Final Report of Major Research Project

(From 2nd May 2013 to 30th April 2017)

"Effect of Bacoside A on Various Organs of Mouse During Aging"

Submitted To

The secretary

University Grant Commission

Bahadur Shah Zafar Marg

New Delhi – 110002.

Submitted By

Dr. Mrs. Sushama S. Pawar
Assistant Professor,
Department of Zoology,
Yashwantrao Mohite College of Arts, Science and Commerce,
Bharati Vidyapeeth (Deemed To Be University)
Erandwane, Pune-38, Maharashtra, India.
zooymc@gmail.com Mob No.9822765229

BHARATI VIDYAPEETH (DEEMED TO BE UNIVERSITY)
YASHWANTRAO MOHITE COLLEGE OF ARTS, SCIENCE AND COMMERCE, PUNE – 411038, MAHARASHTRA (INDIA)

FINAL REPORT

File no. : 42-533/2013(SR), Date: 22nd March 2013

MAJOR RESEARCH PROJECT IN ZOOLOGY
FACULTY OF SCIENCE

Entitled

Effect of Bacoside A on Various Organs of Mouse During Aging

Principal Investigator
Dr. Mrs. Sushama Sunil Pawar
(Assistant Professor)

SUBMITTED TO

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI-110 002
Year -2018

UGC Major Research Project in Zoology (Science)

Final Report

File No. 42-533/2013(SR)
Sanctioned from 22nd March 2013
w. e. f. 1st April 2013
Tenure : 03 years + 01 Year extension
2nd May 2013 - 30th April 2017

Project title

"Effect of Bacoside A on Various Organs of Mouse During Aging"

Principal Investigator : Dr. Mrs. Sushama S. Pawar

Assistant Professor,
Department of Zoology,
Yashwantrao Mohite College of Arts, Science and Commerce,
Bharati Vidyapeeth (Deemed To Be University)
Erandwane, Pune-38, Maharashtra, India.
zooymc@gmail.com Mob No.9822765229

Submitted to

The Secretary,
University Grants Commission,
Bahadur Shah Zafar Marg,
New Delhi – 110 002.

Bharati Vidyapeeth
(Deemed to be University)
YASHWANTRAO MOHITE COLLEGE OF ARTS, SCIENCE & COMMERCE
Erandwane, Pune - 411 038

Founder-Chancellor :
Dr. Patangrao Kadam
M.A., LL.B., Ph.D.

Incharge Principal :
Dr. S. R. Patil
M.Sc., Ph.D.

★ Accredited with 'A+' Grade (2017) by NAAC ★
★ 'A' Grade University Status by MHRD, Govt. of India ★
★ Accredited (2004) & Reaccredited (2011) with 'A' Grade by NAAC ★

Tel. : (020) 25433383, 25424163
Fax : (020) 25440201
E-mail : bvduymc@hotmail.com
ymc@bharatividyapeeth.edu
Website : ymc.bharatividyapeeth.edu

Ref. No. BVDU/YMC/UGC/182/2018-2019 **Date:** 02.08.2018

To,
The Secretary,
University Grants Commission,
Bahadur Shah Zafar Marg,
New Delhi- 110002.

Ref : UGC/ File no: 42-533/2013(SR) Date: 22nd March 2013.
Subject : Submission of Final Report of Major Research Project entitled **"Effect of Bacoside A on Various Organs of Mouse During Aging,"** by P.I. Dr. Mrs. Pawar S. S. in Zoology.

Sir,

We are pleased to forward the completed final report of the major research project in Zoology (Science), as referred above, completed by Principal Investigator Dr. Mrs. Sushama S. Pawar, Assistant Professor, Department of Zoology working in this college. The project entitled **"Effect of Bacoside A on Various Organs of Mouse During Aging"**. I am forwarding herewith the necessary documents for the final report of the project of Dr. Mrs. Sushama S. Pawar as per the UGC guidelines.

Please find enclosed

1. Annexure III -Statement of expenditure
2. Annexure IV-Statement of expenditure incurred on field work(Travelling and Field work)
3. Annexure V -Audited Utilization certificate, Annexure VI, Annexure VII, Annexure VIII
4. Annexure IX

Total dues to be reimbursed from UGC, Delhi, as a receivable amount Rs. **1,63,249/-** (In words Rupees One Lakh Sixty Three Thousand Two Hundred and Forty Nine only).
You are cordially requested to accept and approve the accounts and arrange to send **[a)** Final installment **(Rs. 96,712/-) + b)** an excess expenditure incurred **(Rs. 53,095/-)** + and **c)** HRA there on @ 20% **(Rs. 13,442/-)** as detailed in the certificates attached**]** total receivable amount **1,63,249/- (In words Rupees One Lakh Sixty Three Thousand Two Hundred and Forty Nine only)**. Please approve the same and arrange to send the amount at an early date so as to settle the accounts at the college level and oblige.
Thanking You.

Yours Faithfully,

Dr. Mrs. S. S. Pawar
(Principal Investigator)
Principal Investigator

V. M. College No. F-9-15/95-U3 PUNE-38.

Dr. S. R. Patil
(Dr. S. R. Patil)
Incharge Principal
Yashwantrao Mohite College, Pune-38

Certificate of Declaration

I, Dr. Mrs. Sushama Sunil Pawar, declare that the work presented in this report is original and carried throughout independently by me during the complete tenure of Major Research Project of UGC New Delhi.

Principal Investigator

(Dr. Mrs. Sushama Sunil Pawar)
Assistant Professor and Principal Investigator
Department of Zoology
Bharati Vidyapeeth (Deemed To Be University)
Yashwantrao Mohite College of Arts, Science and Commerce,
Pune-411038. MH, (INDIA)

UGC Sanction Letter

23236351, 23232701, 23237721, 23234116
23235733, 23232317, 23236735, 23239437

विश्वविद्यालय अनुदान आयोग
बहादुरशाह जफर मार्ग
नई दिल्ली-110 002
UNIVERSITY GRANTS COMMISSION
BAHADURSHAH ZAFAR MARG
NEW DELHI-110 002

F. No. **42-533/2013 (SR)**

The Under Secretary (FD-III)
University Grants Commission
New Delhi-110002

22 MAR 2013

Sub:- UGC support for the Major Research Project in Physical Sciences, Bio-Sciences, Maths , Medical, Agricultural Sciences and Engineering & Chemistry to University/College Teachers – Project entitled,

"Effect of bacoside a on various organs of mouse during aging"

Sir,

I am to refer to your letter forwarding the application of **Dr. (Mrs.) Pawar Sushama Sunil** of your institution for financial assistance under the above scheme and to convey the Commission's approval & sanction an on ~~account grant of~~ **Rs. 8,69,300/- (Rupees: eight lakh sixty nine thousand three hundred only)** to the Principal, **Yashwantrao Mohite College, Erandawane, Pune-411038, MS** in r/o Major Research Project of **Dr. (Mrs.) Pawar Sushama Sunil,** Department of **Zoology** for the period of **3** years w.e.f. **1.4.2013** as detailed below:-

S.No	ITEMS	AMOUNT APPROVED	GRANT RELEASED AS Ist INSTALMENT	Category
A.	**Non - Recurring**		**3,80,000/-**	**OPEN**
1.	Books & Journals	30,000/-		
2.	Equipment (as per proposal)	3,50,000/-		
B.	**Recurring**			
1.	Honorarium to Retd. Teacher @ Rs. 12, 000/- p.m.	nil		
2.	Project Fellow @14,000/- p.m. for initial 2 years and Rs. 16,000/- p.m. from the third year onwards.	5,28,000/-		
3.	Chemical/ Glassware / Consumable	1,00,000/-		
4.	Hiring Services	75,000/-	**4,89,300/-**	
5.	Contingency	75,000/-		
6.	Travel/Field Work	45,000/-		
7.	Special Need	nil		
8.	Overhead Charges @ Rs. 10% approved recurring Grant (Except Travel & Field Work)	77,800/-		
	Total (A + B)	**12,80,800/-**	**8,69,300/-**	

The acceptance Certificate in prescribed format (Annexure-I available on the UGC web-site) may be sent to the undersigned within one month from the issue of the award letter failing which the project may be treated as cancelled.

If the terms & conditions are acceptable, as per guideline which are available on UGC web-site www.ugc.ac.in the Demand Draft/ Cheque being sent may be retained. Otherwise the same may be returned in original to the UGC by Registered Post in variably with in 15 days from the receipt of the Demand Draft/Cheque in favour of Secretary, UGC, New Delhi.

Principal Investigators should ensure that the statement of expenditure & utilization Certificate to the effect that the grant has been utilized for the purpose for which it has been sanctioned shall be furnished to the University Grants Commission in time.

The first instalment of the grant shall comprise of 100% of the Non –Recurring including Over Head Charges, and 50% of the total Recurring grant.

1. The sanctioned amount is debitable to the Major Head **4. (i) .a (31) Rs. 4,89,300/- & 4. (i) .a (35) Rs. 3,80,000/-** and is valid for payment during financial year **2012-13**.
2. The amount of the Grant shall be drawn by the Under Secretary (drawing and Disbursing Office), University Grants Commission on the Grants-in-aid Bill and shall be disbursed to and credited to the Principal, **Yashwantrao Mohite College, Erandawane, Pune-411038, MS** through Cheque/Demand Draft/ Mail Transfer.
3. The Grants is subject to the adjustment of the basis of Utilization Certificate in the prescribed performa submitted by the University/Colleges/institution.
4. The University/College shall maintain proper accounts of the expenditure out of the grants which shall be utilized only on approved items of expenditure.
5. The Utilization Certificate of the effect that the grant has been utilized for the purpose for which it has been sanctioned shall be furnished to the University Grants Commission as early as possible after the close of the current financial year.
6. The assets acquired wholly or substantially out of University Grant Commission's grant shall not be disposed or encumbered of utilized for the purposes other that those for which the grant was given, without proper sanctioned of the University Grants Commission and should, at any time the College/University ceased in function such assets shall revert to the University Grant Commission.
7. A Register of assets acquired wholly or substantially out of the grant shall be maintained by the University/College in the prescribed form.
8. The grantee institution shall ensure the utilization of grant-in-aid for which it is being sanction/paid. In case non-utilization/part utilization, the simple interest @ 10% per annum as amended from time to time on unutilized amount from the date of drawl to the date of refund as per provisions contained in General Financial Rules of Govt. of India will be charged.
9. The interest earned by the University/College/Institute on this grants in aid shall be treated as additional grant and may be shown in the Utilization Certificate/Statement of expenditure to be furnished by grantee institution.
10. The University/College/Institute shall follow strictly all the instructions issued by the Government of India from time to time with regard to reservation of posts for Scheduled Castes/Scheduled Tribes/OBC/PH etc.
11. The University/College shall fully implement to Official Language Policy of Union Govt. and comply with the Official Language Act, 1963 and Official Languages (Use for Official purposes of the Union) Rules, 1978 etc.
12 The sanction issues in exercise of the delegation of powers vide Commission Office Order No. 25/92 dated May 01, 1992.
13. An amount of **Rs.** ---------- out the grant of **Rs.** ---------- sanctioned vide letter No. F. **42-533/2013 (SR)** dated has been utilized by University/College/Institution for he purpose for which it was sanctioned. Utilization Certificate for **Rs.** ---------- has already been entered at S. No. ------- now we may enter Utilization Certificate for **Rs.** ---------- S. No ------ and in the U. C. Registrar at page No. -----.
14. It is also certified from the B.C.R. that the funds are available under the scheme. Entered in BCR at S.No ----- 3412 / 1
The above grant is sanctioned against the budget provision of **Rs.** ..... during the current financial year leaving a balance of **Rs.** ---------- under the head of Account **4. (i) .a (31) Rs. 4,89,300/- & 4. (i) .a (35) Rs. 3,80,000/-**
15. The funds to the extent are available under the Scheme.
16. The University/Institution/College is strictly following the UGC regulations on curbing the menace of ragging in Higher Educational Institutions, 2009.

(Dr. (Mrs.) Urmila Devi)
Joint Secretary

Copy forwarded for information and necessary action for:-

1. The Principal, **Yashwantrao Mohite College, Erandawane, Pune-411038, MS,** Acknowledgement for the receipt of DD / Cheque / Mail Transfer for **Rs. 8,69,300/-** may be sent to the Under Secretary, Finance Division III, UGC,
2. **Dr. (Mrs.) Pawar Sushama Sunil,** Principal Investigator, Department of **Zoology** **Yashwantrao Mohite College, Erandawane, Pune, 411038, MS**
3. office of the Director General of Audit, Central Revenues, A. G. C. R. Building, I.P. Estate, New Delhi.
4. The Registrar, **Bharati Vidyapeeth, Pune**

(Pramod Sharma)
Section Officer

UNIVERSITY GRANT COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002
GEN

FD Diary No. 8555
Dated : 21.10.2015

F .No.42-533/2013 (SR)

Dated: November, 2015

The Under Secretary (FD-III)
University Grants Commission
Bahadur Shah Zafar Marg
New Delhi – 110002

17 NOV 2015

Sub: Release of Grant-in aid to **Yashwantrao Mohite College of Arts, Science & Commerce, Pune- 411038 Maharashtra** for the year 2015-16 under plan in respect of Major Research Project entitled **"Effect.....againg"** awarded to **Dr. Mrs. Pawar Sushma Sunil, Department of Zoology,** tenure of project from **01.4.2013 to 31.3.2016.**

Sir,

I am directed to convey the sanction of the University Grants Commission for payment of grant of **Rs. 4,06,403/- (Rupees Four lakh six thousand four hundred three only)** as **2nd installment** for the year 2015-16 towards Major Research Project to **The Principal, Yashwantrao Mohite College of Arts, Science & Commerce, Pune- 411038 Maharashtra** for the plan expenditure to be incurred during 2015-16.

Name of the Item	Amount Allocated	Head of Account	Grant now Being Sanctioned	Grant already Released	Total Grant
Books & Journal	30,000/-	3.A(49)(a).35	.....	30,000/-	30,000/-
Equipment	3,50,000/-		.....	3,50,000/-	3,50,000/-
Honorarium	.....	3.A(49)(a).31	.....	.....	.....
Project fellow	5,11,484/-		1,96,336/-	2,64,000/-	4,60,336/-
HRA	1,02,297/-		92,067/-	.....	92,067/-
Chemicals	1,00,000/-		40,000/-	50,000/-	90,000/-
Contingency	75,000/-		30,000/-	37,500/-	67,500/-
Hiring Services	75,000/-		30,000/-	37,500/-	67,500/-
Travel/field work	45,000/-		18,000/-	22,500/-	40,500/-
Overhead Charges	77,800/-		.....	77,800/-	77,800/-
Additional Grant	.....		.....	.....	.....
Total	13,66,581/-		4,06,403/-	8,69,300/-	12,75,703/-

1. The sanctioned amount is debit able to **Major Research Project head Sector 3.A(49)(a).31** and is valid for payment during the financial year **2015-16** only.

2. The amount of the Grant shall be drawn by the Under Secretary (Drawing and Disbursing Officer) UGC on the Grants-in-aid bill and shall be disbursed to and credited to **The Principal, Yashwantrao Mohite College of Arts, Science & Commerce, Pune- 411038 Maharashtra** through Electronic mode as per the following details:-

(a)	Bank Name & Address of Branch	**State Bank of India, Deccan Gynkhana Branch, Deccan Gymkhana, Pune Shirole Bhavan, PMT Building, Pune, Maharashtra**
(b)	Account No	**31791360494**
(c)	Type of Account : SB /Current /Cash Credit	**SB**
(d)	**IFSC Code**	**SBIN0001110**
(e)	MICR Code	**411002003**
(f)	Whether Bank Branch is RTGS or NEFT enabled : RTGS / NEFT /Both	**YES**
(g)	Name & Address of Account Holder	**The Principal, Yashwantrao Mohite College of Arts, Science & Commerce, Pune- 411038 Maharashtra**

3. The Grant is Subject to the adjustment on the basis of Utilization Certificate in the prescribed proforma submitted by the University / Institution.

4. The University / Institution shall maintain proper accounts of the expenditure out of the Grants, which shall be utilized, only on the approved items of expenditure.

5. The University / Institution may follow the General Financial Rules, 2005 and take urgent necessary action to amend their manuals of financial procedures to bring them in conformity with GFRs, 2005 and those don't have their own approved manuals on financial procedures may adopt the provisions of GFRs, 2005 and instructions / guidelines there under from time to time.

6. The Utilization Certificate to the effect that the grant has been utilized for the purpose for which it has been sanctioned shall be furnished to UGC as early as possible after the close of current financial year.

7. The assets acquired wholly for substantially out of University Grants Commission's Grant shall not be disposed or encumbered or utilized for the purposes other than those for which the grants waayanands given without proper sanction of the UGC and should at any time the University ceased to function, such assets shall revert to the University Grants Commission.

8. A Register of Assets acquired wholly or substantially out of the grant shall be maintained by the University in the prescribed proforma.

9. The grantee institution shall ensure the utilization of grants-in-aid for which it is being sanctioned / paid. In case of non-utilization / part utilization thereof, simple interest @ 10% per annum, as amended from time to time on the unutilized amount from the date of drawal to the date of refund as per provisions contained in General Financial Rules of Govt. of India, will be charged.

10. The University / Institutions shall follow strictly the Government of India / UGC guidelines regarding implementation of the reservation policy [both vertical (for SC, ST & OBC) and horizontal (for persons with disability etc.)] in teaching and non-teaching posts.

11. The University / Institution shall fully implement the Official Language Policy of Union Government and comply with the Official Language Act, 1963 and Official Languages (Use for Official Purposes of the Union) Rules, 1976 etc.

12. The sanction is issued in exercise of the delegation of powers vide UGC Order No. 69/2014 [F.No.10-11/12 (Admn. IA & B)] dated 26/3/2014.

13. The University / Institution shall strictly follow the UGC Regulations on curbing the menace of Ragging in Higher Education Institutions, 2009.

14. The University / Institution shall take immediate action for its accreditation by National Assessment & Accreditation Council (NAAC).

15. The accounts of the University / Institution will be open for audit by the Comptroller & Auditor General of India in accordance with the provisions of General Financial Rules, 2005.

16. The annual accounts i.e. balance sheet, income and expenditure statement and statement of receipts and payments are to be prepared strictly in accordance with the Uniform Format of Accounting prescribed by Government.

17. An amount of **Rs. 8,06,010/-** out the grant of **Rs. 8,69,300/-**..... Sanctioned vide letter No. **F. No. 42-533/2013 (SR)** dated **22-03-2013** has been utilized by University/College/Institution for the purpose for which it was sanctioned. Utilization Certificate for **Rs .....** ....NIL..... has been entered at S. No..... now we may enter Utilization Certificate for **Rs...8,06,010/-** .....S. No.1484 and in the U.C. Register at page No.98..

18. Funds to the extent of Rs are available under the scheme or BE / RBE of the year 2015-16.

19. This issues with the concurrence of IFD vide **Diary No. 4389 (IFD) dated 18.09.2015.**

20. This issues with the approval of Joint Secretary (MRP) vide **Diary No. 15933 dated 01.10.2015.**

Your faithfully,

(G.S. AULAKH)
UNDER SECRETARY

Copy forwarded for information and necessary action for :-

1. **The Principal, Yashwantrao Mohite College of Arts, Science & Commerce, Pune- 411038 Maharashtra**
2. Office of The Principal, General of Audit, Central Revenues, AGCR Building, I.P. Estate, New Delhi.
3. Accountant General, State Govt. of **Maharashtra, Mumbai.**
4. **Dr. Mrs. Pawar Sushma Sunil, Department of Zoology, Yashwantrao Mohite College of Arts, Science & Commerce, Pune- 411038 Maharashtra**
5. **The Registrar, Bharati Vidyapeeth, Pune, Maharashtra.**
6. Guard file.

(ARUN KUMAR SINHA)
(SECTION OFFICER)

Mandate Form

MANDATE FORM
ELECTRONIC CLEARING SERVICE (CREDIT CLEARING)/ REAL TIME GROSS SETTLEMENT (RTGS) FACILITY FOR RECEIVING PAYMENTS

A. DETAILS OF ACCOUNTS HOLDERS:

NAME OF ACCOUNT HOLDER	YASHWANTRAO MOHITE COLLEGE, PUNE
COMPLETE CONTACT ADDRESS	SR. NO. 48/A, ERANDWANE, PAUD ROAD, PUNE- 411 038
TELEPHONE NUMBER/ FAX/ EMAIL	COLLEGE PH.: 020-25433383 FAX NO.: 020-25440201 BVDUYMC@HOTMAIL.COM

B. BANK ACCOUNT DETAILS:

BANK NAME	STATE BANK OF INDIA
BRANCH NAME WITH COMPLETE ADDRESS TELEPHONE NUMBER AND EMAIL	DECCAN GYMKHANA BRANCH, DECCAN GYMKHANA (PUNE), SHIROLE BHAVAN, PMT BUILDING, PUNE
WHETHER THE BRANCH IS COMPUTERIZED? IFSC CODE	YES SBIN0001110
IS THE BRANCH ALSO NEFT ENABLED?	
TYPE OF BANK ACCOUNT	SB
COMPLETE BANK ACCOUNT NUMBER	31791360494
MICR CODE OF BANK	411002003

I hereby declare that the particulars given above are correct and complete. If the transaction is delayed of not effected at all for reasons of incomplete or incorrect information I would not hold the use institution responsible. I have read the option invitation letter and agree to discharge responsibility expected of me as a participant under the scheme.

PRINCIPAL
BHARATI VIDYAPEETH DEEMED UNIVERSITY
YASHWANTRAO MOHITE COLLEGE, PUNE-38

Date :

Certified that the particulars furnished above are correct as per our records.

For STATE BANK OF INDIA

Chief Manager

(Bank Stamp)

Deccan Gymkhana Br., Pune - 4

Date :

PRINCIPAL
BHARATI VIDYAPEETH DEEMED UNIVERSITY
YASHWANTRAO MOHITE COLLEGE, PUNE-38

Annexure III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI-110002

STATEMENT OF EXPENDITURE IN RESPECT OF MAJOR RESEARCH PROJECT

1. Name of Principal Investigator **:** **Dr. Mrs. Sushama Sunil Pawar**
2. Department of Principal Investigator **Department of Zoology**
   University / College : **Bharati Vidyapeeth Deemed To Be University, Yashwantrao Mohite College of Arts, Science and Commerce, Pune-38. Maharashtra, India.**
3. UGC approval Letter No. and Date : **MRP F. No. 42-533/2013(SR) 22nd March 2013**
4. Title of the Research Project : **Effect Of Bacoside A on Various Organs of Mouse During Aging.**
5. Effective date of starting the project : **2nd May 2013**
6. a. Period of Expenditure **:** **From 2nd May 2013** to **30th April 2017**
   b. Details of Expenditure

Sr. No	Items	Amount Approved (RS.)	Expenditure incurred (Rs.)
1.	Books & Journal	30,000/-	34,560/-
2.	Equipments	3,50,000/-	3,50,809/-
3.	Contingency	75,000/-	91157/-
4.	Travel/fieldwork	45,000/-	45000/-
5.	Hiring Services	75,000/-	72276/-
6.	Chemicals & Glassware	1,00,000/-	1,34,293/-
7.	Overhead	77,800/-	77,800/-
8.	Any other items (please specify)	--	--

c. Staff

Date of Appointment: **2nd May 2013**

Sr. No.	Item	From	To	Amount Approved	Expenditure incurred so far
1.	Honorarium to Principal Investigator	--	--	--	--
2.	Staff Appointed : Project fellow @ 14,000/- p.m. for initial 2 years and Rs. 16,000/- p.m. from the third year onwards.	**2nd May 2013**	**30th April 2016**	**5,28,000/-**	**5,27,548/-**

1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.

2. If as a result of check or audit objection some irregularly is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.

3. Payment @ revised rates shall be made with arrears on the availability of additional funds.

7. It is certified that the grant of Rs.**11,83,636/-** (**Rupees Eleven Lakh Eighty Three Thousand Six Hundred Thirty Six Only**) received from the University Grants Commission under the scheme of support for Major Research Project entitled "Effect of Bacoside A on Various Organs of Mouse During Aging.'' vide UGC letter No. F. 42-533/2013(SR) 22nd March 2013 has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

Dr. Mrs. S. S. Pawar
(Principal Investigator)
Principal Investigator

Dr. S. R. Patil
(Dr. S. R. Patil)
Incharge Principal
Yashwantrao Mohite College, Pune-38

M/S Dudhedia & Co.
VIJAYKUMAR A. DUDHEDIA
CHARTERED ACCOUNTANTS
M. No. 13989

V. A. DUDHEDIA & CO. ★ CHARTERED ACCOUNTANTS ★
1249/50, Goodluck Chowk, Deccan Gymkhana, Pune - 411004. F. R. N:-112450W

MAJOR RESEARCH PROJECT

Project Title: Effect of Bacoside A on various organs of mouse during aging.

MRP F. No. 42-533/2013(SR)

STATEMENT OF EXPENDITURE INCURRED

Sr. No.	Items	Amount Sanctioned	Amount Approved	Total grant received	Expenditure incurred	Excess Expenditure incurred	Amount to be Reimbursed from sanctioned amount
A.	**Non -Recurring**						
1	Books & Journal	30,000/-	30,000/-	30,000/-	34,560/- (Excess Rs. 4560 - **Rs. 2724** unutilized transferred from hiring services)	1836/-	--
2	Equipments	3,50,000/-	3,50,000/-	3,50,000/-	3,50,809/-	809/-	--
B.	**Recurring**						
1	Honorarium to Retd. Teacher @ Rs. 12,000/- p.m.	Nil	Nil	Nil	Nil	Nil	Nil
2	Project fellow @ 14,000/- p.m. for initial 2 years and Rs. 16,000/- p.m. from the third year onwards.	5,28,000/-	5,11,484/-	4,60,336/-	5,27,548/-	--	67,212/-
3	Chemicals / Glassware / Consumable	1,00,000/-	1,00,000/-	90,000/-	1,34,293/-	34,293/-	10,000/-
4	Hiring Services	75,000/-	75,000/-	67,500/-	72,276/-	Unutilized amount **Rs.2724/-** transferred under Books & Journals	7500/-
5	Contingency	75,000/-	75,000/-	67,500/-	91,157/-	16,157/-	7500/-
6	Travel/fieldwork	45,000/-	45,000/-	40,500/-	45,000/-	--	4500/-
7	Overhead charges	77,800/-	77,800/-	77,800/-	77,800/-	--	--
	Total Amount	**12,80,800/-**	**12,64,284/-**	**11,83,636/-**	**13,33,443/-**	**53,095/-**	**96,712/-**
8	**HRA**	1,05,600/-	1,02,297/-	92,067/-	**1,05,509/-**	--	13,442/-
				Total expenditure incurred so far	**14,38,952/-**		

Dr. Mrs. S. S. Pawar
(Principal Investigator)
Principal Investigator

Dr. S. R. Patil
(Dr. S. R. Patil)
Incharge Principal
Yashwantrao Mohite College, Pune-38

Y. M. College No. F-9-15/95-U3 PUNE-38.

M/S Dudhedia & Co.
VIJAYKUMAR A. DUDHEDIA
CHARTERED ACCOUNTANTS
M. No. 13989

A. DUDHEDIA & CO. Goodluck Chowk, Deccan Gymkhana, Pune - 411004. F.R.N.:-112450W CHARTERED ACCOUNTANTS

MAJOR RESEARCH PROJECT

Project Title: Effect of Bacoside A on various organs of mouse during aging.

MRP F. No. 42-533/2013(SR)

STATEMENT OF INCOME AND TOTAL EXPENDITURE INCURRED

Particulars	Amount sanctioned	Amount Released	Expenditure Incurred
UGC New Delhi	**12,80,800/-**	1st Installment 8,69,300.00 + 2nd Installment 3,14,336.00 (Excluding HRA) = **11,83,636/-**	**13,33,443/-**
HRA to Fellow	1,05,600/-	92,067/-	**1,05,509/-**
		Total expenditure incurred	**14,38,952/-**
In words Rupees Fourteen Lakh Thirty Eight Thousand Nine Hundred and Fifty Two only.			

Total receivable amount from UGC

Sr No.	Particulars	Expenditure Incurred
a.	Receivable amount by UGC as final installment from sanctioned amount	96,712/-
b.	Excess expenditure amount to be sanctioned and released from UGC	53,095/-
c.	Remaining HRA amount to be reimbursed from UGC	13,442/-
	Total receivable amount from UGC	**1,63,249/-**
In words Rupees One Lakh Sixty Three Thousand Two Hundred and Forty Nine only		

You are cordially requested to release the remaining total receivable amount of Rs.**1,63,249/- (In words Rupees One Lakh Sixty Three Thousand Two Hundred and Forty Nine only)** at the earliest.

Dr. Mrs. S. S. Pawar
(Principal Investigator)
Principal Investigator

Dr. S. R. Patil
(Dr. S. R. Patil)
Incharge Principal
Yashwantrao Mohite College, Pune-38

Y. M. College No. F-9-15/95-U3 PUNE-38.

M/S Dudhedia & Co.
VIJAYKUMAR A. DUDHEDIA
CHARTERED ACCOUNTANTS
M. No. 13989

V.A. DUDHEDIA & CO. 1249/50, Goodluck Chowk, Deccan Gymkhana, Pune - 411004. F. R. N:-112450W CHARTERED ACCOUNTANTS

Annexure IV

MAJOR RESEARCH PROJECT

Project Title: Effect of Bacoside A on various organs of mouse during aging.

MRP F. No. 42-533/2013(SR)

STATEMENT OF EXPENDITURE INCURRED ON FIELD WORK

Name of the Principal Investigator : Dr. Mrs. S. S. Pawar

Sr. No.	Duration of the Visit	Mode of Journey	Expenditure Incurred (Rs.)
1	03.06.2013	Visit to University of Pune to give permission letter for referencing	260.00
2	12.06.2013	Visit to National Chemical Laboratory for library card process	320.00
3	15.07.2013	Visit to University of Pune for referencing	260.00
4	12.08.2013	Visit to University of Pune, Department of Botany for referencing	260.00
5	22.08.2013	Visit to National Chemical Laboratory for referencing	320.00
6	12.09.2013	Visit to National Chemical Laboratory, Central Library for for referencing	320.00
7	08.10.2013	Visit to University of Pune, Jayakar library for referencing	260.00
8	10.02.2014	Maharashtra State Biodiversity Board Katraj, for giving letter	300.00
9	07.03.2014	Visit to University of Pune, National Center for Cell Sciences for taking software copy	260.00
10	21.04.2014	Visit to Agarkar Institute for plant collection	220.00
11	05.05.2014	Maharashtra State Biodiversity Board Katraj, for certificate	300.00
12	14.07.2014	Visit to Ayurvedic medical college Bharati Vidyapeeth for plant collection	300.00
13	15.07.2014	Visit to Ayurvedic medical college Bharati Vidyapeeth for plant sample collection	300.00
14	01.08.2014	Visit to IRSHA about enquiry of animal house	300.00
15	05.08.2014	Visit to Modern analytical laboratory	380.00
16	22.08.2014	Visit to sunrise Agro Industry Services, Wakad for plant material collection	320.00
17	25.08.2014	Visit to National Research Institute of Basic Ayurvedic Science Jawahar Neharu Medicinal Garden for instrument and plant enquiry	140.00

18	09.09.2014	Visit to University of Pune for referencing	260.00
19	06.10.2014	Visit to University of Pune for referencing	260.00
20	07.10.2014	Visit to Agarkar Institute for plant photography	220.00
21	13.10.2014	Visit to National Toxicology Center Sinhgadh Road Pune, for animal enquiry	340.00
22	04.11.2014	Visit to National Research Institute of Basic Ayurvedic Science Jawahar Neharu Medicinal Garden for plant photography	140.00
23	10.11.2014	Visit to Botanical Survey of India for giving Authentication letter	380.00
24	14.11.2014	Visit to Botanical Survey of India for giving Identification permission letter	380.00
25	18.11.2014	Visit to Botanical Survey of India for giving plant specimen herberium sheets	380.00
26	25.11.2014	Visit to Botanical Survey of India for collecting Identification and authentication certificate	380.00
27	03.12.2014	Animal feed and husk purchasing from Bhavani Peth, Pune	150.00
28	04.12.2014	Visit to National Toxicology Center Sinhgadh Road Pune, for giving letter	340.00
29	15.12.2014	Visit to Anchrome Pvt. Ltd. Mumbai for HPTLC analysis traning and sample analysis	4250.00
30	01.01.2015	Autofare from Y. M. college to Bharati Bhavan for audit	50.00
31	02.01.2015	Autofare from Bharati Bhavan to V. A. Dudhedia & CO office, Pune	20.00
32	04.01.2015	Autofare from Rambaug Colony Kothrud to Pune Airport	300.00
33	04.01.2015	Taxifare from Delhi Airport to BVDU Delhi	400.00
34	05.01.2015	Taxifare from BVDU Delhi to UGC office Delhi	600.00
35	05.01.2015	Taxifare from UGC office Delhi to BVDU Delhi	600.00
36	05.01.2015	Taxifare from BVDU Delhi to Delhi Airport	400.00
37	05.01.2015	Autofare from Pune Airport to Rambaug Colony Kothrud	260.00
38	04.01.2015 – 05.01.2015	D.A. for two days, Mid Term Report Presentation at UGC, New Delhi	400.00
39	04.01.2015 & 05.01.2015	Pune-Delhi-Pune by Spice jet, for Mid Term Report Presentation at UGC, New Delhi	11680.00
40	06.04.2015	Visit to University of Savitribai Phule Pune University for referencing	270.00
41	10.04.2015	Visit to National Toxicology Center for animal enquiry	310.00
42	24.04.2015	Visit to Parmar Surgicals for purchasing surgical itoms	110.00
43	25.04.2015	Visit to Metro Chemist for purchasing animal	60.00

		dosing items	
44	28.04.2015	Visit to National Chemical Laboratory for instrumentation facility	320.00
45	11.05.2015	Visit to Vijay Chemicals, Swargate for collecting chemicals	120.00
46	14.05.2015	Visit to Maharashtra State Biodiversity Board Katraj, for follow up regarding permission letter	300.00
47	22.05.2015	Visit to plastic ware house Swargate for purchasing plastic wares	240.00
48	23.05.2015	Prashant Enterprizes Bhavani Peth animal feed delivery	250.00
49	22.06.2015	Visit to National Toxicology Center for sample analysis	310.00
50	02.06.2015	Visit to University of Savitribai Phule Pune University for sample analysis	270.00
51	24.06.2015	Visit to Metro Chemist for purchasing animal dosing itoms	60.00
52	01.07.2015	Visit to Shree Medical for purchasing animal dosing itoms	120.00
53	07.07.2015	Visit to National Toxicology Center for animal purchasing	1200.00
54	13.07.2015	Visit to Neeta Chemicals, Pimpri for purchasing and collecting chemicals	360.00
55	10.08.2015	Visit to University of Savitribai Phule Pune University for referencing	275.00
56	07.09.2015	Visit to University of Savitribai Phule Pune University regarding enquiry for sample analysis	155.00
57	19.10.2015	Visit to University of Savitribai Phule Pune University regarding sample analysis on spectrofluorometer	300.00
58	23.10.2015	Visit to University of Savitribai Phule Pune University regarding sample analysis on spectrofluorometer	310.00
59	27.10.2015	Visit to University of Savitribai Phule Pune University regarding elemental analysis of samples	300.00
60	29.10.2015	Visit to National Chemical Laboratory for attending one day workshop	320.00
61	30.10.2015	Visit to University of Savitribai Phule Pune University for payment against sample analysis	300.00
62	30.11.2015	Visit to National Toxicology Center for animal purchasing	1200.00
63	24.12.2015	Visit to Bharati Vidyapeeth University, Bharati Hospital Biodisposal Department for animal disposal	450.00
64	01.01.2016	Visit to Central Dogma Research Institute, Baner	400.00

		for sample analysis enquiry	
65	04.01.2016	Visit to National Toxicology Center APT Research Foundation for animal enquiry	310.00
66	08.01.2016	Visit to Geneombio Technologies Pvt Ltd, Pune	500.00
67	11.01.2016	Visit to RASA Scientifics Life Sciences, Pune	115.00
68	14.01.2016	Visit to SPPU, Central Instrumentation Facility Department, Pune	155.00
69	13.02.2016	Visit to Bharati Vidyapeeth University, Bharati Hospital Biodisposal Department for animal disposal	450.00
70	16.02.2016	Visit to Abeda Inamdar Senior College of Arts, Science and Commerce for Conference Registration	310.00
71	18.02.2016	Visit to National Toxicology Center APT Research Foundation for payment against sample analysis	350.00
72	22.02.2016	Visit to Parmar Surgicals for purchasing surgical itoms	110.00
73	29.02.2016	Visit to Bharati Vidyapeeth University, Bharati Hospital Biodisposal Department for animal disposal	450.00
74	10.03.2016	Visit to Grafikon, Pune for scientific poster printing	115.00
75	16.03.2016	Visit to National Toxicology Center APT Research Foundation for payment against sample analysis	350.00
76	17.03.2016	Visit to Dhande Pathlab Diagnostics,Pune	100.00
77	02.04.2016	Visit to Dhande Pathlab Diagnostics,Pune	100.00
78	05.04.2016	Visit to NCL, Pashan for doing enquiry about instrumentation facility	320.00
79	07.04.2016	Visit to National Toxicology Center APT Research Foundation for animal enquiry	320.00
80	11.04.2016	Visit to Metro Chemist for purchasing animal dosing itoms	60.00
81	13.04.2016	Visit to National Institute of Bioscience for animals	1200.00
82	18.04.2016	Visit to Savitribai Phule Pune University for sample analysis	270.00
83	21.04.2016	Visit to National Toxicology Center APT Research Foundation for animal enquiry	300.00
84	25.04.2016	Visit to Savitribai Phule Pune University regarding enquiry for sample analysis	155.00
85	26.04.2016	Visit to Bharati Vidyapeeth University, Bharati Hospital Biodisposal Department for animal disposal	450.00
86	27.04.2016	National Institute of Biosciences for Animals	250.00

87	29.04.2016	Prashant Enterprizes Bhavani Peth animal feed delivery	350.00
88	02.05.2016	Visit to Metro Chemist for purchasing animal dosing items	60.00
89	23.05.2016	Prashant Enterprizes Bhavani Peth animal feed delivery	350.00
90	27.05.2016	Visit to Savitribai Phule Pune University for payment against sample analysis	155.00
91	09.06.2016	Visit to Central Dogma Research Institute, Baner for sample analysis enquiry	400.00
92	29.08.2016	Visit to National Institute of Biosciences for experimental Animals	500.00
93	29.09.2016	Visit to Bharati Vidyapeeth University, Bharati Hospital Biodisposal Department for animal disposal	450.00
94	08.12.2016	Visit to Hutatma Rajguru College, Rajgurunagar	650.00
95	09.03.2017	Visit to Shivmalhar Advertising, Pune	25.00
		Rs. Total	**45,000.00**
		In words Rupees Forty Five Thousand only	

Certified that the above expenditure is in accordance with the UGC norms for Major Research Projects.

Dr. Mrs. S. S. Pawar
(Principal Investigator)
Principal Investigator

Dr. S. R. Patil
(Dr. S. R. Patil)
Incharge Principal
Yashwantrao Mohite College, Pune-38

M/S Dudhedia & Co.
VIJAYKUMAR A. DUDHEDIA
CHARTERED ACCOUNTANTS
M. No. 13989

V. A. DUDHEDIA & CO. CHARTERED ACCOUNTANTS 124/50, Goodluck Chowk, Deccan Gymkhana, Pune - 411004. F. R. No.-112450W

Bharati Vidyapeeth
(Deemed to be University)
YASHWANTRAO MOHITE COLLEGE OF ARTS, SCIENCE & COMMERCE
Erandwane, Pune - 411 038

★ Accredited with 'A+' Grade (2017) by NAAC ★
★ 'A' Grade University Status by MHRD, Govt. of India ★
★ Accredited (2004) & Reaccredited (2011) with 'A' Grade by NAAC ★

Founder-Chancellor : Dr. Patangrao Kadam M.A., LL.B., Ph.D.
Incharge Principal : Dr. S. R. Patil M.Sc., Ph.D.

