FINAL REPORT OF THE WORK DONE ON THE MAJOR RESEARCH PROJECT

Micropropagation and germplasm conservation of endangered medicinal plants in Southern India

SUBMITTED TO

University Grant Commission (UGC)
Bahadur Shah Zafar Marg, New Delhi – 100 002
India

BY

Dr. A.ARUMUGAM
Professor
DEPARTMENT OF BOTANY

ALAGAPPA UNIVERSITY
(Accredited with A+ Grade by NAAC (CGPA: 3.64) in the Third Cycle)
Category-I University under MHRD-UGC
KARAIKUDI – 630 003
TAMIL NADU
INDIA

JULY – 2021

FINAL REPORT OF THE WORK DONE ON THE MAJOR RESEARCH PROJECT

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002.

Annexure - I

FINAL STATEMENT OF EXPRENDITURE IN RESPECT OF MAJOR RESEARCH PROJECT

1. Name of Principal Investigator : Dr. A. Arumugam
2. Dept. of the Principal Investigator University : Department of Botany
3. UGC approval letter No. and date : F.No. 37-14/2009 (SR), 24.06.2010
4. Title of the Research Project : Micropropagation and germplasm conservation of endangered medicinal plants in Southern India
5. Project period : 01.02.2010 to 31.1.2013 – 3 years
   Extension period : -
6. Effective date of starting the project : 01.02.2010
7. a. Period of Expenditure: : From 01.02.2010 to 31.01.2013
   b. Details of Expenditure : as below

Sl.No.	Head of the account	Amount allocated Rs.	Grant released			Expenditure Rs.	Unutilized amount Rs.	Remarks
			I installment 2010-11 FY Rs.	II installment 2012-13 FY Rs.	Total grant Rs.			
1	2	3	4	5	6	7	8	9
1	Books & Journals	50000	50,000	-	50000	50000	0	
2	Equipment	200000	200000	-	200000	200000	0	
3	Chemicals	200000	100000	100000	200000	199579	421	
4	Contingency	75000	37500	37500	75000	75000	0	
5	Travel/Field work	50000	25000	25000	50000	47717	2283	
6	Overhead charges	56300	56300	-	56300	56300	0	
7	Interest	-	-	-	-	-	-	
	Total	**631300**	**468800**	**162500**	**631300**	**628596**	**2704**	

PTO

c. Staff: Date of appointment 09-04-2010

Sl.No.	Head of the account	Amount allocated	Grant released			Expenditure	Unutilized amount	Remarks
			First installment 2010-11 FY	Second installment 2012-13 FY	Total grant			
		Rs.	Rs.	Rs.	Rs.	Rs.	Rs.	
1	2	3	4	5	6	7	8	9
8	**Project Fellow** Period: from 01.02.2010 to 31.01.2013 Non-GATE/NON-NET: Rs.14,000/- p.m. for initial 2 years and Rs.16,000/- p.m. for the 3rd year.	491733	1,44,000	266060	410060	410060	0	Unutilized grant including interest Rs.3146 /- has been transferred through RTGS to the UGCs bank account vide Cheque No. 300733 Dated: 16.02.2017
	Grand total (b & c)	**1123033**	**612800**	**428560**	**1041360**	**1038656**	**2704**	

1. It is certified that the appointment had 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 irregularity is noticed at later date, action will be taken to refund, adjust or regularize the objected amount.
3. Payment at revised rates shall be made with arrears on the availability of additional funds.
4. It is certified that the grant of **Rs. 11,23,033/-** (Rupees eleven lakhs, twenty-three thousand and thirty-three only) sanctioned by the University Grants Commission under the scheme of support for Major Research Project entitled "Micropropagation and germplasm conservation of endangered medicinal plants in Southern India" vide UGC sanction letter No. F.No. 37-14/2009 (SR), dated 24.06.2010. A sum of **Rs. 10,41,360/-** (Rupees ten lakhs, forty-one thousand, three hundred and sixty only) had been received from the University Grants Commission. A sum of **Rs. 10,38,656/-** (Rupees ten lakhs, thirty-eight thousand and six hundred and fifty-six only) had been utilised for the purpose for which it was sanctioned and in accordance with the terms and condition laid down by the University Grants Commission. An unspent amount of **Rs. 3,146/-** (Chemicals: Rs. 421/- ; Travel/Fieldwork: Rs. 2,283/- and interest Rs. 442/- at a total of Rs. 3146/-) has been transferred through RTGS to the UGC account.

Principal Investigator
DR. A. ARUMUGAM M.Sc., Ph.D.,
PROFESSOR,
DEPARTMENT OF BOTANY (GREEN CAMPUS),
ALAGAPPA UNIVERSITY,
THONDI CAMPUS.

Finance Officer
FINANCE OFFICER
ALAGAPPA UNIVERSITY
KARAIKUDI - 630 003.
19/7/21 19/7/21

Registrar 20/7/21
REGISTRAR
ALAGAPPA UNIVERSITY
KARAIKUDI

(Auditor / Chartered Accountant)

Annexure - II

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

UTILIZATION CERTIFICATE

Certified that the grant of **Rs. 11,23,033/-** (Rupees: Eleven lakhs twenty-three thousand and thirty-three only) sanctioned by the University Grants Commission under the scheme of support for Major Research Project entitled "Micropropagation and germplasm conservation of endangered medicinal plants in Southern India" vide UGC sanction letter No. **F. No. 37-14/2009 (SR)** dated **24.06.2010.** A sum of **Rs. 10,41,360/-** (Rupees ten lakhs, forty-one thousand, three hundred and sixty only) had been received from the University Grants Commission. A sum of **Rs. 10,38,656/-** (Rupees ten lakhs, thirty-eight thousand and six hundred and fifty-six only) had been utilised for the purpose for which it was sanctioned and in accordance with the terms and condition laid down by the University Grants Commission. An unspent amount of **Rs. 3,146/-** (Chemicals: Rs. 421/- ; Travel/Fieldwork: Rs. 2,283-/ and interest Rs. 442/- at a total of Rs. 3,146/-) has been transferred through RTGS to the UGC account.

Principal Investigator
DR. A. ARUMUGAM M.Sc., Ph.D.,
PROFESSOR,
DEPARTMENT OF BOTANY (GREEN CAMPUS),
ALAGAPPA UNIVERSITY,
THONDI CAMPUS.

Finance Officer
FINANCE OFFICER
ALAGAPPA UNIVERSITY
KARAIKUDI - 630 003.
19/7/21

Registrar
REGISTRAR
ALAGAPPA UNIVERSITY
KARAIKUDI

Auditor/Chartered Accountant
D. BHARADHWAJ, B.Com., F.C.A.
KARAIKUDI - 2
M.No.220529
CHARTERED ACCOUNTANT

Annexure – III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002.

Final Report of the work done on the Major Research Project. (Report to be submitted within 6 weeks after completion of each year)

1.	Project report No. 1 st/2nd /3rd /final	:	**Final**
2.	UGC Reference No. F. No.	:	**F. No. 37-14/2009 (SR)**
3.	Period of report	:	**01.02.2010 to 31.1.2013**
4.	Title of research project	:	**Micropropagation and germplasm conservation of endangered medicinal plants in Southern India**
	(a) Name of the Principal Investigator	:	**Dr. A. Arumugam**
5.	(b) Dept of University / College where work has progressed	:	**Department of Nanoscience & Technology, Division Biotechnology, Alagappa University, Karaikudi.**
6.	Effective date of starting of the project	:	**01.04.2010**
	Grant approved and expenditure incurred during the period of the report		
7.	(a) Total amount approved	:	**Rs. 10,41,360/-**
	(b) Total expenditure	:	**10,38,656/-**

Report of the work done: (Please attach a separated sheet)

(I) **Brief objectives of the project**

1. To develop micropropagation systems for selected endangered medicinal plants and to propagate them throughout tissue culture techniques.

Name of Endangered Medicinal Plants

(i) *Withania somnifera*
(ii) *Aristolochia indica*
(iii) *Gloriosa superba*
(iv) *Cassia alata*
(v) *Plumbago zeylanica*
(vi) *Rauvolfia tetraphylla*
(vii) *Tinospora cordifolia*

Action Plan:

Following action plan was prepared to carry out the work.

1. Identification of proper explants and season and different plant tissues for *in vitro* responses.

2. Multiplication of the initiated cultures and optimization of the medium and culture conditions.

3. The method of regeneration through tissue culture has been standardized.

4. Primary and secondary hardening nursery of tissue culture.

5. Transfer of plants from *in vitro* to *in vivo*

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

Annexure - IV

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE INCURRED ON TRAVEL & FIELD WORK

Name of the Principal Investigator: Dr.A.Arumugam

Name of the places visited	Duration of the visit		Mode of journey	Expenditure incurred Rs.
	From	To		
-	-	-	-	47717
We have utilized an amount of Rs.47,717/- towards field work for the purchase of Metal- tissue culture rack, drip irrigation pipe, polyethylene bags, sun shade net, fencing nets, water sprayer, PVC pipes, T-pipes and water controllers and seed germination tray have been purchased for medicinal plants propagation purpose. Aforementioned items were successfully utilized on the travel field work grant due to the necessity.			**Total**	**47,717/-**

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

Principal Investigator
DR. A. ARUMUGAM M.Sc., Ph.D.,
PROFESSOR,
DEPARTMENT OF BOTANY (GREEN CAMPUS),
ALAGAPPA UNIVERSITY,
THONDI CAMPUS.

