	65
5. RESULTS AND DISCUSSION	66-102
5.1. Chlorophyll content	66-68
5.2. Histochemical Analysis	69-85
5.2.1. In-leaf estimation and visualization of ROS (O_2^- , H_2O_2 , OH^-)	69-74
5.2.1.1. Histochemical analysis of leaf tissue by NBT staining method	69-71
5.2.1.2. Histochemical analysis of DAB staining method	72-73

5.2.1.3.	Histochemical analysis of lipid peroxidation	73-74
5.2.1.4.	Histochemical analysis of Evan's blue staining method	75-76
5.2.2.	Visualization of cell membrane permeability	76-78
5.2.3.	In-situ localization of Phenolics/Flavonoids	78-79
5.2.4.	In-situ localization of viable cells	80-82
5.2.5.	Scanning Electron Microscopy reports (SEM)	82-85
5.3.	Super oxidase dismutase	85-87
5.4.	Nutraceutical profiling	87-95
5.4.1.	Carbohydrate content	87-88
5.4.2.	Impact on protein content	89-90
5.4.3.	Total Phenolic content (TPC)	90-91
5.4.4.	Total Flavonoid content (TFC)	92-93
5.4.5.	Antioxidant Activity	93-95
5.5.	HPTLC Semi-quantification for phenolic/flavonoid compound	96-99
5.6.	Identified phenolics/flavonoids of <i>A. cruentus</i> under PAH induced oxidative stress	99-102
5.7.	GC-FID Analysis of PAHs	101-102
6.	CONCLUSION	103-104
7.	REFERENCES	105-111
8.	ANNEXURES	