Tel. : (020) 25433383, 25424163
Fax : (020) 25440201
E-mail : bvduymc@hotmail.com
ymc@bharatividyapeeth.edu
Website : ymc.bharatividyapeeth.edu

Ref. No. BVDU/YMC/UGC/182 /2018-2019 **Date:** 02.08.2018

Annexure V

M/S Dudhedia & Co.
Chartered Accounts
CTS No. 1249/50,
Goodluck Chowk,
Deccan Gymkhana,
Pune- 411 038.

Date : 02.08.2018

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110002

UTILIZATION CERTIFICATE

Certified that the grant of **(Rs. 11,83,636/-)** (Rupees: Eleven Thousand Eighty Three Thousand Six Hundred Thirty Six Only) received from the University Grants Commission under the scheme of support for Major Research Project entitled "Effect of Bacoside A on various organs of mouse during aging." vide UGC Letter No. F. 42-533 /2013 dated 22nd March 2013, out of which Rs. **14,38,952/- (In words Rupees Fourteen Lakh Thirty Eight Thousand Nine Hundred and Fifty Two only)** has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

Dr. Mrs. S. S. Pawar
(Principal Investigator)
Principal Investigator

Dr. S. R. Patil
(Dr. S. R. Patil)
Incharge Principal
Yashwantrao Mohite College, Pune-38

Y. M. College No. F-9-151 95-U3 PUNE-38.

M/S Dudhedia & Co.

V. A. DUDHEDIA & CO. 1249/50, Goodluck Chowk, Deccan Gymkhana, Pune - 411004. F. R. No.:112450W CHARTERED ACCOUNTANTS

JAYKUMAR A. DUDHEDIA
CHARTERED ACCOUNTANTS
M. No. 13969

Annexure VI

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

UGC FILE NO. F. 42-533/2013 (SR) YEAR OF COMMENCEMENT 2 2 0 3 2 0 1 3

TITLE OF THE PROJECT : Effect Of Bacoside A on Various Organs of Mouse During Aging.

1.	Name of the Principal Investigator	Dr. Mrs. Sushama Sunil Pawar				
2.	Name of the University/ College	Bharati Vidyapeeth (Deemed To Be University) Yashwantrao Mohite College of Arts, Science and Commerce, Erandwane, Pune- 411 038.				
3.	Name of the Research Personnel Appointed	Miss. Jadhav Manmohini Gangadharrao				
4.	Academic qualification	Sr. No.	Qualification	Year	Marks	%
		1.	M.Sc.	2008	699	61.83
		2.	Ph.D.	Registered		
5.	Date of joining	2nd May 2013				
6.	Date of Birth of Research Personnel	17th Jan 1986				
7.	Amount of HRA, if drawn	1,05,509/-				
8.	Number of candidates applied for the post	Six				

CERTIFICATE

This is certified that all the rules and regulations of UGC Major Research Project outlined in the guidelines have been followed. Any lapse on the part of the University will be liable to terminate the said UGC project.

Dr. Mrs. S. S. Pawar
(Principal Investigator)
Principal Investigator

Head of the Dept.
Dr. Mrs. S. S. Pawar
Department of Zoology
Bharati Vidyapeeth University
Yashwantrao Mohite College,
Pune - 411 038.

Dr. S. R. Patil
(Dr. S. R. Patil)
Incharge Principal
Yashwantrao Mohite College, Pune-38

BHARATI VIDYAPEETH (DEEMED TO BE UNIVERSITY)
YASHWANTRAO MOHITE COLLEGE OF ARTS, SCIENCE AND COMMERCE, PUNE-411038.

UGC Major Research Project in Zoology (Science)

File No. 42-533/2013(SR)
2nd May 2013-30th April 2017

Final Report

Project title: "Effect of Bacoside A on various organs of mouse during aging''.

CERTIFICATE

UTILIZATION OF OVERHEAD CHARGES

Certified that the overhead charges of the project amounting **Rs. 77,800/-** (In words Rupees Seventy Seven Thousand Eight Hundred only) have been fully utilized for the purpose for which it was sanctioned in accordance with UGC norms.

Dr. Mrs. S. S. Pawar
Principal Investigator
Principal Investigator

Y. M. College No. F-9-15/95-U3 PUNE-38.

Dr. S. R. Patil
(Dr. S. R. Patil)
Incharge Principal
Yashwantrao Mohite College, Pune-38

Authentication Certificate

टेलीफोन/ Tel. 020-26122125, (Direct) 26124139, 26141491, 26139512
email : bsi _wrcpune@yahoo.co.in
GOVERNMENT OF INDIA
MINISTRY OF ENVIRONMENT & FORESTS
BOTANICAL SURVEY OF INDIA
WESTERN REGIONAL CENTRE,.
KOREGAON ROAD, PUNE - 411001

सत्यमेव जयते

तार / Telegram : BOTSURVEY
फैक्स / Fax : 020-26124139
भारत सरकार
पर्यावरण और वन मंत्रालय
भारतीय वनस्पति सर्वेक्षण
पश्चिमी क्षेत्रीय केंद्र
७, कोरेगांव रोड, पुणे- ४११ ००१

No. BSI/WRC/Tech./2014/ Date 14-11-2014

CERTIFICATE

This is to certify that the plant specimen brought by **Miss. Manmohini. G. Jadhav**, JRF of Department of Zoology, from **Yashwantrao Mohite College, Bharti Vidyapeeth University, Erandwane, Pune;** is identified as:

Number	Name	Family
MGJ - 1	***Bacopa monnieri*** (L.) Wettst.	Scrophulariaceae

14/11/14

(A. Benniamin)
Scientist 'D' & H.o.O

Maharashtra State Biodiversity Board Approval

MAHARASHTRA STATE BIODIVERSITY BOARD

(Govt. of Maharashtra)

B-13/3 & 13/4, Govt. Employees Colony, Near C.P. & Berar High School,
Ravinagar, Nagpur-440001 ☎ : 0712-2053473 / 74

No. : MSBB /A-53/ 10 / 14-15
Nagpur.

Date : 02.04.2014

Y. M. College, Pune-38.
Inward No. 17
Date 25/4/2014
File No.

To,
Princ. K. D. Jadhav,
Bharati Vidyapeeth Deemed University,
Yashwantrao Mohite College,
Pune-411038

Sub: Permission for access to biological resources and associated traditional knowledge for purely research and academic purpose.
Title: Effect of Bacoside A on various organs of mouse during aging

Ref: Your application received in Form I dated 25/01/2014

With reference to the above subject it is to inform that Maharashtra State Biodiversity Board has no objection for access to biological resources i.e. Fresh aerial part of Bacopa monniera (Brahmi) from Garden of Yashwantrao Mohite College, Erandwane, Pune, and associated traditional knowledge for purely research and academic purpose within time span of one year (April 2014- March 2015) under below mentioned terms and condition. This permission is subject to compliance of all other existing acts, rules etc.

i. MSBB reserve the right to inspect the collection sites and the research laboratory as and when there will be reason to do so. For this the Applicant shall communicate commencement date and site-wise schedule of collection of bio-resources with name of contact person at the site in first instance.
ii. The applicant shall submit the inventory of Biological resources on completion of their studies indicating their locations, possible uses and future plan.
iii. In case any new species is found, details of the same with exact location would be sent to MSBB.
iv. Applicant should deposit collected specimen to National Biodiversity Authority notified repository viz The Director, Botanical Survey Of India, Complex 3rd,MSO Building, Block F, DL Block, sector 1,Salt Lake City, Kolkata-700 064 under intimation to this MSBB Nagpur office.
v. The applicant has indicated present scope of collection of bio-resources is solely for research purpose and findings are not intended for transfer to any foreign national or obtaining Intellectual Property Rights (IPR) or for commercial use. Should such a need arise in the course of time, the applicant shall seek proper prior permission from the NBA/ MSBB as envisaged under the provision of BD Act 2002 and Maharashtra Biological Diversity Rules 2008, whichever will be applicable.

(Dr. Dilip Singh IFS)
Addl. Principal Chief Conservator of Forests &
Member Secretary
Maharashtra State Biodiversity Board
Nagpur

Dr. V. P. Sapre
Discuss
Hon 24/4/14

Copy to: 1. Chairman, Maharashtra State Biodiversity Board, Pune Office for information.

Website : www.maharashtrabiodiversityboard.gov.in
e-mail : msbb.ngp@gmail.com

F.No. 25/21/2013-AWD
Government of India
Ministry of Environment & Forests
***

8th floor, Jeevan Prakash Building.
25, Kasturba Gandhi Marg, New Delhi-110 001
Dated: 15/11.2014

To,

Dr. K. R. Mahadik, Principal,
Poona College of Pharmacy,
Bharati Vidyapeeth Deemed University,
Erandwane, Pune – 411038, Maharashtra

Subject: Constitution of Institutional Animal Ethics Committee (IAEC)-regarding.

Sir,

With reference to your letter on the above mentioned subject, this is to inform you that your establishment has been registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for the purpose of **"Research"**. The Registration number **1703/PO/c/13/CPCSEA** is valid upto **16th June, 2016**. The above registration number should be quoted in all future correspondence with this office.

2. CPCSEA hereby nominates the following members to the Institutional Animals Ethics Committee (IAEC) of your establishment/ institute.-

S.No.	Name	Designation
(i)	Dr. Arun Baburao Gadge "Pasaydan" 28/3/21, Samarth Colony Jagatap Dairy Pimdale Milakh, Pune-411 027, Maharashtra Mobile: 09881977751	Main Nominee
(ii)	Dr. R.P. Deolankar, National Institute of Virology, 20 A, Dr. Ambedkar Road, Pune Tele: 020-26006233, Mob:09604372189 Email: oonnatie@yahoo.com	Link Nominee

3. The IAEC members nominated by your institute are as per Rule 13 of the Breeding of and Experiments on Animals (Control and Supervision), as amended. Therefore, CPCSEA has accepted the following nominees recommended by you as member of IAEC:-

.No	Name	Designation
1.	Dr. Mrs. V.B. Pokharkar	Biological Scientist
2.	Dr. Mrs. Arulmozi. S	Scientist from different discipline
3.	Dr. S.L. Bodhankar	Scientist from different discipline
4.	Dr. S.P. Atakare	Veterinarian
5.	Dr. A. M. Harsulkar	Scientist Incharge
6.	Dr. A.M. Majumdar	Scientist from outside the institute
7.	Mrs. P. Parihar	Non scientific socially aware member

Please note that any change in IAEC members can be made only with prior approval of CPCSEA.

Dr Bodhankar
17/12/2014

Bharati Vidyapeeth Deemed University
POONA COLLEGE OF PHARMACY
Inward No. 58
Date 17/12/2014

4. Further, you are requested to convene the meeting of the constituted IAEC at the earliest and forward the Minutes to the O/o CPCSEA, Delhi.

5. This supersedes our previous communications on the subject. Kindly acknowledge the receipt of the letter to the CPCSEA.

Yours faithfully,

(M. Geethanjali)
Dy. Secretary (AW) & Member-Secretary, CPCSEA
Tel No. 011 – 23318553/23327398

Copy to: Dr. Arun Baburao Gadge – with the request to ensure the conduct of IAEC meeting as stipulated in the SOI and also to furnish the minutes of the meeting to the O/o CPCSEA. with in 30 days of the conduct of the meeting.

Approval from CPCSEA Committee

Certificate

This is to certify that the project entitled as 'Effect of Bacoside A on various organs of mouse during aging' has been approved by the IAEC of Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandawane, Pune – 411 038, India.

Proposal number: - CPCSEA/PCL 23/2014 - 2015

Date first received: - 28.08.2014

Date received after modification (if any): -

Date received after second modification: -

Name of Chairman/ Member Secretary IAEC:

Name of CPCSEA nominee:

Signature with date

(Dr. K.R. Mahadik)
30/8/2014

(Dr. R.P. Deolankar)
30/8/2014

Chairman/ Member Secretary of IAEC: ***CPCSEA nominee:***

Certificate

This is to certify that the project entitled as 'Effect of Bacoside A on various organs of mouse during aging' has been approved by the IAEC of Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandawane, Pune – 411 038, India.

Proposal number: - CPCSEA/PCL 24/2014-2015

Date first received: - 28.08.2014

Date received after modification (if any): -

Date received after second modification: -

Name of Chairman/ Member Secretary IAEC:

Name of CPCSEA nominee:

Signature with date

(Dr. K. R. Mahadik)
30/8/2014

(Dr. R.P. Deolankar)
30/8/2014

Chairman/ Member Secretary of IAEC: ***CPCSEA nominee:***

Approval from Institutional Ethics Committee

BHARATI VIDYAPEETH DEEMED UNIVERSITY
YASHWANTRAO MOHITE COLLEGE, PUNE - 411038

Hon. Dr. Patangrao Kadam
M.A., LL.B., Ph.D.
Chancellor

Prof. Dr. Shivajirao Kadam
M.Sc., Ph.D.
Vice-Chancellor

Principal : Dr. K. D. Jadhav
M.Sc., M.Phil., Ph.D.

REACCREDITED WITH 'A' GRADE BY NAAC

Ref. : BVDU / YMC / Ethic/ 01/475/2014-15 Date : 01/ 01 /2015

INSTITUTIONAL ETHICS COMMITTEE

Chairman
Dr. A. N. Kowale
M.D.
Professor of Physiology &
Deputy Dean,
B. J. Medical College and
Sasson General Hospital,
Pune.

Member Secretary
Dr. K. D. Jadhav
M.Sc., M.Phil., Ph.D.
Principal,
Y. M. College, Pune.

Members
Dr. P. M. Bulakh
Ph.D.
Prof. of Biochemistry,
Medical College, Pune.

Dr. M. B. Sarda
LL.M., Ph.D.
Principal,
New Law College, Pune.

Dr. G. R. Rathod
Ph.D.
Director,
Social Sciences Centre,
Pune.

Dr. V. V. Dhapte
M.Sc., Ph.D.
Dept. of Chemistry,
Y. M. College, Pune.

Dr. M. G. Bodhankar
M.Sc., Ph.D.
Dept. of Microbiology,
Y. M. College, Pune.

Dr. Mrs. S. S. Pawar
M.Sc., Ph.D.
Dept. of Zoology,
Y. M. College, Pune.

CERTIFICATE

The Institutional Ethics Committee is pleased to inform you that your UGC Major research proposal entitled **"Effect of Bacoside A on various organs of mouse during aging"** has been approved in the Ethics Committee meeting held on 31st December, 2014.

Name of the Principal Investigator :- Dr. Mrs. Sushama S. Pawar
Department :- Zoology

Y. M. College No. F-9-15/95-U3 PUNE-38.

(Prin. Dr. K. D. Jadhav)
Secretary
Institutional Ethics Committee
PRINCIPAL
YASHWANTRAO MOHITE COLLEGE
PUNE - 38.

Annexure -VIII

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110002

Final Report of the work done on the Major Research Project

1. **Project Report No. :** Final

2. **UGC Reference No. :** MRP F. No. 42-533/2013(SR) 22nd March 2013

3. **Period of report: from :** 2nd May 2013 to 30th April 2017

4. **Title of research project :** Effect of Bacoside A on Various Organs of Mouse During Aging.

5. **(a) Name of the Principal Investigator :** Dr. Mrs. Sushama Sunil Pawar

   (b) Dept. : Department of Zoology

   (c) University/College where work has progressed: Bharati Vidyapeeth (Deemed To Be University), Yashwantrao Mohite College of Arts, Science and Commerce, Pune-38, Maharashtra, India.

6. **Effective date of starting of the project** : 2nd May 2013

7. **Grant approved and expenditure incurred during the period of the report:**

   a. **Total amount approved Rs. :** 12,80,800/-

   b. **Total amount received Rs. :** 11,83,636/-

   c. **Total expenditure Rs. :** 14,38,952/-

   d. **Amount to be reimbursed Rs: (Receivable amount)** 1,63,249/-

   e. **Report of the work done:** (Separate sheet attached)

Report of the work done

i. Brief objective of the project

The proportion of old age people in the human population is increasing throughout the world at an alarming rate. It is the fastest growing section of the population due to awareness of health, advances in medicine and change in style of living. But olds are not vital, the prevalence of age associated diseases such as cancer, cardio-vascular diseases, stroke, Alzheimer's disease, arthritis, osteoporosis and other related conditions are also on rise. Now a day, due to urbanization, pollution, mechanization and environmental toxicity people are facing adverse effects on body even in young age.

With the progress in aging, a variety of age related diseases may chase our health. Large numbers of people are suffering from dementia and Alzheimer's disease in their old age. These disorders are due to progressive accumulation of lipofuscin granules in brain cells. The progressive accumulation of lipofuscin in post mitotic cells is thought to contribute to a wide variety of age related and pathological conditions.

As the age progresses, the natural defense system of antioxidant enzymes starts to decline due to oxidative stress. Antioxidants are the agents that disable free radicals produced in the body (Borek, 1993, Waterhouse, 1995). Tripathi *et al* (1996) reported that antioxidant properties of *Bacopa monnieri* L. offer the protection from free radical damage in cardiovascular diseases, certain types of cancer and helps to prevent induced lipid peroxidation. To overcome the increasing free radical formation in the cells it becomes necessary to supplement the diet with antioxidant rich edible sources. No one can control former, but it is possible to affect later, by having nutritious food, including high amount of antioxidant-rich fruits, vegetables and supplements (Borek, 1995).

Therefore it has been decided to use natural bioconstituent, Bacoside A from *Bacopa monnieri* L. to delay and prevent the pathogenesis of age related chronic diseases in various organs like brain, heart, liver, muscle and prostate gland and formation of lipofuscin granules during aging. Hence, the aim of the project was to study effect of Bacoside A on various organs of mouse during aging.

ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication).

A. Introduction :

A large number of scientists have studied the aging in various fields of biology, such as genetics, physiology, cell biology and molecular biology, which can be grouped together into the gerontology. The diseases of old age constitute a major challenge for bio-medical research. Therefore efforts have been made to give better life to aged people, so that they should not be burden on the family. They should lead a healthy, happy life and be useful to the society. However the biologists have attempted to cover the fundamentals of aging process, clinicians approached geriatrics from patient's points of view and sociologists have presented their revolution of individual problems of the elders in the society.

Aging is described as a progressive deterioration of physical and mental functions after the growth period is over. The life span of all multicellular organisms is characterized by a smooth transition from the developmental phase to the reproductive phase, which is followed by a period of senescence (aging) and death. The different systems that serve our body and mind undergo alteration during the aging process as unavoidable part of life .This process starts, according to some researchers, with birth and accelerate with advancing age, leading to changes that are sometimes obvious but frequently go unnoticed for the long time. The external factors like toxic agents, environmental factors and certain diseased conditions also play equally important role in aging (Alvarez *et al*, 1987; Sanocka and Kurpisz, 2004). Thus aging is multifactorial (Semsei, 2001). Each cell type, organ and system of the body may have its own pattern of characteristic changes with aging, but a basic set of underlying principles govern the actual phenomena of reduced physiological capacity, cell damage causing cell death.

All animals suffer the phenomenon of aging, including the morphologic and functional changes with degeneration (Lee & Park, 1997). Aging is a natural process, which involves seemingly an inevitable decline in physiological functions that occur overtime (Herman, 1981). A number of cellular function declines progressively with age, which may lead to cell death. The morphological alterations in aging cells include irregular nuclei, diminished

Golgi, accumulation of lipofuscin granules (Semsei, 2000), Advanced Glycation End product (AGE) etc.

Several theories explain mechanism of aging, like membrane hypothesis (Zs-Nagy, 1978), DNA mutation theory (Strehler and Freeman, 1980), cross-linking theory of aging (Miquel, 2002), free radical theory (Herman, 1981) etc. Herman in 1957 proposed, the free radical theory is perhaps the strongest and best established theory scientifically, to account for many age related changes.

Our body is formed of three types of cells fixed post mitotic highly differentiated permanent cells like nerve cells, muscle cells and red blood cells. The cells from second category are also highly differentiated but divide sometimes. Kidney, liver, salivary glands are the examples of this type. Stem cells are less specialized, are grouped into third category. They renew themselves and share accumulated waste. The cells of first and second category do not get rid of accumulated waste. It remains in the form of lipofuscin granules and accumulated in these cells. The granule accumulation in nerve cells is most consistent change of aging of nerve cells. It is associated with a progressive injurious effect to the metabolism of cells. It has been strongly stated that loss of neurons and Nissl substances during aging may because of accumulation of lipofuscin pigments. After lot of research and debates it has been proved that lipofuscin granules are nothing but residual bodies of non functional lysosomes.

Free radicals are short lived reactive chemical intermediates, highly reactive because of unpaired electron that oxidise lipid, amino acid, carbohydrates (Semsei, 2000). These free radicals or reactive oxygen species such as hydroxyl radical ($OH^{\cdot}$), superoxide anions ($O^{\cdot}2$) and nitric oxides (NO) inactivate enzymes and damage important cellular component causing tissue injury through covalent binding and lipid peroxidation. Free radicals damage the molecules from which they take electrons, leading to cell damage or cell death. The prime molecules in the body that are damaged by free radicals are DNA, lipids and proteins.

Free radical damage to DNA can overwhelm the repair mechanism in cells, leading to impaired cellular functioning or even cell death. Free radicals can also alter some part of DNA, provoking uncontrolled cell growth, resulting in cancer (Floyd, 1990), a process that

occurs with increased frequency with age. Damage to DNA in mitochondria can cause diminished ability of some parts of body to produce adequate energy for high demands (Beal, 1997; Hansford *et al,* 1997).

Free radical damage to lipids could encourage the oxidation of low density lipoproteins (LDLs), leading to artery-clogging plaques (atherosclerosis) (Steinberg *et al*, 1989; Salonen *et al,* 1992). At the same time, free radial damage to cell lining the blood vessels (endothelial cells) reduces their ability to react quickly and efficiently to maintain proper blood flow to vital organs. The consequences of these problems are increased heat strokes, kidney failure and high blood pressure (hypertension), to name a few of the more dangerous outcomes.

Free radicals also promote a harmful complexing of proteins and carbohydrates called as glycosylation. This process is greatly accelerated in diabetes and also appears to contribute to cataract formation, damage to arteries, reduced movement of the joints and other chronic problems (Romero *et al,* 1998). Environmental factors can increases the risk of free radical generation (Witkop, 1985). Smoking, exposure to toxic pollutants in the environment does similar damage by producing free radicals. Ultraviolet light is particularly able to stimulate the generation of free radicals in skin, leading to premature wrinkling and loss of texture, as well as an increased risk of skin cancer (Malhotra and Pushpadevi, 2005).

An antioxidant defense system of the body has natural defense system to fight against reactive oxygen species (Bendich, 1985; McCord, 1987). The superoxide radicals are scavenged by superoxide dismutase (Forman and Kennedy, 1976). The unscavenged free radicals or their uncontrolled production exert cytotoxic effect on the cells and are considered to be the mediators of the etiology of pathological conditions such as myocardial infarction, rheumatoid arthritis, cardiovascular diseases, neurodegenerative diseases, cancer etc. (Halliwell, 1994; Beal, 1995; Usmar *et al,* 1995; Weiseman and Halliwell, 1995, 1996). Imminent free radical damage in a cell is membrane damage associated with lipid peroxidation (Tappel, 1980; Donato and Sohal, 1981; Schroeder, 1984; Lee *et al,* 1997).

Lipid peroxidation in biological membranes mainly mitochondrial membrane and lysosomes causes impairment of membrane functioning decreased fluidity, inactivation of membrane bound receptors and enzymes and increased non-specific permeability to ions, the

most popular product of lipid peroxidation is malondialdehyde (MDA) (Jones *et al,* 1979; Machlin and Bendich 1987; Reichter, 1987; Esterbauer *et al,* 1988; Esterbauer *et al,* 1990; Aitken *et al,* 1993). The excessive peroxidation of membrane lipids have been linked with lysosomal impairment which leads to inefficiency and instability of lysosomes (Nakamura *et al,* 1989) and accumulation of lipofuscin granules in the cells (Tappel, 1975; Patro *et al* 1988).

In the post-mitotic cells, rate of accumulation of age-pigments (lipofuscins) is increased (Ivy *et al,* 1984; Patro and Patro, 1992). Lipofuscin is an autofluorescent material which accumulates progressively with age within secondary lysosomes and damage lysosomal membrane and enzymes. Lipofuscin-overload lysosomes might be unable to handle peroxidized material formed during oxidative stress, which would increase lipid peroxidation such as MDA, critical cellular functions including mitochondria (Brunk *et al,* 1992).

Antioxidants like centrophenoxine, prostaglandin, quinolic acid, lipoic acid, phosphatidyl serine, dihydroepiandrosterone, piracetam etc. are in the use against lipofuscin formation but these antioxidants are not strongly effective in decreasing and preventing lipofuscin granules formation. As the age progresses due to oxidative stress, the natural defense system of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase starts to decline (Tsay *et al,* 2000). To overcome the increasing free radical formation in the cells it becomes necessary to take supplement in the diet with antioxidant rich edible sources like onion, beet, cabbage, lettuce, parsley etc. having rich phytoconstituents (Hermann, 1976). *Bacopa monnieri* L. is traditionally used as a nerve tonic and to treat various ailments. It was reported that *Bacopa monnieri* L. posseses anti inflammatory, antipyretic, free radical scavenging, anti lipid peroxidative and antioxidant properties. *Bacopa monnieri* L. has rich source of secondary metabolites namely, saponins, flavonoids, terpenoids, phenols, alkaloids and bacosides. It offered protection to the brain by stabilizing the membrane and thereby retaining the structural and functional integrity of the membrane (Sumathi,2011). Bacoside A administration improved the antioxidant status and maintained the level of trace elements and ionic equilibrium. (Krishna *et al,* 2011). One of the recent study showed the protective role of bacoside A against chronic cigarette smoking induced oxidative damage in rat brain. (Anbarasi 2006). Bacoside A is the dammarene type triterpenoid saponine isolated from

Bacopa monnieri L. Recent studies revealed that natural antioxidants such as flavonoids, tannins and phenols are increasingly attracting because these are disease preventing, health promoting and antiaging substances. Therefore present study has been carried out by using Bacoside A from *Bacopa monnieri* L.

B. Materials and Methods :

Approvals taken for the experimentation (Documents were attached separately)

- Authentication of *Bacopa monnieri* (L) was obtained from Botanical Survey of India, Pune, Maharashtra.
- Permission for access to Biological resources and associated traditional knowledge for purely research and academic purpose has been obtained after perseverance from, The member secretary, Maharashtra State Biodiversity Board, Nagpur (Government of Maharashtra).
- The project has been approved by the IAEC of Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandawane, Pune–411 038, India.
  Approved proposal number : CPCSEA/PCL /23/2014-15; CPCSEA/PCL/24/2014-15
- Institutional Ethics Committee (IEC), Yashwantrao Mohite College of Arts, Science and Commerce, Pune approved this UGC Major Research Project.

1. Plant material :

Bacopa monnieri L. is obtained from Ayurmed Biomed Pvt. Ltd. Mumbai. *Bacopa monnieri* L. was cultivated and maintained in the Botanical garden of institute. The whole plant material was collected and shade dried (Plate No. 3) at room temperature and kept in oven for 40ºC to remove moisture. The dried plant was then finely grinded. The powder obtained was sieved and kept in air tight container for experimentation. *Bacopa monnieri* L., Family Scrophulariaceae (Plantaginaceae) fresh plant was collected from botanical garden of the institute (Plate No. 1). The sample herbarium was prepared and authentication has been obtained from Scientist D and HOD, Botanical Survey of India, Pune, Maharashtra. The specimen (MGJ-1) was kept to herbarium department in Botanical Survey of India (Plate No. 2).

Plate No. 1 : *Bacopa monnieri* L

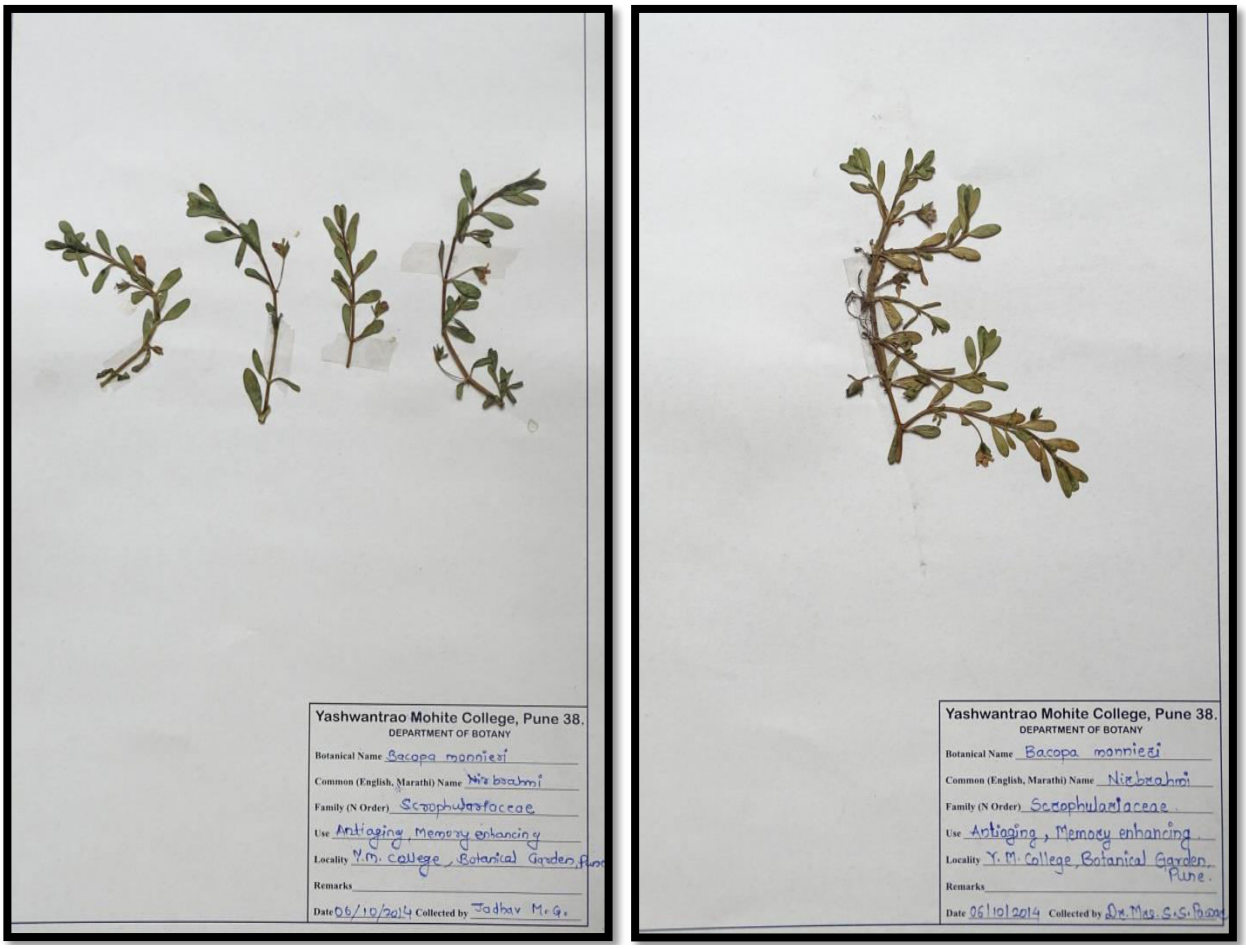


Plate No. 2 : Herbarium of *Bacopa monnieri* L

Plate No. 3 : Shed dried *Bacopa monnieri* (L)

2. Animals :

According to CPCSEA guidelines, approved male Mice (*Mus musculus*) **(Approved proposal number : CPCSEA/PCL /23/2014-15; CPCSEA/PCL/24/2014-15)** were reared in the animal house. They were housed in appropriate numbers (3-5) per cage. They were supplied with rat/mice feed obtained from Pranav Agro Pvt. Ltd., Pune and water Ad libitum. Adult, adult old and old male mice were used for the present study. Quantity of dose and duration was decided experimentally on the basis of LD_{50}. 5% D-galactose was given subcutaneously to induce aging. Dose of Bacoside A was given to adult old mice for 45 days as mg/kg body weight of the animal. Following experimental groups were used for the study.

	Group I	Group II	Group III	Group IV	Group V
Groups used for the study	Adult	Adult old	Old	D-galactose treated	D-galactose + Bacoside A treated
	6-8 Months old	12-16 Months old	18-24 Months old	12-16 Months old	12 Months old

Treated groups received D-galactose subcutaneously for induced aging and Bacoside A orally. Group II received water. After completion of dose the animals from all groups were sacrificed according to guidelines. Cerebral hemisphere, cerebellum, heart, liver, muscle and prostate gland were separated and homogenized. Homogenate was subjected to cold centrifugation to isolate nuclear, mitochondrial, lysosomal and cytosol fraction for biochemical estimations.

C. Results

1. Preliminary physicochemical analysis of *Bacopa monnieri* L.

a. Ash value and Loss on drying

The ash value and loss on drying of *Bacopa monnieri* L was determined and represented in Table No. 1. and showed in Plate No. 4. Total ash value, acid insoluble ash value, and water soluble ash value of *Bacopa monnieri* (L) was 13.5%, 5.5% and 2.5% respectively. The loss on drying was calculated as 1.5%.

Table No. 1 Ash value and loss on drying of *Bacopa monnieri* L

Total ash value	13.5%
Acid insoluble ash value	5.5%
Water soluble ash value	2.5%
Loss on Drying	1.5%

b. Extractive values

The extractive value and color of extracts of *Bacopa monnieri* L was investigated and represented in Table No. 2 and Fig No.1. From the present study it was found that, the extractive value of *Bacopa monnieri* L in methanolic extract was maximum (10.1%) as compared to other extracts. The ethanolic extract showed slightly lower extractive value (8.6%) than methanolic extract of *Bacopa monnieri* L. The extractive value of *Bacopa monnieri* L in aqueous extract was 7.6% followed by chloroform extract (2%) and acetone extract (1.5%), dichloromethane (0.6%), ethyl acetate (0.5%), petroleum ether (0.5%). The ethyl acetate and petroleum ether extract showed lower (0.5 %) extractive value than all other extracts. The color of extracts observed were yellowish green in methanol, green in ethanol, dark brown in aqueous, light green in petroleum ether and chloroform, green in acetone, dichloromethane and ethyl acetate depicted in Plate No. 4.

Table No. 2 Extractive value (%) of *Bacopa monnieri* L

Solvents	Weight of Plant material (g)	Colors of extract	Extractive value (%)
Methanol	2	Yellowish green	10.1
Ethanol	2	Green	8.6
Aqueous	2	Dark brown	7.6
Chloroform	2	Light green	2
Acetone	2	Green	1.5
Dichloromethane	2	Light green	0.6
Ethyl acetate	2	Green	0.5
Petroleum Ether	2	Colorless	0.5

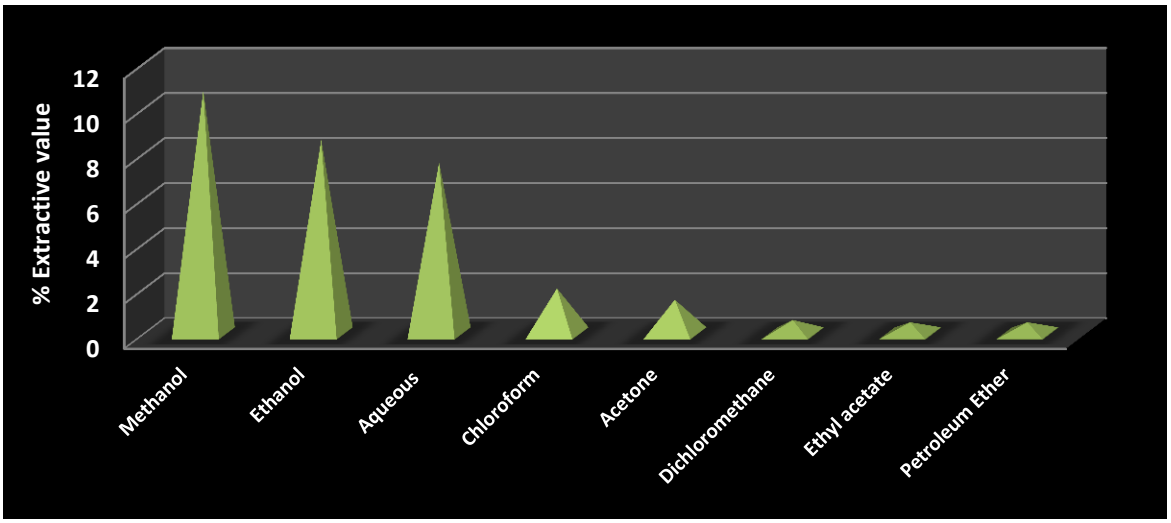


Fig No. 1 Extractive value (%) in different extracts of *Bacopa monnieri* L

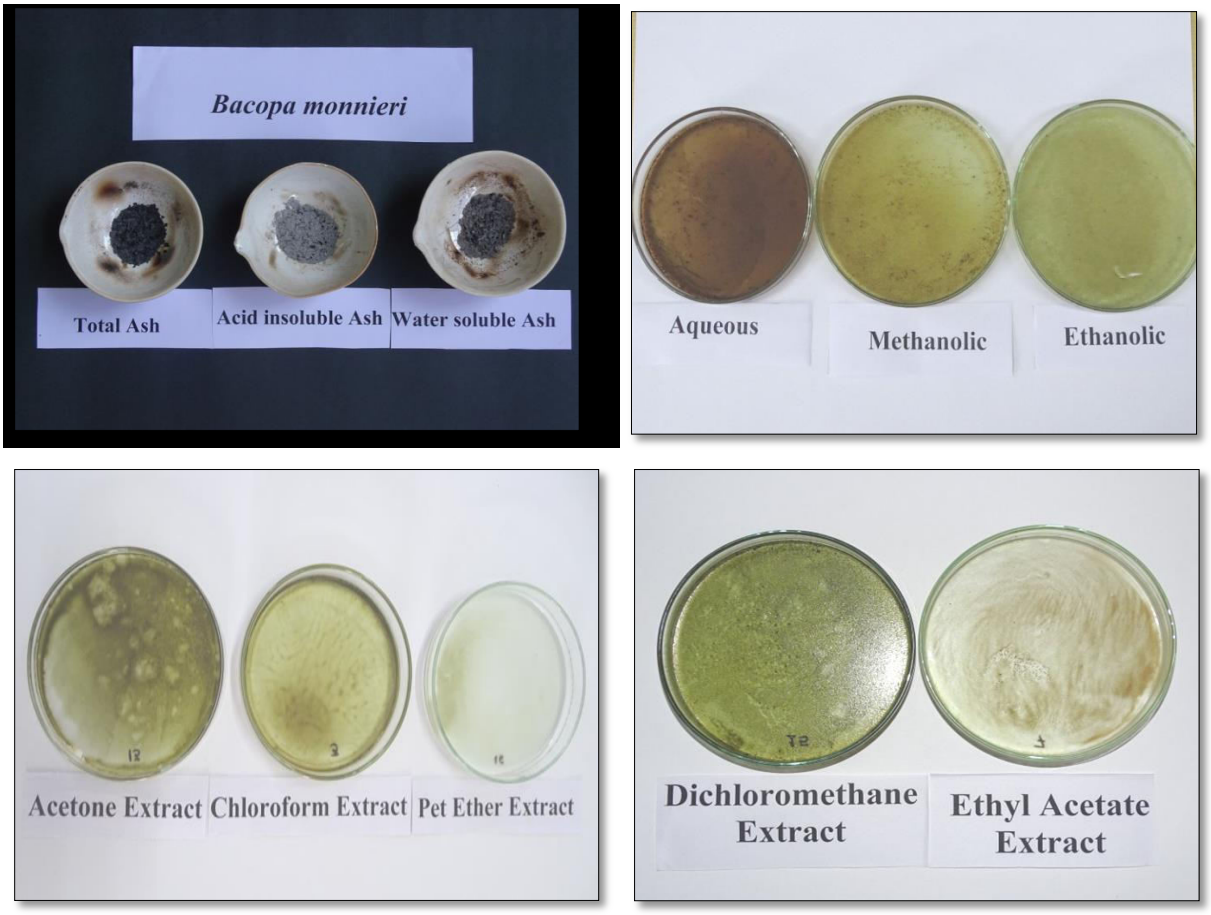


Plate No. 4 : Different extracts of *Bacopa monnieri* L

- **Extract preparation**
  Methanolic extract of *Bacopa monnieri* L was prepared by maceration method showed in Plate No. 5.