Registrar
REGISTRAR
ALAGAPPA UNIVERSITY
KARAIKUDI
19/7/21
19/7/21

Annexure - V

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG, NEW DELHI – 110 002

PROFORMA FOR SUPPLYING THE INFORMATION
IN RESPECT OF THE STAFF APPOINTED
UNDER THE SCHEME OF MAJOR RESEARCH PROJECT

UGC **F. No. 37-14/2009 (SR)** Year of Commencement: **01.02.2010**

Title of the Project: Micropropagation and germplasm conservation of endangered medicinal plants in Southern India

1	Name of the Principal Investigator	Dr. A. Arumugam
2	Name of the University/College	Alagappa University, Karaikudi
3	Name of the Research personal appointed	K. Gopinath
4	Academic qualification	1. M.Sc. – 64.40% (2008) 2. Ph.D. – Completed
5	Date of joining	09.04.2010
6	Date of birth of the Research personal	02.06.1986
7	Amount of HRA, if drawn	Not applicable
8	No. of candidates applied for the post	8

CERTIFICATE:

This is to certify 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 liable to terminate of said UGC Project.

Principal Investigator
DR. A. ARUMUGAM M.Sc., Ph.D.,
PROFESSOR,
DEPARTMENT OF BOTANY (GREEN CAMPUS),
ALAGAPPA UNIVERSITY,
THONDI CAMPUS.

Registrar
REGISTRAR
ALAGAPPA UNIVERSITY
KARAIKUDI

Annexure - VI

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG, NEW DELHI – 110 002

Month-wise and Year-wise detailed Statement of Expenditure towards salary of Project fellow appointed under MRP

Title of the Project : "Micropropagation and germplasm conservation of endangered medicinal plants in Southern India"
UGC File No. : 37-14/2009 (SR), dated 24.06.2010
Project Period : 01.02.2010 to 31.1.2013

Amount allocated : Rs.4,10,060/- for Project fellow

Month & Year	Due Rs.		Drawn Rs.	Balance to be released by UGC
09.04.2010 to 30.04.2010		10066/-	10066/-	
May 2010		14000/-	14000/-	
June 2010		14000/-	14000/-	
July 2010		14000/-	14000/-	
August 2010		14000/-	14000/-	
September 2010		14000/-	14000/-	
October 2010		14000/-	14000/-	
November 2010		14000/-	14000/-	
December 2010		14000/-	14000/-	
January 2011		14000/-	14000/-	
February 2011		14000/-	14000/-	
March 2011		14000/-	14000/-	
April 2011		14000/-	14000/-	
May 2011		14000/-	14000/-	
June 2011		14000/-	14000/-	
July 2011		14000/-	14000/-	
August 2011		14000/-	14000/-	
September 2011		14000/-	14000/-	
October 2011		14000/-	14000/-	
November 2011		14000/-	14000/-	
December 2011		14000/-	14000/-	
January 2012		14000/-	14000/-	
February 2012		14000/-	14000/-	
March 2012		14000/-	14000/-	
01.04.2012 to 08.04.2012	3,733/-			
09.04.2012 to 30.04.2012	11,733/-	15,466/-	15,466/-	
May 2012		16000/-	16000/-	
June 2012		16000/-	16000/-	
July 2012		16000/-	16000/-	
August 2012		16000/-	14528/-	
Total		**410060/-**	**410060/-**	**Nil**

Principal Investigator
DR. A. ARUMUGAM M.Sc., Ph.D.,
PROFESSOR,
DEPARTMENT OF BOTANY (GREEN CAMPUS),
ALAGAPPA UNIVERSITY,
THONDI CAMPUS.

Registrar
REGISTRAR
ALAGAPPA UNIVERSITY
KARAIKUDI

Annexure VII

Date: 16.07.2021

CERTIFICATE

It is certified that the executive summary of the UGC Major Research Project entitled "Micropropagation and germplasm conservation of endangered medicinal plants in Southern India" vide UGC sanction letter No. F.No. 37-14/2009 (SR), dated 24.06.2010 had been uploaded in our university website (www.alagappauniversity.ac.in) and a copy is kept in the Central Library of Alagappa University for reference.

Principal Investigator

DR. A. ARUMUGAM M.Sc., Ph.D.,
PROFESSOR,
DEPARTMENT OF BOTANY (GREEN CAMPUS),
ALAGAPPA UNIVERSITY,
THONDI CAMPUS.

19.7.2021

Librarian

LIBRARIAN i/c
Alagappa University
KARAIKUDI - 630 003

20/7/21

Registrar

REGISTRAR
ALAGAPPA UNIVERSITY
KARAIKUDI

19/7/21

19/7/21

ALAGAPPA UNIVERSITY
(Reaccredited with 'A' Grade by NAAC)
KARAIKUDI – 630 003
FINANCE SECTION

Ref.No. AU/F5/EMF/UGC refund/013/2017 Date:17.02.2017

S.Murugaraj,
Finance Officer

SPECIAL OFFICER ★ ALAGAPPA UNIVERSITY ★ KARAIKUDI - 630003

To
The Manager,
Canara Bank,
Karaikudi.

Sir,

Sub: Refund of unspent amount along with interest to UGC, New Delhi – reg.
Ref: UGC MRP F.No.37-14/2009(SR), dt 18..01.2010

****

By direction, I am to inform that a sum of Rs3,146/- (Rupees Three thousand one hundred and forty six only) may be transferred through RTGS to UGC, New Delhi as per details given below from Earmarked Fund SB A/c. No.20751. The relevant cheque No.300733 / 16.02.2017 is enclosed herewith for the below transfer.

Account Holder	:	**Secretary, UGC, New Delhi -110 002**
Branch Name	:	**Canara Bank, UGC Office, New Delhi – 110 002**
A/c.No	:	**8627101002122**
Type of A/c.	:	**Savings**
IFSC Code	:	**CNRB0008627**
MICR Code	:	**110015170**

FINANCE OFFICER
FINANCE OFFICER
ALAGAPPA UNIVERSITY
KARAIKUDI - 630 003.

Copy to:
1. S.O. (Planing & Development), Alagappa University, Karaikudi
- unspent amount along with interest in UGC-Major Research Project- has been refunded to UGC. This may kindly be informed to UGC.

2. Dr.A. Arumugam , Principal Investigator,
Associate Professor, Dept. of Botany, Alagappa University, Karaikudi.

UGC –
Sanction order

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. **37-14/2009 (SR)**

008479

24 JUN 2010

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

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.
"Micro-propagation and germplasm conservation of endangered medicinal plants in Southern India ."

Sir,

I am to refer to your letter forwarding the application of **Dr. A. Arumugam** of your institution for financial assistance under the above scheme and to convey the Commission's approval & sanction an on account grant of **Rs. 6,12,800/- (Rupees: Six lakh twelve thousand eight hundred only)** to the , **Alagappa University, Karaikudi, TN** in r/o Major Research Project of **Dr. A. Arumugam,** Department of **Biotechnology** for the period of **3** years w.e.f. **1.2.2010** as detailed below:-

S.No.	ITEMS	AMOUNT APPROVED	GRANT RELEASED AS Ist INSTALMENT
A.	**Non - Recurring**		**Non-Rec 100 %**
1.	Books & Journals	50,000/-	50,000
2.	Equipment (as per proposal)	2,00,000/-	200000
B.	**Recurring**		**Rec 50 %**
1.	Honorarium to Retd. Teacher @ Rs. 12, 000/- p.m.	nil	
2.	Project Fellow @ 8, 000/- p.m.	2,88,000/-	
3.	Chemical/ Glassware	2,00,000/-	100000 -
4.	Hiring Services/ Consumable	nil	
5.	Contingency	75,000/-	37,500
6.	Travel/Field Work	50,000/-	25000
7.	Overhead Charges @ Rs. 10% approved recurring Grant (Except Travel & Field Work)	56,300/-	**100 %**
	Total (A + B)	**9,19,300/-**	**6,12,800/-**

The acceptance Certificate in prescribed format attached Annexure 1 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 commission by Registered Post in variably with in 15 days from the receipt of the Demand Draft/Cheque.

Principal Investigators should ensure that 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 the University Grants Commission in time.

The final report of the project may be submitted in typed form as well as electronically i.e. in floppy/CD

548.00

4-21 6

1. The sanctioned amount is debitable to the Major Head 4. (i).a and is valid for payment during financial year **2010-11**.
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 University/College, **Alagappa University, Karaikudi, TN** 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. **37-14/2009 (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 ------ 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**
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.

(K.C. Pathak)
Joint Secretary

Copy forwarded for information and necessary action for:-

1. The , **Alagappa University, Karaikudi, TN-630003,**
Acknowledgement for the receipt of DD / Cheque / Mail Transfer for **Rs. 6,12,800/-** may be sent to the Under Secretary, Finance Division III, UGC,
2. **Dr. A. Arumugam,** Principal Investigator, Department of **Biotechnology**
Alagappa University, Karaikudi, TN, 630003
3. office of the Director General of Audit, Central Revenues, A. G. C. R. Building, I. P. Estate, New Delhi.