Step 1- Dry plant powder kept for maceration in methanol solvent.

Step 2 - Filtrate obtained

Step 3 – Dry methanolic extract of *Bacopa monnieri* L

Plate No. 5 : Extract preparation of *Bacopa monnieri* L

2. Phytochemical screening of *Bacopa monnieri* L.

Bacopa monnieri L. have beneficial therapeutic effects in traditional Indian system of medicine. Various phytochemicals that are present in it are responsible for these therapeutic effects. The quantitative phytochemical analysis of *Bacopa monnieri* L. was carried out. (Table No. 3) It revealed the presence of saponins, flavonoids, alkaloids, tannins, carbohydrates, proteins and steroids in methanolic, ethanolic and aqueous extracts. Anthraquinone glycosides were absent in methanolic, aqueous and ethanolic extracts. The aqueous extract of *Bacopa monnieri* L showed the presence of amino acids and methanolic, ethanolic extracts showed absence of amino acids.

Table No. 3 Phytochemical screening of *Bacopa monnieri* L in different extracts

Sr. No.	Secondary metabolites	Phytochemical tests	Methanol	Ethanol	Aqueous
1.	Carbohydrates	Molisch's Test	+	+	+
2.	Proteins	Millon's Reagent Test	+	+	+
3.	Amino acid	Ninhydrin Test	-	-	+
4.	Steriod	Liebermann Burchard Reaction	+	+	+
5.	Glycosides a)Cardiac glycosides	Legal's Test	+	+	+
	b)Anthraquinone glycosides	Borntrager's Test	–	–	–
	c)Saponin glycosides	Foam Test	+	+	+
6.	Flavonoids	Sodium hydroxide test	+	+	+
7.	Alkaloids	Mayer's Test	+	+	+
		Wagner's Test	+	+	+
		Hager's Test	+	+	+
8.	Tannins	Dilute Nitric acid Test	+	+	+

3. Total phenolic and flavonoid contents in *Bacopa monnieri* L.

Estimation of total phenolic and flavonoid contents in *Bacopa monniri* L. was carried out. As observed in Fig. No. 2 the total phenolic contents were estimated in the range from 12.92 to 28.37 mg gallic acid equivalent per gram of extract. The total flavonoid contents were observed in the range from 21.37 to 44.89 mg quercetin equivalent per gram of extract. The phenolic and flavonoid contents in methanolic extract have been found highly significant as compared to all other extracts.

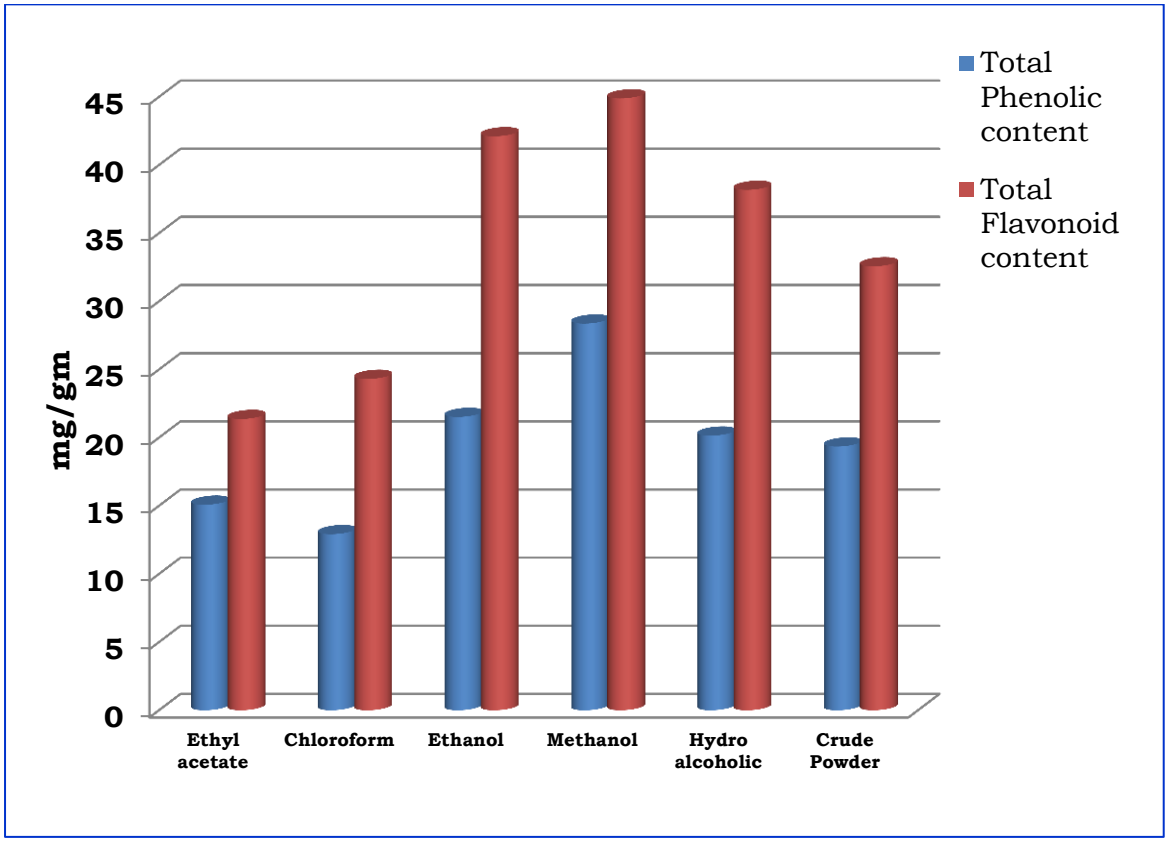


Fig. No. 2 Total phenolic and flavonoid contents in *Bacopa monnieri* L.

4. Characterization of *Bacopa monnieri* L

a. Determination of elements in *Bacopa monnieri* L by AAS :

Atomic Absorption Spectroscopy revealed the presence of 05 elements showed in Table No. 4.

Table No. 4 Concentration of elements in *Bacopa monnieri* L

Elements	Unit	Concentration
Calcium	%	0.42
Magnesium	%	0.32
Copper	ppm	Less than 0.1
Manganese	%	0.03
Zinc	%	0.0025

b. Determination of elements in *Bacopa monnieri* L by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) technique :

Elemental concentrations in *Bacopa monnieri* L dry powder was determined in ppm by ICP-MS technique depicted in Fig. No. 3 and 4. In the present study 26 elements (Na, Mg, K, Ca, Cr, Mn, Fe, Co, Cu, Zn, Se, Mo, Li, Be, B, Al, P, Ni, As, Ag, Cd, Sn, Ba, Pb, Hg and Bi) of biological importance were observed in varying concentrations. It was observed that *Bacopa monnieri* L showed important elements these are Na (2248.9 ppm), Mg (4995.3 ppm), K (2749.1 ppm), Ca (527.2 ppm), Cr (6.10 ppm), Mn (767.6 ppm), Fe (363.6 ppm), Co (1.68 ppm), Cu (21.4 ppm), Zn (43.03 ppm), Se (231.6 ppm), Mo (0.516 ppm), Li (234.4 ppm), Be (10.2 ppm), B (44.7 ppm), Al (3265.2 ppm), P (3718.4 ppm), Ni (7.62 ppm), As (0.35 ppm), Ag (6.50 ppm), Cd (1.76 ppm), Sn (123.5ppm), Ba (98.7 ppm), Pb (6.29 ppm), Hg (1.53 ppm) and Bi (4.62 ppm).

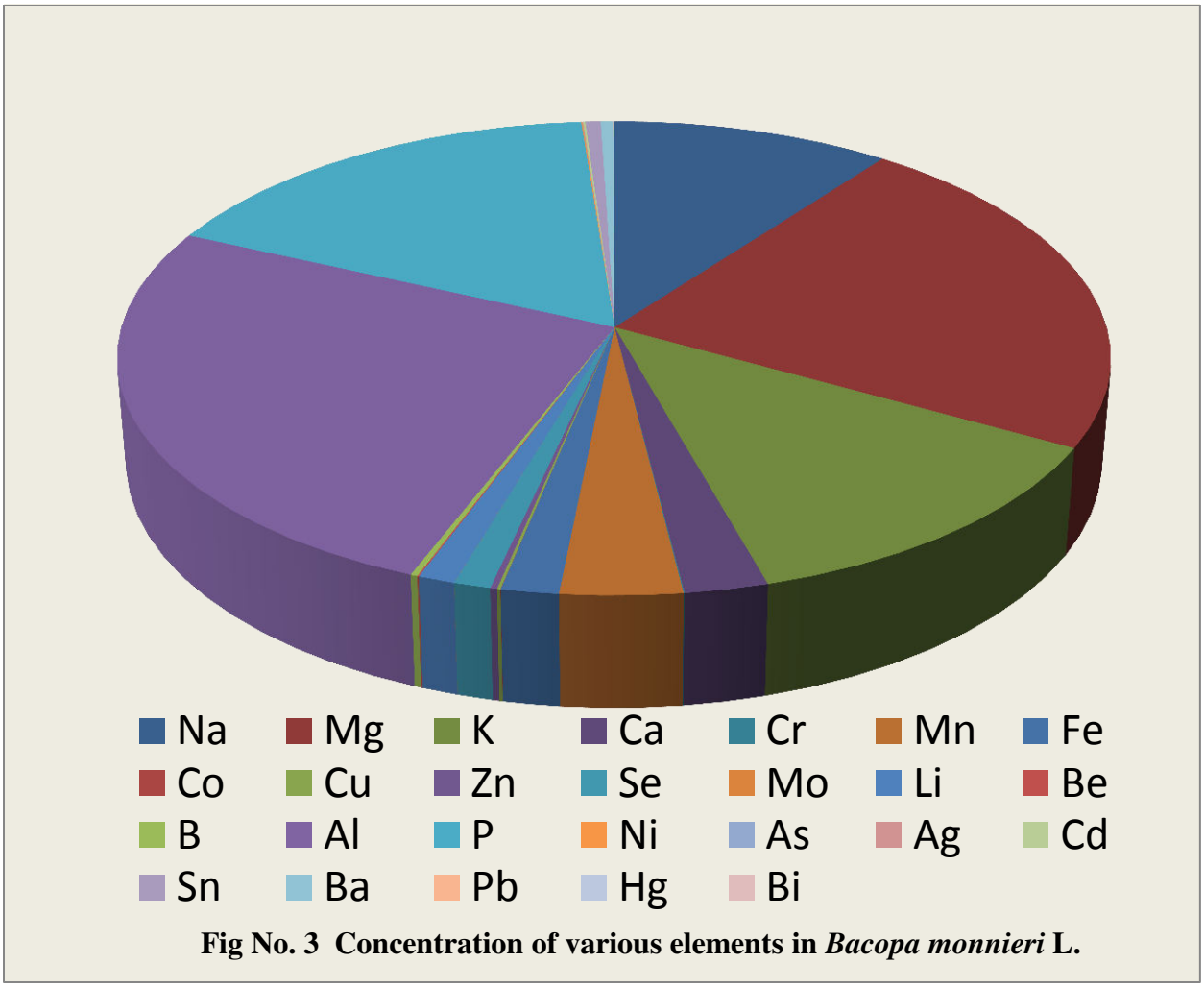


Fig No. 3 Concentration of various elements in *Bacopa monnieri* L.

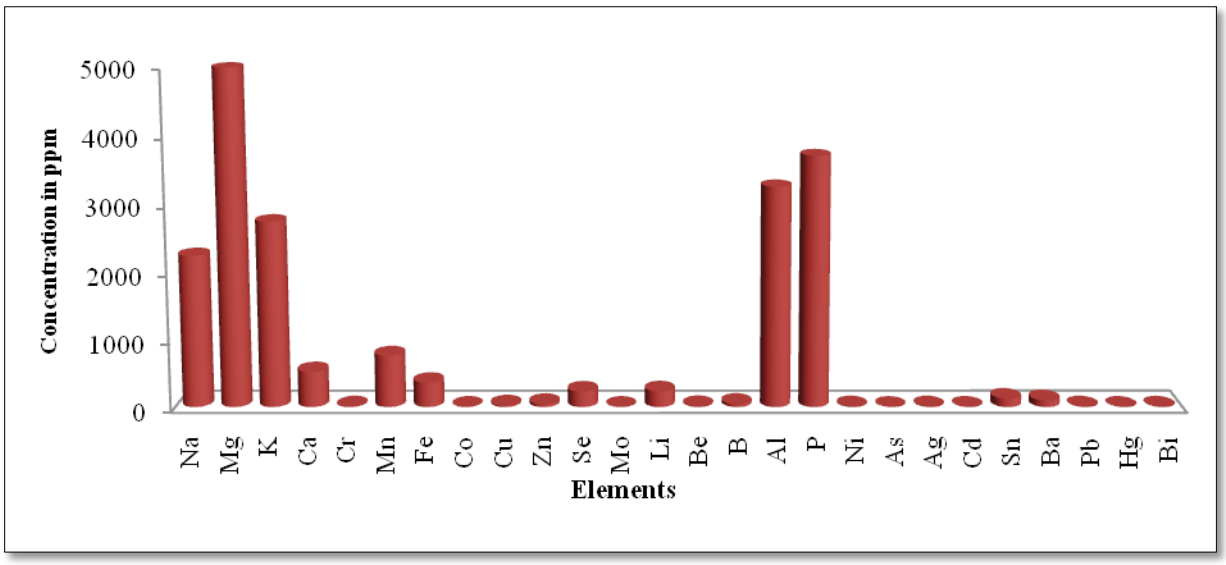


Fig. No. 4 Concentration of various elements (in ppm) in *Bacopa monnieri* L.

c. Field emission scanning electron microscopy (FESEM) and EDAX elemental analysis :

The result of elemental composition of *Bacopa monnieri* L crude powder using FESEM and EDAX technique showed in Table No. 5. Total eleven elements were detected in *Bacopa monnieri* L. From detected eleven elements C and O are observed in higher amount while Cl, K, Na, Ca, Mg, Si observed as moderate and Se, S, Pt found in trace amount.

Table No. 5 Elemental composition of *Bacopa monnieri* L.

Element	Atomic Number	Atomic %
C	6	44.59
O	8	39.38
Cl	17	2.38
K	19	1.23
Na	11	1.46
Ca	20	0.2
Se	34	0.11
Mg	12	0.25
Si	14	0.19
S	16	0.11
Pt	78	0.1
Total		100

d. Fourier transform infrared spectroscopy (FTIR)

Samples were analyzed using Fourier Transform Infrared Spectroscopy (Shimadzu, Japan 8000 series). The observations of FTIR spectroscopic study revealed the presence of various chemical constituents in crude powder, maceration and Soxhlet extracts of *Bacopa monnieri* L. which showed variations in their peaks.

The peak at 3244 and 2920 cm^{-1} are corresponding to Hydroxyl and CH stretching frequency respectively. The band 1651 cm^{-1} is corresponded to carbonyl carbon. The strong peak at 2852 cm^{-1} assigned to the C-H stretching which means that some alkane compounds existed in this plant.

The band between 3000 to 2800 cm^{-1} represent C-H stretching vibration that are mainly generated by lipids. The more intense bands observed at 1732 cm^{-1}, 1683 cm^{-1}, 1777 cm^{1}, corresponds to C=O stretching indicate the presence of ketone, aldehyde, carboyl, esters and amides in *Bacopa monnieri* L crude powder, macerated and Soxhlet extracts respectively.

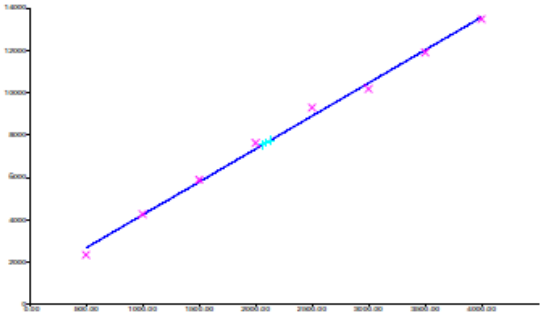
5. Determination and quantification of Bacoside A from *Bacopa monnieri* L by High Performance Thin Layer Chromatography (HPTLC)

The method described utilizes Silica gel 60F254 HPTLC plates as stationary phase and Toulene : Ethylacetate: Methanol: Glacial Acetic acid (3:4:3:1v/v) as mobile phase which gives good separation of Bacoside A (Rf = 0.31). The calibration curve was linear in the range of 0.5 - 4 μg/spot as shown in Fig. No. 5. The correlation coefficient (r) was determined it was found to be 0.9977 indicating good linearity between concentration and peak area. Well defined bands were obtained which are shown in Fig. No. 6 and Plate No. 6. The percentage coefficient of variance (CV) for peak area was found to be 1.766. The HPTLC chromatograms of standard Bacoside A and methanolic extract of *Bacopa monnieri* L are presented in Fig. No. 7 and Fig. No. 8.

Substance: Bacoside A @ 540 nm

Regression via area: Linear Y = 1039 + 3.108 * X r = 0.99774 sdv = 3.45

Track	Vial	Rf	Amount	Height	X(Calc)	Area	X(Calc)		SampleID/Remark
1	2	0.33	500.00 ng			2256.76			
2	2	0.32	1000.00 ng			4180.88			
3	2	0.32	1.500 µg			5796.00			
4	2	0.32	2.000 µg			7536.99			
5	2	0.32	2.500 µg			9211.71			
6	2	0.32	3.000 µg			10088.88			
7	2	0.32	3.500 µg			11805.48			
8	2	0.32	4.000 µg			13381.45			
9	1								Not used
10	1								Not used
11	1	0.31				7542.90	2.093 µg	Exttract	
12	1	0.31				7436.65	2.058 µg	Exttract	
13	1	0.31				7666.35	2.132 µg	Exttract	
14	1								Not used



winCATS summary report

Calibration results per Analysis

Sample from vial 1: Exttract

Result via area

Substance	Rf	X(average)	CV [%]	n	Regression	Remark
Bacoside A	0.31	2.094 µg	1.766	3	Linear	

Fig. No. 5 Calibration results per analysis

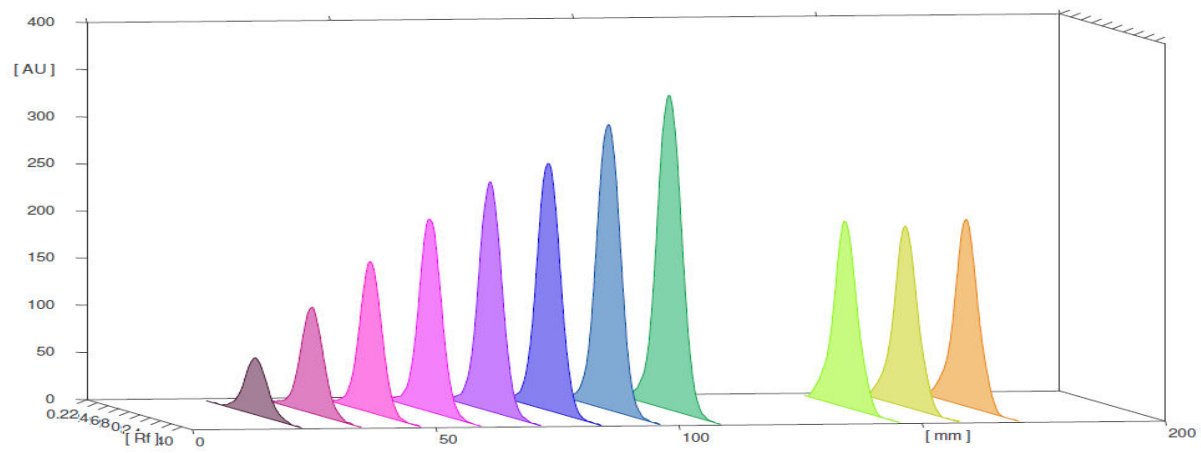


Fig. No. 6 Chromatograph for standard bacoside A and methanolic extract of *Bacopa monnieri* (L.) sample

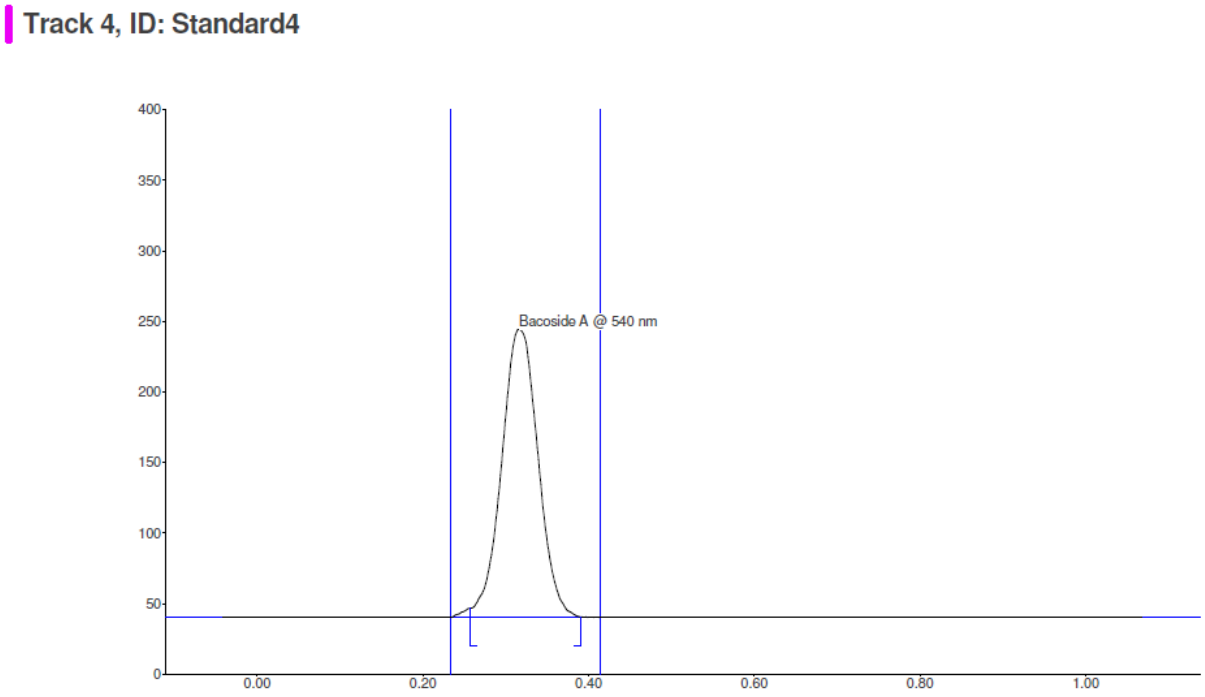


Fig. No. 7 Chromatograph for standard Bacoside A

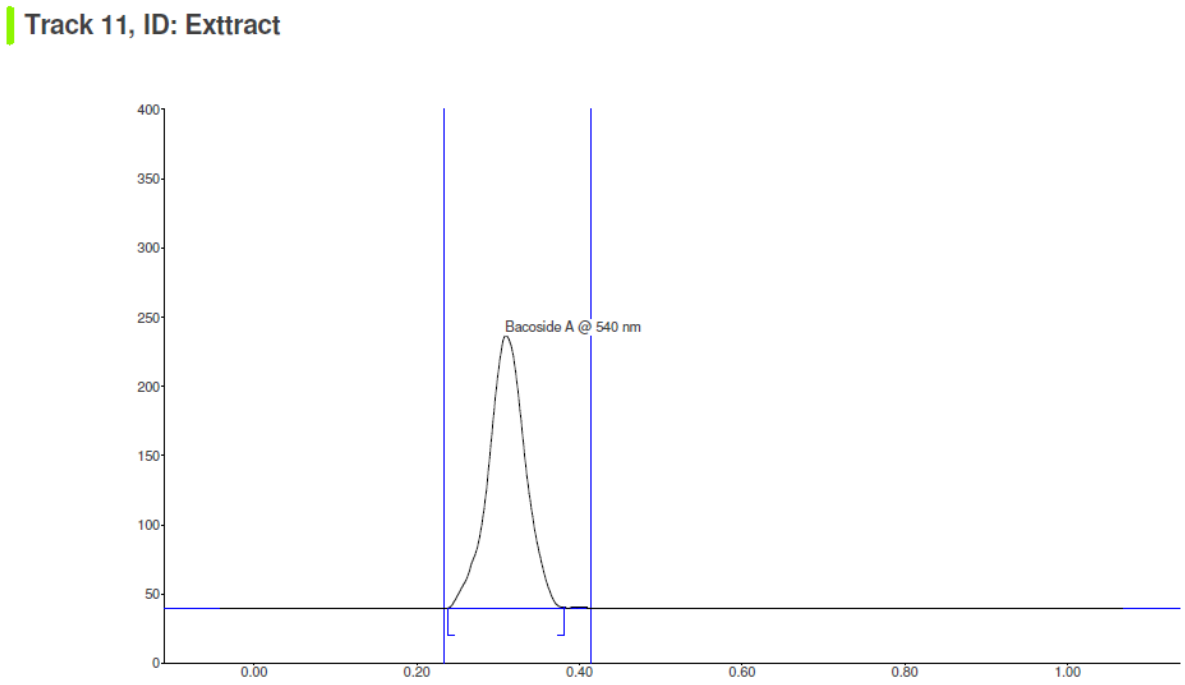


Fig. No. 8 Chromatograph for methanolic extract of *Bacopa monnieri* (L)

Plate No. 6 : TLC Plate Visualized under CAMAG Visualizer : 150503 White remission showing separation of Bacoside A compound

6. Antimicrobial activity of *Bacopa monnieri* L.

Antimicrobial activity of *Bacopa monnieri* L aqueous and methanolic extracts are showed in Table No. 6 &7 and presented in Plate No. 7. The antimicrobial activity study revealed that, the pattern of inhibition varied with the plant extract and the organism tested. The highest antifungal activity was observed in methanolic extract and maximum zone of inhibition was observed against *Aspergillus niger* and *Candida albicans*, where as in aqueous extract no antifungal activity was observed. The zone of inhibition of methanolic extract was highest for *Candida albicans* at 10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml concentrations, while for *Aspergillus niger* the highest zone of inhibition was observed at 2.5mg/ml and 1.25mg/ml concentrations. No antibacterial activity was observed against *Staphylococus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* in aqueous and methanolic extracts of *Bacopa monnieri* L. in these used concentrations.

Table No. 6 Labels and each well used during the experiment for antimicrobial activity *Bacopa monnieri* L

Sr.No.	Plate Label	Extract used	Test Organism	Well No.	Concentration of extract
1	SF1	Aqueous	*Staphylococcus aureus*	1,2,3	10mg/ml,5mg/ml, control respectively
2	SF1	Aqueous	*Staphylococcus aureus*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
3	SF2	Methanolic	*Staphylococcus aureus*	1,2,3	10mg/ml,5mg/ml, control respectively
4	SF2	Methanolic	*Staphylococcus aureus*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
5	ST1	Aqueous	*Bacillus subtilis*	1,2,3	10mg/ml,5mg/ml, control respectively
6	ST1	Aqueous	*Bacillus subtilis*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
7	ST2	Methanolic	*Bacillus subtilis*	1,2,3	10mg/ml,5mg/ml, control respectively
8	ST2	Methanolic	*Bacillus subtilis*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
9	EC1	Aqueous	*Escherichia coli*	1,2,3	10mg/ml,5mg/ml, control respectively
10	EC1	Aqueous	*Escherichia coli*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
11	EC2	Methanolic	*Escherichia coli*	1,2,3	10mg/ml,5mg/ml, control respectively
12	EC2	Methanolic	*Escherichia coli*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
13	P1	Aqueous	*Pseudomonas aeruginosa*	1,2,3	10mg/ml,5mg/ml, control respectively
14	P1	Aqueous	*Pseudomonas aeruginosa*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
15	P2	Methanolic	*Pseudomonas aeruginosa*	1,2,3	10mg/ml,5mg/ml, control respectively
16	P2	Methanolic	*Pseudomonas aeruginosa*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
17	A1	Aqueous	*Aspergillus niger*	1,2,3	10mg/ml,5mg/ml, control respectively
18	A1	Aqueous	*Aspergillus niger*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
19	A2	Methanolic	*Aspergillus niger*	1,2,3	10mg/ml,5mg/ml, control respectively
20	A2	Methanolic	*Aspergillus niger*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
21	C1	Aqueous	*Candida albicans*	1,2,3	10mg/ml,5mg/ml, control respectively
22	C1	Aqueous	*Candida albicans*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
23	C2	Methanolic	*Candida albicans*	1,2,3	10mg/ml,5mg/ml, control respectively
24	C2	Methanolic	*Candida albicans*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively

Well No 3 and 6 are control in each plate, Well No.1: 10 mg/ml, Well No.2: 5 mg/ml, Well No.4 : 2.5mg/ml, Well No.5 : 1.25mg/ml

Table No. 7 Antibacterial and antifungal activity of aqueous and methanolic extract of *Bacopa monnieri* L						
Diameter of Growth Inhibition Zone (mm)						
Extract and its concentration	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Candida albicans*
Aqueous extract						
10mg/ml	*No*	*No*	*No*	*No*	*No*	*No*
5 mg/ml	*No*	*No*	*No*	*No*	*No*	*No*
2.5mg/ml	*No*	*No*	*No*	*No*	*No*	*No*
1.25mg/ml	*No*	*No*	*No*	*No*	*No*	*No*
Methanolic extract						
10mg/ml	*No*	*No*	*No*	*No*	*No*	**23**
5 mg/ml	*No*	*No*	*No*	*No*	*No*	**23**
2.5mg/ml	*No*	*No*	*No*	*No*	**35**	**22**
1.25mg/ml	*No*	*No*	*No*	*No*	25	**22**
Control	*No*	*No*	*No*	*No*	25	20

Note : Values including diameter of wells. No : No inhibition was observed.

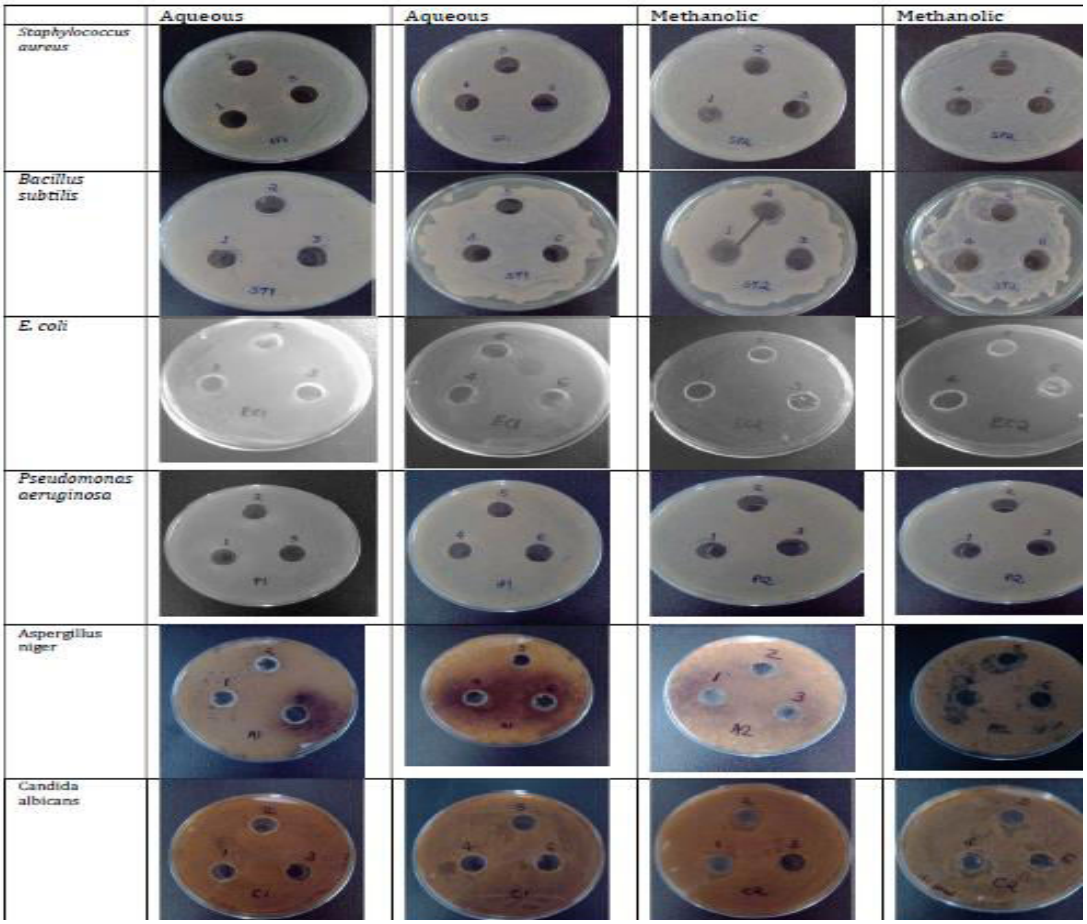
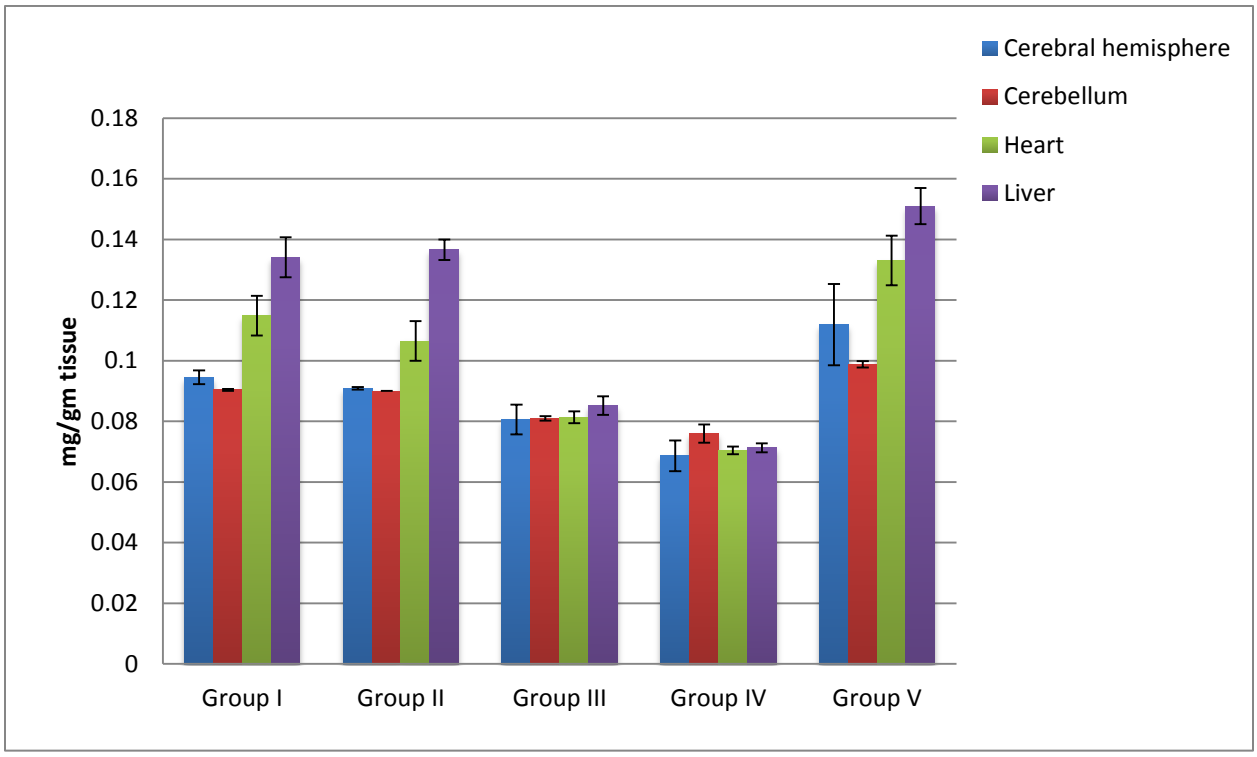


Plate No. 7 : Antimicrobial activity of *Bacopa monnieri* (L) in aqueous and methanolic extract

7. Estimation of protein

Effect of Bacoside A was evaluated against age induced, natural aging and treated mice for estimation of protein in various organs of mouse. Results were shown in Fig. No. 9. The protein content was significantly reduced during induced aging and natural aging, after Bacoside A treatment in Group V it was increased significantly in all organs. In liver significant increase in protein content was observed than other organs.



*All values of D-galactose, induced, D-galactose+BacosideA treated group, adult and natural aging group were compared with respect to Group II -adult old. Values were expressed as mean ± SE. (n=6 mice), $p<0.01$.
In graph where, Gr I Adult, Gr II Adult old, Gr III Old, Gr IV D-galactose, Gr V D-galactose+Bacoside A.

Fig. No. 9 Effect of Bacoside A on protein concentration in various organs of mouse during aging

8. Electrophoretic separation of protein

The electrophoretic separation and scanning of protein in the brain, heart, liver and muscle was illustrated using SDS-PAGE slab gel and gel documentation unit Biorad make and showed as follows.

Plate No. 8 : Electrophoretic separation and scanning of protein in brain of
1] Adult, 2] Adult old, 3] Old, 4] D-galactose induced, 5] Bacoside A treated group.

Plate No. 9 : Electrophoretic separation and scanning of protein in heart of
1] Adult, 2] Adult old, 3] Old, 4] D-galactose induced, 5] Bacoside A treated group.

Plate No. 10 : Electrophoretic separation and scanning of protein in liver of
1] Adult, 2] Adult old, 3] Old, 4] D-galactose induced, 5] Bacoside A treated group.

Plate No. 11 : Electrophoretic separation and scanning of protein in muscle of
1] Adult, 2] Adult old, 3] Old, 4] D-galactose induced, 5] Bacoside A treated group.

The electrophoretic separation of protein in brain, heart, liver and muscle from all groups showed different banding pattern. In Bacoside A treated group proteins were separated more intensely in all organs as compared to old and D-galactose aging induced mice. In D-galactose aging induced and old groups in all organs, the intensity was much reduced as compared to other groups. In case of natural aging group the electrophoretic separation of proteins from all organs showed low intensity of bands as compared to adult, adult old and Bacoside A treated group.

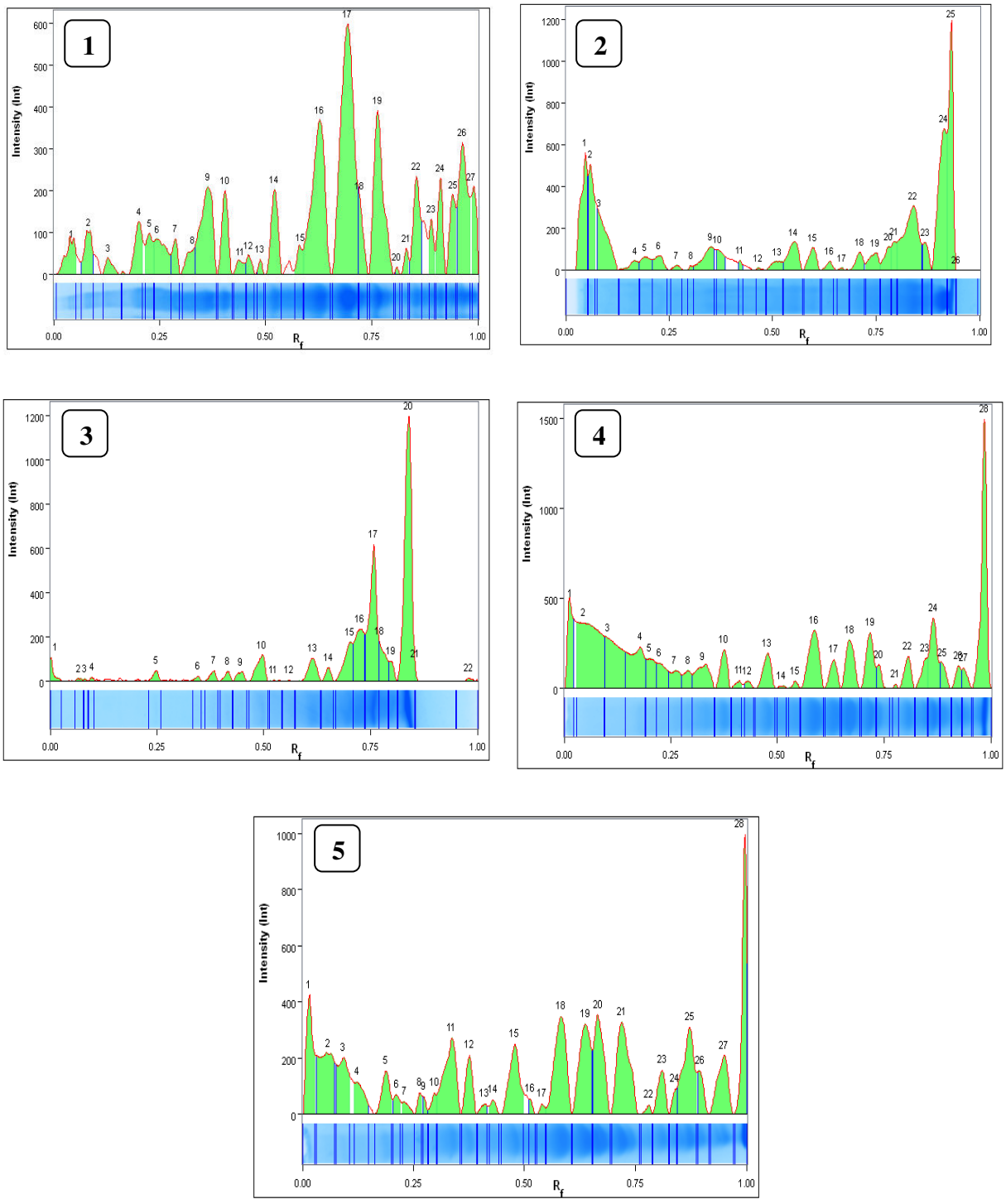


Plate No. 8 : Effect of Bacoside A on electrophoretic separation of protein in brain during aging

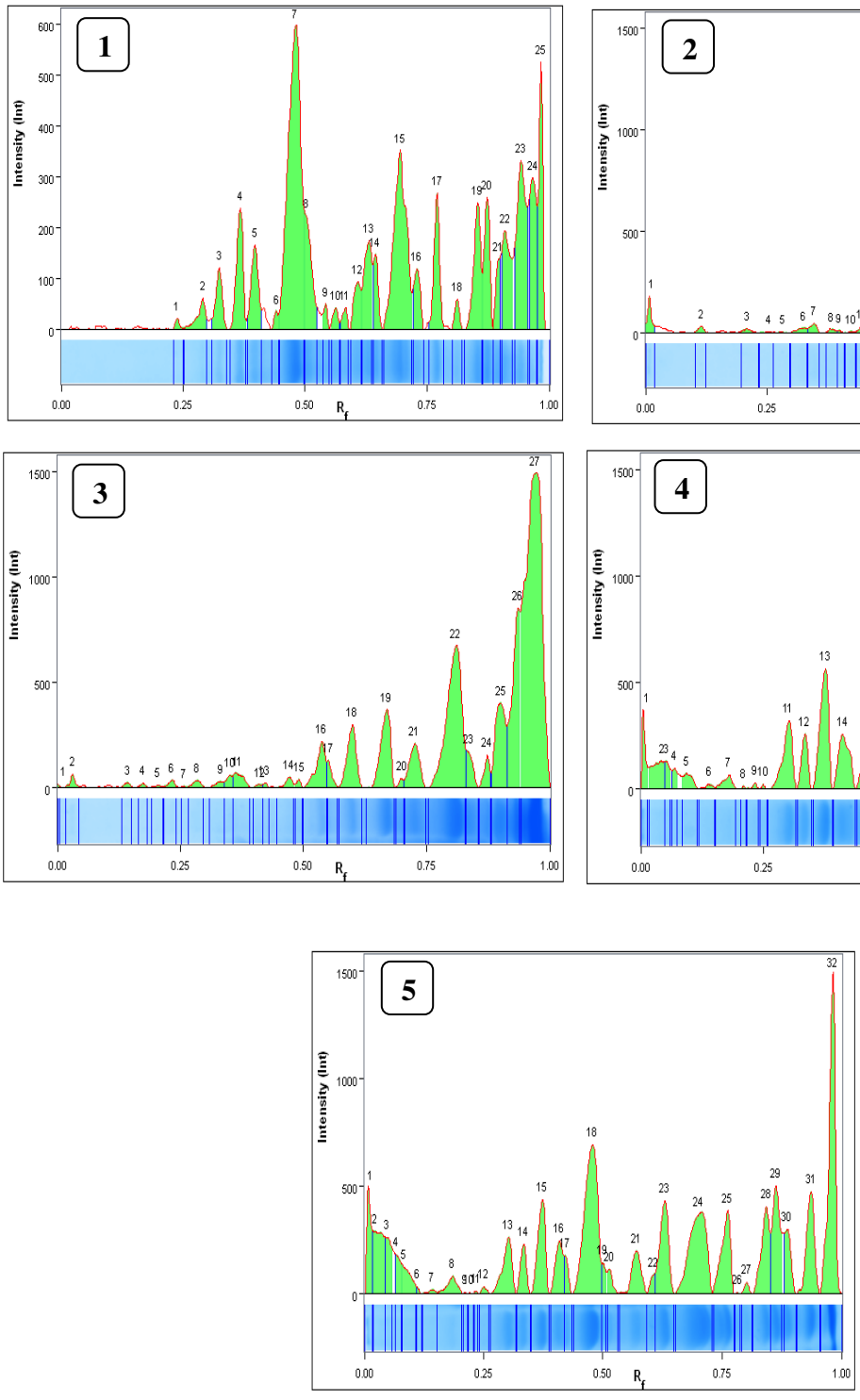


Plate No. 9 : Effect of Bacoside A on electrophoretic separation of protein in heart during aging

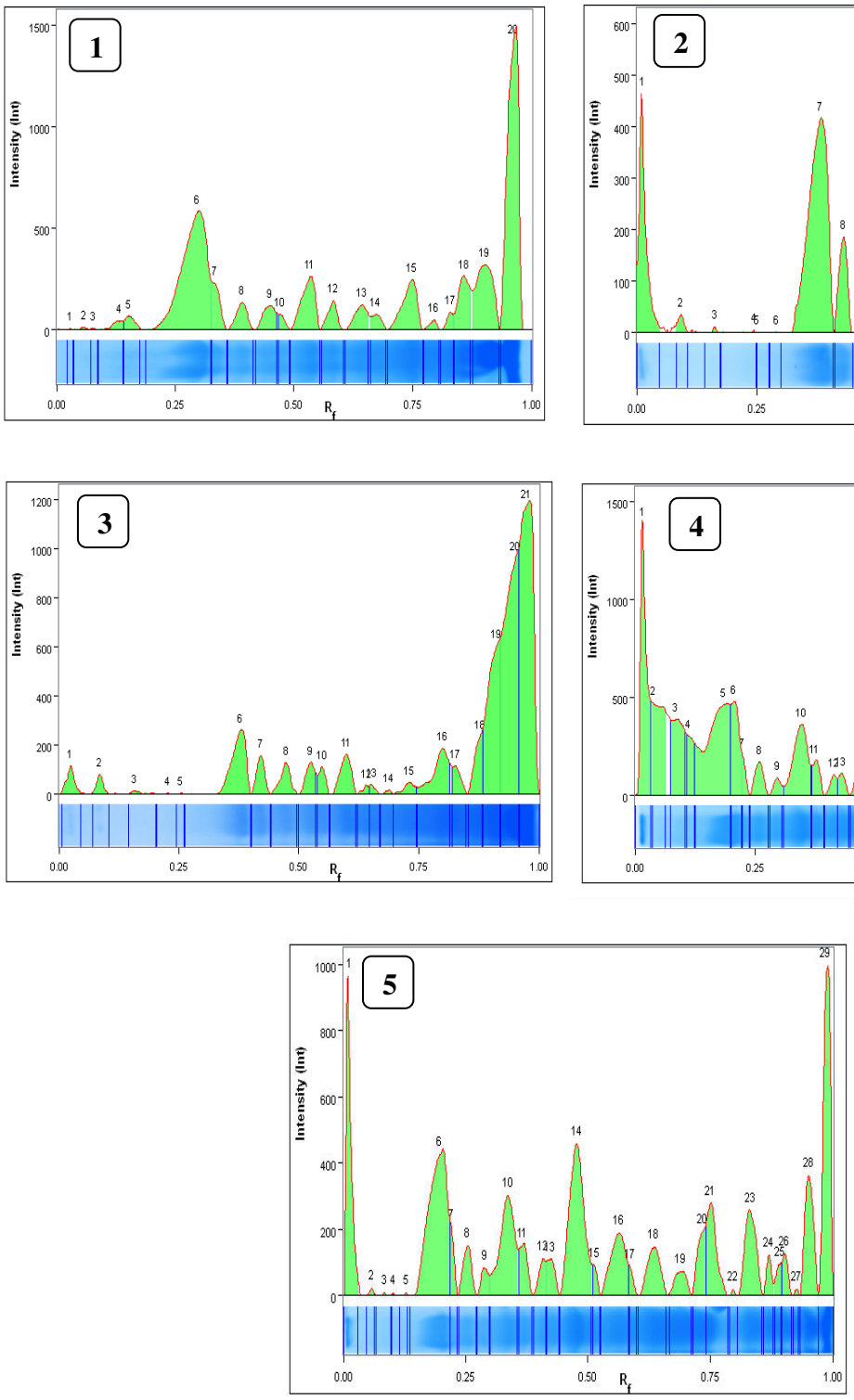


Plate No. 10 : Effect of Bacoside A on electrophoretic separation of protein in liver during aging

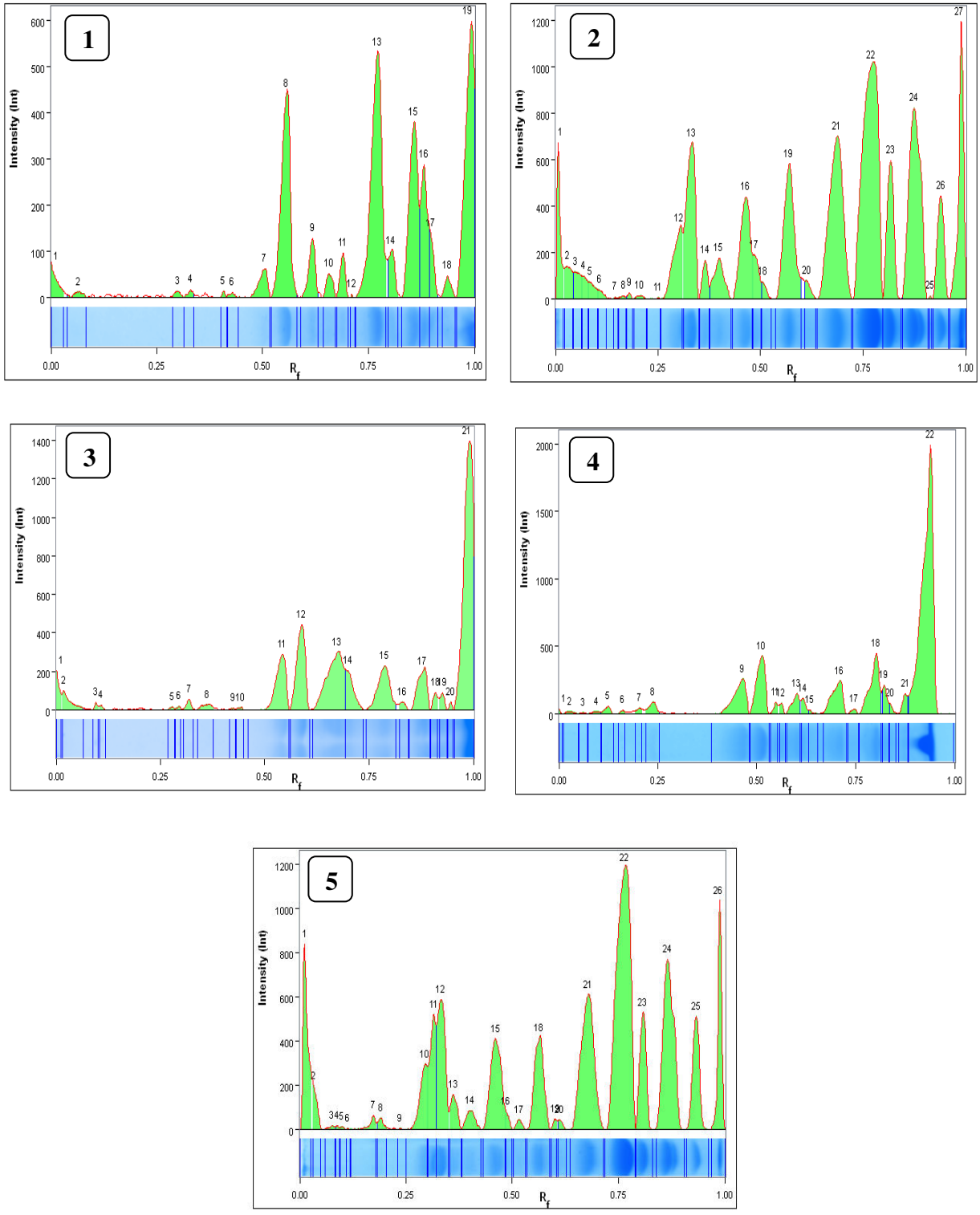
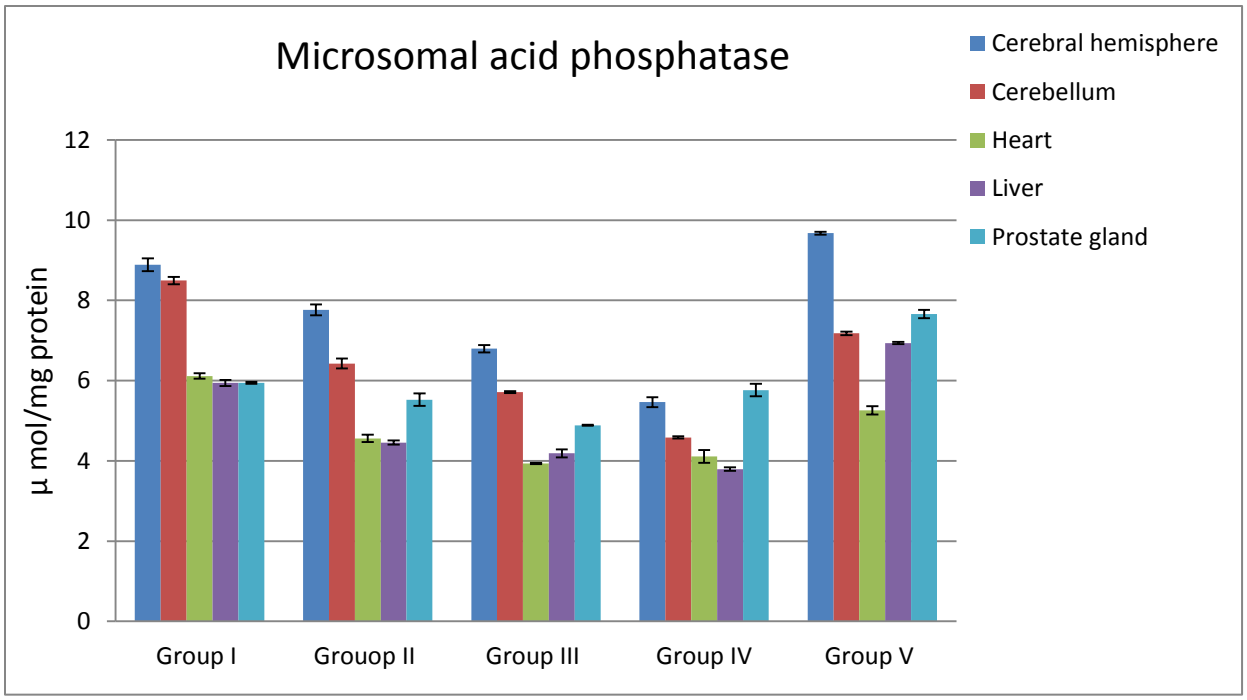


Plate No. 11: Effect of Bacoside A on electrophoretic separation of protein in muscle during aging

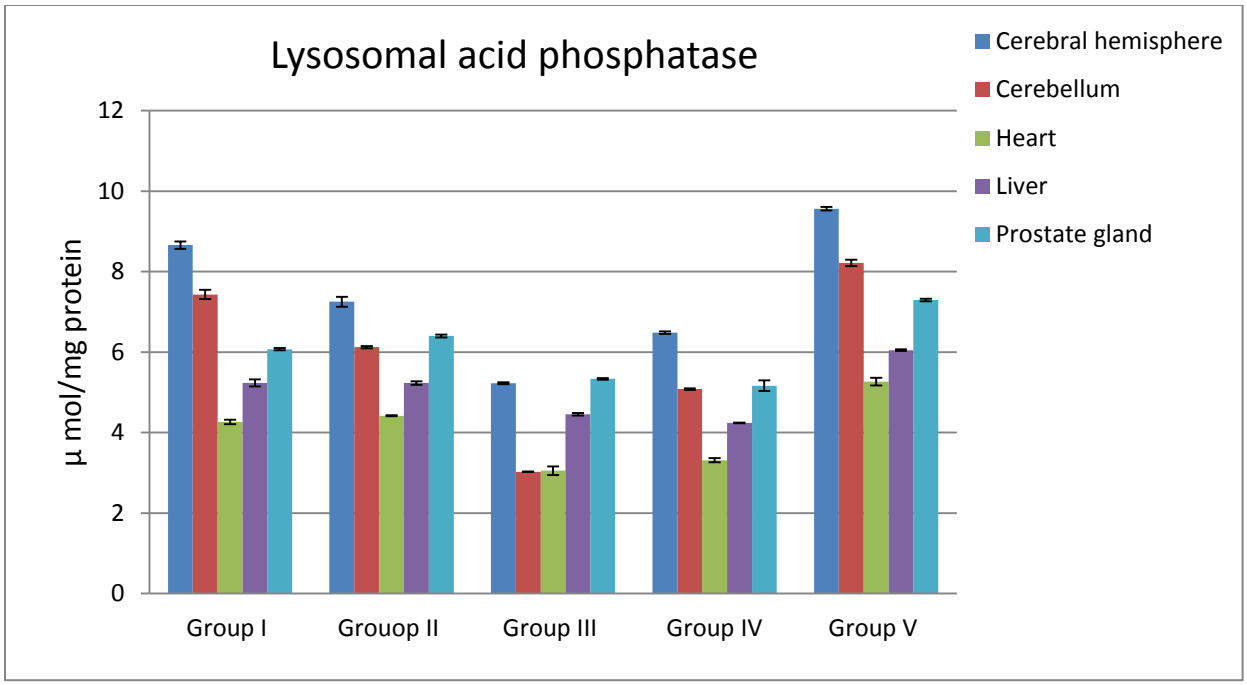
9. Estimation of acid phosphatase

Estimation of acid phosphatase was carried out and measured at 405nm using spectrophotometer Fig. No. 10 and 11 illustrates the effect of Bacoside A on microsomal and lysosomal acid phosphatase activity (µ mols/mg protein) in cerebral hemisphere, cerebellum, heart, liver and prostate gland of male mice. The significant decrease in acid phosphatase activity in cerebral hemisphere, cerebellum, heart and liver of D-galactose-treated aging-induced mice was observed as compared to adult old group. In Bacoside A treated group of mice, the acid phosphatase enzyme activity was significantly increased as compared to induced aging and natural aging groups. In cerebral hemisphere, both the microsomal and lysosomal enzyme activity was maximum in all groups except Group IV. In Group V microsomal and lysosomal acid phosphatase enzyme activity reveals adult old i.e. Group II and significantly increased in all organs except heart.



*All values of D-galactose, induced, D-galactose+BacosideA treated group, adult and natural aging group were compared with respect to Group II -adult old. Values were expressed as mean ± SE. (n=6 mice), $p<0.001$.
In graph where,Gr I Adult, Gr II Adult old, Gr III Old, Gr IV D-galactose treated, Gr V D-galactose+Bacoside A treated.

Fig. No. 10 Effect of Bacoside A on microsomal acid phosphatase concentration in various organs of mouse during aging



*All values of D-galactose, induced, D-galactose+BacosideA treated group, adult and natural aging group were compared with respect to Group II -adult old. Values were expressed as mean ± SE. (n=6 mice), $p<0.001$.
In graph where,Gr I Adult, Gr II Adult old, Gr III Old, Gr IV D-galactose treated, Gr V D-galactose+Bacoside A treated.

Fig. No. 11 Effect of Bacoside A on lysosomal acid phosphatase concentration in various organs of mouse during aging

10. Kinetic study of acid phosphatase

Effect of substrate concentration on microsomal and lysosomal acid phosphatase activity in cerebral hemisphere, cerebellum and liver of adult, adult old, old, D-galactose and Bacoside A treated mice was depicted in Fig. No. 12 to 17.

Fig. No. 12 showed K_m and V_{max} value for adult microsomal acid phosphatase in cerebral hemisphere. Low K_m was observed in Group I, Group II and Group V and it was minimum in Group III. In Group IV maximum K_m (1.30±0.045 X 10^{-3} M) was recorded as compared to other groups. V_{max} was more in Group V and less in Group IV it was 6.610±0.0671µmol/mg protein.
Lysosomal acid phosphatase in cerebral hemisphere showed 0.399±0.024 X 10^{-3} M and 0.304±0.021 X 10^{-3} M K_m value (Fig. No. 13), in adult and Bacoside A treated mice respectively, in old group K_m was slightly more (0.515±0.036 X 10^{-3} M). Maximum K_m was recorded in D-galactose aging induced mice, it was 0.897±0.057 X 10^{-3} M. In Group V highest V_{max} (9.716±0.08419 µmol/mg protein) was observed and it was minimum in Group III and Group IV animals.

In cerebellum microsomal acid phosphatase was revealed the maximum Km value in D-galactose aging induced mice. In adult lowest K_m value was recorded that is 0.376±0.0284 X 10^{-3} M. Maximum V_{max} was observed in Group I followed by Group V and minimum in Group IV (D-galactose mice), depicted in Fig. No. 14.
Fig. No. 15 showed lysosomal acid phosohatase K_m and V_{max} value in cerebellum for all groups. In Bacoside A treated group the Km value was lowest (0.343±0.02865 X 10^{-3} M) as compared to all other groups. High K_m was observed in Group III (0.572±0.050 X 10^{-3} M) followed by Group IV. Maximum V_{max} was recorded in Group V (8.487±0.093 µmol/mg protein) and minimum in Group IV, it was 5.871±0.0617 µmol/mg protein.

Microsomal acid phosphatase in liver showed more or less same K_m values in Group I and Group V that is 0.536±0.056 X 10^{-3} M and 0.549±0.060 X 10^{-3} M respectively. Highest (2.509±0.3129 X 10^{-3} M) K_m was observed in Group IV. In adult old and old animals K_m values were 1.023±0.104 X 10^{-3} M and 1.235±0.122 X 10^{-3} M. V_{max} was more in Bacoside A treated microsomal acid phosphatase than old micosomal acid phosphatase showed in Fig. No. 16.
Fig. No. 17 represents effect of substrate concentration on lysosomal acid phosphatase in liver. Lowest K_m was observed in Bacoside A treated mice and it was maximum in D-galactose aging induced mice. More K_m value was recorded (1.075±0.122 X 10^{-3} M) in Group IV. In Bacoside A treated group maximum V_{max} (6.418±0.112 µmol/mg protein) was observed as compared to other groups.

a. Effect of substrate concentration on acid phosphatase activity

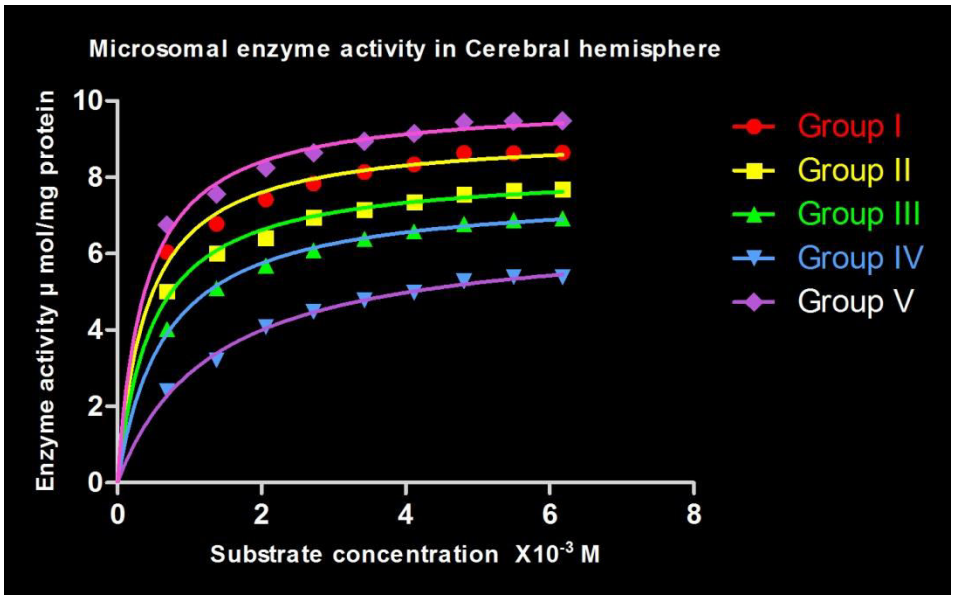


Fig. No. 12 Effect of substrate concentration on microsomal acid phosphatase activity in cerebral hemisphere of mouse during aging

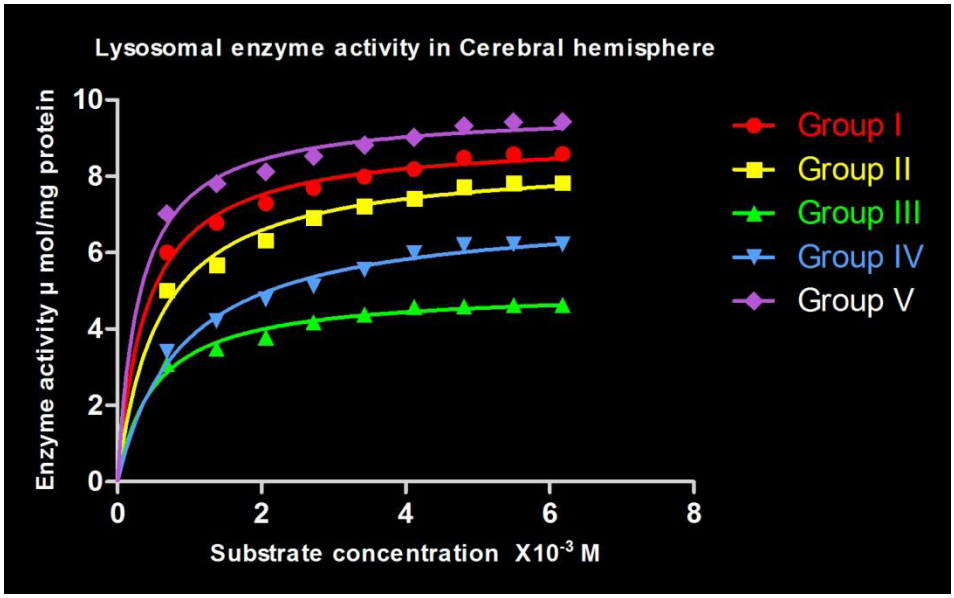


Fig. No. 13 Effect of substrate concentration on lysosomal acid phosphatase activity in cerebral hemisphere of mouse during aging

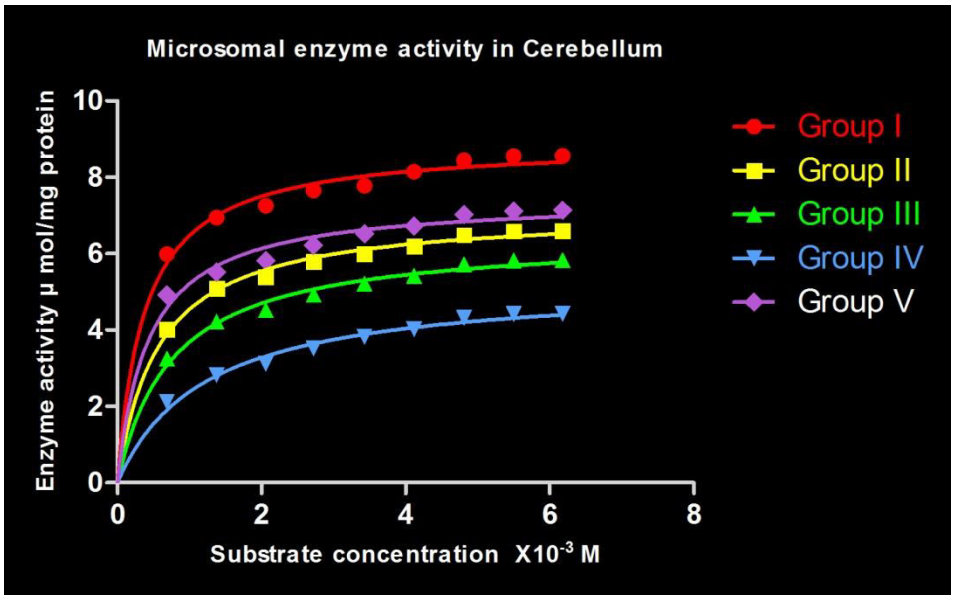


Fig. No. 14 Effect of substrate concentration on microsomal acid phosphatase activity in cerebellum of mouse during aging

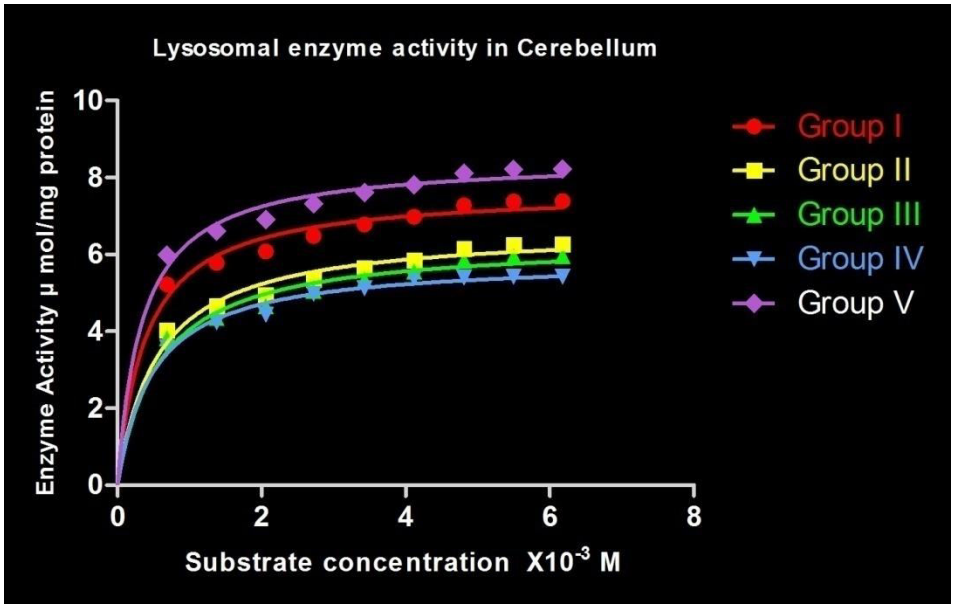


Fig. No. 15 Effect of substrate concentration on lysosomal acid phosphatase activity in cerebellum of mouse during aging

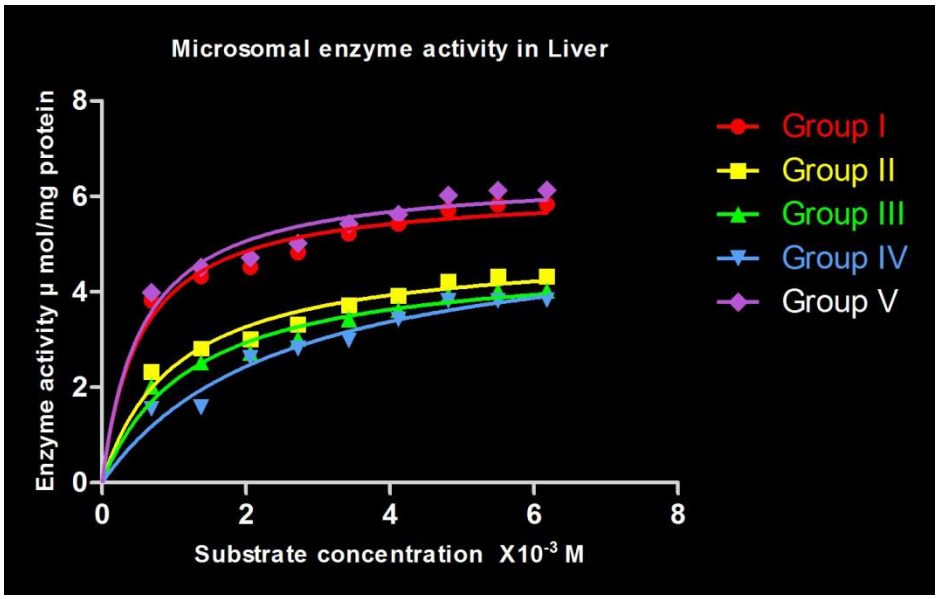


Fig. No. 16 Effect of substrate concentration on microsomal acid phosphatase activity in liver of mouse during aging

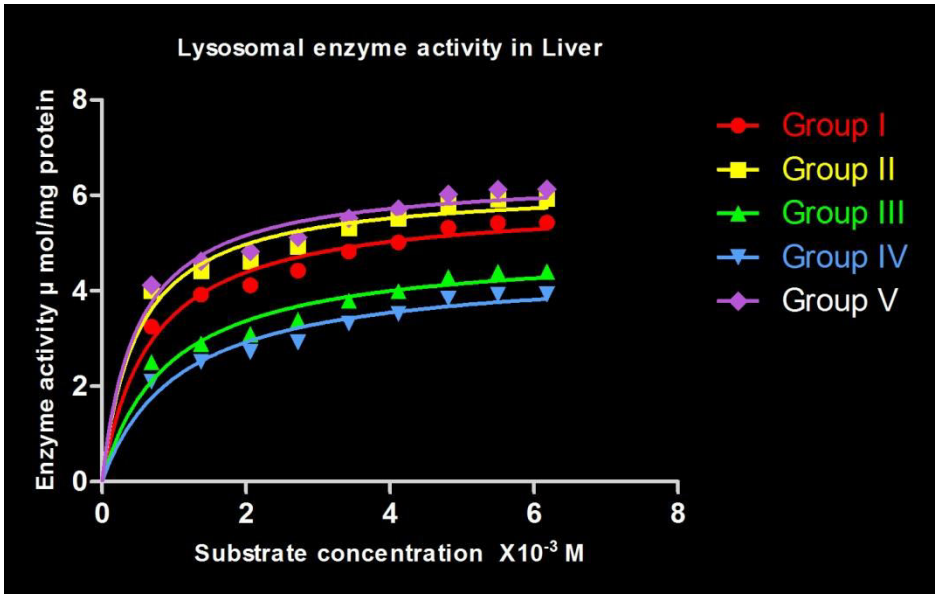


Fig. No. 17 Effect of substrate concentration on lysosomal acid phosphatase activity in liver of mouse during aging

b. Effect of pH on acid phosphatase activity

Effect of pH on acid phosphatase activity in cerebral hemisphere, cerebellum and liver of adult, adult old, old, D-galactose treated and Bacoside A treated mice was studied and illustrated in Fig. No. 18-23. pH optima of acid phosphatase was studied using 0.05 M citrate buffers having a range of pH from 3 to 7 was selected for the experiments.

Alterations in the microsomal acid phosphatase activity at different pH in the cerebral hemisphere of adult, adult old, old, D-galactose and Bacoside A treated group were represented in Fig No. 18. pH optima for all groups was recorded and found to be in the range from 4.5 and decreased from 5.5 onwards.

Fig. No. 19 showed the effect of pH on lysosomal acid phosphatase activity in cerebral hemisphere in all groups. Maximum acid phosphatase enzyme activity in Group I, Group II, Group III, Group IV and Group V was observed at the pH optima 4.5.

The alteration in the microsomal acid phosphatase enzyme activity of cerebellum in Group I, Group II, Group III Group IV and Group V was plotted in Fig. No.20. pH optima in all groups was observed at pH 4.6 and the activity was decreased from pH 5.5.

The lysosomal acid phoaphatse enzyme activity of cerebellum in Group I, Group II, Group III, Group IV and Group V was found to be 7.56, 6.37, 4.64, 5.02 and 8.99 µmols/mg protein, depicted in Fig. No. 21. Maximum enzyme activity was recorded at the pH optima 4.5 to 5.

pH optima for microsomal acid phosphatase in liver of adult, adult old, old, D-galactose aging induced and Bacoside A treated group was showed in Fig. No. 22, it was between pH 4.6 to 5.5. Optimum enzyme activity was recorded at 4.6 pH and from pH 5.5 onwards the activity showed substantial decrease upto pH 7.

In liver all the groups of mice i.e. adult, adult old, old, D-galactose and Bacoside A treated showed pH optima at 4.5 to 5.5 (Fig. No.23). At these pH optima alteration in the enzyme activity in liver was observed i.e. 5.38, 5.90, 4.73, 4.23 and 6.81 µmols/mg protein in respective groups.