CEBH 9018011100 / 6,12,800
D^ 3. July 2010

(Kanta Batra)
Under Secretary

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. **37-14/2009 (SR-)**

Dated 28 MAY 2012

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

Sub:- UGC assistance for the Major Research Project entitled, **"Micro.....India."**
Dr. A. Arumugam, Department of **Biotechnology,** tenure of the project from **1.2.2010** to **31.1.2013.**

Sir,

I am directed to convey the sanction of the University Grants Commission to the payment of **Rs. 4,28,560/- (Rupees: Four lakh twenty eight thousand five hundred sixty only)** to **Alagappa University, Karaikudi - 630003** for the Major Research Project of **Dr. A. Arumugam,** Department of **Biotechnology,** as detailed below:

Purpose	Amount Allocated	Amount already released	Amount being released	Total grant sanctioned including the present Instalment	Category
	Rs.	Rs.	Rs.	Rs.	
Books & journals	**50,000/-**				
Equipment	**2,00,000/-**				
*** Project Fellow**	**4,91,733/-**				
Travel/ Field work	**50,000/-**	6,12,800/-	4,28,560/-	10,41,360/-	Gen
Chemicals	**2,00,000/-**				
Contingency	**75,000/-**				
Overhead Charges	**56,300/-**				
Total	**11,23,033/-**	**6,12,800/-**	**4,28,560/-**	**10,41,360/-**	

1. The sanctioned amount is debitable to the Major Head **4. (i). a (31)** and is valid for payment during financial year **2012-2013** only.
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 **Alagappa University, Karaikudi - 630003** through Cheque/Demand Draft/ Mail Transfer.
3. The Grants is subject to the adjustment of the basis of Utilization Certificate in the prescribed proforma 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.

* **As per revised rates w.e.f. 1.4.2010.**

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. 5,77,507/-** out the grant of **Rs. 6,12,800/-** sanctioned vide letter No. F. **37-14/2009** dated **24.6.2010** has been utilized by University/College/Institution for the 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. 5,77,507/-** S. No. ....420... and in the U. C. Registrar at page no. ....148....

14. It is also certified from the B.C.R. that the funds are available under the scheme. Entered in BCR at P.48 S. No ....420... The above grant is sanctioned against the budget provision of **Rs. 41 Crore** during the current financial year leaving a balance of **Rs.** ..... under the head of Account **4. (i) . a (31)**

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.

(Balbir Bhatia)
Under Secretary

Copy forwarded for information and necessary action for:-

1. The Registrar, **Alagappa University, Karaikudi - 630003 .**
   Acknowledgement for the receipt of DD / Cheque / Mail Transfer for **Rs. 4,28,560/-** may be sent to the Under Secretary, Finance Division III, UGC,
2. Dr. A. Arumugam, Principal Investigator,
   Department of **Biotechnology,**
   Alagappa University, Karaikudi - 630003 .
3. Office of the Director General of Audit, Central Revenues, A. G. C. R. Building, I. P. Estate, New Delhi.
4.

(Pramod Sharma)
Section Officer

Summary of the Project Findings

Report of the work done: (Please attach a separated sheet)

(I) **Brief objectives of the project**

1. To develop micropropagation systems for selected endangered medicinal plants and to propagate them throughout tissue culture techniques.

Name of Endangered Medicinal Plants

(i) *Withania somnifera*
(ii) *Aristolochia indica*
(iii) *Gloriosa superba*
(iv) *Cassia alata*
(v) *Plumbago zeylanica*
(vi) *Rauvolfia tetraphylla*
(vii) *Tinospora cordifolia*

Action Plan:

Following action plan was prepared to carry out the work.

1. Identification of proper explants and season and different plant tissues for *in vitro* responses.

2. Multiplication of the initiated cultures and optimization of the medium and culture conditions.

3. The method of regeneration through tissue culture has been standardized.

4. Primary and secondary hardening nursery of tissue culture.

5. Transfer of plants from *in vitro* to *in vivo*

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

Publication Details

Published articles

1. **A. Arumugam**, and **K. Gopinath**. 2011. Micro propagation and tissue culture of theendangeredmedicinal plant *Withania somnifera* by the direct shoot and root initiation method. *International Journal of Applied biology and Pharmaceutical Technology*. 2 (3): 315-321.
2. **A. Arumugam** and **K. Gopinath**, 2012. *In vitro* Micropropagation using Corm Bud Explants:An Endangered Medicinal Plant of *Gloriosa superba* L. *Asian Journal of Biotechnology*, 4:120- 128.
3. **Kasi Gopinath** and **Ayyakannu Arumugam**, 2012. Micropropagation and in vitro micro rhizome initiation of *Gloriosa superba* L. (an endangered medicinal plant). *Asian Pacific Journal of Tropical Biomedicine*. 1: 1-6.
4. **K. Gopinath** and **A. Arumugam,** 2012**.** Effect of Temperature and pH on the *Gloriosa superba* L. pollen fertility. *Advanced BioTech*:12(06):15-18.
5. **A. Arumugam** and **K. Gopinath,** 2013. *In vitro* regeneration of an endangered medicinal plant of *Withania somnifera* using four different explants. Plant Tissue Cult. & Biotech. 23(1): 79-85.
6. **K. Gopinath**, K.S. Venkatesh, R. Ilangovan, K. Sankaranarayanan, **A. Arumugam**, 2013. Green synthesis of gold nanoparticles from leaf extract of *Terminalia arjuna*, for the enhanced mitotic cell division and pollen germination activity. Industrial crops and products, 50, pp.737-742.
7. **K. Gopinath**, V. Karthika, S. Gowri, **A. Arumugam**, 2014. In vitro Morphogenetic Regeneration from Root Explant of *Gloriosa superba* L. for Enhanced Crop Production. The Scitech Journal, 1, pp.27-30.
8. **K. Gopinath**, C. Karthikeyan, A.H. Hameed, K. Arunkumar, **A. Arumugam**, 2015. Phytochemical synthesis and crystallization of sucrose from the extract of *Gloriosa superba*. Research Journal of Phytochemistry, 9, 144-160.
9. S. Umavathi, **K. Gopinath**, M.S. Manjula, B. Chinnasamy**, A. Arumugam**, 2020. *Gloriosa superba* L: A critical Review of Recent Advances. Abasyn Journal of Life Sciences, 3(2), 48-65.

Final Report of the Project

13. SUMMARY OF THE FINDINGS: :
(IN 500 WORDS)

* *Withania somnifera, Aristolochia indica, Gloriosa superba Cassia alata, Plumbago zeylanica Rauvolfia tetraphylla* and *Tinospora cordifolia,* this seven endangered medicinal plants have been successfully regenerated in to the micro propagation methods.

* Different explants viz., leaf, stem, root, nod, apical meristem, corm bud, coytyledon, hypocotyls, epicotyls were used in the tissue aspect.

* *In vitro* regenerated plnatlets were transferred into the polythene bags which contain a sterile cow dung, sand, and red soil in the ratio of 1:2:3 (or) vermi compost, sand, and red soil in the ratio of 1:2:2 and were kept in a mist house. After acclimatization in the mist house for 2-months, plantlets were hardened in the greenhouse and successfully transferred to the field.

* Dried samples (stem, leaf, root and rhizome) were ground in a grinder with a 2 mm diameter mesh. Dried and powdered sample 20g and 350 ml of extraction with four different solvents like methanol (65°C), acetone (57°C), chloroform (62°C) and petroleum ether (35°C).

* Isolated phytocompound were analyzed by UV, FTIR and GC. UV- vis absorption band to indicates the secondary metabolites of withanolides and colchicine. The FTIR analysis to find out the functional groups (or) organic molecules present in the samples (phytocompounds) and GC analysis to find the presence of alkaloids and secondary metabolites.

* We have successfully isolated for the sucrose crystal derived from the hot MeOH extract of the rhizome powder of *G. superba*. This condensed extract has been used to grow single crystals from aquseous MeOH solvent. The grown crystal has been characterized with single crystal X-ray diffraction, X-ray diffraction, NMR spectroscopy, TG-DTA, Micro Raman, FTIR and UV-visible spectroscopy studies, To our knowledge, this is the first report for the phytochemical synthesis of new sucrose crystal derived from the hot MeOH extract of the rhizome powder of *G. superba.*

Plant Tissue Culture

Methodology

Plant Collection and identification

Fist the endangered medicinal plants and seeds were collected from the wild and cultivate places.

1. Irula Tribe Women's Welfare Society (ITWWS) in 2011, Kanchipuram
2. Nathapalayam, Tamil Nadu,
3. Karaikudi, Tamil Nadu,
4. Arimalam, (Pudukkottai) Tamil Nadu.

The taxonomic identification was made by Dr. S. John Britto, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Campus), Tiruchirappalli, Tamil Nadu, India. The voucher specimen was numbered and herbarium kept in our Department of Nanoscience and Technology.