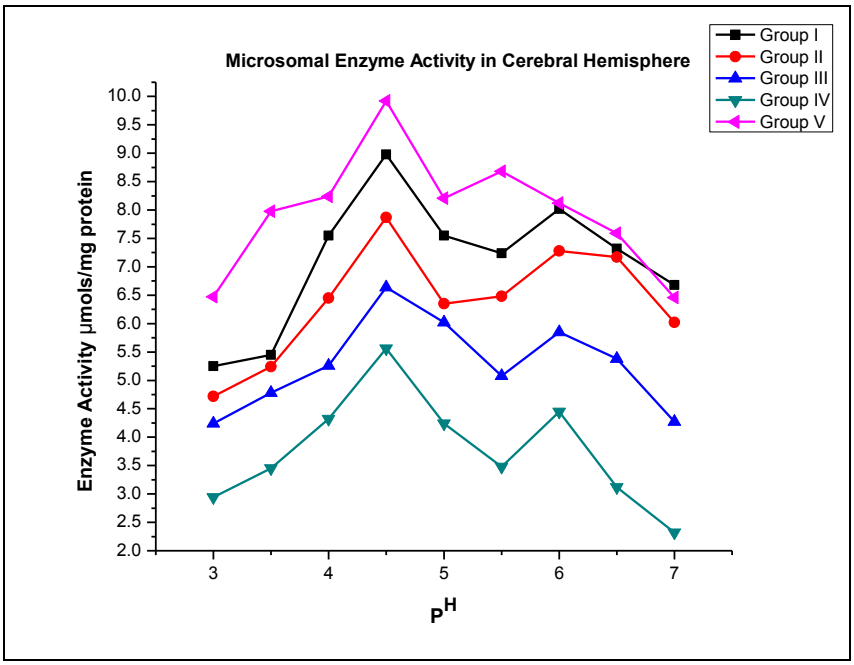


Fig. No. 18 Effect of pH on microsomal acid phosphatase activity in cerebral hemisphere of mouse during aging

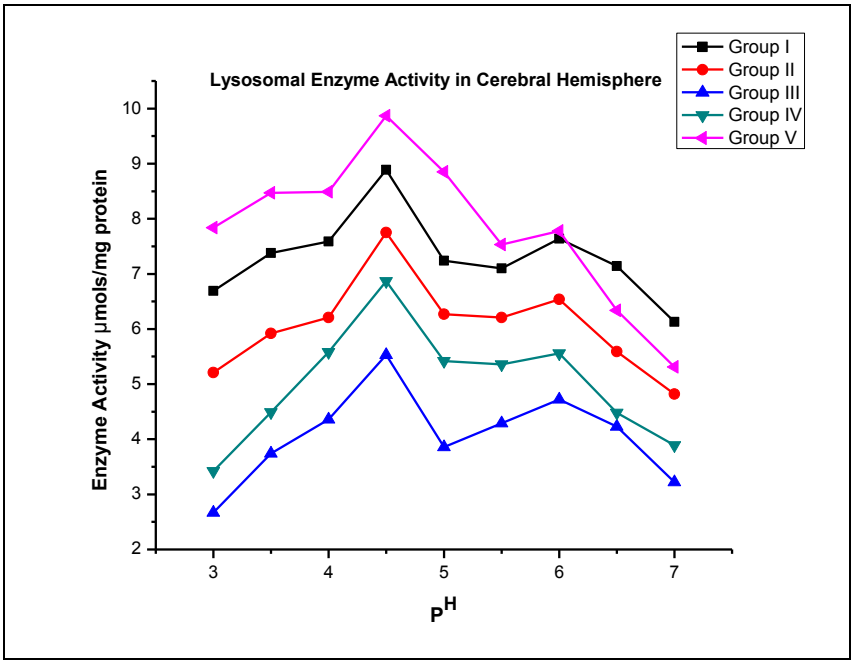


Fig. No. 19 Effect of pH on lysosomal acid phosphatase activity in cerebral hemisphere of mouse during aging

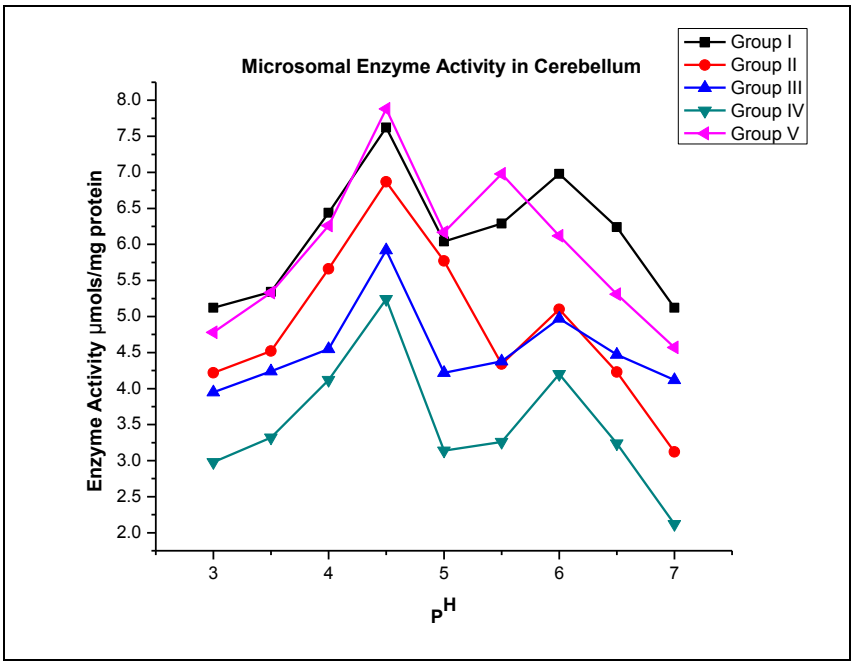


Fig. No. 20 Effect of pH on microsomal acid phosphatase activity in cerebellum of mouse during aging

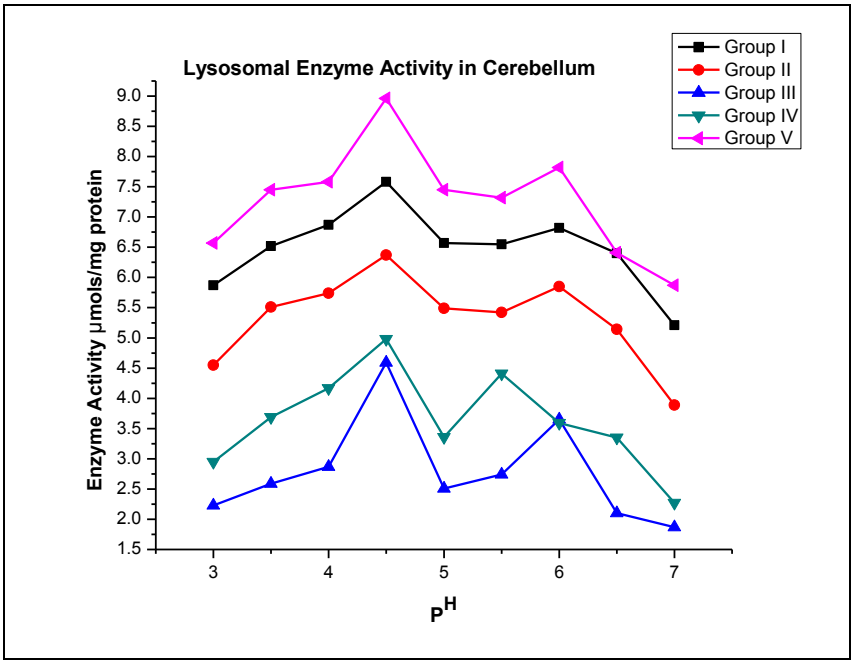


Fig. No. 21 Effect of pH on lysosomal acid phosphatase activity in cerebellum of mouse during aging

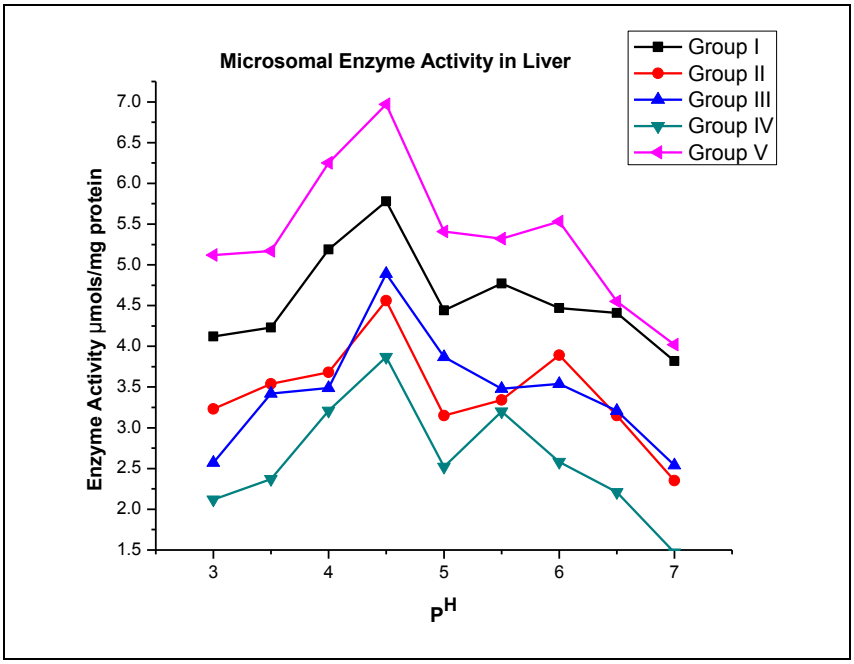


Fig. No. 22 Effect of pH on microsomal acid phosphatase activity in liver of mouse during aging

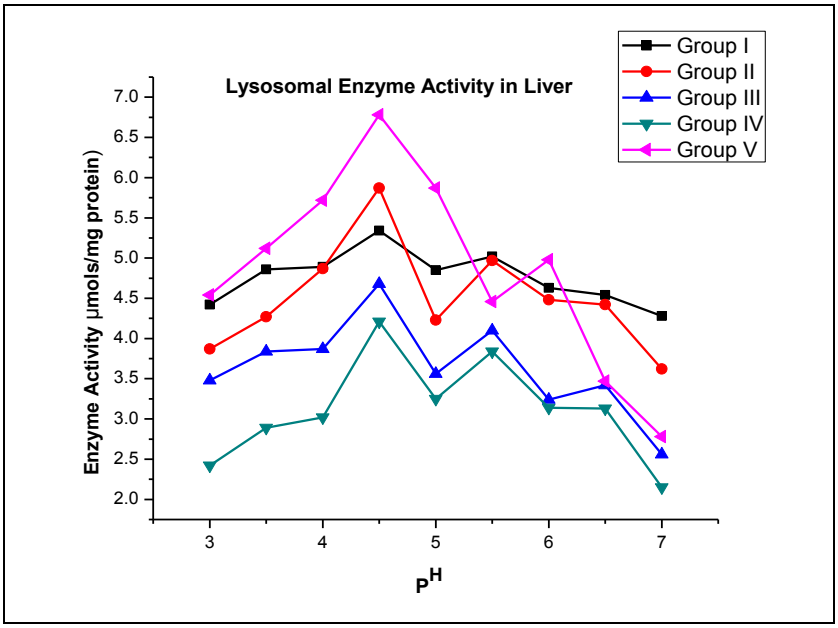
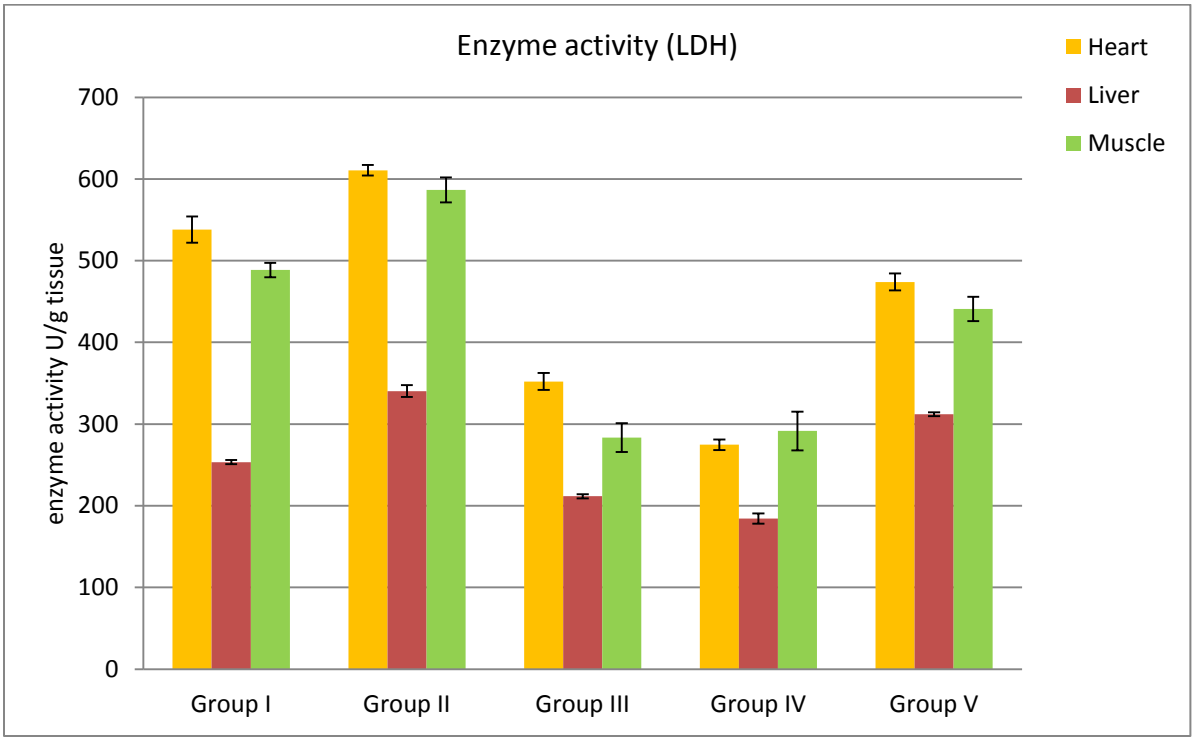


Fig. No. 23 Effect of pH on microsomal acid phosphatase activity in liver of mouse during aging

11. Estimation of lactate dehydrogenase (LDH enzyme)

Lactate dehydrogenase enzyme was carried out and activity was measured at 340 nm. Effect of Bacoside A on lactate dehydrogenase enzyme activity in heart, liver and muscle of mouse during aging is depicted in Fig. No. 24. In all organs less LDH enzyme activity was observed in natural aging group (Group III) and D-galactose induced aging group. In Bacoside A treated group LDH enzyme activity was increased significantly in heart, liver and muscle as compared to old and D-galactose treated group. Group II (Adult old) showed comparatively highly significant increase in the enzyme activity. In old and D-galactose aging induced animals significantly less enzyme activity was observed. Lactate dehydrogenase enzyme activity was more in heart and muscle than in liver. It was maximum in Group I, Group II and Group V.



*All values of D-galactose, induced, D-galactose+BacosideA treated group, adult and natural aging group were compared with respect to Group II -adult old. Values were expressed as mean ± SE. (n=6 mice), $p<0.001$.
In graph where, Gr I Adult, Gr II Adult old, Gr III Old, Gr IV D-galactose treated, Gr V D-galactose+Bacoside A treated.

Fig. No. 24 Effect of Bacoside A on lactate dehydrogenase enzyme activity in heart, liver and muscle during aging

12. Electrophoretic separation of lactate dehydrogenase

The electrophoretic separation and scanning of lactate dehydrogenase in the heart and muscle was illustrated using SDS-PAGE slab gel and scanning was carried on gel documentation unit (Biorad make) and showed as follows.

Plate No.12 : Electrophoretic separation and scanning of LDH in heart of
1] Adult, 2] Adult old, 3] Old, 4] D-galactose induced, 5] Bacoside A treated group.

Plate No.13 : Electrophoretic separation and scanning of LDH in muscle of
1] Adult, 2] Adult old, 3] Old, 4] D-galactose induced, 5] Bacoside A treated group.

Electrophoretic study in adult, adult old, old, D-galactose and Bacoside A treated group showed clear separation of lactate dehydrogenase enzyme in respective groups. In Bacoside A treated group high intensity of band was observed in both organs. Lactate dehydrogenase enzyme was separated intensely in Bacoside A treated group as compared to old and D-galactose aging induced group. Similar band pattern was observed in adult, adult old and Bacoside A treated group. In old group intensity of band was low in heart as compared to all other groups. In case of liver, low intensely separated bands were observed in D-galactose and old group than adult, adult old and Bacoside A treated group.

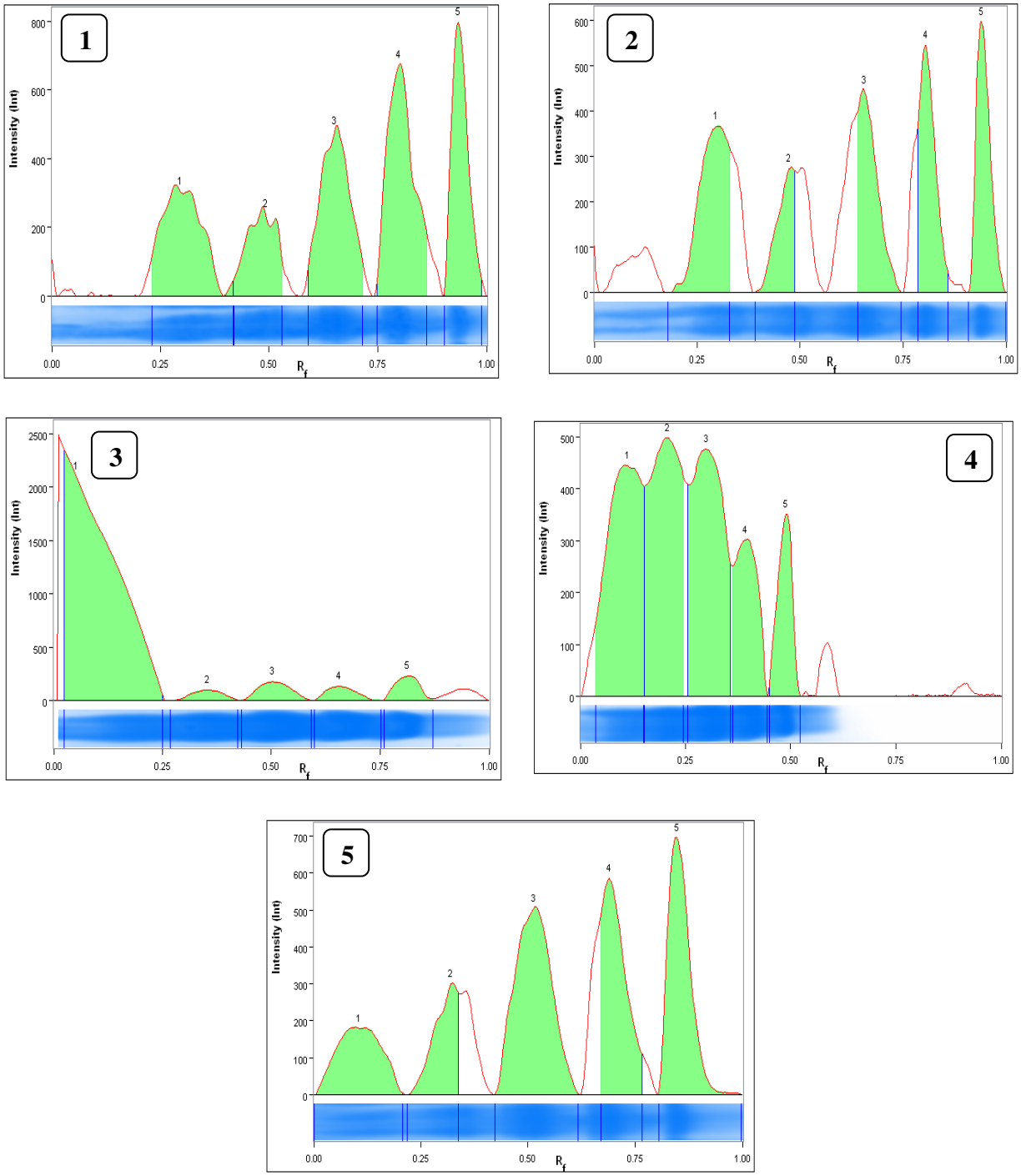


Plate No. 12 Effect of Bacoside A on electrophoretic separation of Lactate dehydrogenase enzyme in heart during aging

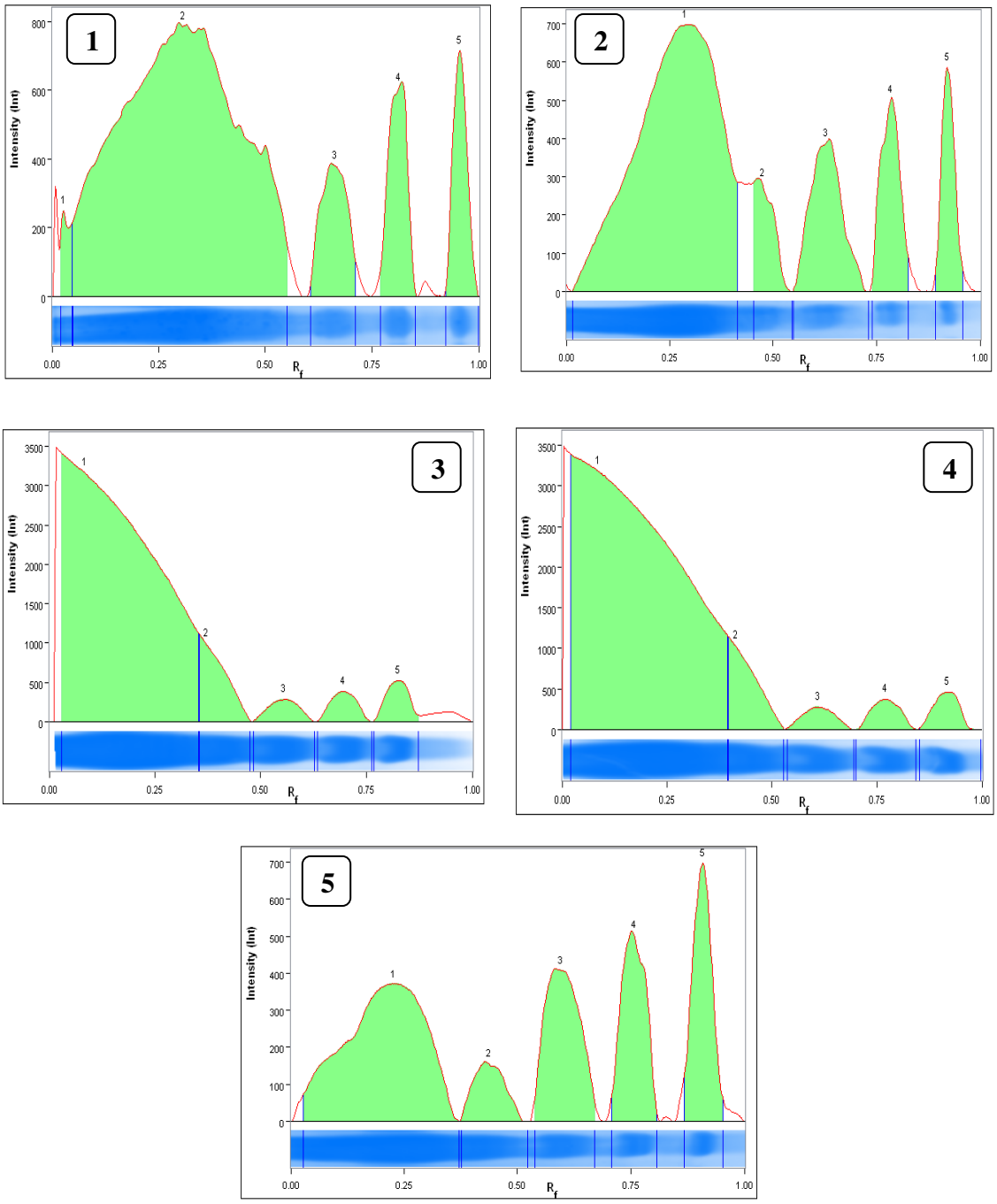
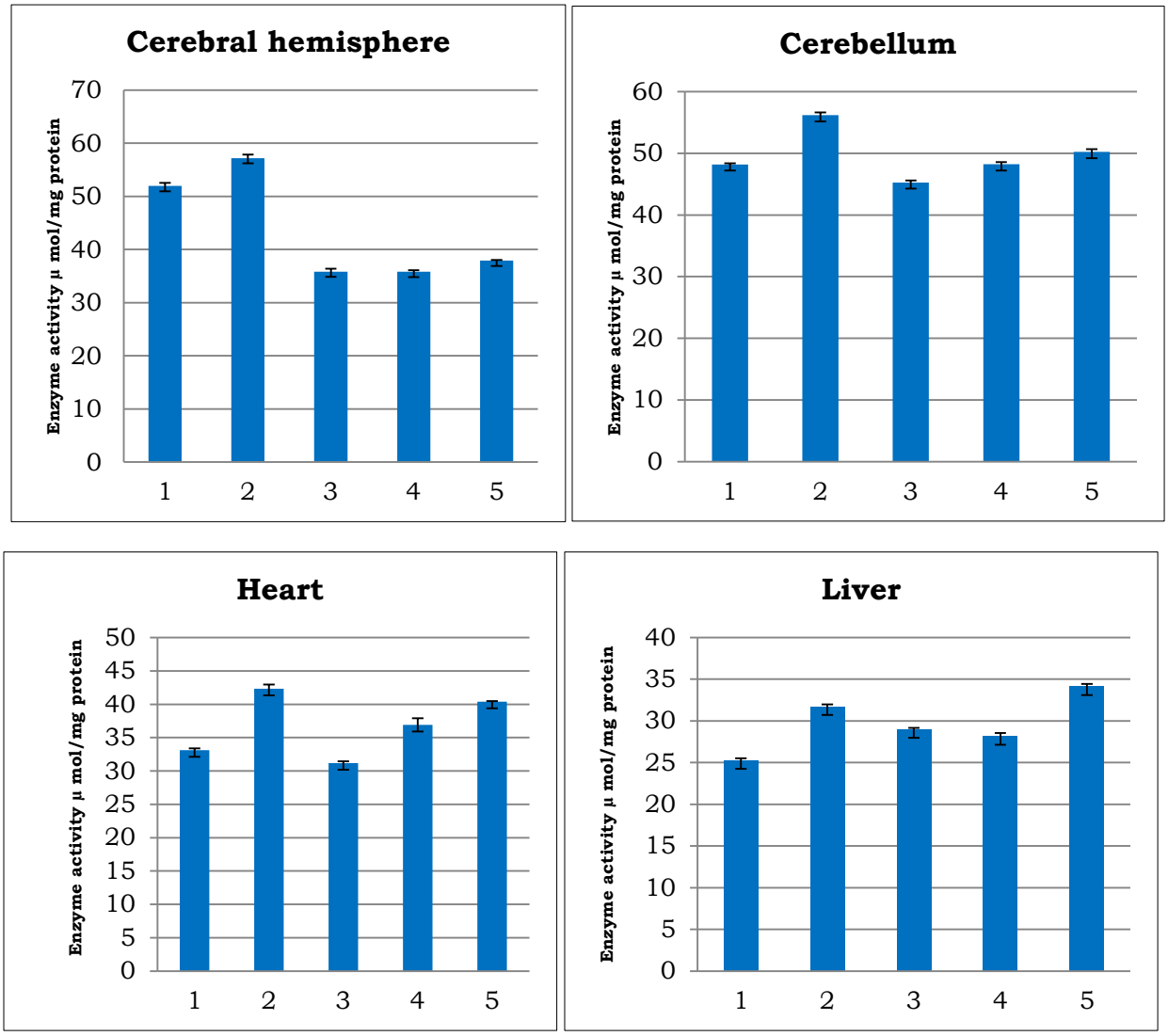


Plate No. 13 Effect of Bacoside A on electrophoretic separation of Lactate dehydrogenase enzyme in muscle during aging

13. Estimation of non specific esterase enzyme activity

Estimation of non specific esterase enzyme activity was carried and measured at 400nm. The statistical data obtained were analyzed using one way ANOVA, and results were expressed as mean ±SE in Fig. No.25. Non specific esterase enzyme activity was decreased in induced age group in all organs while in Bacoside A treated group highly significant increase in non-specific esterase activity was observed in the cerebral hemisphere, cerebellum, heart and liver. Treatment of Bacoside A significantly elevated the non-specific esterase enzyme activity compared to all group.



*All values of D-galactose, induced, D-galactose+BacosideA treated group Adult and Natural aging group were compared with respect to Group II -adult old. Values were expressed as mean ± SE. (n=6 mice), $p<0.001$.
In graph where, 1=Gr I Adult, 2=Gr II Adult old, 3=Gr III Old, 4=Gr IV D-galactose treated, 5=Gr V D-galactose+Bacoside A treated

Fig No. 25 Effect of Bacoside A on Non-specific esterase enzyme activity in various organs of mouse during aging

14. Estimation of lipid peroxidation

In the present study, the level of lipid peroxidation in brain, heart and liver of experimental groups was measured at 532nm. The lipid peroxidation in the form of MDA formation in cerebral hemisphere, cerebellum, heart and liver was measured and depicted in Table No. 8 and Fig. No. 26. In Bacoside A treated group the lipid peroxidation level in heart, brain and liver was significantly decreased ($p<0.001$) which some extent resembles as in normal adult. Significant increase ($p<0.0001$) in the level of lipid peroxidation was observed in D-galactose induced mice. In natural aging group highly significant increase ($p<0.0001$) in initial lipid peroxidation, ascorbate dependent lipid peroxidation and spontaneous lipid peroxidation was observed.

The results from our study revealed that, concentration of MDA in brain regions, heart and liver of D-galactose treated group was elevated as compared to control group. In the animals which received Bacoside A along with D- galactose, MDA level was significantly less in brain regions, heart and liver as compared to D-galactose treated group. The results show that administration of Bacoside A brings about alterations in the level of lipid peroxidation in different tissues.

Table No. 8 Formation of TBA reacting products in spontaneous, ascorbate dependant and initial lipid peroxidation (n mols malondialdehyde/mg tissue/1hr) in Cerebral hemisphere, Cerebellum, Heart and Liver

Organ / Group	Cerebral hemisphere			Cerebellum			Heart			Liver		
	X1	X2	X3	X1	X2	X3	X1	X2	X3	X1	X2	X3
Normal Adult	39.20 ±0.1006	9.502 ±0.0213	0.8263 ±0.0075	45.68 ±0.0138	6.678 ±0.0060	1.063 ±0.0245	31.49 ±0.1407	4.770 ±0.0057	0.6728 ±0.0014	41.13 ±0.2493	5.832 ±0.0144	1.345 ±0.0585
D-galactose induced	43.92 ±0.0482	11.65 ±0.0135	1.513 ±0.0016	48.30 ±0.0338	14.87 ±0.0100	1.873 ±0.0024	39.95 ±0.0135	5.125 ±0.0120	1.013 ±0.0010	46.98 ±0.0076	9.055 ±0.0088	2.067 ±0.0007
D-galactose +Bacoside A treated	39.77 ±0.1545	6.147 ±0.01145	0.5380 ±0.0006	42.12 ±0.0788	11.67 ±0.0114	0.8865 ±0.0050	29.33 ±0.0130	4.858 ±0.0600	1.090 ±0.0025	31.65 ±0.1174	7.237 ±0.0311	1.649 ±0.0017
Natural aging	49.78 ±0.0065	14.29 ±0.0061	1.816 ±0.1239	52.90 ±0.0057	17.07 ±0.0202	2.328 ±0.0543	48.45 ±0.1869	6.702 ±0.1269	1.519 ±0.0386	50.47 ±0.0589	11.38 ±0.1216	3.169 ±0.0040

All values of D-galactose, induced, D-galactose + Bacoside A treated group and Natural aging group are compared with respect to normal adult (control) group.

Values are expressed as Mean ± SE (n=6 mice).

X1- The rate of spontaneous lipid peroxidation in the homogenates nmoles of malondialdehyde formation during 1hr.

X2- The rate of ascorbate dependant nonenzymatic peroxidation in the homogenates nmoles of malondialdehyde formation during 1hr.

X3- The amount of malondialdehyde in the initial homogenate

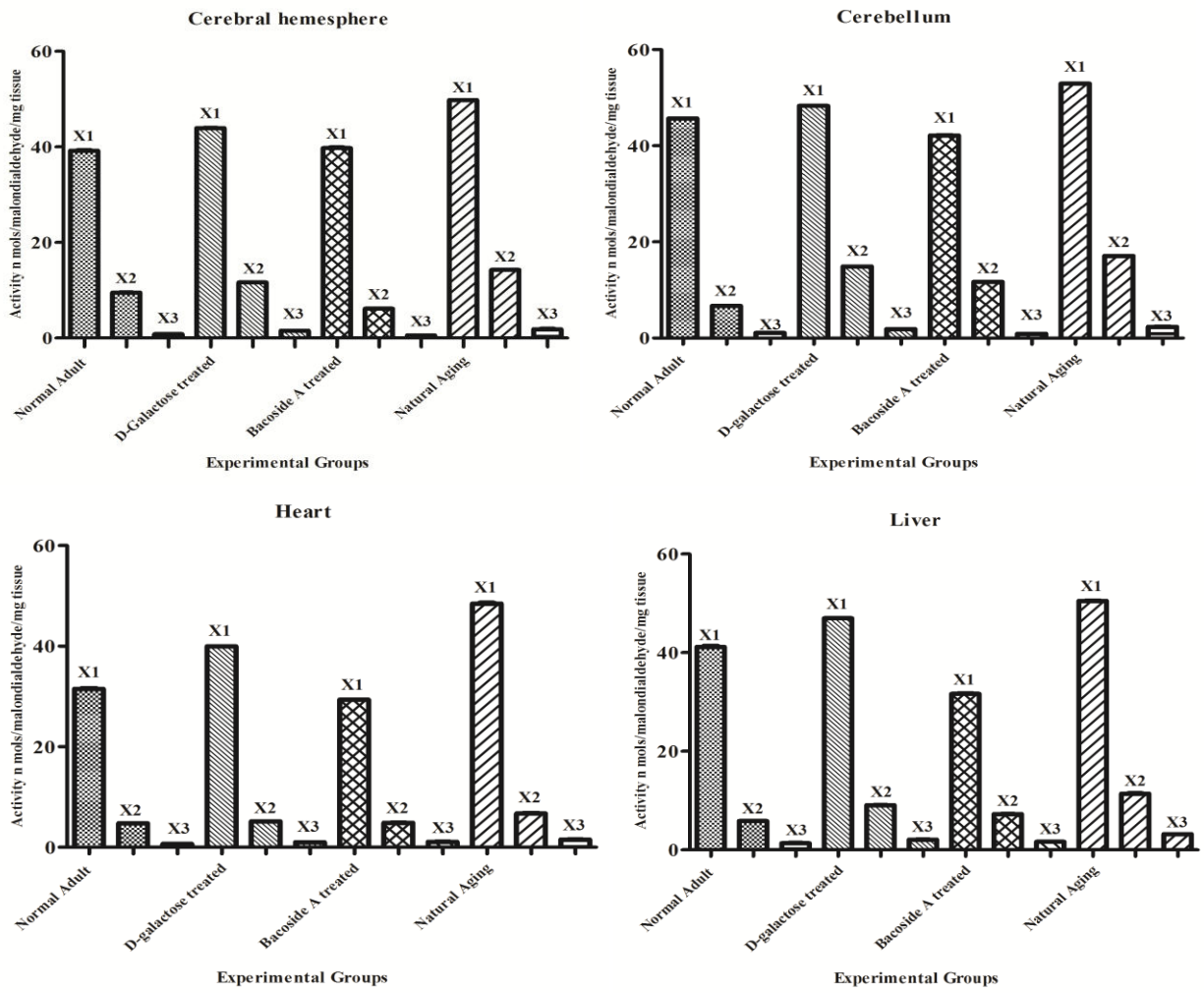
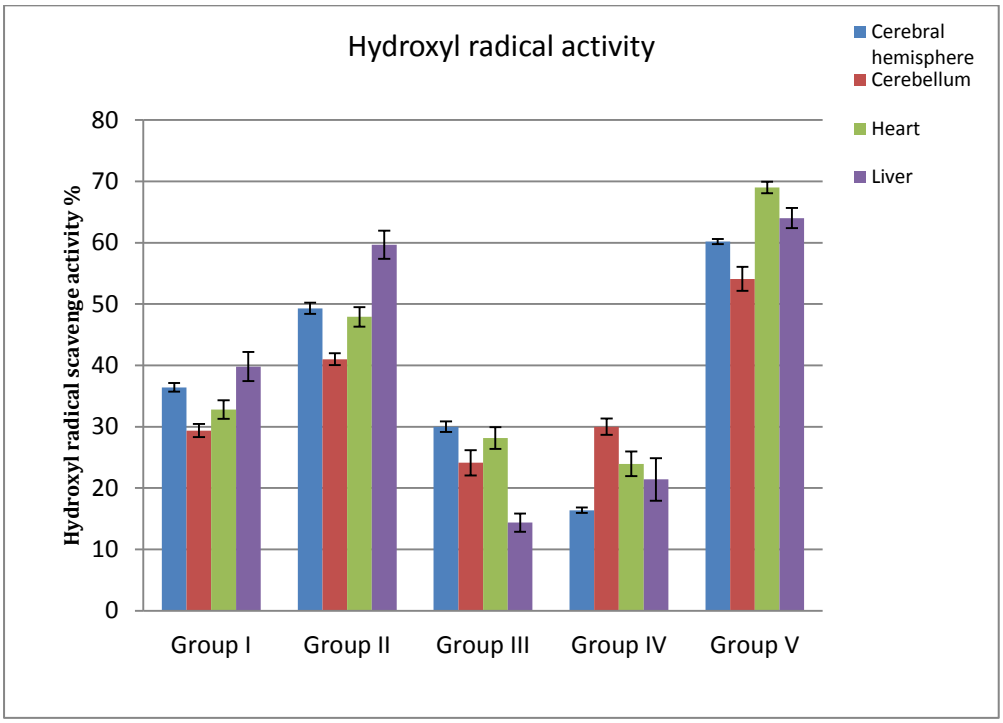


Fig. No. 26 Formation of TBA reacting products in spontaneous, ascorbate dependant and initial lipid peroxidation (n mols malondialdehyde/mg tissue/1hr) in cerebral hemisphere, cerebellum, heart and liver

15. Estimation of hydroxyl radical scavenging activity

Estimation of hydroxyl radical scavenging activity was carried and measured at 532nm Age dependant changes were observed in the hydroxyl radical activity showed in Fig. No.27. In the present study administration of Bacoside A to the D-galactose aging induced mice significantly inhibited the hydroxyl radical activity in cerebral hemisphere, cerebellum, heart and liver with a maximum inhibition. The potential of Bacoside A to inhibit hydroxyl radical mediated deoxyribose damage in aging induced mice was assessed by the treatment of Bacoside A. Highly significant increase in hydroxyl radical activity was observed in Bacoside A treated animals i.e. Group V than all other groups.



*All values of D-galactose, induced, D-galactose + BacosideA treated group Adult and Natural aging group were compared with respect to Group II -adult old. Values were expressed as mean ± SE. (n=6 mice), $p<0.001$.
In graph where, Gr I Adult, Gr II Adult old, Gr III Old, Gr IV D-galactose treated, Gr V D-galactose+Bacoside A treated

Fig. No. 27 Effect of Bacoside A on hydroxyl radical activity in various organs of mouse during aging

16. Study of fluorescence products

The statistical data obtained were analyzed using one way ANOVA, control vs other groups and results were expressed as mean ±SE and illustrated in Fig. No.28. Study of fluorescence product was carried out using photofluorometer and spectroflurometer. The spectra of fluorescence obtained were depicted in Fig. No. 29-30. In cerebral hemisphere, cerebellum, heart, liver and prostate gland fluorescence product was significantly increased during induced aging. In Bacoside A treated group significant decrease in fluorescence product was observed in all organs.

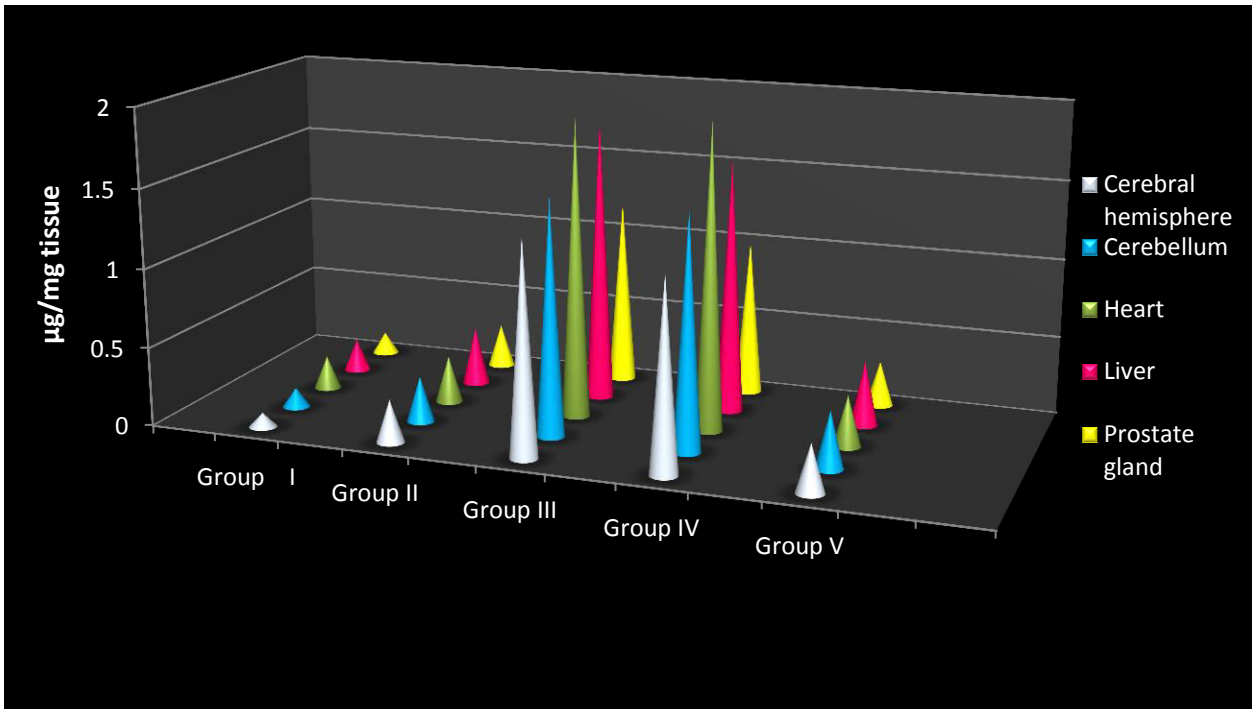


Fig No. 28 Effect of Bacoside A on fluorescence product in Cerebral hemisphere, cerebellum, heart, liver and prostate gland of mouse during aging

- **Measurement of fluorescence spectra**

The fluorescence spectra in adult, adult old, old, D-galactose and Bacoside A treated mice in cerebral hemisphere, cerebellum, heart, liver and prostate gland was measured by using Elico-SL174- Japan, spectrofluorometer. Fluorescence spectra of cerebral hemisphere, cerebellum, heart, liver and prostate gland was recorded at excitation wavelength 360 nm respectively depicted in Fig. No. 29 to 33. In cerebral hemisphere of Group III and Group IV animals, emission spectra was at 428 nm and 449 nm. Highest intensity was recorded in cerebral hemisphere of D-galactose aging induced mice followed by old mice. In cerebral hemisphere of Adult (Group I) mice lowest intensity at emission spectra 396 nm was observed. In cerebral hemisphere of Bacoside A treated mice intensity of fluorescence spectra was low as compared to Group III and Group IV. In cerebellum intensity of fluorescence was increased in Group III, decreased in Group V and lowest intensity was observed in Group I. In heart of all animals fluorescence spectra was at 398 nm but in Group III emission was at 428 nm. Fluorescence spectra in heart revealed highest intensity in Group III followed by Group IV and lowest in Group I. In heart fluorescence intensity was equivalent in some extent in Group II (5.7 AU) and V (6.75 AU). In liver and prostate gland fluorescence intensity was high in Group IV and Group III. Group V showed low fluorescence intensity in liver as well as in prostate gland as compared to Group III and Group IV. In all organs low intensity of fluorescence spectra was observed in Bacoside A treated group than old and D-galactose treated mice.

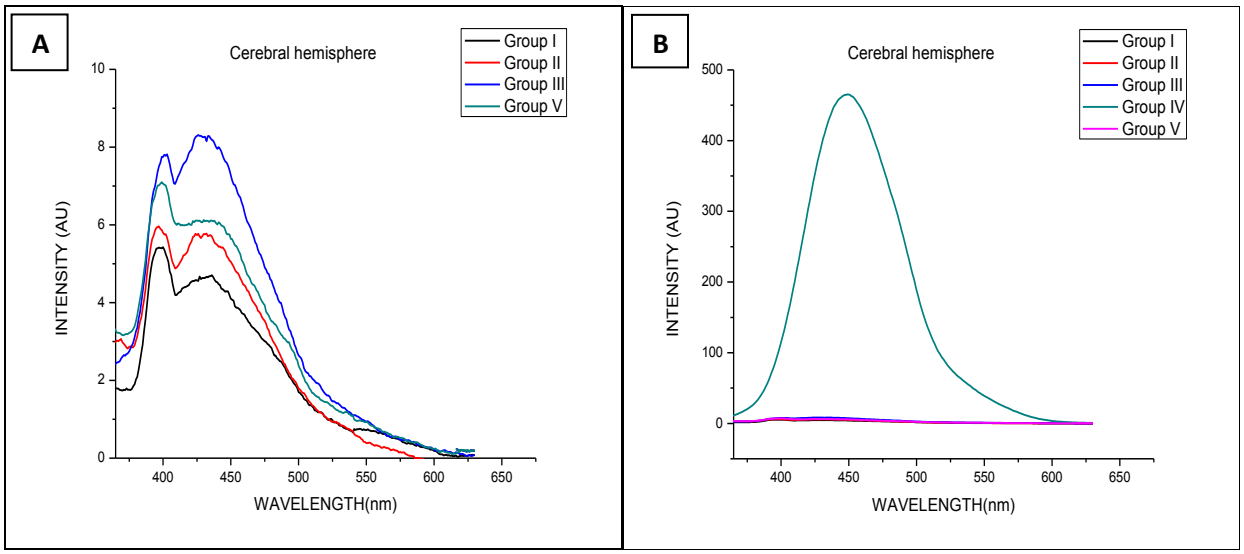


Fig No. 29 A] Fluorescence spectra of cerebral hemisphere in adult, adult old, old, Bacoside A B] Fluorescence spectra of cerebral hemisphere in D-galactose treated mice

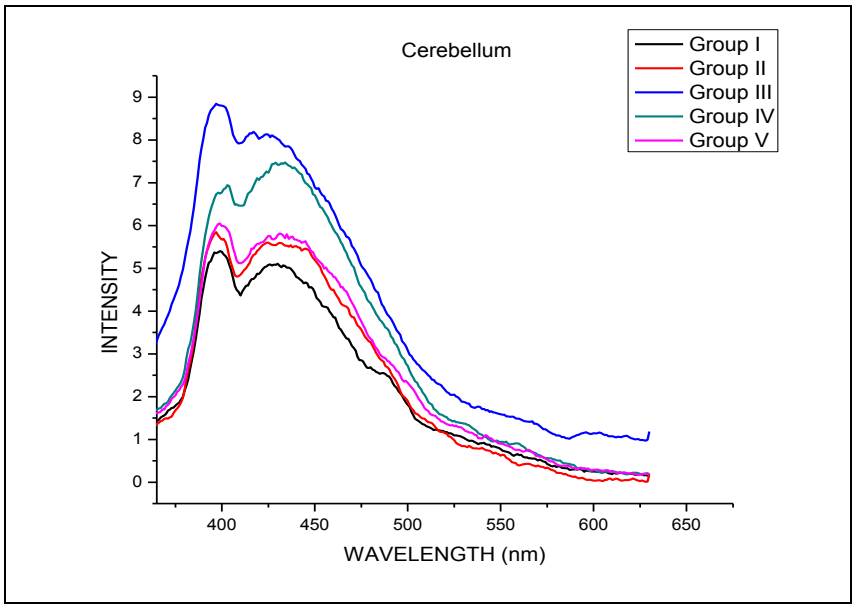


Fig No. 30 Fluorescence spectra of cerebellum in adult, adult old, old, Bacoside A and D-galactose treated mice

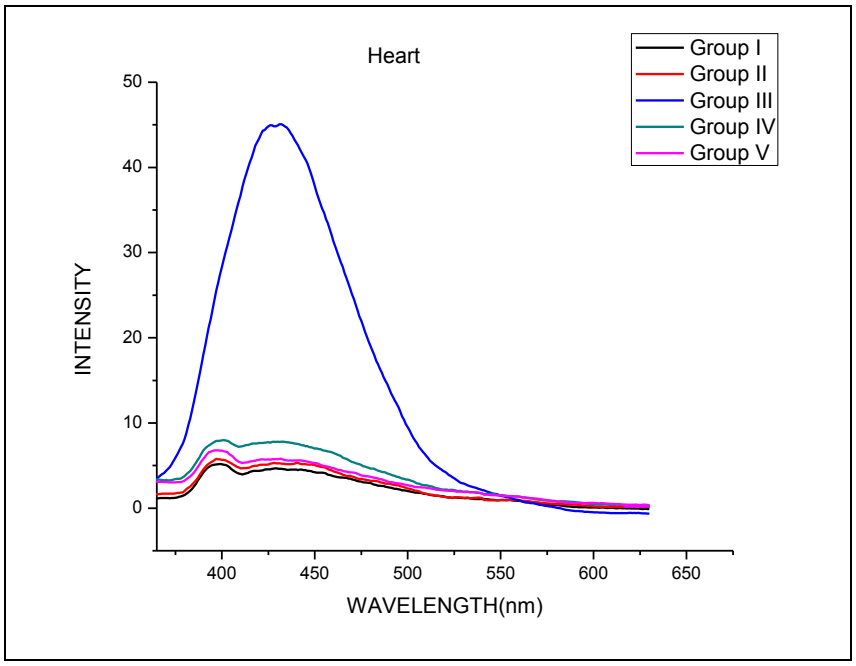


Fig No. 31 Fluorescence spectra of heart in adult, adult old, old, Bacoside A and D-galactose treated mice

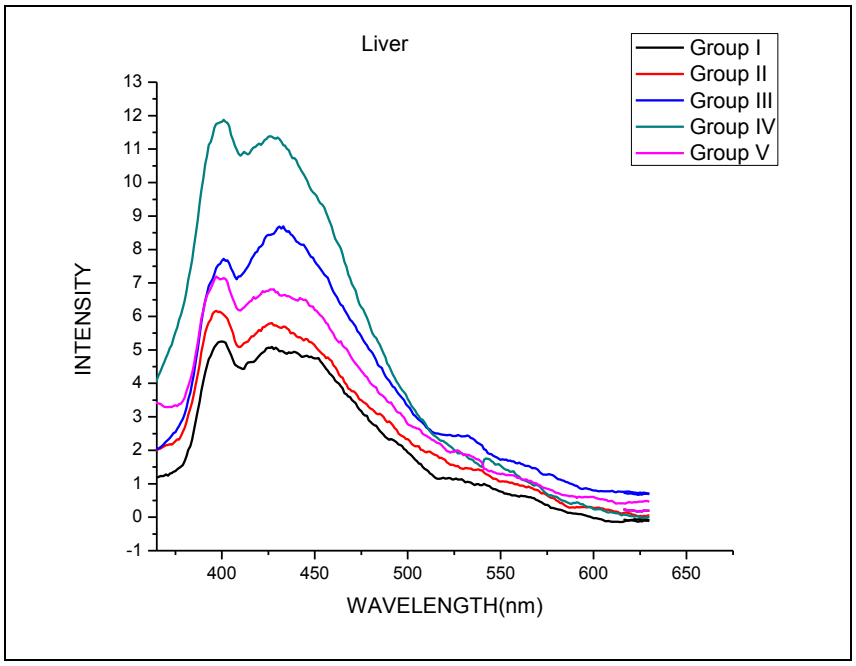


Fig No. 32 Fluorescence spectra of liver in adult, adult old, old, Bacoside A and D-galactose treated mice

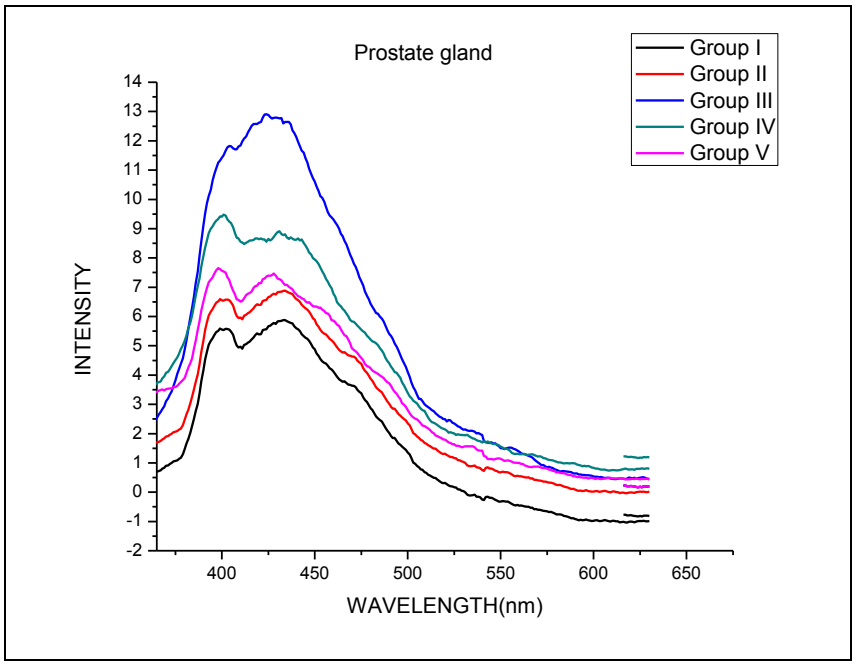


Fig No. 33 Fluorescence spectra of prostate gland in adult, adult old, old, Bacoside A and D-galactose treated mice

17. Estimation of antioxidant enzymes

Estimation of catalase, superoxide dismutase, glutathione peroxidase was carried out and activity was measured at 240nm, 560nm, 340nm respectively. Effect of Bacoside A on antioxidant enzyme activity in the brain, heart and liver was recorded in Fig. No. 34-36.

1. Catalase activity:

Statistically significant decrease in catalase activity was found in Group II and Group III and it was elevated in Bacoside A treated group. Maximum catalase activity was observed in brain. Group IV showed significantly high catalase activity in brain and heart than liver.

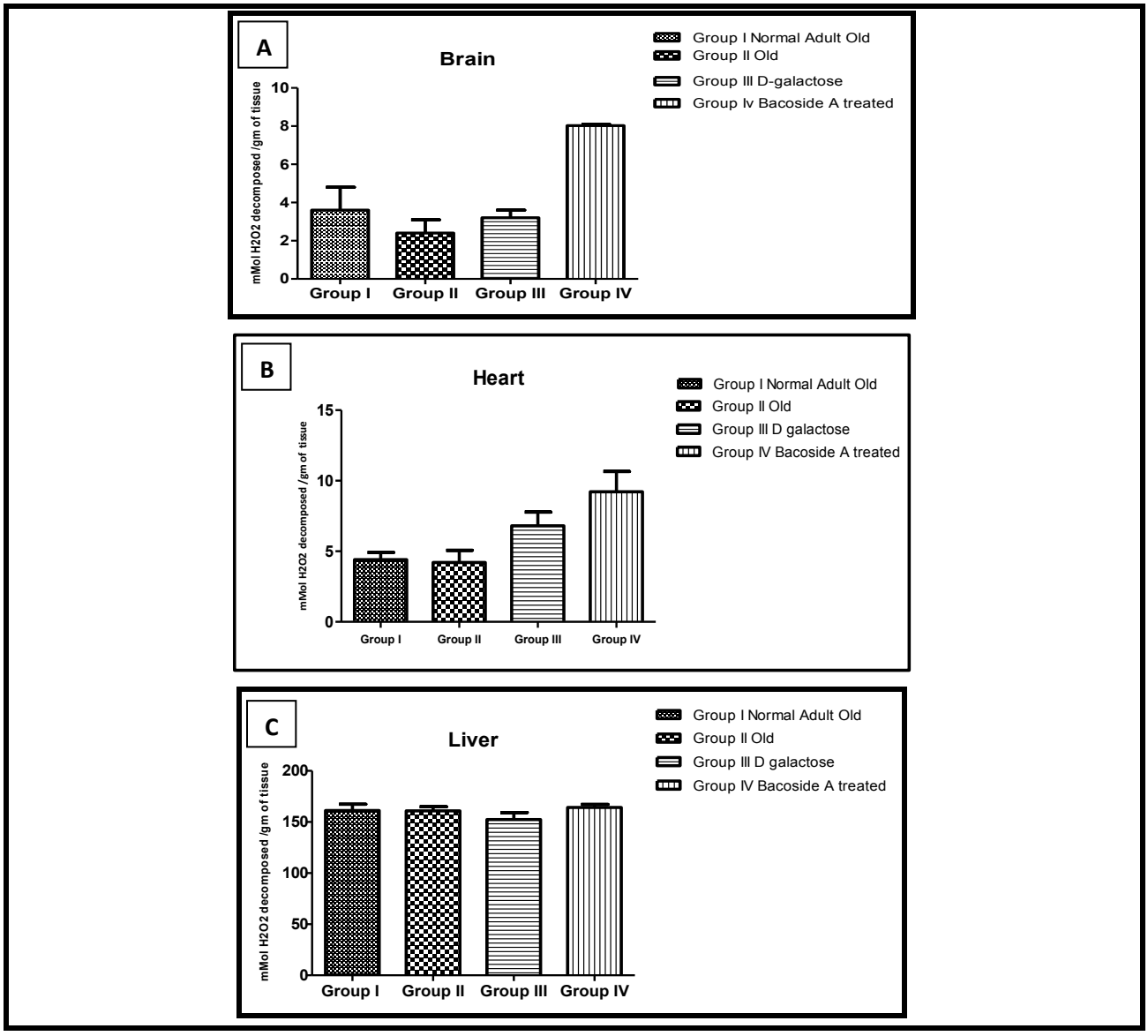


Fig. No. 34 Effect of Bacoside A on catalase activity in brain [A], heart [B], Liver [C]

2. Superoxide dismutase activity :

In the brain, heart and liver superoxide dismutase enzyme activity increased markedly in Group IV. Significant decrease in SOD activity was observed in old and D-galactose aging induced mice than normal adult old and Bacoside A treated mice. Maximum activity was observed in heart after treatment of Bacoside A.

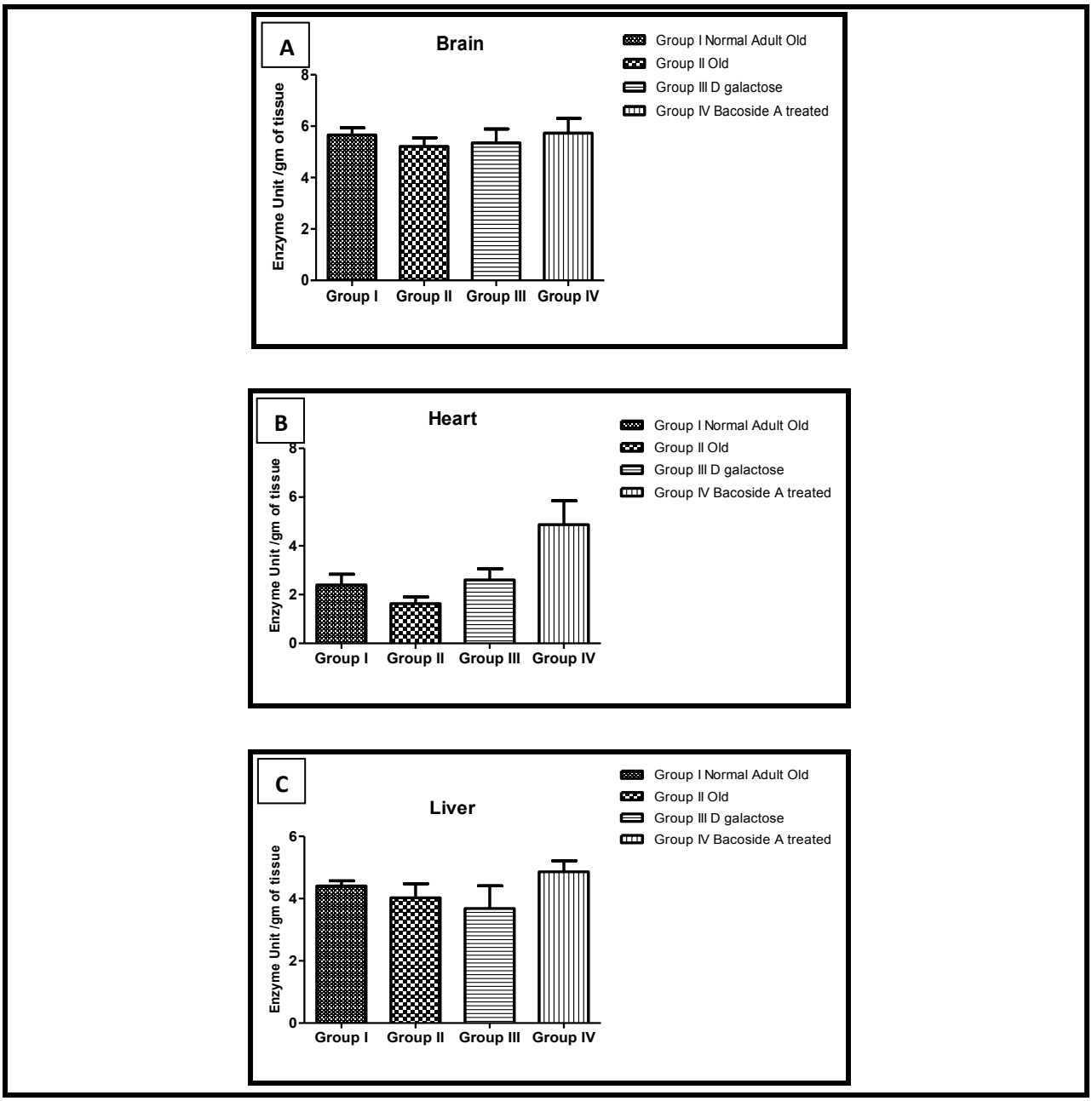


Fig. No. 35 Effect of Bacoside A on superoxide dismutase activity in brain [A], heart [B], Liver [C]

3. Glutathione peroxidase activity :

The GPx activity decreased significantly in Group II and Group III. The GPx activity in all organs was increased significantly in Group IV as compared to old and D-galactose aging induced group. GPx activity was maximum in liver in Group IV as compare to heart and brain. Bacoside A treated group showed the resemblance with normal adult old group.

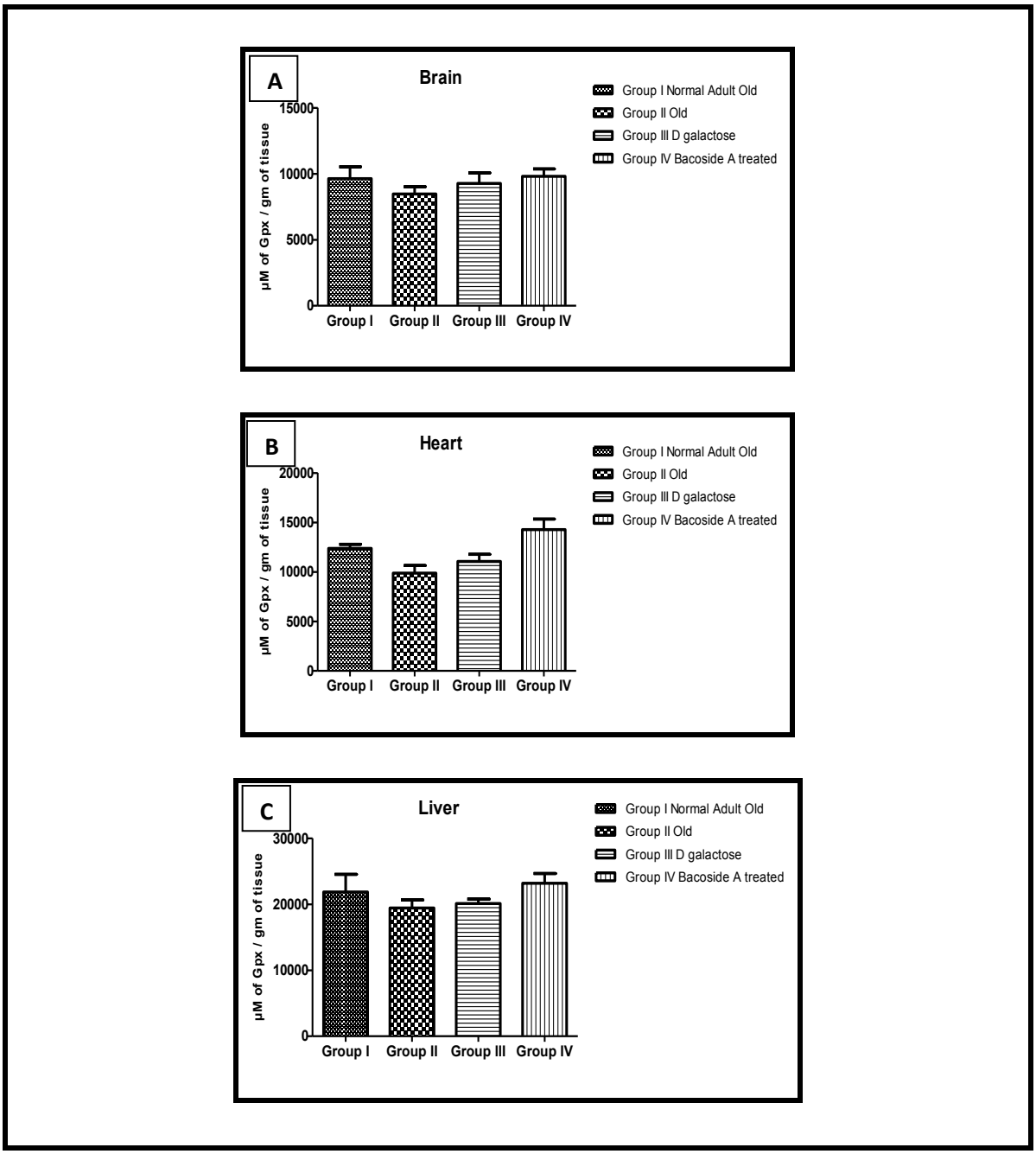


Fig. No. 36 Effect of Bacoside A on glutathione peroxidase activity in brain [A], heart [B], Liver [C]

18. Haematology study

The haematological study, lipid profile and estimation of serum B12 vitamin and Vitamin D was carried out. The statistical data obtained were analyzed using one way ANOVA, control vs other groups and results were expressed as mean ±SE.

The investigations carried out under haematological and lipid profile was observed in expected reference ranges. The PCV, Hb, RBC and other essential blood components were significantly increased in Bacoside A treated group.

19. Lipid profile

Bacoside A caused significant reduction in total cholesterol and LDL cholesterol in a dose dependant manner in treated group while in old and D-galactose induced group it was significantly increased.

20. Estimation of Serum B12 and Vitamin D

Serum Vitamin B12 and Vitamin D level was significantly high in Bacoside A treated group than old and D-galactose induced aging mice.

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iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons:

Yes, the progress has been according to original plan of work and towards achieving the objective.

iv. Please indicate the difficulties, if any, experienced in implementing the project:

No.

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet:

Major Research Project is completed in the given tenure of project.

vi. If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to University Grants Commission:

Summary of the findings of the study is attached in Annexure IX. One bound copy of the final report of work done is sent to University Grants Commission, New Delhi.

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as

a) **Manpower trained** : Project fellow appointed and trained. . Project fellow attended one day training program at ANCHROM Pvt. Ltd. Mumbai, Maharashtra (Skill- HPTLC) and workshop at BioEra, to learn various advanced techniques in biochemistry and cell biology.

b) **Ph. D. awarded:** Nil

c) **Publication of results :** Following 04 research papers were published in UGC approved, SCOPUS indexed, Peer reviewed International Journals.

Sr. No	Title of the Article/ Paper published	Authors	Title of Journal	Volume Pages ,Year	ISSN/ ISBN No.	Impact Factor
1	Determination and quantification of Bacoside A from *Bacopa monnieri* (L) by High Performance Thin Layer Chromatography.	**S.S. Pawar,** M.G. Jadhav	International Journal of Pharmacognosy and Phytochemical Research (International)	Vol. 7 (5) pp. 1060-65. 2015	0975-4873	1.846
2	Determination of Extractive value and Phytochemical analysis of *Bacopa monnieri* (L).	**S.S. Pawar,** M.G. Jadhav	Proceeding of State level seminar on Recent Advances in Life Sciences.	pp 71-76. 2015	978-93-84916-59-6	Nil
3	Study of Phytochemical Screening, Physicochemical Analysis and Antimicrobial Activity of *Bacopa monnieri* (L) Extracts	**S.S. Pawar,** M.G. Jadhav, T. G. Deokar	International Journal of Pharmaceutical and Clinical Research	Volume 8 Issue 8 pp 1222-1229 August 2016	ISSN- 0975 1556	1.668
4	Effect of Bacoside A on lipid peroxidation in D- galactose induced aging mice	**S.S. Pawar,** M.G. Jadhav	International Journal of Pharmacy and Pharmaceutical Sciences	Volume 9, Issue 9, Pp 12-15, Sept 2017	0975-1491	0.54

(c) other impact, if any

Following 09 research papers were presented at the National International Conferences/ Workshop / Seminars.

Sr. No.	Title of the Paper presented	Name of the Conference / Seminar/ Workshop/ Symposia	Organized by	Date
1	Determination of Extractive value and Phytochemical analysis of *Bacopa monnieri* (L) **(Bagged 1st PRIZE)**	State level seminar on Recent Advances in Life Sciences	Jointly organized Savitribai Phule Pune University, Pune and Department of Zoology, Bharatiya Jain Sanghatana's Arts, Science and Commerce College, Wagholi, Pune	4-5th Feb 2015
2	Determination of elements in *Bacopa monnieri* (L) by ICP-MS technique.	International Conference on Validation of Medicinal Plants and Traditional Medicines – Global Perspectives	Department of Pharmaceutical Sciences, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur, Maharashtra, India.	20th – 22nd Feb 2015
3	Preliminary Physicochemical Evaluation of Ethnomedicinal Herb : *Bacopa monnieri* (L).	International Conference on Advances in Asian Medicine Symposium on Ethno pharmacology – Validation of Traditional Medicine	Bharati Vidyapeeth University –Poona College of Pharmacy In association with Society for Ethnopharmacology, India (SFE -India) Affiliated to International Society for Ethnopharmacology, UK	4th January 2016
4	Characterization of *Bacopa monnieri* (L) by using FESEM and FTIR spectroscopy.	International Conference on Functional Eco-friendly Smart Emerging Materials (FESEM)	Pune District Education Association's Baburaoji Gholap College, Sangavi, Pune. Maharashtra. YASHADA Pune.	10th -12th March 2016

5	Quantitative determination of elements in *Bacopa monnieri* (L) extracts by ICP- MS technique	6th International Science Congress (ISC-2016) Theme : Digitization in Research for cultural, commercial and scientific development	International Science Community Association Under the association of Hutatma Rajguru Mahavidyalaya, Pune, Maharashtra, India	8th - 9th December 2016
6	Quantitative Estimation of Phenolic and Flavonoid Contents in *Bacopa monnieri* L.	National Seminar Environmental Issues And Biodiversity	Lal Bahadur Shastri College of Arts, Science and Commerce, Satara, Maharashtra	24th - 25th January 2017
7	Effect of Bacoside A on Lipid Peroxidation in D-Galactose Induced Aging Mice	INNOPHARM2 2nd International Conference on Bridging Innovations in Pharmaceutical, Medical and Biosciences	Innovare Academic Sciences, Bhopal, MP, India	11th -12th February 2017
8	Effect of Bacoside A on fluorescence product in different organs of mouse during induced aging **(Got 2nd PRIZE)**	International Conference on Frontiers in Life Sciences and Earth Sciences	Prof. Ramkrishna More Arts, Commerce and Science College, Akurdi Pune. India	18th -19th January 2018
9	Effect of Bacoside A on Non-specific esterase activity during induced aging in *Mus musculus*	International Conference on Recent Trends in Life Sciences	Modern College of Arts, Science and Commerce. Ganeshkhind, Pune MH, India	2nd-3rd February 2018

Dr. Mrs. Pawar S. S.
Principal Investigator

Head of the Dept.
Dr. Mrs. S. S. Pawar
Department of Zoology
Bharati Vidyapeeth University
Yashwantrao Mohite College,
Pune - 411 038.

Dr. S. R. Patil
(Dr. S. R. Patil)
Incharge Principal
Yashwantrao Mohite College, Pune-38

Annexure – IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI- 110002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. **TITLE OF THE PROJECT:**
   "Effect of Bacoside A on various Organs of mouse During Aging."

2. **NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR :**
   Dr. Mrs. Sushama Sunil Pawar,
   Flat No. 303,Shivraj Residency,
   Bharat Colony, Behind Kakade City,
   Karve Nagar, Pune-52.

3. **NAME AND ADDRESS OF THE INSTITUTION :**
   Dr. Mrs. Sushama Sunil Pawar,
   Yashwantrao Mohite College of Arts, Science and Commerce,
   Bharati Vidyapeeth (Deemed To Be University),
   Erandwane, Pune -411038. Maharashtra, India.

4. **UGC APPROVAL LETTER NO. AND DATE :**
   UGC File No. 42-533/2013(SR) 22nd March 2013

5. **DATE OF IMPLEMENTATION :** 2nd May 2013

6. **TENURE OF THE PROJECT :** 2nd May 2013 to 30th April 2017

7. **TOTAL GRANT ALLOCATED :** 12,80,800/-

8. **TOTAL GRANT RECEIVED :** 1st Installment Rs. 8,69,300.00 +
   2nd Installment Rs. 3,14,336.00 +
   HRA of fellow Rs. 92,067.00
   Total Amount Rs =12,75,703/-

9. **FINAL EXPENDITURE :** 14,38,952/-

10. **TITLE OF THE PROJECT :** "Effect of Bacoside A on various Organs of mouse During Aging."

11. OBJECTIVES OF THE PROJECT :

a. To separate Bacoside A from *Bacopa monnieri* L plant extract.
b. Study of distribution and chemistry of lipofuscin granules.
c. Estimation of lipid peroxidation.
d. Estimation of Lactate dehydrogenase enzymes.
e. Estimation of lysosomal enzymes.
f. Kinetic study of acid phosphatase.
g. Study of Antioxidant enzymes.
h. Estimation, isolation and separation of proteins.
i. To promote the research in this field.
j. To translate the research in practical utility for the welfare of the society.

12. WHETHER OBJECTIVES WERE ACHIEVED :

Yes, the objectives of the project were achieved totally. As per the objectives all work was successfully completed. Some extra work was also been carried out as an interdisciplinary approach. Preliminary physicochemical, phytochemical, antimicrobial, activity, quantification and characterization of *Bacopa monnieri* L were done. Blood related parameters in all groups were conducted and results were recorded.

13. ACHIEVEMENTS FROM THE PROJECT :

04 research papers were published in reputed UGC approved, SCOPUS indexed, Peer reviewed International Journals and one research paper is in the process of publication. **09** research papers were presented in National and International conferences, seminars and workshops. Got Best Poster Presentation award and Prize (Momentum) for two research papers presented in the conferences. Got an opportunity to understand and carry advanced research and to find out recent aspects related to the project. Principal Investigator has got an opportunity to attend National and International seminars and conferences and also chaired as a chair person in the National conference. Project fellow attended one day training program at ANCHROM Pvt. Ltd. Mumbai, Maharashtra (Skill- HPTLC) and workshop at BioEra, to learn various advanced techniques in biochemistry and cell biology.

14. SUMMARY OF THE FINDINGS :

Authentication of *Bacopa monnieri* L. plant was obtained from Botanical Survey of India, Pune. Maharashtra. Permission for access to Biological resources and associated traditional knowledge for purely research and academic purpose has been obtained after perseverance from, The Member Secretary, Maharashtra State Biodiversity Board, Nagpur (Government of Maharashtra). The project has been approved by the IAEC of Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandawane, Pune–411038, India. (Approved proposal number: CPCSEA/PCL/23/2014-15; CPCSEA/PCL/24/2014-15). Approval (dated 31st Dec 2014) was taken from Institutional Ethics Committee (IEC), Yashwantrao Mohite College of Arts, Science and Commerce, Pune. Preliminary physicochemical analysis of *Bacopa monnieri* L. was carried out for Ash value, loss on drying and extractive value using different solvents to determine best solvent for extraction. From which methanol solvent was selected for the extraction of *Bacopa monnieri* L. Phytochemical screening of *Bacopa monnieri* L. revealed the presence of Saponins, Flavonoids, Alkaloids, Tannins, Carbohydrates, Amino acids, Proteins and Steroids which may be responsible for the medicinal property of *Bacopa monnieri* L.. Total Phenolic and flavonoid contents in various extracts were determined spectrophotometrically, methanolic extract showed the significant concentration of total phenols and flavonoids which may be useful to evaluate the biological activity of *Bacopa monnieri* L. Characterization of the *Bacopa monnieri* L. was carried out as an extra work in which essential macro, micro and trace elements were detected using AAS, ICP-MS, FESEM and EDAX technique. The antioxidant enzymes require micronutrients cofactors such as selenium, iron, copper, zinc and manganese for their activity. It has been suggested that inadequate dietary intake of these trace minerals may also lead to low antioxidant activity. In *Bacopa monnieri* L. all these minerals were observed which may helpful to prove antioxidant property of Bacoside A by increasing antioxidant enzymes after treatment of Bacoside A. FTIR analysis was used to detect the functional groups present in the *Bacopa monnieri* L. The observed peaks from FTIR analysis were more characteristic of flavonoids and tannins. As the result of all

above parameters methanolic extract was observed as an eminent solvent therefore used for final extraction. This extract was used for the determination and quantification of Bacoside A. New method was developed for the determination and quantification of Bacoside A by using HPTLC. *Bacopa monnieri* L. was investigated for its antimicrobial activity in aqueous and methanolic extracts in order to use it as a possible source for new antimicrobial substances against human pathogens. Methanolic extract showed significant antifungal activity and maximum zone of inhibition against *Candida albicans* and *Aspergillus niger*, which reveals that *Bacopa monnieri* L. may help in curing skin diseases and fungal diseases. Various organs like brain, heart, liver, prostate gland and muscle from the maintained animals were dissected out to carry the biochemical study. Significant increase in protein was observed in Bacoside A treated group than all other groups. It showed that Bacoside A effectively protects proteins in all organs against oxidative damage during induced aging. Lysosomal enzymes like acid phosphatase and non specific esterase were estimated, highly significant increase in the level of these enzyme activity clearly indicate that Bacoside A increases the lysosomal membrane integrity and decreases the membrane leakage. In the present study induced aging affects on the level of LDH enzyme as it was decreased in old, D-galactose age induced mice and significant increase in LDH enzyme was observed in Bacoside A treated mice. An explosive release of energy for muscular contraction even in the absence of oxygen is achieved due to the lactate dehydrogenase. This is precisely the reaction that occurs in liver, heart tissues and in skeletal muscle during periods of low availability of oxygen. Thus in the present study, those animals treated with Bacoside A showed positive effect and have potential to reduce oxidation which may prevent the formation of free radicals. Administration of Bacoside A significantly elevated the level of antioxidant enzymes in all organs. Excessive production of reactive oxygen species and reduced antioxidant defense with age significantly contribute to aging. In the present study the reversal of altered antioxidant enzymes status and peroxidative damage in various organs by Bacoside A proved its antioxidant and antiperoxidative property and hence reveals its potential to play a crucial role in defense against free radicals and aging. Antioxidants are capable of stabilizing or deactivating free radicals before they attack cells.

Developing natural and pharmacological agents capable of increasing the antioxidative protection and modulating the endogenous defense and repair mechanisms may potentially improve health, increase longevity and contribute to treatment of degenerative age related diseases, such as cardiovascular and neurodegenerative disorders and cancer. Strengthening the antioxidants system may be able to extend the healthy lifespan. Results suggest that Bacoside A prevent the formation of malondialdehyde and lipofuscin granules in the form of fluorescence products which are indicators of aging. Bacoside A was able to attenuate the D-galactose aging induced increased lipid peroxidation and fluorescence products. Tissue damage may be prevented by the supplementation of Bacoside A, which may implies beneficial effects of *Bacopa monnieri* L. against aging. The protective effect of Bacoside A can be correlated directly with its ability to reduce the fluorescence product. Hydroxyl radical can damage virtually all types of macromolecules like carbohydrates, proteins, lipids. After treatment of Bacoside A the hydroxyl radical scavenging activity was significantly increased. It shows protective and antioxidant nature of Bacoside A. Kinetic study of enzymes was carried out to find out whether the affinity of the enzyme was substrate concentration dependant, Acid phosphatase have gained importance as clinical diagnostical tools in the detection of gynecological conditions, metastasizing prostate cancer, bone conditions including rheumatic osteoblastoma, bone cancer etc. The increase in enzyme activity with increasing substrate concentration and get stable in some extent suggested positive cooperation interaction. Thus kinetics of Acid phoaphatase enzyme can hydrolyze the substrate with lesser efficacy if substrate concentration is low and can act more efficiently if the substrate level is sufficient. Our results showed that pH and substrate concentration affect the activity of Acid phasphatase. The kinetic constants can help to explain how enzymes work and assist in the prediction of the behavior of enzymes in living organisms. V_{max} and K_m both play a key role in understanding the metabolism of the human body. Knowledge of the enzyme kinetics allows us to gain a better understanding of the enzymes and processes that take place in human metabolism. This knowledge could then be used for medical purposes to improve elderly patient health outcomes. Electrophoretic separation (PAGE) of protein and lactate dehydrogenase enzyme

revealed the highly intense bands in Bacoside A treated group which supports the biochemical study. In addition haematology, lipid profile and vitamin analysis in blood serum of all groups were carried out. Thus setting control on low-density lipoprotein and cholesterol levels is important point for normal functioning of body. The life style changes like regular physical activity, increased intake of fruits, vegetables and reduced calorie intake may improve health and increase cellular resistance to stress. Natural antioxidant supplements may help to correct the high levels of oxidative stress that cannot be controlled by the synergy of endogenous antioxidant system. The findings of this study suggest that Bacoside A, bioconstituent of *Bacopa monnieri* L. can be used as a safe, cheap and effective alternative chemo preventive and protective agent for the management of early aging disorders. Such research may also assist in our understanding of how to improve sound health in elderly. In all objectives were achieved successfully and we got promising results, which will be published in upcoming years.