S.No	Name of Endangered Medicinal Plants	Voucher Specimen Number
1	*Withania somnifera*	KG-003
2	*Aristolochia indica*	KG-002
3	*Gloriosa superba*	KG-001
4	*Cassia alata*	KG-006
5	*Plumbago zeylanica*	KG-004
6	*Rauvolfia tetraphylla*	KG-005
7	*Tinospora cordifolia*	KG-007

Surface sterilization

Different explants viz., leaf, stem, root, nod, apical meristem, corm bud, coytyledon, hypocotyls, epicotyls were used. This explants were washed thoroughly with running tap water for 20 minutes to remove microbes and soil particles, then they were cleansed with liquid detergent Tween 20 (1% v/v) for 5-10 minutes and rinsed with sterile double distilled water. They were then surface sterilized with 0.01% ($HgCl_2$ w/v) solution for 1-2 minutes and again washed well in distilled water 3-4 times to remove all traces of $HgCl_2$ (Mercuric chloride).

Inoculation

The surface sterilized explant were then aseptically inoculated on sterile MS medium comprising 3% sucrose as carbon source and 0.8% agar as solidifying agent. The medium was also supplemented with various growth regulators (2,4-D, NAA, IAA, IBA, BAP, IPA, BA, GA_3, Zen, AC and CW) adjusted to pH 5.8 ± 0.1 with 0.1 N NaOH or 0.1 N HCl before autoclaved at 121°C and 15 lb for 20 minutes.

Culture conditions

All cultures were maintained at 22±1°C under 16 hours photoperiod at a photosynthetic flux of 12.6µ mol m^{-2} s^{-1}, provided by cool daylight fluorescent lamps.

Hardening

The rooted plantlets were removed from the culture flasks. They were gently washed with sterile distilled water to remove the adhering agar and transferred into the polythene bags which contain a sterile cow dung, sand, and red soil in the ratio of 1:2:3 (or) vermi compost, sand, and red soil in the ratio of 1:2:2 and were kept in a mist house. After acclimatization in the mist house for 2-months, plantlets were hardened in the greenhouse and successfully transferred to the field.

Statistical analysis

All the experiments were repeated for five times. The data shown represent the mean ± SE. The data were statistically analyzed using the one-way analysis of variance (ANOVA) and significant differences between the means.

Phytochemical Analysis

Material and Methods

Glasswares

Borosil / Corning brand glassware's were used. Glassware's were washed in detergent and soaked in acidified potassium dichromate solution before washing thoroughly in running tap water. They were then rinsed with distilled water and dried in hot air oven.

Water

Double distilled water from a borosil glass distillation unit was used for media preparations and other experimental purposes.

Chemicals

Potassium Bromide (KBr), Methanol, Acetone, Chloroform and Petroleum ether, were bought from SRL chemicals were used for all experiments.

Sterilization

Dried glassware and media were sterilized in an autoclave for 15 min. at 15 lb. /sq inch pressure.

Sample collection and Soxhlet extraction

Tissue culture regenerated endangered medicinal plant material was collected and all parts of the plant were removed carefully and shaken to remove unwanted particles like sand and soil. Drying was done under shade, because shade drying was preferred to avoid denaturing of phytochemical constitution. Dried samples (stem, leaf root and rhizome) were ground in a grinder with a 2 mm diameter mesh. Dried and powdered sample 20g and 350 ml of extraction with four different solvents like methanol (65°C), acetone (57°C), chloroform (62°C) and petroleum ether (35°C). The powdered plant material was subjected to extraction in a soxhlet apparatus for 6 hours, each using methanol, acetone, chloroform and petroleum ether. The excess solvent was evaporated with the rotary

evaporator. Final concentration 60 ml of solvent mixed compound were filtered through Whatman No. 1 filter paper and stored at 30 ml of vials.

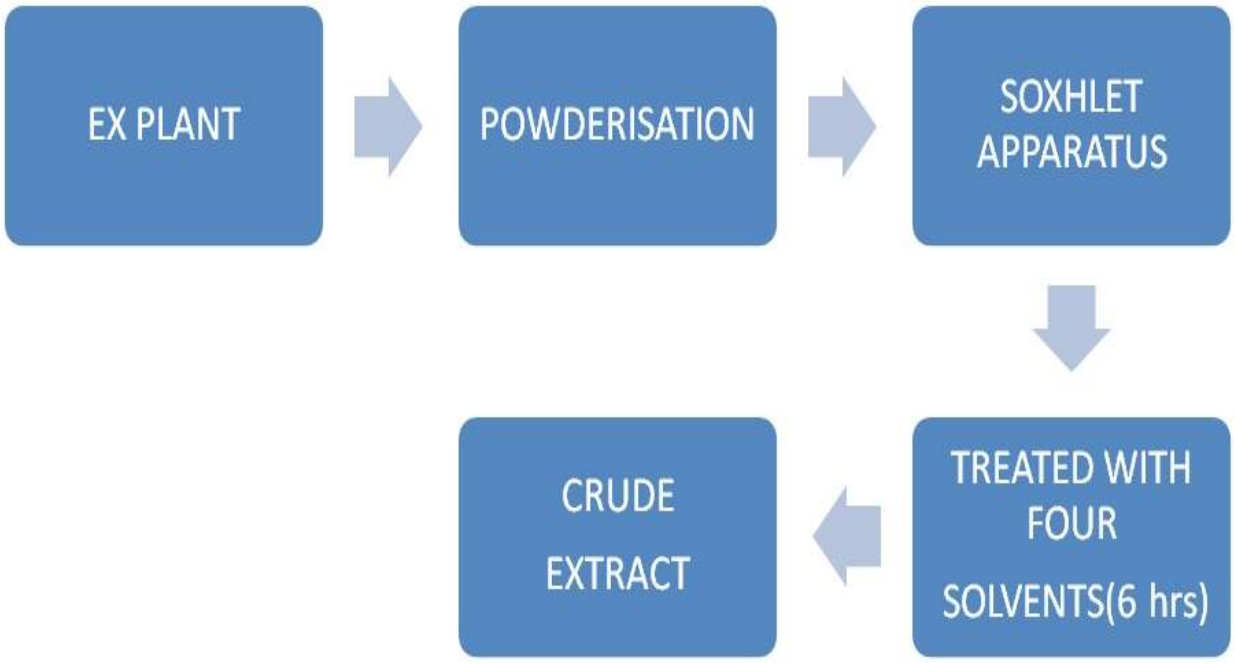
Crystal Growth

Different crystals were successfully grown by the slow cooling method. It was initiated by temperature at a rate of 3°C per day down to 45°C, More then 40 colorless single crystals (0.35 x 0.30 x 0.20 mm size) and different size of crystal were obtained from after 7 days.

Characterization

UV – visible spectra of the phytosynthesized samples were obtained for the wavelength in the range of 200–800 nm using Shimadzu spectrophotometer (Model UV-1800) operating at a resolution of 1 nm. Fourier Transform Infrared Spectroscopy (FTIR) measurements were prepared by different sample powder with KBr. This powder is then compressed into a thin pellet 12 mm in diameter and about 15 mg in weight. Spectrum was recorded in the 4000–400 cm^{-1} region. GC-MS and GC chromatograph analysis measures the content of various components in a sample.

Graphical abstract :



1. Plant Name : *Withania somnifera* (L.) Dunal

2. Botanical classification :

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Solanales
Family	Solanaceae
Genus	*Withania*
Species	*somnifera*

3. Vernacular Names:
Ashwagandha, winter cherry, Ashgandh, Achuvagandi, Amikkira-gadday, Amkulang-kalang, Amukkira-kilzhangu, Amukran-kizhangu, Asagandha, Asana, Asgandh, Asundha, Asvagandhi, Fatarfoda, Hirimaddina-gadday, Hirre- gadday, Penneroo-gadda, Pevette, Sogade-beru.

4. Major Alkaloids:
Somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropino, pseudotropine, choline, cuscohygrine, isolettetierine, anaferine, anahydrine, 3-alpha-gloyloxy tropane.

5. Description Medicinal Property:
According to Ayurveda, the root is bitter, acrid, heating, aphrodisiac, tonic, alternative, anthelmintic and useful in treatment of inflammations, psoriasis, bronchitis, asthma, consumption, ulcers, scabies, marasmus of children, insomnia, senile debility etc. According to Unani system of medicine, the root is bitter, tonic, aphrodisiac, emmenagogue, good in asthma, inflammations, leucoderma, bronchitis, lumbago, thirst etc.

Ashwagandha is a rejuvenating drug used for rheumatism, hypertension, and as a tonic. It shows antitumour, bradycardic, respiratory stimulant and antispasmodic properties. Withanolides also show antitumour, antiarthritic and immunosuppressive properties (Sudhir *et al,* 1986)

6. Useful parts:
Root,leaves,green-berries and seeds.

7. Micropropagation aspects:
In our study the result shows *W. somnifera* micropropagation and multiplication protocol was developed by leaf explants.

8. Results

* The leaf and stem explant shows most significant effect of 100% response in callus initiation at MS+2,4-D (3 & 4 mg/l)+IAA (2.5 & 3.5mg/l).

* But, 88% of multiple root formation observed with leaf callus on media containing NAA (5 mg/l) and 85% of multiple shoot formation observed with leaf callus on media containing BAP (4 mg/l).

* The regenerated plants (rooted shootlets) were successfully hardened and acclimatized, 90% of plantlets survived well under natural conditions after transplantation.

* Finally, In our study the result shows *W. somnifera* micropropagation and multiplication protocol was developed by leaf explant.

* This protocol can be used as an efficient tool for rapid multiplication, conservation and maintenance of germplasm of this important medicinal plant species.