15. CONTRIBUTION TO THE SOCIETY :

The stressful lifestyle, adverse environmental impacts and disturbed routine life, provokes the physiological changes and mental disorders in human being. The process of growing old begins at conception and ends with death. The process of aging is associated with several disorders. The challenge of a larger proportion of older than of younger people in the population demands that societies reorient themselves toward the care of a large, dependant population at the end of life rather than at the beginning. There is continuous progress in advances in medical knowledge and practices but the general population depends on the easily available medicinal plants for their primary health care needs. Plant based drugs remain an important source of therapeutic agents because of the availability, relatively cheaper cost and non toxic nature. From our findings study of brain, heart, muscle, liver, prostate gland at cellular and enzyme level showed satisfactory improvement after treatment of Bacoside A. Project helps the budding scientists to carry further research on phytochemicals and aging to promote healthy aging. Bacoside A can be used in the herbal formulations, as an effective bioconstituent which may help to reduce the problems in elderly in some extent.

Bacoside A may increase immunity, prevents aging disorders and chronic diseases. Cultivation of *Bacopa monnieri* L is easy and cheaper as a medicinal plant. One can easily provide it as a raw material for herbal formulation. One can easily cultivate *Bacopa monnieri* L in the house garden and may include in the diet as supplement in small quantity to overcome the problems arise during aging.

16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT:

Nil

17. NO. OF PUBLICATIONS OUT OF THE PROJECT :

a) Publications

Sr. No	Title of the Article/ Paper published	Authors	Title of Journal	Volume Pages ,Year	ISSN/ ISBN No.	Impact Factor
1	Determination and quantification of Bacoside A from *Bacopa monnieri* (L) by High Performance Thin Layer Chromatography.	**S.S. Pawar,** M.G. Jadhav	International Journal of Pharmacognosy and Phytochemical Research (International)	VoL 7 (5) pp. 1060-65. 2015	0975-4873	1.846
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3	Study of Phytochemical Screening, Physicochemical Analysis and Antimicrobial Activity of *Bacopa monnieri* (L) Extracts	**S.S. Pawar,** M.G. Jadhav, T. G. Deokar	International Journal of Pharmaceutical and Clinical Research	Volume 8 Issue 8 pp 1222-1229 August 2016	ISSN- 0975 1556	1.668
4	Effect of Bacoside A on lipid peroxidation in D- galactose induced aging mice	**S.S. Pawar,** M.G. Jadhav	International Journal of Pharmacy and Pharmaceutical Sciences	Volume 9, Issue 9, Pp 12-15, Sept 2017	0975-1491	0.54

b) Papers Presented at National-International Conferences/ Seminars

Sr. No.	Title of the Paper presented	Name of the Conference / Seminar/ Workshop/ Symposia	Organized by	Date
1	Determination of Extractive value and Phytochemical analysis of *Bacopa monnieri* (L) **(Got 1st PRIZE)**	State level seminar on Recent Advances in Life Sciences	Jointly organized Savitribai Phule Pune University, Pune and Department of Zoology, Bharatiya Jain Sanghatana's Arts, Science and Commerce College, Wagholi, Pune	4-5th Feb 2015
2	Determination of elements in *Bacopa monnieri* (L) by ICP-MS technique.	International Conference on Validation of Medicinal Plants and Traditional Medicines – Global Perspectives	Department of Pharmaceutical Sciences, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur, Maharashtra, India.	20th – 22nd Feb 2015
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5	Quantitative determination of elements in *Bacopa monnieri* (L) extracts by ICP- MS technique	6th International Science Congress (ISC-2016) Theme :Digitization in Research for cultural, commercial and scientific development	International Science Community Association Under the association of Hutatma Rajguru Mahavidyalaya, Pune, Maharashtra, India	8th - 9th December 2016
6	Quantitative Estimation of Phenolic and Flavonoid Contents in *Bacopa monnieri* L	National Seminar Environmental Issues And Biodiversity	Lal Bahadur Shastri College of Arts, Science and Commerce, Satara, Maharashtra	24th - 25th January 2017
7	Effect of Bacoside A on Lipid Peroxidation in D-Galactose Induced Aging Mice	INNOPHARM2 2nd International Conference on Bridging Innovations in	Innovare Academic Sciences, Bhopal, MP, India	11th -12th February 2017

		Pharmaceutical, Medical and Biosciences		
8	Effect of Bacoside A on fluorescence product in different organs of mouse during induced aging **(Got 2nd PRIZE)**	International Conference on Frontiers in Life Sciences and Earth Sciences	Prof. Ramkrishna More Arts, Commerce and Science College, Akurdi Pune. India	18th -19th January 2018
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Dr. Mrs. Pawar S. S.
Principal Investigator

Dr. S. R. Patil

(Dr. S. R. Patil)
Incharge Principal
Yashwantrao Mohite College, Pune-38

State Level Seminar on "Recent Advances in Life Sciences" ISBN: 978-93-84916-59-6

Determination of Extractive value and Phytochemical analysis of *Bacopa monnieri* (L)

Pawar S.S. [*1], Jadhav M.G.[2],
[* 1,2] Department of Zoology,
Yashwantrao Mohite College, Erandwane, Pune.
Bharati Vidyapeeth Deemed University, Pune (India) – 411 038.
zooymc@gmail.com [*1], mgjvvk11@yahoo.com[2]

Abstract

Bacopa monnieri (L) popularly known as Brahmi is an important nervine herb in an ayurvedic medicine, belongs to the family Scrophulariaceae. It is used to treat several diseases like nervous disorder, leprosy, respiratory problem, insomnia etc. It also shows antioxidant, antiaging, antibacterial, antidepressant, anticancer activity.The present study was carried out to determine extractive values and to investigate the phytochemical constituents of *Bacopa monnieri* (L). The methanolic extractive value (10.1%) was found more followed by ethanol (8.6%), aqueous (7.6%), chloroform (2%), acetone (1.5%) and petroleum ether (0.5%) of *Bacopa monnieri* (L). Phytochemical investigation of *Bacopa monnieri* (L) revealed the presence of various important secondary metabolites such as carbohydrates, proteins, amino acids, steroid, glycosides, flavonoids, alkaloids and tannins in methanolic, ethanolic and aqueous extracts.

Keywords **:** *Bacopa monnieri* (L), phytochemical constituents, extractive values, secondary metabolite.

Introduction

According to World Health Organization (WHO) majority of the world's population use traditional medicines for their primary health care needs. Medicinal plants are the most important resource of life saving drugs. Plant secondary metabolites possesses biological properties such as antioxidant, antiapoptosis, antiaging, anticarcinogen, antiinflammatory, antiatherosclerosis, cardiovascular protection, inhibition of angiogenesis and cell proliferation activity (Han X. et al. 2007).

Bacopa monnieri (L) belongs to the family Scrophulariaceae, is a creeping, glabrous, succulent herb grows in marshy areas throughout India. It has been traditionally used to treat anxiety, anger, nerve pain, insomnia, learning problems and concentration difficulties (Mukherjee D. G. et al. 1966). It has been reported that the plant is used in the treatment of epilepsy and asthma (Chopra R. N. 1958). The plant has also been used as a cardio tonic, digestive aid and to improve respiratory function (Nadkarni K. M. 1988). Natural antioxidants such as flavonoids, tannins and phenols are increasingly attracting because they are disease preventing, health promoting and antiaging substances (Farnsworth N. R. 1991). Tripathi Y. B. et al. (1996) reported that antioxidant properties of *Bacopa monnieri* (L) offer the protection from free radical damage in cardiovascular diseases, certain types of cancer and helps to prevent induced lipid peroxidation Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening is an important tool in bioactive compound analysis (Sasidharan et al. 2011). Therefore the objective of the present study was to determine the effective solvent for extraction of *Bacopa monnieri* (L) using water, methanol, ethanol, acetone, chloroform and petroleum ether. and to study the phytochemical screening in methanolic, ethanolic and aqueous extracts of *Bacopa monnieri* (L).

Materials and Methods

Plant material

Plant material of *Bacopa monnieri* (L) was obtained from Sunrise Agro Services, Pune. The whole plant material was shade dried at room temperature and kept in oven for 40ºC to remove moisture. The dried plant was then finely grinded by mechanical grinder mannually. The powder

obtained was then sieved and kept in air tight containers. The herbarium of *Bacopa monnieri* (L) was prepared and authentication has been obtained from Scientist D and HOD, Botanical survey of India, Pune, Maharashtra. The specimen (MGJ - 1) was kept to herbarium department in Botanical Survey of India,Pune.

Determination of Extractive value of *Bacopa monnieri* (L) (Khandelwal K.R. 2002)

The dry powdered plant material of *Bacopa monnieri* (L) was extracted with water, methanol, ethanol, acetone, chloroform and petroleum ether using a maceration process. 2 gm of the coarsely powdered plant material was weighed in a weighing bottle and transferred into a dry 250 ml conical flask. Then the flask was filled with different solvents (30 ml) separately. The flasks were corked and kept aside for 24 hrs at room temperature, shaking frequently. The mixtures were filtered through Whatmann No. 1 filter paper into a 50 ml measuring cylinder. After the filtrate has obtained, it was then transferred into a weighed petry plates. The obtained extracts were concentrated to dryness by keeping filtrate for complete evaporation of solvent.

The extractive value in percentage was calculated by using following formula and recorded.

$$\text{Extractive value (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100$$

Solvents	Weight of Plant material (g)	Colors of extract	Extractive value (%)
Aqueous (Water)	2	Dark brown	7.6
Methanol	2	Yellowish green	10.1
Ethanol	2	Green	8.6
Acetone	2	Green	1.5
Chloroform	2	Light green	2
Petroleum Ether	2	Colorless	0.5

Table 1. Extractive value (%) of *Bacopa monnieri* (L)

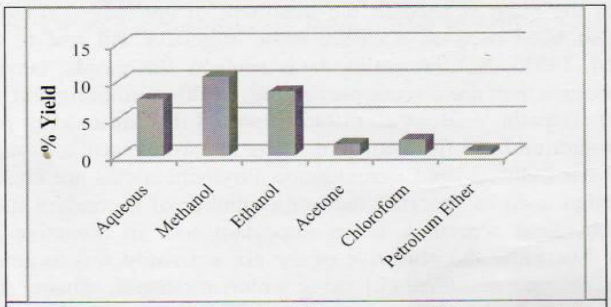
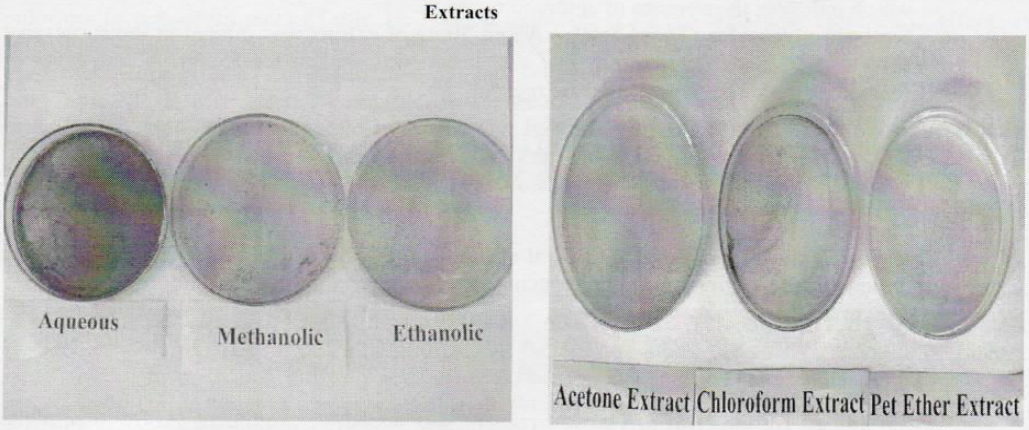


Fig No. 1 Extractive value (%) different extract of *Bacopa monnieri* (L)



Aqueous, methanolic, ethanolic, acetone, chloroform and petroleum ether extract of *Bacopa monnieri* (L)

Phytochemical screening of methanolic, ethanolic and aqueous extracts of *Bacopa monnieri* (L) (Khandelwal K.R. 2002)

The different phytochemical tests were performed for establishing the profile of plant extract for its phytochemical constituents. The phytochemical screening of *Bacopa monnieri* (L) for Carbohydrates, Proteins, Amino acids, Steroids, Glycosides, Cardiac glycosides, Anthraquinone glycosides, Saponin glycosides, Flavonoids, Alkaloids and Tannins was carried out. The extract obtained from the methanol, ethanol and aqueous was used for the phytochemical screening.

1. **Test for carbohydrates :**
   a. **Molisch's test** – 3ml of extract was taken in a test tube 2 drops of alcoholic alpha naphthol solution was added, shaked well and then 1ml of concentration sulfuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicated the presence of carohydrates.
2. **Test for proteins :**
   a. **Millon's test** – About 3ml of sample extract was treated with 5ml of Million's reagent. White pricipitate was obtained. The mixture was then warmed, pricipitate turned to brick red. It indicates the presence of proteins.
3. **Test for amino acids :**
   a. **Ninhydrin test -** About 3ml of plant extract solution was heated followed by addition of 3 drps of 5% ninhydrin solution. The test tubes with this solution were kept in boiling waterbath for 10 minutes. The purple color was observed. It indicated the presence of amino acids.
4. **Test for steroids :**
   a. **Leibermann- Burchard reaction** – To 3ml extract 10ml chloroform was added followed by 2ml of acetic anhydride. Then 2 drops of conc. Sulfuric acid was added from the side of the test tube. The blue green color indicated the presence of steroids.
5. **Test for glycosides :**
   a. **Cardiac Glycosides (Legal's Test)** – To the 3ml extract 1ml pyridine was added by frequent shaking followed by 1ml sodium nitroprusside. Pink to red color appeared. It indicates the presence of cardiac glycosides.
   b. **Anthraquinone glycosides (Borntrager's test)** – To 3ml extract dil. Sulfuric acid was added. The solution was then filtered. Then equal volume of chloroform was added to the filtrate. After shaking organic solvent was separated. Finally equal volume of ammonia

solution was added. No bright pink, red or violet color was developed in the upper layer which indicates the absence of anthraquinones.

c. **Saponin glycosides (Foam test)** – About 50mg of extract was diluted in the successive solvents and made up to 20ml. The suspension was shaken for 15min. A 2cm layer of foam appeared. That is saponins are present.

6. **Test for flavonoids :**
   a. **Sodium hydroxide test** – To 3ml of extract increasing amount of sodium hydroxide was added it showed colouration, which was decolorised after addition of dil. hydrochloric acid.
7. **Test for alkaloids :**
   Solvent free extract was stirred with 10ml of dilute hydrochloric acid and filtered. The filtrate was tested with following alkaloidal reagents as follows :
   a. **Mayer's test –**
   To 3ml filtrate two drops of Mayer's reagent was added by the sides of the test tube. A white creamy precipitate was observed. It indicated positive test.
   b. **Hager's test –**
   To 3ml of filtrate, 1ml of Hager's reagent was added. A prominent yellow precipitate occurred. It indicated the presence of alkaloids.
   c. **Wagner's test –**
   To 3ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish brown precipitate confirmed the presence of alkaloids.
8. **Test for Tannins :**
   a. **Nitric acid test** – 3ml of extract was taken in test tube, a few drops of dilute nitric acid was added. The reddish yellow color indicated the presence of tannins.

Table No. 2. Phytochemical screening of aqueous, methanolic and ethanolic extracts of *Bacopa monnieri* (L)

Sr. No.	Secondary metabolites	Phytochemical tests	Methanol	Ethanol	Aqueous
1.	Carbohydrates	Molisch's Test	+	+	+
2.	Proteins	Millon's Reagent Test	+	+	+
3.	Amino acid	Ninhydrin Test	-	-	+
4.	Steriod	Liebermann Burchard Reaction	+	+	+
5.	Glycosides a)Cardiac glycosides	Legal's Test	+	+	+
	b)Anthraquinone glycosides	Borntrager's Test	–	–	–
	c)Saponin glycosides	Foam Test	+	+	+
6.	Flavonoids	Sodium hydroxide test	+	+	+
7.	Alkaloids	Mayer's Test	+	+	+
		Wagner's Test	+	+	+
		Hager's Test	+	+	+
8.	Tannins	Dilute Nitric acid Test	+	+	+

+ Present, - Absent.

Result

The extractive value and color of extracts of *Bacopa monnieri* (L) was investigated and represented in Table No. 1. From the present study it was found that, the extractive value of *Bacopa monnieri* (L) in methanolic extract was maximum (10.1%) as compare to other extracts. The ethanolic extract showed slightly lower extractive value (8.6%) than methanolic extract of *Bacopa monnieri* (L). The extractive value of *Bacopa monnieri* (L) in aqueous extract was 7.6% followed by chloroform extract (2%) and acetone extract (1.5%). The petroleum ether extract showed lower (0.5 %) extractive value than all other extracts. The color of extracts observed were yellowish green in

methanol, green in ethanol, dark brown in aqueous, light green in chloroform, green in acetone, and colorless in petroleum ether (Table 1). *Bacopa monnieri* (L) have beneficial therapeutic effects in traditional Indian system of medicine. Various phytochemicals that are present in it are responsible for these therapeutic effects. The quantitative phytochemical analysis of *Bacopa monnieri* (L) was carried out.(Table No. 2) It revealed the presence of saponins, flavonoids, alkaloids, tannins, carbohydrates, proteins and steroids in methanolic, ethanolic and aqueous extracts. Anthraquinone glycosides were absent in methanolic, aqueous and ethanolic extracts. The aqueous extract of *Bacopa monnieri* (L) showed the presence of amino acids and methanolic, ethanolic extracts showed absence of amino acids. (Table No. 2)

Discussion

Estimation of extractive value determines the amount of the active constituents in a given amount of plant material when extracted with solvent. Azad A. K. et al.(2012) observed the yield percentage of *Bacopa monnieri* (L) in alcoholic extract. In the present study the extractive value of *Bacopa monnieri* (L) in methanol, ethanol, aqueous, chloroform, acetone and petroleum ether extract was determined. The methanolic extractive value of *Bacopa monnieri* (L) was observed more (10.1%) than ethanolic (8.6%), aqueous (7.6%), chloroform (2%), acetone (1.5%) and petroleum ether extract (0.5%). Plant synthesises a broad range of primary and secondary metabolites with different functional groups (Sharanabasappa et al. 2007). Phytochemical screening is an important tool in bioactive compound analysis. It is quick, inexpensive and simple procedure that shows the various types of phytochemicals present in plant. The presence of phytochemicals is a marker that the plant can be a prospective source of precursors in the formation of synthetic drug. (Ayoola et al. 2008). Gupta A. (2013) reported that carbohydrates, phenols glycosides and anthraquinones were present in petroleum ether and ethanolic extract of *Bacopa monnieri* (L).

The in vitro phytochemical analysis of roots of *Bacopa monnieri* (L) was carried out, the study showed presence of alkaloids, anthraquinone, cardiac glycosides, flavanoids, phenols, saponin, steroids, tannins, terpenoids, alkaloids in ethanolic, methanolic, chloroform, petroleum ether and ethyl acetate extract (Bhoomi B. 2013). According to Subashri B. (2014), terpenoids and steroids were predominately found in ethanol, aqueous, chloroform, acetone and ethyl acetate extracts. Shah et al. 2012 observed the presence of phenols, flavanoids, glycosides, alkaloids and carbohydrates in aqueous and hydroalcoholic extracts of *Bacopa monnieri* (L). The phytochemical analysis of leaf callus of *Bacopa monnieri* (L) was carried out by Singh S. K. (2002), he observed the presence of tannins, saponins, terpenoids, steroids in ethanol and aqueous extract and absence of anthraquinone glycosides and phenols in same extract.

From the present study phytochemical screening revealed that saponins, flavonoids, alkaloids, tannins, carbohydrates, proteins and steroids were present in methanolic, aqueous and ethanolic extracts of *Bacopa monnieri* (L). The aqueous extract of *Bacopa monnieri* (L) showed the presence of amino acids and methanolic, ethanolic extracts showed absence of amino acids. Anthraquinone glycosides were absent in methanolic, aqueous and ethanolic extracts of *Bacopa monnieri* (L).

Conclusion

From the present study it can be concluded that the extractive value is useful to find the effective solvent for extraction process. It gives idea about the nature of the chemical constituents present in a plant material. Extractive value is also useful for the estimation of constituents extracted with the solvent used for the extraction. We found methanol is the best solvent for extraction process to yield more extract. The phytochemical screening is helpful for confirmation of bioactive phytochemical constituents in *Bacopa monnieri* (L).

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Review Article

Determination and Quantification of Bacoside A From *Bacopa monnieri* (L) By High Performance Thin Layer Chromatography

Pawar S.S.*, Jadhav M.G.

Bharati Vidyapeeth Deemed University, Yashwantrao Mohite College, Department of Zoology, Pune (India) – 411 038.

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ABSTRACT

Bacopa monnieri (L) has been used in Ayurvedic medicine as nervine tonic for promoting mental health and improving brain function. The plant has been reported to contain several phytoconstituents mainly flavonoids (luteolin and apigenin), betulic acid, stigmastarol, beta sistosterol, bacopasaponins and the minor components like bacopasaponin F, bacopasaponin E, bacopaside N1, bacopaside III, IV, V. Bacoside A is a major bacopasaponin constituent of *Bacopa monnieri* (L).

The aim of the present investigation was to develop a simple, sensitive and reproducible HPTLC method for the determination of Bacoside A from the methanolic extract of *Bacopa monnieri* (L) .

The HPTLC method developed for separation of Bacoside A by TLC on stationary phase i.e. Silica gel 60 F_{254} with a solvent system Toulene:Ethylacetate:Methanol:Glacial Acetic acid (3:4:3:1v/v) and detection of Bacoside A was carried out by scanning and quantifying the peak at 540 nm by winCATS Planar Chromatography Manager.

The calibration curve was linear in the range of 0.5 - 4 µg/spot with correlation coefficient 0.9977. The coefficient of variance was found to be 1.766. The proposed HPTLC method was found precise and can be used for monitoring, detection, identification and quantification of Bacoside A from the methanolic extract of *Bacopa monnieri* (L).

Keywords : *Bacopa monnieri* (L), Phytoconstituents, Bacoside A, HPTLC, TLC, Quantification

INTRODUCTION

Bacopa monnieri (L) belongs to the family Scrophularieaceae. It is a medicinal herb found throughout the Indian subcontinent in wet, damp and marshy area[1]. *Bacopa monnieri* (L) is used in the indigenous systems of medicine for the treatment of various nervous system ailments such as insomnia, anxiety, epilepsy, hysteria and in improving intellect, memory[2-4]. In addition to the memory boosting activity, it also claimed to be useful in the treatment of cardiac, respiratory, neuropharmacological disorder like insanity, depression, psychosis and stress[5,6]. It was reported that *Bacopa monnieri* (L) to posses anti inflammatory, analgesic, antipyretic, sedative, free radical scavenging, antioxidant and anti lipid peroxidative activities[7,8,9].

Triterpenoid saponins, the major components in Brahmi, were reported to be responsible for the cognitive enhancing activity of Brahmi[3,6]. The major bioactive dammarene type triterpenoid saponin isolated from the *Bacopa monnieri* (L)[10], that carries the neuropharmacological activities, is Bacoside A, which is a mixture of Bacoside A3, Bacoside II, Bacopasaponin C and an isomer of Bacopasaponin C[11]. Bacoside A is held in high repute as a potent nerve tonic[12]. Now a days standardization and quantification of medicinal plant extracts is essential for formulation[13]. As the number of aged people suffering from cognitive problems, the memory boosters have gained immense importance and there is an urgent need to develop sensitive and reliable quality control techniques to establish the authenticity and purity of memory boosting drugs[14]. The use of medicinal plants in both crude and prepared forms has increased substantially. Use of chromatography for standardization of plant products was introduced by the WHO and is accepted as a strategy for identification and evaluation of the quality of plant products[15-17].

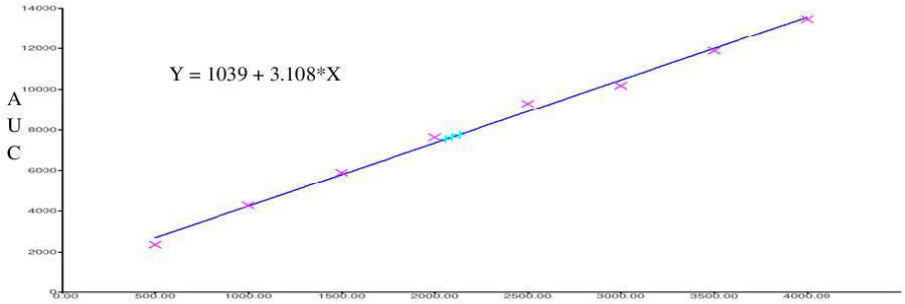
Methods described in the literature for analysis of bacosides are mainly based on UV Spectroscopy[4,18], Thin Layer Chromatography[19] and HPLC[11,20,21]. Therefore in the present study a suitable, sensitive and reliable quantitative High Performance Thin Layer Chromatography method has been developed for qualitative and quantitative estimation of the phytochemical marker namely Bacoside A from methanolic extract of *Bacopa monnieri* (L).

MATERIAL AND METHOD

Material

The herbarium of *Bacopa monnieri* (L) was prepared and authentication has been obtained from Scientist D and HOD, Botanical survey of India, Pune, Maharashtra. The specimen (MGJ-1) was kept to herbarium department in Botanical Survey of India, Pune. The whole plant material was shade dried at room temperature and kept in oven for 40ºC to remove moisture. The dried plant was then finely grinded by mechanical grinder manually. The powder obtained was then sieved and kept in air tight containers for further extraction process. The reference standard of Bacoside A (C_{41} H_{68} O_{13}) was purchased from M / S

*Author for Correspondence



Result via area

Substance	Rf	X(average)	CV [%]	n	Regression	Remark
Bacoside A	0.31	2.094 µg	1.766	3	Linear	

Figure 1: Linearity curve for standard Bacoside A.

Natural Remedies Pvt. Ltd. Banglore, India and used for quantification.

Method

Extraction of Bacopa monnieri (L)

The methanolic extract of *Bacopa monnieri* (L) was prepared by traditional maceration method for the determination and quantification of Bacoside A. *Bacopa monnieri* (L) dry plant powder was dissolved in methanol in the glass jar and it was wrapped by aluminium foil and covered well to avoid evaporation. Mixture was kept for 72 hrs at room temperature with occasional shaking. After completion of the maceration, the supernatant was decanted and the mixture was filtered through the muslin cloth. The extract was concentrated to dryness by keeping filtrate for complete evaporation of solvent. After evaporation of solvent the extract was weighed and kept in air tight glass container for further use.

Preparation of plant extract solution

The methanolic extract was taken and dissolved in methanol and sonicated for 15min. Then from this, stock solution was pipette out and methanol was added to make final dilution.

Preparation of standard solution

The reference standard stock solution of Bacoside A was prepared in methanol and sonicated for 15 min.

Chromatographic conditions

The following chromatographic conditions were used to quantify the Bacoside A :

Stationary phase : Silica gel 60 F_{254} (E. Merck) precoated TLC plates

Mobile phase : Toulene:Ethylacetate:Methanol:Glacial Acetic acid (3:4:3:1v/v)

Developing chamber – Twin trough glass chamber

Sample volume : 2µl

Mode of application : Band

Saturation time : 30min

Temperature : Ambient room temperature

Migration distance: 8mm

Detection wavelength: 540nm

Assay / development of chromatogram/ procedure

For determination of Bacoside A content in the methanolic extract of *Bacopa monnieri* (L), 2µl sample was used in High Performance Thin Layer Chromatography (HPTLC). Standard and sample solutions were applied to the Silica gel 60 F_{254} (E. Merck) precoated TLC plates as sharp bands by means of CAMAG Linomat V sample applicator. The spots were dried in a current of air. Chromatography was carried out in a glass chamber (CAMAG). The mobile phase Toulene : Ethylacetate:Methanol:Glacial Acetic acid (3:4:3:1v/v) was poured into a twin trough glass chamber whole assembly was left to equilibrate and for presaturation for 30 min. The plate was then developed until the solvent front had travelled at a distance of 80mm above the base of the plate, at 20ºC and 50% relative humidity. The plate was visualized by immersing it in vanillin–sulphuric acid-ethanol (1g:5ml:95ml) solution, using an automatic immersion device (CAMAG), followed by heating on CAMAG TLC plate heater for few min. For detection and quantification TLC spots corresponding to Bacoside A were scanned at 540nm using CAMAG TLC scanner. The percentage of Bacoside A present in *Bacopa monnieri* (L) extract was calculated by comparison of the areas measured for the sample and standard solution.

Linearity

Linearity was performed by applying solution at different concentrations ranging from 0.5 to 4 µg/spot on 20×10 cm HPTLC plates, precoated with Silica gel 60F_{254} (E. Merck) in the form of sharp 8mm bands. The distance between two adjacent bands was 12.3mm. The plates were developed in a solvent system of Toulene:Ethylacetate:Methanol:Glacial Acetic acid (3:4:3:1v/v), upto a distance of 80mm, at room temperature. The plates were dried in air.

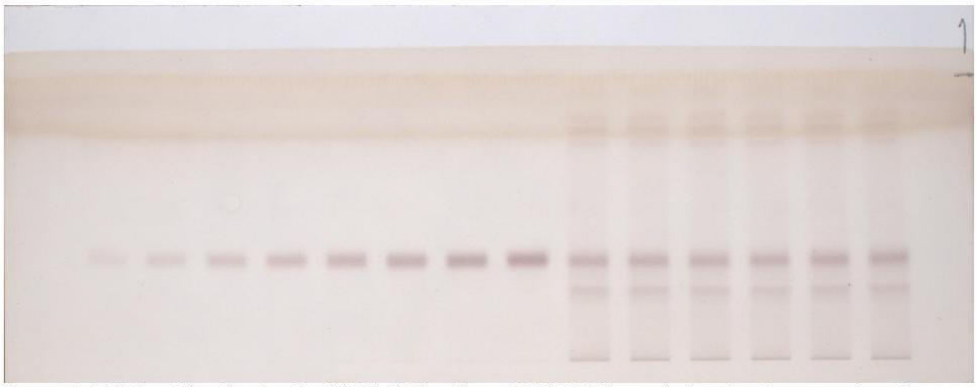
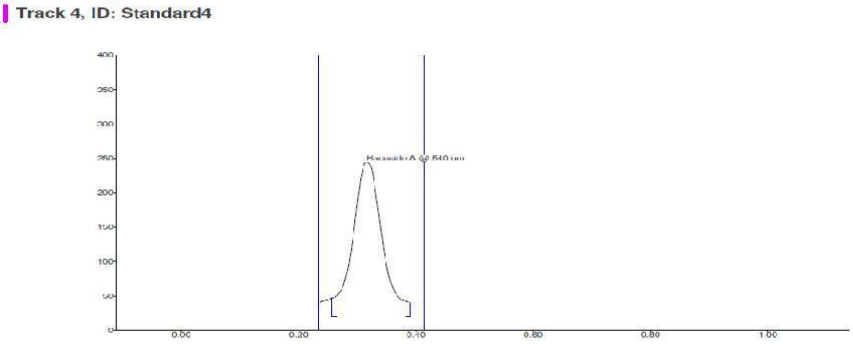


Figure 2: TLC Plate Visualized under CAMAG Visualizer : 150503 White remission showing separation of Bacoside A compound



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.26	6.6	0.32	204.3	100.00	0.39	0.5	7537.0	100.00	Bacoside A

Solvent system: Toulene:Ethylacetate:Methanol:Glacial Acetic acid (3:4:3:1v/v) Peak for Bacoside A

Figure 3: HPTLC Chromatogram of Standard Bacoside A

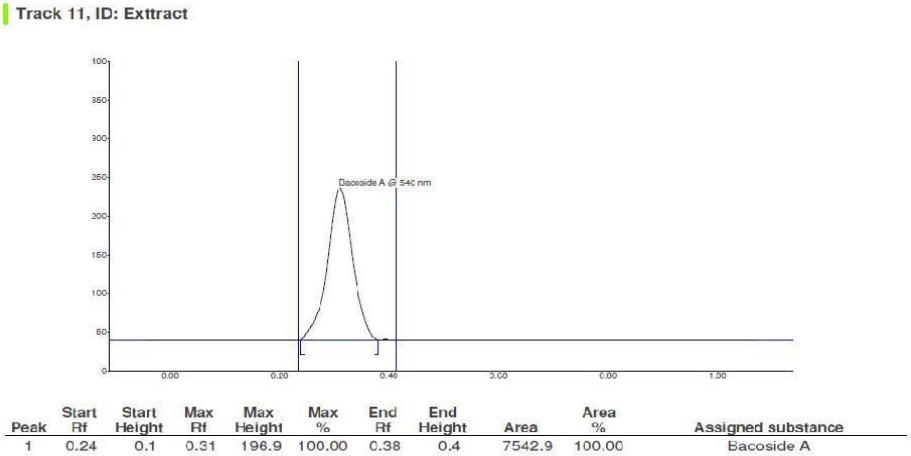
The detector response for Bacoside A was measured for each band at wavelength 540nm, using CAMAG TLC scanner and with WinCAT software. The peak areas of Bacoside A were recorded for each concentration. The linearity curve of Bacoside A was obtained by plotting a graph of peak area of Bacoside A υs applied concentration of Bacoside A.

RESULT

The method described utilizes Silica gel $60F_{254}$ HPTLC plates as stationary phase and Toulene : Ethylacetate: Methanol: Glacial Acetic acid (3:4:3:1v/v) as mobile phase which gives good separation of Bacoside A (R_f = 0.31). The calibration curve was linear in the range of 0.5 - 4 µg/spot as shown in fig 1. The correlation coefficient (r) was determined it was found to be 0.9977 indicating good linearity between concentration and peak area. Well defined bands were obtained which are shown fig 2. The percentage coefficient of variance (CV) for peak area was found to be 1.766. The HPTLC chromatograms of standard Bacoside A and methanolic extract of *Bacopa monnieri* (L) are presented in fig. 3 and fig. 4.

DISCUSSION

Sample preparation and development of suitable mobile phase or solvent system are two important stages in development of the analytical procedures, which becomes more significant for herbal drugs because of their complexity of the chemical compounds and their affinity towards different solvent systems. In the present study by using various mobile phase compositions, a better resolution of Bacoside A with symmetrical and reproducible peak was achieved with Toulene:Ethylacetate:Methanol:Glacial Acetic acid (3:4:3:1v/v). With the developed HPTLC method the R_f value of Bacoside A was found to be 0.31. Linearity range was found to be in the range of 0.5 - 4 µg/spot with a



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.24	0.1	0.31	196.9	100.00	0.38	0.4	7542.9	100.00	Bacoside A

Solvent system: Toulene:Ethylacetate:Methanol:Glacial Acetic acid (3:4:3:1v/v) Peak for Bacoside A

Figure 4: HPTLC Chromatogram of *Bacopa monnieri* methanolic extract

coefficient of variance of 1.766 indicating good linearity between concentration and peak area.

Several kinds of techniques were employed for plant analysis. Amit *et al*. (2012) developed the HPTLC method for the quantification of Bacoside A and fingerprinting of the in–house mother tincture (*Bacopa monnieri*) and marketed samples of homeopathic medicines in India by using Dichloromethane: Methanol : Water as a solvent system[22]. Rastogi *et al*. (1994) isolated and characterized bacoside A3 from *Bacopa monnieri* (L)[23]. Chakravarty *et al*. (2001) isolated two saponins, bacoside I and II from *Bacopa monnieri* (L) by 2D NMR technique[24]. Pal *et al.* (1998) performed quantitative analysis of bacoside by HPLC[25]. Shrikumar *et al*. (2003) determined and analysed the Bacoside A in the *Bacopa monnieri* (L) and its commercial monoherbal capsule formulation by HPTLC[26]. Deepak *et al.* (2005) performed quantitative analysis of Bacoside A from *Bacopa monnieri* (L) by HPLC[27]. Kawai *et al*. (1978) performed acid hydrolysis of Bcoside A from *Bacopa monnieri* (L) and obtained ebelin lactone and bacogenin A[28]. Agrawal *et al*. (2006) carried out separation of Bacoside A_3 and Bacopaside II which are the major triterpenoid saponins in *Bacopa monnieri* (L) by HPTLC and Super Critical Fluid Chromatography techniques[29]. Watoo *et al*. (2007) determined the saponin glycosides in *Bacopa monnieri* (L) by reversed phase High Performance Thin Layer Chromatography[30]. Five major saponins namely bacoside A3, bacopaside II, bacopasaponin C isomer, bacopasoponin C and bacopaside I in the extracts of *Bacopa monnieri* (L) were determined by using HPLC technique[31]. Shinde *et al*. (2011) developed the HPTLC method for the simultaneous determination of Withanolide A and Bacoside A in spansules from *Withania somnifera* and *Bacopa monnieri* (L)[32]. An HPLC – UV method was developed by Mishra *et al.* (2013) for the standardization of Brahmi Vati and simultaneous quantitative estimation of Bacoside A3 and Piperine which are the major constitutes of *Bacopa monnieri* (L) and *Piper longum* respectively[33].

CONCLUSION

According to Ayurvedic literature, *Bacopa monnieri* (L). is ethnically used in various diseases in humans and animals. Various separation techniques have been reported for separation and quantification of specific phytoconstituents in the medicinal plant. HPTLC is becoming a routine analytical technique because of its advantages of low operating cost, high sample throughput, simplicity and speed, the need for minimum sample clean up, reproducibility, accuracy, reliability and robustness. Multiple numbers of samples can be analyzed in a single run allows one to save time and thus cost of analysis and the sample preparation requirements are often minimal because the stationary phase is disposable. From the present study it can be concluded that, the developed HPTLC method is fast, precise and reliable and may be useful for quantitative monitoring of Bacoside A in methanolic extract of *Bacopa monnieri* (L).

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Research Article

Study of Phytochemical Screening, Physicochemical Analysis and Antimicrobial Activity of *Bacopa monnieri* (L) Extracts

Pawar S S*, Jadhav M G, Deokar T G

Department of Zoology, Bharati Vidyapeeth Deemed University Yashwantrao Mohite College of Arts, Science and Commerce, Pune (India) – 411 038.

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ABSTRACT

Bacopa monnieri (L) popularly known as Brahmi is an important nervine herb in an ayurvedic medicine. It belongs to the family Scrophulariaceae. It has been traditionally used as ethno medicine and is useful to treat anxiety, anger, insomnia, nerve pain, concentration difficulties and learning problems. It has also been used as a cardio tonic, digestive aid and improves respiratory function. It shows antioxidant, antiaging, antidepressant, anticancer and antibacterial activity. The present study was carried out to determine the phytochemical constituents and physicochemical values according to the pharmacopoeial method. The antimicrobial activity of *Bacopa monnieri* (L) was also investigated by using aqueous and methanolic extracts against two gram positive, two gram negative bacteria and two fungal organisms at 1.25 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml concentrations. The methanolic extractive value of *Bacopa monnieri* (L) (10.1%) was found highest followed by ethanol (8.6%), aqueous (7.6%), chloroform (2%), acetone (1.5%), dichloromethane (0.6%), ethyl acetate (0.5%) and petroleum ether (0.5%) extract. Phytochemical investigation of *Bacopa monnieri* (L) revealed the presence of various important secondary metabolites such as carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, alkaloids and tannins in methanolic, ethanolic and aqueous extracts. No activity was observed against bacterial strains like *Staphylococus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* when subjected to aqueous and methanolic extract of *Bacopa monnieri* (L). Methanolic extract showed significant antifungal activity against *Candida albicans* and *Aspergillus niger* at 2.5mg/ml and 1.25mg/ml concentrations.

Keywords: *Bacopa monnieri* (Linn), Extractive value, Physicochemical, Antidepressant, Antimicrobial.