Effect of different concentration of 2,4-D, IAA and NAA callus induction in leaf and stem explants of *W. somnifera.*

Two different explants of *Withania somnifera* were used at different concentrations of 2, 4-D, IAA and NAA in MS for callus initiation. Combination of (3.0 -10 mg/L) 2,4-D and (2.5- 5.0mg/L) IAA in MS produced maximum number of callus (Table-1). In addition of NAA to inhibited the callus formation only minimum number of calls were produced leaf and stem explant (Table - 1). The multiple callus were observed at MS medium with combination of (4.0 + 2.00 + 1.0 + 1.25 mg/L) BA+BAP+IAA+NAA (Table -2).

Effect of various explants used to different contraction of 2, 4-Dichlorophenoxyactic acid with MS media on callus induction (%):

Callus initiation was observed within 3 to 4 weeks on MS media supplemented with 2, 4-D (0.1-20 mg/L). The response shown by different explants varied widely depending on the concentration of 2-4-D (Table-3). The overall callus induction frequency on MS medium varied from 42-93 percent with leaf explants, while in hypocotyl, epicotyl and cotyledon the frequency range was observed in the order of 36-84, 25-89 and 32-80 percent respectively. The optimum frequency of callus induction was elicited with the supplementation of 1.5-3.0 mg/L of 2, 4-D more or less uniformly for all the explants (Figure: A & B). Among the 4 explants maximum callus induction was observed with leaf explants followed by the L > E > H > C. Different concentration of 2, 4-Dichlorophenoxyactic acid with MS

media - Leaf induction of callus fresh and dry weight: The explants of leaf maximum callus yield was obtained on MS medium with different concentration of 2,4-D after 4 week (Table-4). The fresh and dry weight data indicated that, the optimum levels of 2, 4-D required for maximum yield of callus ranged from 1.5-3.0 mg/L.

Effect of different explants and Benzyl Aminopurine (BAP) with MS media on Shoots Induction (%):

Benzyl amino purine (BAP) is a synthetically produced N6-adenine compound. BAP is a synthetic cytokinin with a capacity to promote cell division and influences steps in the cell cycle for a long time has been considered. The addition of BAP is necessary to obtain callus and shoot growth. The level of BAP required for optimum shoot formation may be (3.0-5.0mg/L) but this depends upon the type of explants (Figure 1: C) Anatomical study of callus cross section developed on the shoot (Figure 1: G & H), The leaf explants callus produced highest shoot formation 6-85% (Table-6). Compare to order of 23-80, 8-52 and 6-19 respectively L > C> H >E.

Effect of different explants and Naphthalene acetic acid (NAA) with Ms media on Roots induction (%):

Callus initiation occurred in leaf, cotyledon, hypocotyl and epicotyl explants on MS media supplemented with different concentrations of NAA (0.1-10.0mg/L) (Table-5). After 4 weeks of induction, different explants were varied in callus roots induction response depending on the levels of NAA. In lower concentration of NAA (0.1-0.5mg/L) callus did not produced root induction. A highest root formation was obtained by leaf explants up to 88% at 5-10mg/L NAA concentration (Figure1: D). Compare to hypocotyl, epicotyl and cotyledon the frequency range was observed in the order of 0-85, 0-87 and 0-50 respectively L > E > H > C.

Histological analysis of shoot development:

Histologial analysis of the regenerating shoots was performed on explants at different stages of development and showed that the shoot buds had emerged from epidermal parenchymal cells, with no intermediate callus formation (Figure 1-G). After 14 days of culture, the shoot buds showed well developed leaf primordia and apical meristem (Figure 1-H). The meristematic cell were much samllar than the surrounding cells, which consisted of closely arranged and highly cytoplasmic cells.

Table 1. Effect of different concentration of 2,4-D, IAA and NAA callus induction in leaf and stem explants of *W. somnifera*

S.No	**MS medium with 2,4-D+ IAA (mg/l)**	**Leaf Callus initiation %**	**Leaf callus morphology**	**Stem callus initiation %**	**Stem callus morphology**
1	1.0 + 0.5	**50**	**B.C**	**50**	**B.C**
2	1.5 + 1.0	**50**	**B.C**	**50**	**B.C**
3	2.0 + 1.5	**50**	**B.C**	**50**	**B.C**
4	2.5 + 2.0	**50**	**B.C**	**50**	**B.C**
5	3.0 + 2.5	**100**	**B.C**	**100**	**B.C**
6	3.5 + 3.0	**50**	**B.C**	**25**	**B.C**
7	4.0 + 3.5	**100**	**B.C**	**100**	**B.C**
8	4.5 + 4.0	**50**	**B.C**	**25**	**B.C**
9	5.0 + 4.5	**50**	**B.C**	**100**	**B.C**
10	10.0 + 5.0	**100**	**B.C**	**100**	**B.C**
	MS medium with 2,4-D + IAA + NAA (mg/l)				
11	0.5 + 0.5 + 1.0	**50**	**G.C**	**50**	**B.C**
12	1.0 + 1.0 + 1.5	**-**		**-**	
13	2.0 + 1.5 + 2.0	**-**		**-**	
14	3.0 + 2.0 + 2.5	**-**		**-**	
15	4.0 + 3.0 + 3.0	**50**	**B.C**	**50**	**B.C**
16	5.0 + 4.0 + 3.5	**50**	**B.C**	**50**	**B.C**
17	7.5 + 5.0 + 4.0	**-**		**-**	
18	10.0 + 7.5 + 4.5	**-**		**-**	
19	15.0 + 10.0 + 5.0	**-**		**-**	
20	20.0 + 15.0 + 10.0	**-**		**-**	

G.C: Green Callus **B.C**: Brown Callus

Table 2. Effect of different concentration growth regulators and callus multiplication with leaf and stem explant

S.No	MS medium with BA+BAP+IAA+NAA (mg/l)	Leaf callus initiation %	Stem callus initiation %
1	0.5 + 0.50 + 1.0 + 0.50	75	25
2	1.0 + 0.75 + 1.0 + 0.75	**100**	25
3	2.0 + 1.00 + 1.0 + 0.90	50	50
4	3.0 + 1.50 + 1.0 + 1.00	50	50
5	4.0 + 2.00 + 1.0 + 1.25	**100**	**100**
6	5.0 + 2.50 + 1.0 + 1.50	25	-

– - Not response

Table 3. Effect of various explants used to different concentration 2,4-Dichlorophenoxyactic acid with MS media on callus induction (%)

S.No	MS medium with 2,4-D (mg/l)	L - callus initiation %	C - callus initiation %	H - callus initiation %	E - callus initiation %
1	0.1	42	42	62	25
2	0.5	58	48	48	39
3	1	72	45	62	45
4	1.5	80	60	65	72
5	2	92	62	72	82
6	3	93	80	84	89
7	4	80	65	62	87
8	5	69	48	43	78
9	10	65	32	36	46
10	20	-	-	-	-
	SD ± SE	**16..28 ± 5.85**	**14.47 ± 5.16**	**14.84 ± 5.32**	**23.84 ± 7.94**

L- Leaf, C -Cotyledon, H - Hypocotyl, E – Epicotyl, – - Not response. SD – Standard Deviation, SE – Standard Error

Table 4. Different concentration 2,4-Dichorophenoxyactic acid with MS media leaf explants induction of callus fresh and dry weight.

S.No	MS medium with 2,4-D (mg/l)	Leaf callus F.W/g	Leaf callus D.W/g
1	0.1	169.5	25.03
2	0.5	325.2	31.2
3	1	366.2	50.2
4	1.5	354.2	54.3
5	2	337.8	58.2
6	3	375.2	48.3
7	4	325.2	34.8
8	5	332	35.4
9	10	323.2	25.1
10	20	-	-
	SD ± SE	**58.79 ± 19.43**	**12.64 ± 4.49**

F.W – Fresh weight, D.W – Dry weight , – - Not response, SD – Standard Deviation, SE – Standard Error.

Table 5. Effect of different explants and Naphthalene acetic acid (NAA) with ms media on roots induction (%)

S.No	MS medium with NAA (mg/l)	L - Roots initiation %	C - Roots initiation %	H - Roots initiation %	E - Roots initiation %
1	0.1	-	-	-	-
2	0.5	-	-	22	-
3	1	-	10	54	50
4	1.5	25	12	54	54
5	2	52	18	65	70
6	3	54	22	70	72
7	4	65	47	82	75
8	5	88	45	85	87
9	10	88	50	70	85
10	20	26	24	26	22
	SD ± SE	**25.83 ± 9.47**	**16.31 ± 5.71**	**22.33 ± 7.46**	**21.54 ± 7.66**

L- Leaf, C -Cotyledon, H - Hypocotyl, E – Epicotyl, – - Not response. SD – Standard Deviation, SE – Standard Error.

Table 6. Effect of different explnts and Benzyl Aminopurine (BAP) with MS media on shoots induction (%)

S.No	MS medium with BAP (mg/l)	L- Shoots initiation %	C - Shoots initiation %	H - Shoots initiation %	E - Shoots initiation %
1	0.1	-	23	25	-
2	0.5	22	65	26	-
3	1	25	25	32	6
4	1.5	28	58	35	8
5	2	34	65	52	12
6	3	52	49	48	13
7	4	85	80	39	19
8	5	82	72	20	18
9	10	16	50	12	14
10	20	6	52	8	6
	SD ± SE	**28.24 ± 9.11**	**18.57 ± 6.03**	**14.39 ± 4.65**	**5.04 ± 2.06**

L- Leaf, C -Cotyledon, H - Hypocotyl, E – Epicotyl, – - Not response. SD – Standard Deviation, SE – Standard Error.

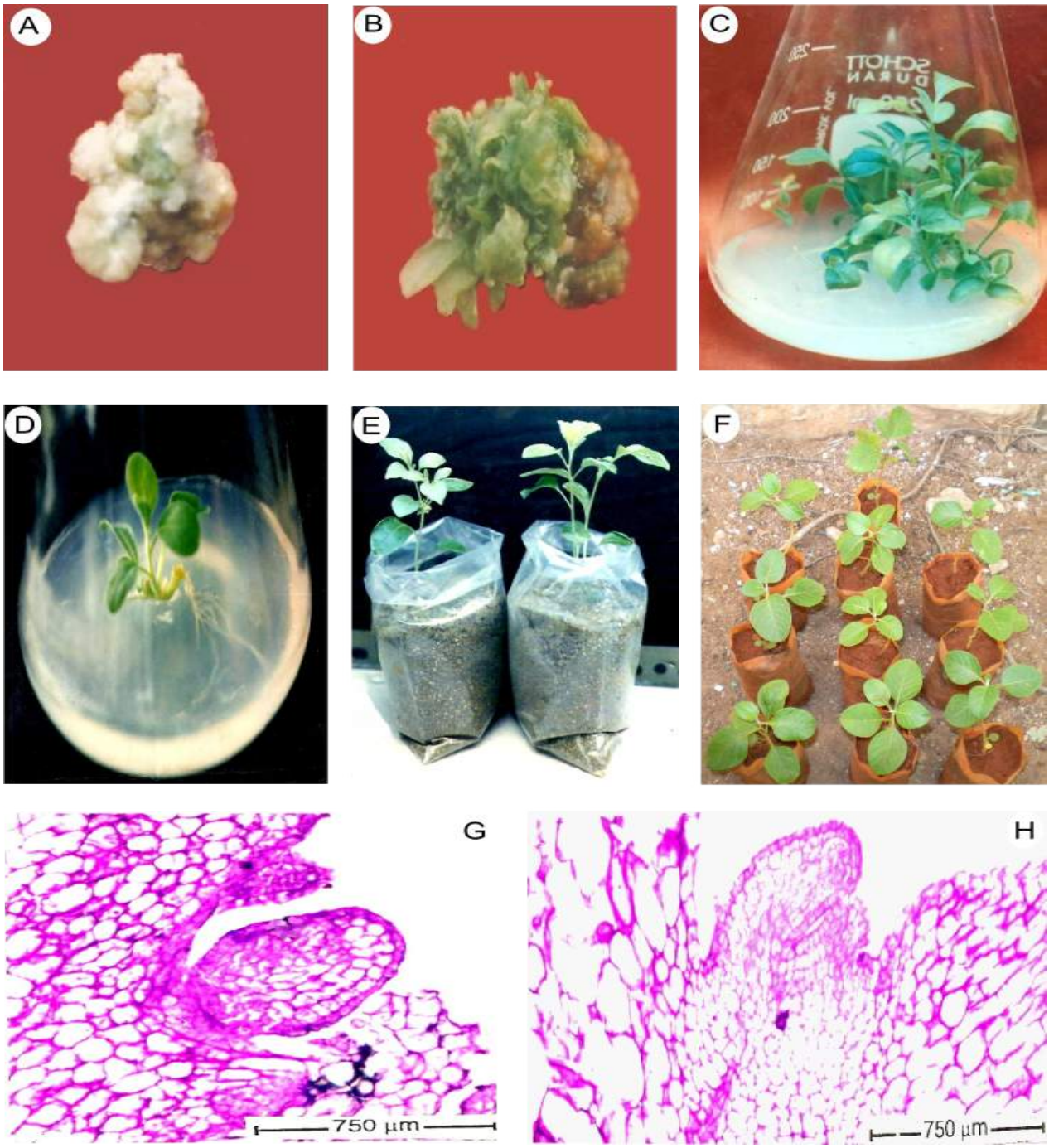
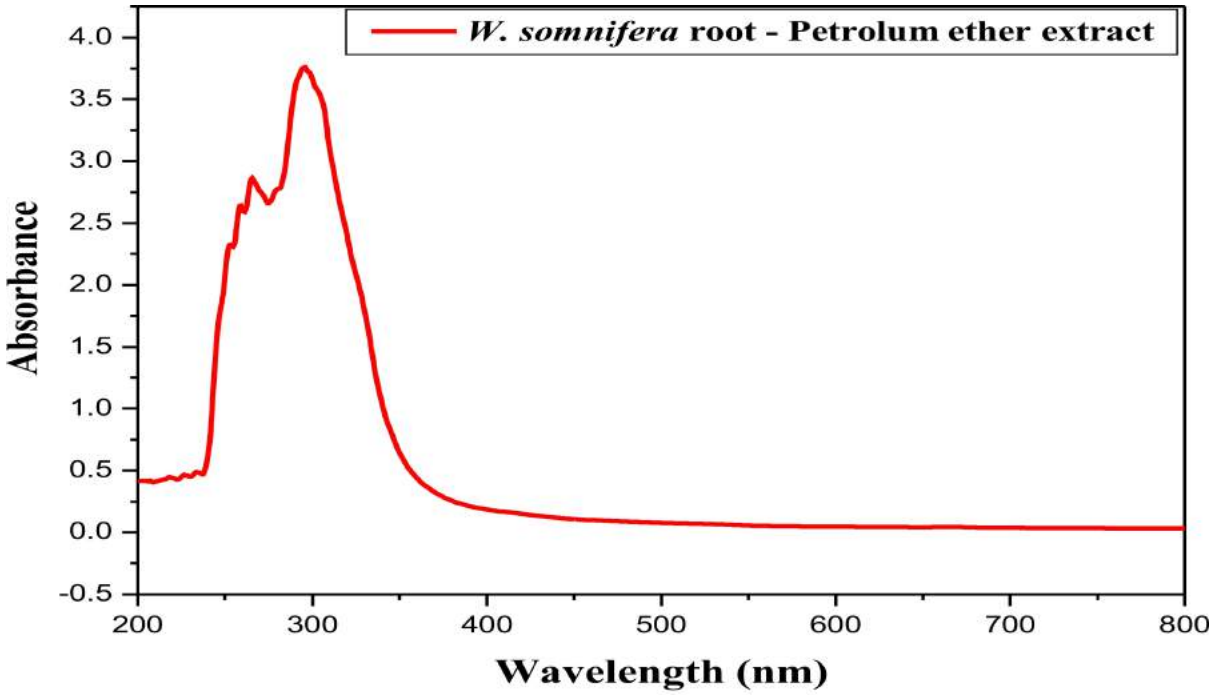


Figure: A – *Withania somnifera* leaf callus, **B** - Callus regeneration, **C** - Multiple shoots induction, **D** - Roots induction, **E** - Plantlets were transplanted with polythene bags to acclimatization for 2 month in mist house, **F** - Plant hardening for in vivo condition, **G –** Cross section showing meristematic region after two weeks culture, **H** - Cross section of shoot bud connected to leaf explant showing leaf primodia and the shoot eapical meristem,