INTRODUCTION

According to World Health Organization (WHO) majority of the world's population use traditional medicines for their primary health care needs. Medicinal plants are the most important natural resource of life saving drugs. Secondary metabolites of plants possesses biological properties such as antioxidant, antiapoptosis, antiaging, anticarcinogen, antiinflammatory, antiatherosclerosis, cardiovascular protection, inhibition of angiogenesis and cell proliferation activity[1-7]. *Bacopa monnieri* (L) belongs to the family Scrophulariaceae, is a creeping, glabrous, succulent herb grows in marshy areas throughout India. It has been traditionally used to treat anxiety, anger, nerve pain, insomnia, learning problems and concentration difficulties[8,9]. It has been reported that it is used in the treatment of epilepsy and asthma[10]. It has also been used as a cardio tonic, digestive aid and to improve respiratory function[11]. Natural antioxidants such as flavonoids, tannins and phenols are increasingly attracting because they are disease preventing, health promoting and antiaging substances[12]. It was reported that antioxidant properties of *Bacopa monnieri* (L) offer the protection from free radical damage in cardiovascular diseases, certain types of cancer and helps to prevent induced lipid peroxidation[3]. Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening is an important tool in bioactive compound analysis[13]. Identification, separation, quantification and standardization of major phytochemical compounds were carried out by many researchers using advance techniques[14-16]. In India, infectious diseases account for high proportions of health problems. Infections are due to variety of bacterial etiologic agents, such as pathogenic *Staphylococus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa, Shingella dysenteriae* are most common[17]. Considering the increased incidence of severe fungal and bacterial infections in immunologically deficient patients, there is a great need in finding new classes of natural products that may be effective against antibiotic resistant bacteria and fungi. Therefore, the objective of the present study was to determine the effective solvent for extraction of *Bacopa monnieri* (L), to determine the ash value, to study the phytochemical screening in methanolic, ethanolic and aqueous extracts of *Bacopa monnieri* (L) and to investigate the antimicrobial activity of *Bacopa monnieri* (L) extracts in order to use it as a possible source for new antimicrobial substances against human pathogens.

*Author for Correspondence: zooymc@gmail.com

Table 1: Extractive value (%) of *Bacopa monnieri* (L).

Solvents	Weight of Plant material (g)	Colors of extract	Extractive value (%)
Methanol	2	Yellowish green	10.1
Ethanol	2	Green	8.6
Aqueous	2	Dark brown	7.6
Chloroform	2	Light green	2
Acetone	2	Green	1.5
Dichloromethane	2	Light green	0.6
Ethyl acetate	2	Green	0.5
Petroleum Ether	2	Colorless	0.5

Table 2: Ash value and loss on drying of *Bacopa monnieri* (L).

Total ash value	13.5%
Acid insoluble ash value	5.5%
Water soluble ash value	2.5%
Loss on Drying	1.5%

MATERIAL AND METHODS

Plant material

The herbarium of *Bacopa monnieri* (L) was prepared and authentication has been obtained from Scientist D and HOD, Botanical survey of India, Pune, Maharashtra. The specimen (MGJ-1) was deposited to herbarium department in Botanical Survey of India, Pune. The whole *Bacopa monnieri* (L) plant material was shade dried at room temperature and kept in oven at 40ºC to remove moisture. The dried plant was then finely ground by mechanical grinder. The powder obtained was then sieved and kept in air tight containers for further extraction process.

METHODS

Extraction of Bacopa monnieri (L)

The extracts of *Bacopa monnieri* (L) were prepared by traditional maceration method. *Bacopa monnieri* (L) dry plant powder was dissolved in methanol, ethanol and water. Each mixture was kept separately for 72 hrs at room temperature with occasional shaking. After completion of the maceration, the supernatant was decanted and the mixture was filtered. The extract was concentrated to dryness by keeping filtrate for complete evaporation of the solvent. After evaporation of solvent the extract was weighed and kept in air tight glass container for the determination of phytochemical constituents and antimicrobial activity. Aqueous extract was reconstituted in sterile distilled water with the required concentration for antimicrobial activity. Methanolic extracted powder was suspended in absolute methanol to prepare the desired concentration of extract solution. Four concentration stocks 1.25 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml were prepared and used for antimicrobial activity.

Physicochemical Analysis[18]

Determination of Extractive value of Bacopa monnieri (L)

The dry powdered plant material of *Bacopa monnieri* (L) was extracted with water, methanol, ethanol, acetone, chloroform, dichloromethane, ethyl acetate and petroleum ether using a maceration process. The coarsely powdered plant material was weighed and transferred into a dry conical flask. Then each flask was filled with different solvents separately. The flasks were corked and kept aside for 24 hrs at room temperature, shaking frequently. The mixtures were filtered through Whatmann No. 1 filter paper. After the filtrate has obtained, it was then transferred into a weighed petry plates. The obtained extracts were concentrated to dryness by keeping filtrate for complete evaporation of solvent.

The extractive value in percentage was calculated by using following formula.

$$\text{Extractive value (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100$$

Determination of Ash value of Bacopa monnieri (L)

Total ash

Two gram coarsely powdered dry plant material of *Bacopa monnieri* (L) was weighed in a previously ignited crucible and ignited gradually by heating to 500-600ºC until it become white. Cooled in desiccator and weighed. The content of total ash in terms of percentage was calculated.

Acid-Insoluble Ash

It is residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. 25ml of dilute HCl was added to the crucible containing total ash and boiled gently for 5min. The insoluble matter was collected on ashless filter paper, washed with hot water then filter paper was ignited, cooled in desiccator and weighed. The content of acid insoluble ash in terms of percentage was calculated.

Water Soluble Ash Values

The total ash was boiled for five minutes with 25 ml of water, the soluble matter was collected in a crucible, ignited and weighed. The content of water soluble ash in terms of percentage was calculated.

Loss on Drying

Loss on drying is the loss of mass expressed as percent w/w. The test for loss on drying determines both water and volatile matter in the crude drug. Moisture is an inevitable component of crude drug, which must be eliminated as far as possible. An accurately weighed quantity of about 2 g of *Bacopa monnieri* (L) powder was taken in glass petri dish. The Petri dish was kept open in vacuum oven and dried at a temperature between 100 to 105°C for 2 h until a constant weight is recorded. Then cooled in a desiccator to room temperature, weighed and recorded. Percent loss on drying was calculated using the following formula.

Table 3: Phytochemical screening of aqueous, methanolic and ethanolic extracts of *Bacopa monnieri* (L).

S. No.	Secondary metabolites	Phytochemical tests	Methanol	Ethanol	Aqueous
1.	Carbohydrates	Molisch's Test	+	+	+
2.	Proteins	Millon's Reagent Test	+	+	+
3.	Amino acid	Ninhydrin Test	-	-	+
4.	Steriod	Liebermann Burchard Reaction	+	+	+
5.	Glycosides	Legal's Test	+	+	+
	a)Cardiac glycosides				
	b)Anthraquinone glycosides	Borntrager's Test	-	-	-
	c)Saponin glycosides	Foam Test	+	+	+
6.	Flavonoids	Sodium hydroxide test	+	+	+
7.	Alkaloids	Mayer's Test	+	+	+
		Wagner's Test	+	+	+
		Hager's Test	+	+	+
8.	Tannins	Dilute Nitric acid Test	+	+	+

+ Present, - Absent.

Loss on drying (%) =

$$\frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \times 100$$

In the present investigation the highest extractive value was observed in methanolic extract followed by ethanol and aqueous extract of *Bacopa monnieri* (L), therefore these extracts were subjected to further phytochemical screening.

Phytochemical screening of methanolic, ethanolic and aqueous extracts of Bacopa monnieri (L)[18]

The different phytochemical tests were performed for establishing the profile of plant extract for its phytochemical constituents. The phytochemical screening of *Bacopa monnieri* (L) for Carbohydrates, Proteins, Amino acids, Steroids, Glycosides, Cardiac glycosides, Anthraquinone glycosides, Saponin glycosides, Flavonoids, Alkaloids and Tannins was carried out. The extract obtained from methanol, ethanol and aqueous was used for phytochemical screening.

Test for carbohydrates

Molisch's test

3ml of extract was taken in a test tube, 2 drops of alcoholic alpha naphthol solution was added, shaked well and then 1ml of concentrated sulfuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicated the presence of carohydrates.

Test for proteins

Millon's test

About 3ml of sample extract was treated with 5ml of Million's reagent. White pricipitate was obtained. The mixture was then warmed, pricipitate turned to brick red. It indicated the presence of proteins.

Test for amino acids

Ninhydrin test

About 3ml of plant extract solution was heated followed by addition of 3 drops of 5% ninhydrin solution. The test tubes with this solution were kept in boiling waterbath for 10 minutes. The purple color was observed. It indicated the presence of amino acids.

Test for steroids

Leibermann- Burchard reaction–

To 3ml extract 10ml chloroform was added followed by 2ml of acetic anhydride. Then 2 drops of concentrated sulfuric acid were added from the side of the test tube. The blue green color appearance indicated the presence of steroids.

Test for glycosides

Cardiac Glycosides (Legal's Test)

To the 3ml extract 1ml pyridine was added by frequent shaking followed by 1ml sodium nitroprusside. Pink to red color appeared. It indicated the presence of cardiac glycosides.

Anthraquinone glycosides (Borntrager's test)

To 3ml extract dil. Sulfuric acid was added. The solution was then filtered. Then equal volume of chloroform was added to the filtrate. After shaking organic solvent was separated. Finally equal volume of ammonia solution was added. No bright pink, red or violet color was developed in the upper layer which indicated the absence of anthraquinones.

Saponin glycosides (Foam test)

About 50mg of extract was diluted in the successive solvents and made up to 20ml. The suspension was shaken for 15min. A 2cm layer of foam appeared. Appearance of foam indicated the presence of saponins.

Test for flavonoids

Sodium hydroxide test

To 3ml of extract increasing amount of sodium hydroxide was added it showed colouration, which was decolorised after addition of dil. hydrochloric acid. Decolorization showed presence of flavonoids.

Test for alkaloids

Solvent free extract was stirred with 10ml of dilute hydrochloric acid and filtered. The filtrate was tested with following alkaloidal reagents as follows:

Mayer's test

To 3ml filtrate two drops of Mayer's reagent were added by the sides of the test tube. A white creamy precipitate was observed. It indicated positive test.

Hager's test

To 3ml of filtrate, 1ml of Hager's reagent was added. A prominent yellow precipitate occurred. It indicated the presence of alkaloids.

Wagner's test

Table 4: Labels and each well used during the experiment for antimicrobial activity *Bacopa monnieri* (L).

S. No.	Plate Label	Extract used	Test Organism	Well No.	Concentration of extract
1.	SF1	Aqueous	*Staphylococcus aureus*	1,2,3	10mg/ml,5mg/ml, control respectively
2.	SF1	Aqueous	*Staphylococcus aureus*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
3.	SF2	Methanolic	*Staphylococcus aureus*	1,2,3	10mg/ml,5mg/ml, control respectively
4.	SF2	Methanolic	*Staphylococcus aureus*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
5.	ST1	Aqueous	*Bacillus subtilis*	1,2,3	10mg/ml,5mg/ml, control respectively
6.	ST1	Aqueous	*Bacillus subtilis*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
7.	ST2	Methanolic	*Bacillus subtilis*	1,2,3	10mg/ml,5mg/ml, control respectively
8.	ST2	Methanolic	*Bacillus subtilis*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
9.	EC1	Aqueous	*Escherichia coli*	1,2,3	10mg/ml,5mg/ml, control respectively
10.	EC1	Aqueous	*Escherichia coli*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
11.	EC2	Methanolic	*Escherichia coli*	1,2,3	10mg/ml,5mg/ml, control respectively
12.	EC2	Methanolic	*Escherichia coli*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
13.	P1	Aqueous	*Pseudomonas aeruginosa*	1,2,3	10mg/ml,5mg/ml, control respectively
14.	P1	Aqueous	*Pseudomonas aeruginosa*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
15.	P2	Methanolic	*Pseudomonas aeruginosa*	1,2,3	10mg/ml,5mg/ml, control respectively
16.	P2	Methanolic	*Pseudomonas aeruginosa*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
17.	A1	Aqueous	*Aspergillus niger*	1,2,3	10mg/ml,5mg/ml, control respectively
18.	A1	Aqueous	*Aspergillus niger*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
19.	A2	Methanolic	*Aspergillus niger*	1,2,3	10mg/ml,5mg/ml, control respectively
20.	A2	Methanolic	*Aspergillus niger*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
21.	C1	Aqueous	*Candida albicans*	1,2,3	10mg/ml,5mg/ml, control respectively
22.	C1	Aqueous	*Candida albicans*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
23.	C2	Methanolic	*Candida albicans*	1,2,3	10mg/ml,5mg/ml, control respectively
24.	C2	Methanolic	*Candida albicans*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively

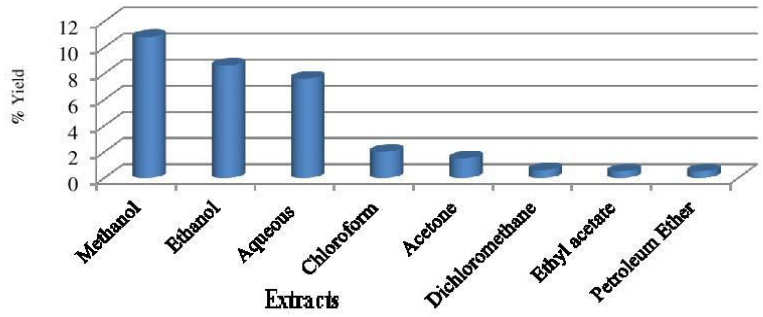

Figure 1: Extractive value (%) different extracts of *Bacopa monnieri* (L).

To 3ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish brown precipitate confirmed the presence of alkaloids.

Test for Tannins

Nitric acid test

3ml of extract was taken in test tube, a few drops of dilute nitric acid were added. The reddish yellow color indicated the presence of tannins.

Investigation of antimicrobial activity

Microorganism strains

Four bacterial and two fungal test organisms were selected for this activity. Identification of the isolates was established by 16S rDNA and Internal Transcribed Spacer (ITS) sequencing respectively. Following strains of microorganisms were used for antimicrobial activity.

1. *Staphylococus aureus* (Gram +ve)
2. *Bacillus subtilis* (Gram +ve)
3. *Escherichia coli* (Gram -ve)
4. *Pseudomonas aeruginosa* (Gram -ve)
5. *Aspergillus niger*
6. *Candida albicans*

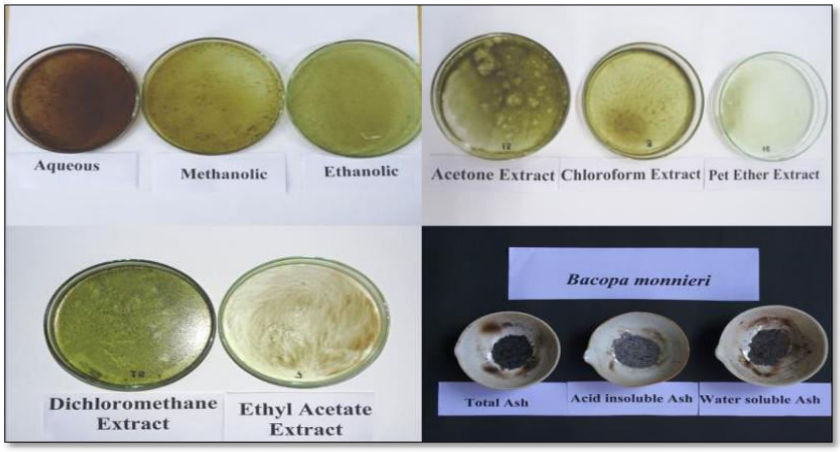

Plate No. 1: Aqueous, methanolic, ethanolic, acetone, chloroform, petroleum ether, dichloromethane and ethyl acetate extract of *Bacopa monnieri* (L).

Screening for antimicrobial activity of Bacopa monnieri (L)

Antimicrobial activity was performed by the agar well diffusion method. In this method, pure isolate of each microbe was sub cultured on the nutrient agar media plates at 37ºC for 24 h. For fungal cultures YPD agar medium was used. One plate of each microorganism was taken and a minimum of four colonies were touched with a sterile loop and transferred into normal saline (0.85%) under aseptic conditions. Density of each microbial suspension was adjusted equal to that of 10^6 cfu/ml (standardized by 0.5 McFarland standard) and used as the inoculum for performing agar well diffusion assay. One hundred microlitre (100µl) of inoculum of each test organism was spread onto the agar plates so as to achieve a confluent growth. The agar plates were allowed to dry and wells of 10 mm were made with a sterile borer in the inoculated agar. A 100µl volume of each extract was propelled directly into the wells of the inoculated agar plates for each test organism. The plates were allowed to stand for 1hr for diffusion of the extract into the agar and incubated at 37ºC for 24h. Sterile water and methanol was used as negative control in each respective plate for analysis. The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone of inhibition was greater than 10 mm.

RESULTS

Following are the labels and each well used during the experiment for antimicrobial activity of *Bacopa monnieri* (L).

Well No 3 and 6 are control in each plate, Well No.1: 10 mg/ml, Well No.2: 5 mg/ml, Well No.4: 2.5mg/ml, Well No.5: 1.25mg/ml

RESULTS AND DISCUSSION

In the present study the extractive value of *Bacopa monnieri* (L) in methanol, ethanol, aqueous, chloroform, acetone, dichloromethane, ethyl acetate and petroleum ether extract was determined. The extractive value and color of extracts of *Bacopa monnieri* (L) was investigated and represented in Table No. 1. From the present study it was found that, the extractive value of *Bacopa monnieri* (L) in methanolic extract was maximum (10.1%) as compared to other extracts. The ethanolic extract showed slightly less extractive value (8.6%) than methanolic extract of *Bacopa monnieri* (L). The extractive value of *Bacopa monnieri* (L) in aqueous extract was 7.6% followed by chloroform extract (2%), acetone extract (1.5%) and dichloromethane (0.6%). The ethyl acetate and petroleum ether extract showed very less (0.5 %) extractive value. The color of extracts observed was yellowish green in methanol, green in ethanol, acetone and ethyl acetate extract, dark brown in aqueous, light green in chloroform, dichloromethane and colorless in petroleum ether extract (Table 1). From the literature review it was observed that more yield percentage of *Bacopa monnieri* (L) was obtained in alcoholic extract[19]. The total ash, acid insoluble, water soluble ash value and loss on drying are depicted in Table No. 2. The physicochemical analysis of *Bacopa monnieri* (L) are presented in Plate No. 1. Plant synthesises a broad range of primary and secondary metabolites with different functional groups[20]. Phytochemical screening is an important tool in bioactive compound analysis. It is quick, inexpensive and simple procedure that shows the various types of phytochemicals present in plant. The presence of phytochemicals is a marker that the plant can be a prospective source of precursors in the formation of synthetic drug[21]. It was reported that carbohydrates, phenols glycosides and anthraquinones were present in petroleum ether and ethanolic extract of *Bacopa monnieri* (L)[22]. The in vitro phytochemical analysis of roots of *Bacopa monnieri* (L)

Table 5: Antibacterial and antifungal activity of aqueous and methanolic extract of *Bacopa monnieri* (L).

Diameter of Growth Inhibition Zone (mm)						
Extract and its concentration	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Candida albicans*
Aqueous extract						
10mg/ml	*No*	*No*	*No*	*No*	*No*	*No*
5 mg/ml	*No*	*No*	*No*	*No*	*No*	*No*
2.5mg/ml	*No*	*No*	*No*	*No*	*No*	*No*
1.25mg/ml	*No*	*No*	*No*	*No*	*No*	*No*
Methanolic extract						
10mg/ml	*No*	*No*	*No*	*No*	*No*	23
5 mg/ml	*No*	*No*	*No*	*No*	*No*	23
2.5mg/ml	*No*	*No*	*No*	*No*	35	22
1.25mg/ml	*No*	*No*	*No*	*No*	25	22
Control	*No*	*No*	*No*	*No*	25	20

Note: Values including diameter of wells.
No: No inhibition was observed.

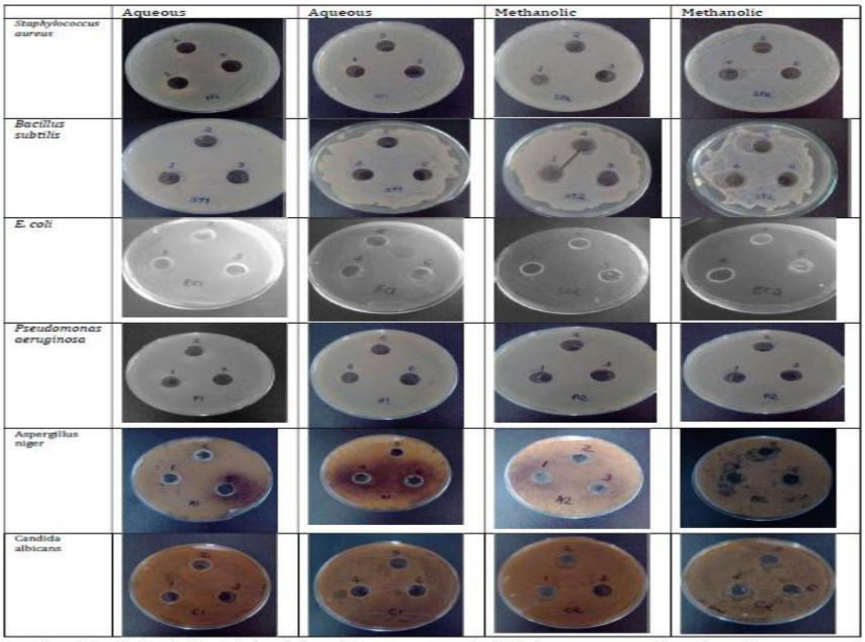

Plate No. 2: Antimicrobial activity of *Bacopa monnieri* (L) in aqueous and methanolic extract.

was carried out, the study showed presence of alkaloids, anthraquinone, cardiac glycosides, flavanoids, phenols, saponin, steroids, tannins, terpenoids, alkaloids in ethanolic, methanolic, chloroform, petroleum ether and ethyl acetate extract[17]. *Bacopa monnieri* (L) was reported to possess terpenoids and steroids predominately in ethanol, aqueous, chloroform, acetone and ethyl acetate extracts[23]. The aqueous and hydroalcoholic extracts of *Bacopa monnieri* (L) were reported for the presence of phenols, flavanoids, glycosides, alkaloids and carbohydrates[24]. The phytochemical analysis of leaf callus of *Bacopa monnieri* (L) was carried out by Singh, he observed the presence of tannins, saponins, terpenoids, steroids in ethanol and aqueous extract and absence of anthraquinone glycosides and phenols in same extracts[25]. From the present study phytochemical screening revealed that saponins, flavonoids, alkaloids, tannins, carbohydrates, proteins and steroids were present in methanolic, aqueous and ethanolic extracts of *Bacopa monnieri* (L). The aqueous extract of *Bacopa monnieri* (L) showed the presence of amino acids and methanolic, ethanolic extracts showed absence of amino acids. Anthraquinone glycosides were absent in methanolic, aqueous and ethanolic extracts of *Bacopa monnieri* (L). (Table No. 3). The antimicrobial activity study revealed that, the pattern of inhibition varied with the plant extract

and the organism tested. The highest antifungal activity was observed in methanolic extract and maximum zone of inhibition was observed against *Aspergillus niger* and *Candida albicans*, where as in aqueous extract no antifungal activity was observed. The zone of inhibition of methanolic extract was highest for *Candida albicans* at 10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml concentrations, while for *Aspergillus niger* the highest zone of inhibition was observed at 2.5mg/ml and 1.25mg/ml concentrations. No antibacterial activity was observed against *Staphylococus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* in aqueous and methanolic extracts of *Bacopa monnieri* (L) in these used concentrations. Antimicrobial activity of *Bacopa monnieri* (L) aqueous and methanolic extract are presented in Table No. 5. and Plate No. 2. Earlier studies have reported that, methanolic extracts of *Bacopa monnieri* (L) was found to possess maximum inhibitory effects against gram positive and gram negative organisms tested compared to chloroform and ethanolic extract[26]. Therapeutic value of medicinal plants and bioactivity of extract lies in the various phytochemicals present in it, plant rich in tannins have antimicrobial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane[27]. Flavonoids are the major group of phenolic compounds reported to have antimicrobial activity[28]. The extracts of seeds of *Vitexagnus-castus* was reported to possess antimicrobial activity which is associated with its alkaloid, saponin, taninn, flavonoid and glycoside contents[29]. Phenolic compounds such as coumarin and quercetin had extended protection to gastroenteritis disease causing microbes[30].The antimicrobial activity of *Bacopa monnieri* (L) extract as recorded in the present study may, therefore, be attributed to the phytoconstituents present in it.

CONCLUSION

From the present study it can be concluded that, the extractive value is useful to find the effective solvent for extraction process. It gives idea about the nature of phytochemical constituents present in the plant material. In the present study methanol has been found to be pre-eminent solvent used for extraction. The ash value helps to determine purity of a crude plant material and foreign inorganic matter present as an impurity. For further analytical study of *Bacopa monnieri* (L) ash value is useful as it removes all traces of organic matter which may interfere further experimentation. Loss on drying test is effective to measure the amount of moisture content and volatile matters in a sample. Higher water content may prone to have chemical and microbial decomposition of crude drug. The phytochemical screening is helpful for confirmation of bioactive phytochemical constituents in *Bacopa monnieri* (L). The loss on drying has been found less in *Bacopa monnieri* (L). No inhibitory effect was observed against bacterial strains like *Staphylococus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* when subjected to aqueous and methanolic extract of *Bacopa monnieri* (L) with four concentration stocks used for this study perhaps it may show antibacterial activity in some other concentrations which need further investigation.The promising anti fungal activity of *Bacopa monnieri* (L) may be due to presence of phytochemicals such as alkaloids, phytosterols, proteins, Flavonoids, tannins etc.. which will be helpful in future to treat certain fungal diseases and will be used in therapeutic natural drugs.

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Original Article

EFFECT OF BACOSIDE A ON LIPID PEROXIDATION IN D-GALACTOSE INDUCED AGING MICE

SUSHAMA PAWAR*, MANMOHINI JADHAV

Department of Zoology, Bharati Vidyapeeth Deemed University, Yashwantrao Mohite College of Arts, Science and Commerce, Pune 411038, Maharashtra, India
Email: zooymc@gmail.com

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ABSTRACT

Objective: Bacoside A is a major bioactive constituent of *Bacopa monnieri* L having antioxidant property. The objective of this study was to evaluate the effect of Bacoside A, on lipid peroxidation in brain, heart and liver during induced aging.

Methods: Male Swiss albino mice, *Mus musculus* was used for the present investigation. Four experimental groups were used as Group I-Normal adult, Group II-D-galactose induced, Group III–D-galactose induced plus Bacoside A treated and Group IV–Natural aging. The effect of Bacoside A was studied against lipid peroxidation during induced aging. The level of lipid peroxidation in the form of MDA formation was determined and measured in brain, heart and liver.

Results: The statistical data obtained were analyzed using one way ANOVA, control vs other groups and results were expressed as mean±SE. In Bacoside A treated group the lipid peroxidation level in heart, brain and liver was significantly decreased ($p<0.001$) compared to control group. A significant increase ($p<0.0001$) in the level of lipid peroxidation was observed in D-galactose induced mice. In natural aging group highly significant increase ($p<0.0001$) in initial lipid peroxidation, ascorbate dependent lipid peroxidation and spontaneous lipid peroxidation was observed.

Conclusion: The observations revealed that, lipid peroxidation was reversed in Bacoside A treated group which may be due to antioxidant property of Bacoside A. Thus Bacoside A is able to ameliorate the stress induced changes in lipid peroxidation during aging. The findings also provide a theoretical basis for the development of novel therapeutic formulations, such as antioxidant supplementation to boost antioxidant defenses in the body.

Keywords: Oxidative stress, Aging, Lipid peroxidation, D-galactose, Bacoside A, Antioxidant

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INTRODUCTION

Lipid peroxidation is the product of chemical damage done to the lipid component of cell membranes by oxygen free radicals [1-3]. This damage is thought to be a basic mechanism underlying many diverse disorders such as atherosclerosis, cancer, aging, rheumatic diseases, cardiac and cerebral ischemia, various liver problems and toxicity induced by harmful environmental factors. It has been considered that the lipid peroxidation damage is involved in aging and pathological disorders [4, 5]. Reactive Oxygen Species (ROS) are constantly generated in living cells as a part of intracellular metabolic processes and induce oxidative damage to cell membranes, lipids, proteins and nucleic acids [6, 7]. The free radicals that contribute to the aging process are derived directly or indirectly from oxygen. As lipid peroxidation traditionally has been regarded as the major process that produces damage to oxygen radicals and the oxidized lipid residues are major components of lipofuscin, the fluorescent pigment that accumulates with age in most tissues [8-10]. Post mitotic cells are very susceptible to the oxidative damage due to their high consumption of oxygen and presence of fatty acids that are prone to peroxidation. Thus damage due to lipid peroxidation is evident in a wide range of degenerative diseases and aging [6, 11]. A reducing sugar, D-galactose reacts with free amino groups of amino acids and proteins to form insoluble agglomerates called as advanced glycation end products, AGE's [12, 13]. The advanced glycation end products formed due to D-galactose accumulates in cells and provokes the formation of free radicals which are responsible for the pathogenesis of various diseases and aging as well [14].

Many researchers have focused on natural antioxidants, numerous crude extracts and pure natural compounds present in plants. A large amount of secondary metabolites are present in medicinal plants. Flavonoids, saponins and polyphenols in plants are a versatile group of antioxidants that protect against damage caused due to lipid peroxidation by directly neutralizing reactive oxygen species [15-22]. It is therefore important to study the effect of secondary metabolites on a particular tissue. *Bacopa monnieri* (L) traditionally used to treat various human ailments. It has been reported to have possible medicinal attributes as an antioxidative, antimicrobial, anti-aging and free radical scavenging activity [23-28]. Triterpenoid saponins, the major components in Brahmi, were reported to be responsible for the cognitive enhancing activity of Brahmi [24, 26, 29]. The major bioactive dammarene type triterpenoid saponin isolated from the *Bacopa monnieri* (L), that carries the neuropharmacological activities, is Bacoside A [30]. In this context, the study has been carried out to evaluate the effect of Bacoside A on lipid peroxidation in D-galactose induced aging mice.

MATERIALS AND METHODS

Chemicals and reagents

D Galactose, Potassium chloride (KCl), Ascorbic acid, Ammonium ferrous sulphate (Mohr's salt), Trichloroacetic acid (TCA), Thiobarbituric acid (TBA) were purchased from LOBA chemie Pvt. Ltd., Mumbai, India.

Animals

Male mice (*Mus musculus*) of Swiss albino strain of different age groups were reared in animal house. Approval was obtained by the CPCSEA and IAEC (CPCSEA/PCL/23/2014-15). All instructions and rules of CPCSEA were followed. Animals were kept under a 12:12 hr L: D cycle and fed *ad libitum* a commercial diet provided by Pranav Agro Industries, Pune. Animals were divided into the following four experimental groups, six animals each group:

Group I-Normal adult (control) (12 Mo Age)

Group II-D-galactose induced (12 Mo Age)-injected with D-galactose subcutaneously for 56 d

Group III–D-galactose induced+Bacoside A treated (12 Mo Age)-injected with D-galactose subcutaneously and administered Bacoside A orally at a dose of mg/kg body weight for 56 d

Group IV–Natural aging (18-20 Mo Age).

Plant

The Bacoside A ($C_{41}H_{68}O_{13}$) was purchased from M/S Natural Remedies Pvt. Ltd. Banglore, India.

Preparation of homogenate

The experimental animals were sacrificed after completion of doses. The brain, heart and liver were dissected out. The brain tissues were separated as cerebral hemisphere and cerebellum. The homogenates were prepared by using 0.9% KCL and centrifuged at 3000 rpm at 10 °C. The obtained homogenates were used for further study.

Lipid peroxidation assay

The rate of lipid peroxidation was measured by the method of Strove and Makarova 1980. The level of lipid peroxidation in the form of MDA formation was determined and measured in brain regions, heart and liver at 532 nm.

Statistical analysis

The results obtained were analyzed by the SPSS software package version 20. The mean values obtained for the different groups were compared by one-way ANOVA, followed by Dunnett test, Normal adult (control) vs other groups. The results were expressed as mean±SE and P<0.001 was considered as highly significant.

RESULTS AND DISCUSSION

In the present study, the level of lipid peroxidation in brain, heart and liver of experimental groups was measured. The lipid peroxidation in the form of MDA formation in cerebral hemisphere, cerebellum, heart and liver were depicted in table 1 and Graph No. 1,2,3,4. In Bacoside A treated group the lipid peroxidation level in heart, brain and liver was significantly decreased (p<0.001) which some extent resembles as in the normal adult. A significant increase (p<0.0001) in the level of lipid peroxidation was observed in D-galactose induced mice. In natural aging group highly significant increase (p<0.0001) in initial lipid peroxidation, ascorbate dependent lipid peroxidation and spontaneous lipid peroxidation was observed.

In vivo lipid peroxidation has been identified as a basic deteriorative reaction in cellular mechanisms of aging processes [5, 31]. As highly reactive free radicals are not removed from the cell, the propagation of free radical reaction may be increasing exponentially in old age. The pattern of damage to proteins induced by peroxidizing lipid is similar to radiation damage [32]. Effect of age on lipid peroxidation in various parts of the rat brain was studied by Koudelova and Mourk, a significant increase in MDA production in cerebral cortex was observed [33]. Age related changes in lipid peroxidation were observed by Pawar, the lipid peroxidation was increased in old male mice than in adult male mice [34]. It was reported that feeding of *Murraya koenigii* and *Brassica Juncea* decreased the level of lipid peroxidation in liver and heart of rats [15]. The significant reduction in the level of lipid peroxidation was observed in rats liver and heart treated with methanol extract of *Teramnus labialis* [19].

When the level of D-galactose increases above the normal, it gets oxidized into hydrogen peroxide and aldehydes [35]. Increased level of it induces premature aging due to increased advanced glycation end products, decreases motor activity and stimulates diabetes [36]. It has been reported that D-galactose responsible to deflate immune responses, accelerate oxidative stress by increasing lipid peroxidation and decline antioxidant enzyme activities by prevailing degeneration [37]. The study on aging showed that in D-galactose treated mice increased levels of lipid peroxidation indicates ageing associated changes since during aging there is increased production of reactive oxygen species, hence increased lipid peroxidation [38]. It was observed that the animals administered *Petroselinum crispum* extract along with D-galactose showed the significantly low level of MDA in the brain as compared to the D-galactose treated mice [11]. Increased malondialdehyde is an indication of increased lipid peroxidation in D-galactose treated mice that results due to increased oxidative stress [39, 40].

The results from our study revealed that the concentration of MDA in brain regions, heart and liver of D-galactose treated group was elevated as compared to control group. In the animals which received Bacoside A along with D-galactose, MDA level was significantly less in brain regions, heart and liver as compared to D-galactose treated group. The results show that administration of Bacoside A brings about alterations in the level of lipid peroxidation in different tissues. The concentration of MDA decreased significantly in the brain, heart and liver of Bacoside A treated group hence Bacoside A has ameliorative effects on D-galactose induced mice. The level of lipid peroxides in Bacoside A administered group suggests that it can able to revert the effects caused due to D-galactose and can maintain the concentration of MDA at the normal level during induced ageing.

Table 1: Formation of TBA reacting products in spontaneous, ascorbate dependent and initial lipid peroxidation (n mols malondialdehyde/mg tissue/1h.) in cerebral hemisphere, cerebellum, heart and liver

Organ	Cerebral hemisphere			Cerebellum			Heart			Liver		
group	X1	X2	X3	X1	X2	X3	X1	X2	X3	X1	X2	X3
Normal Adult	39.20 ±0.100 6	9.502 ±0.0213	0.8263 ±0.007 5	45.68 ±0.013 8	6.678 ±0.006 0	1.063 ±0.024 5	31.49 ±0.140 7	4.770 ±0.005 7	0.6728 ±0.001 4	41.13 ±0.249 3	5.832 ±0.014 4	1.345 ±0.058 5
D-galactose induced	43.92 ±0.048 2	11.65 ±0.0135	1.513 ±0.001 6	48.30 ±0.033 8	14.87 ±0.010 0	1.873 ±0.002 4	39.95 ±0.013 5	5.125 ±0.012 0	1.013 ±0.001 0	46.98 ±0.007 6	9.055 ±0.008 8	2.067 ±0.000 7
D-galactose+Bacoside A treated	39.77 ±0.154 5	6.147 ±0.0114 5	0.5380 ±0.000 6	42.12 ±0.078 8	11.67 ±0.011 4	0.8865 ±0.005 0	29.33 ±0.013 0	4.858 ±0.060 0	1.090 ±0.002 5	31.65 ±0.117 4	7.237 ±0.031 1	1.649 ±0.001 7
Natural aging	49.78 ±0.006 5	14.29 ±0.0061	1.816 ±0.123 9	52.90 ±0.005 7	17.07 ±0.020 2	2.328 ±0.054 3	48.45 ±0.186 9	6.702 ±0.126 9	1.519 ±0.038 6	50.47 ±0.058 9	11.38 ±0.121 6	3.169 ±0.004 0

All values of D-galactose, induced, D-galactose+Bacoside A treated group and Natural aging group are compared with respect to the normal adult (control) group, Values are expressed as mean±SE (n=6 mice), X1-The rate of spontaneous lipid peroxidation in the homogenates nmoles of malondialdehyde formation during 1h. X2-The rate of ascorbate dependent non-enzymatic peroxidation in the homogenates nmoles of malondialdehyde formation during 1h. X3-The amount of malondialdehyde in the initial homogenate

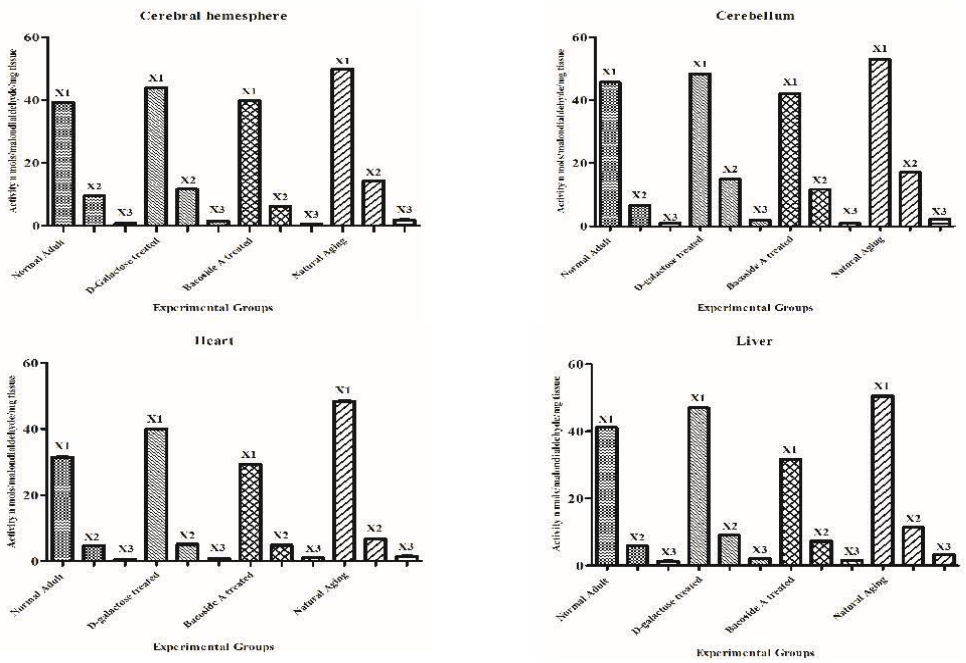

Fig. 1: Formation of TBA reacting products in spontaneous, ascorbate dependent and initial lipid peroxidation (n mols malondialdehyde/mg tissue/1h.) in Cerebral hemisphere, cerebellum, heart and liver

CONCLUSION

The present study concluded that increased level of lipid peroxidation may be due to formation advanced glycation end products by D-galactose. Maximum protection against lipid peroxidation damage can be achieved by using sufficient concentrations of natural antioxidants. Thus, the present results suggest that Bacoside A may have the potential to reduce the formation of lipid peroxidation during induced aging.

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AUTHORS CONTRIBUTION

Authors are equally contributed in this work.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest

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