1. UV analysis

Figure 1.1



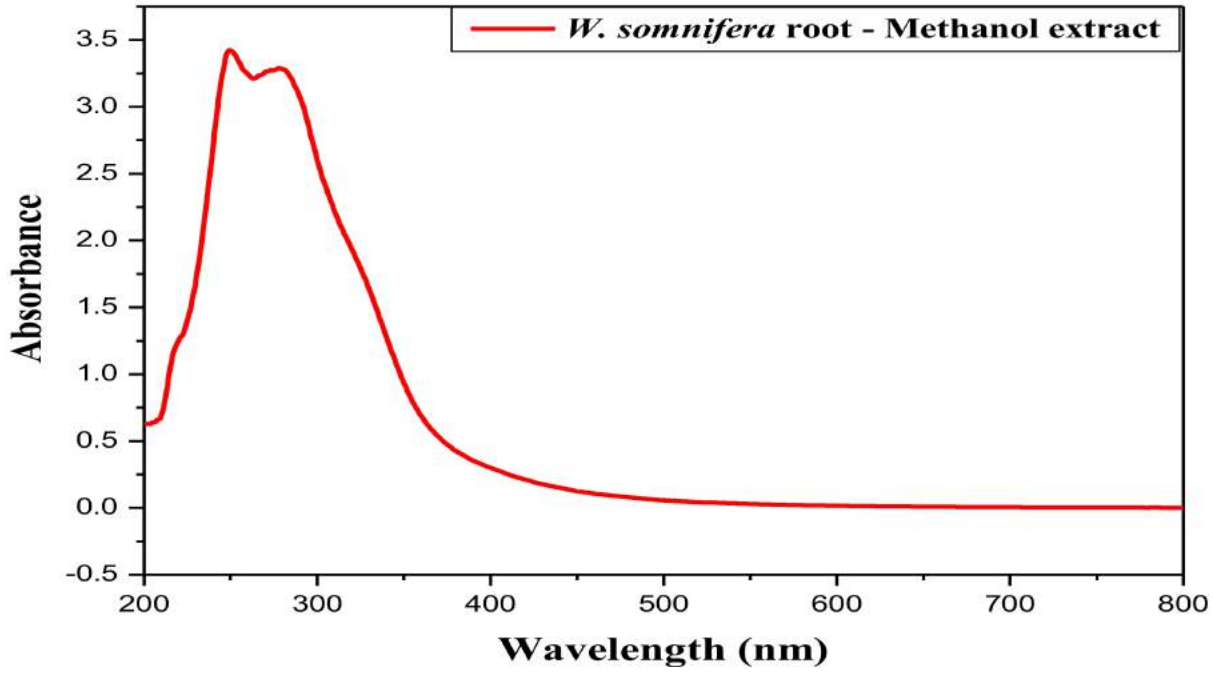


Figure 1.2

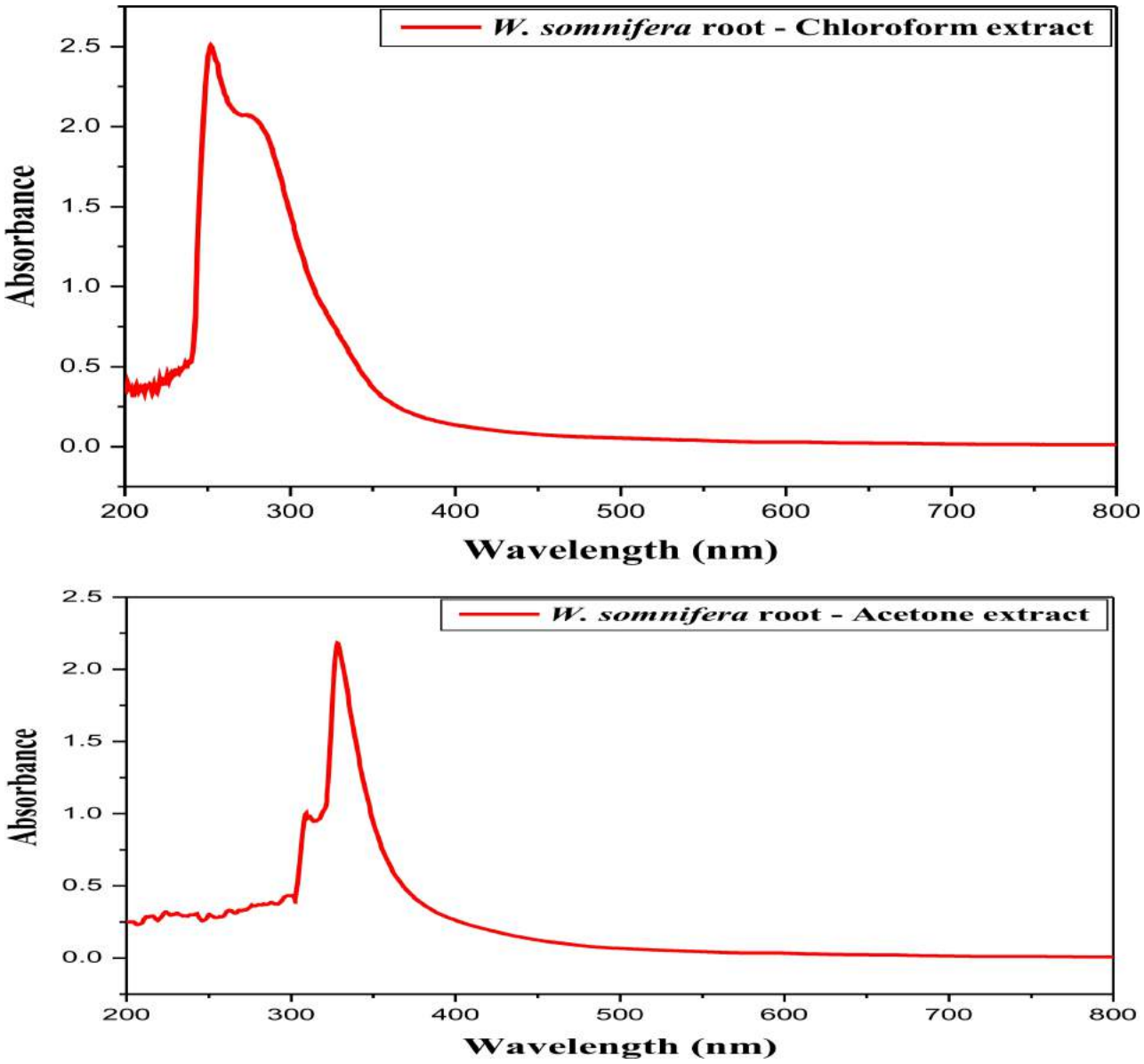
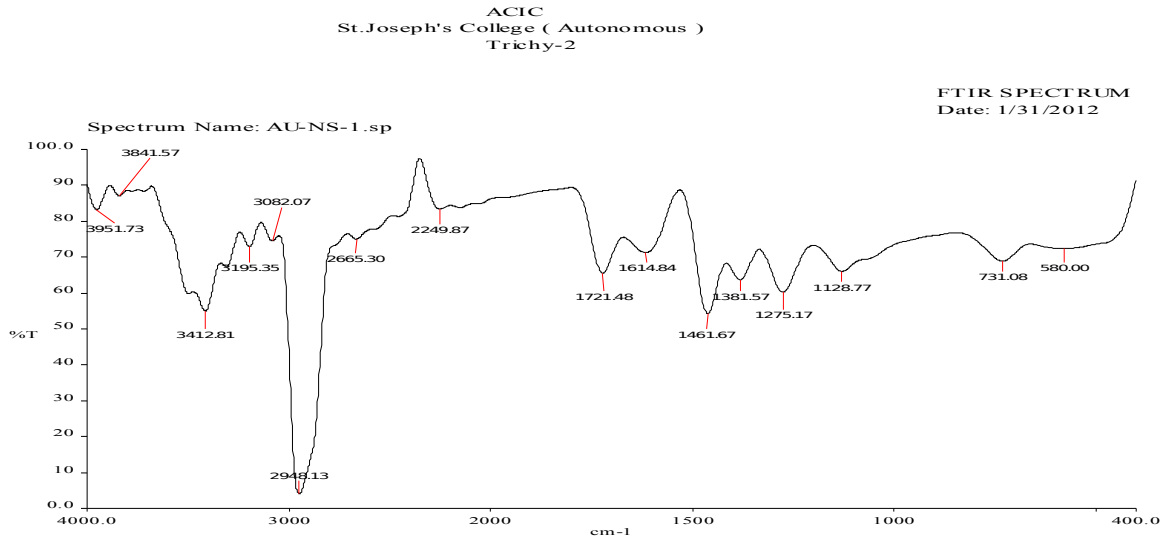


Fig 1.1 -1,2 *Withania somnifera* root extract in four different solvents were subjected to UV–vis spectroscopy analysis in the recorded spectra and it showed a observable peaks at 205 - 395 nm which corresponds to the withanolides. Various reports have established that the resonance peak of appears around this region for 27-Hydroxy withanone, 17-hydroxy withaferin A, 17-hydroxy 27-deoxy withaferin A, withaferin A, withanolide D, 27-hydroxy withanolide B, withanolide A, withanone, 27-deoxy withaferin A.

2 FTIR analysis

Figure 2.1 *Withania somnifera* Root - Petroleum ether extract

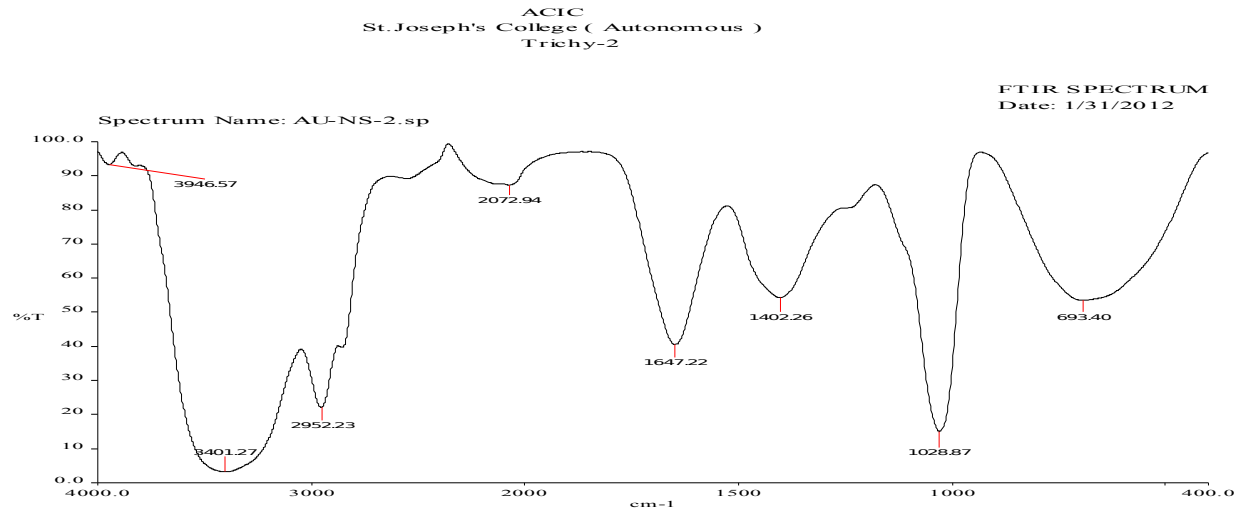


AU-NS-1.pk

AU-NS-1.sp 1801 4000.00 400.00 4.00 97.40 4.00 %T 18 1.00

REF 4000 90.23 2000 86.30 600
3951.73 83.23 3841.57 87.07 3412.81 55.00 3195.35 73.02 3082.07 74.50
2948.13 4.00 2665.30 75.03 2249.87 83.45 1721.48 65.51 1614.84 71.35
1461.67 54.26 1381.57 63.65 1275.17 60.29 1128.77 66.02 731.08 68.91
580.00 72.37

Figure 2.2 *Withania somnifera* Root - Methanol extract

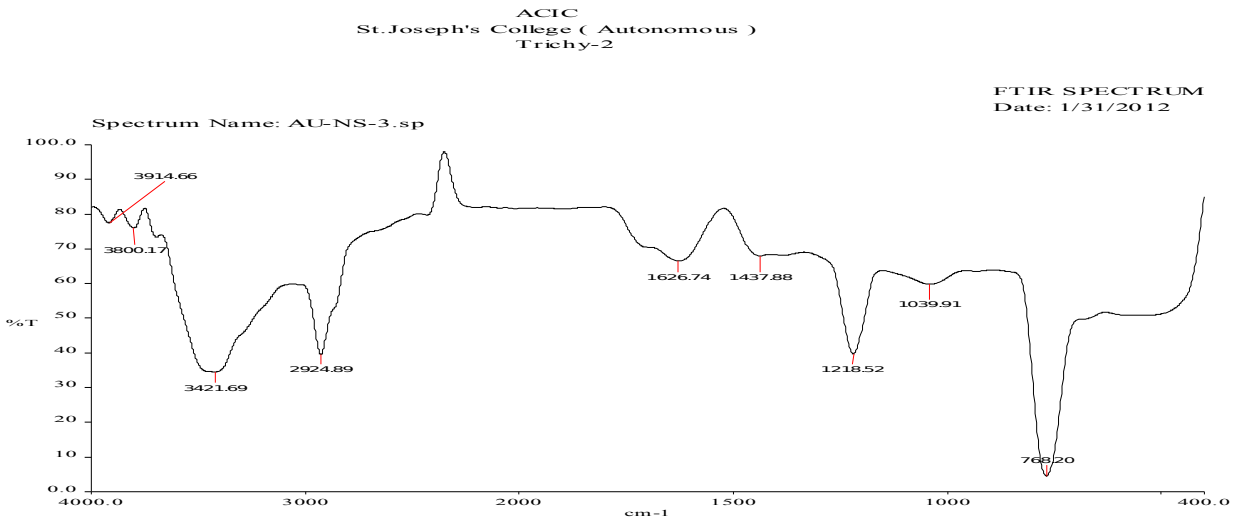


AU-NS-2.pk

AU-NS-2.sp 1801 4000.00 400.00 3.19 99.31 4.00 %T 18 1.00

REF 4000 97.15 2000 92.02 600
3946.57 93.32 3401.27 3.19 2952.23 22.13 2072.94 87.28 1647.22 40.57
1402.26 54.28 1028.87 15.09 693.40 53.50

Figure 2.3 *Withania somnifera* Root - Chloroform extract

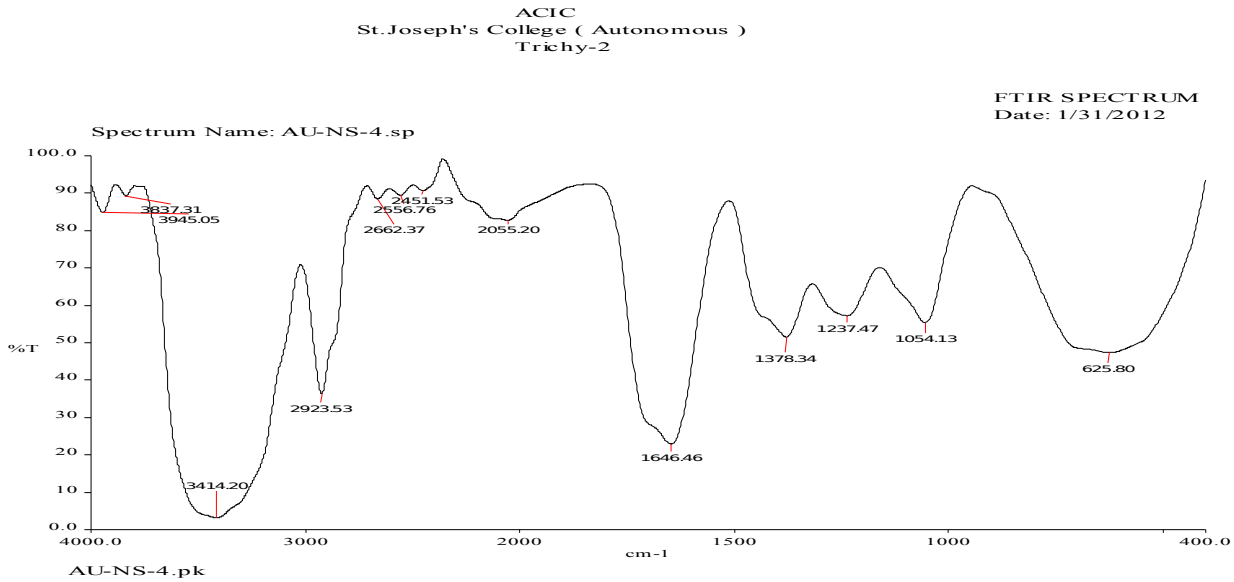


AU-NS-3.pk

AU-NS-3.sp 1801 4000.00 400.00 4.49 97.97 4.00 %T 18 1.00

REF 4000 82.02 2000 81.66 600
3914.66 77.48 3800.17 76.01 3421.69 34.55 2924.89 39.54 1626.74 66.47
1437.88 67.97 1218.52 39.75 1039.91 59.77 768.20 4.49

Figure 2.4 *Withania somnifera* Root - Acetone extract



AU-NS-4.pk

AU-NS-4.sp 1801 4000.00 400.00 3.22 99.23 4.00 %T 18 1.00

REF 4000 92.28 2000 85.38 600
3945.05 84.88 3837.31 89.28 3414.20 3.22 2923.53 36.46 2662.37 88.45
2556.76 89.39 2451.53 90.68 2055.20 82.76 1646.46 22.93 1378.34 51.50
1237.47 57.20 1054.13 55.33 625.80 47.30

FTIR - Result

Fig 2.1 - 2.4 FTIR analysis was performed to identify the possible biomolecules present in the *Withania somnifera* root extract in four different solvent in methanol, acetone, chloroform and petroleum ether extract. The strong IR bands were observed at 3382, 2922, 2337, 1614, 1384, 1070 and 590 cm^{-1}. The bands appeared at 3382 and 2922 cm^{-1} are corresponds to -OH stretching and aliphatic -C-H stretching respectively. The bands at 2337 and 1613 cm^{-1} are due to the CO_2 and C=C stretching respectively. The IR bands observed at 1384 and 1070 cm^{-1} may be ascribed to -C-O and -C-O-C stretching modes respectively. The low band at 590 cm^{-1} corresponds to C-Cl stretching.

1. Plant Name : *Aristolochia indica* L.

2. Botanical classification :

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Aristolochiales
Family	Aristolochiaceae
Genus	*Aristolochia*
Species	*indica*

3. Vernacular Names:
Birthwort, *Aristolochia indica*, Indian Birthwort, Ishvari, Gandhnaakuli, Naagadamani, Adagam, IsarmuulaI, srola, Adu theenda palai.

4. Chemical Constituents:
Aristolochic acids, volatile oil and tannins. Root: A crystalline substance-probably a glucoside, isoaristolochic acid, allantoin, 0.05% carbonyl compounds and a small amount of an oil, with the odour of isovanillin, ishwarone, ishwarane, aristolochene.

5. Description Medicinal Property:
Oxytocic, abortifacient, emmenagogue. Roots possess emmenagogue, antiarthritic and emitic properties and are also used against leprosy. Aristolochic acid is found to be anticancerous but also causes renal damage. Aristolic acid showed antifertility effected in rabbits, Aristolochic acid is not a true isoquinoline alkaloid, but because it is presumed to be fromed due to oxidation of an aporphine nucleus,.

6. Useful parts:
Root and aerial parts.

7. Micropropagation aspects:
In our methods shows that *Aristolochia indica* micropropagation and mass propagation by using the leaf explants.

8. Results

* The leaf and stem explant were used for callus initiation medium. But, only leaf explant alone shows most significant effect of 100% response in callus initiation at MS+2,4-D (0.5-5.0mg/l) +IAA (0.5 - 4.0mg/l)+NAA (1 -3.5mg/l).

* BAP, Kinetin and Coconut water combination was not useful for shoot formation, only for callus initiation of leaf and stem explant. BAP (2 -3mg/l) plays good role in greenish shoot initiation as well as shoot elongation.

* The regenerated rooted shootlets were successfully hardened and acclimatized, 95% of plantlets survived well under natural conditions after transplantation.

* Finally, In our study the result shows *A. indica* micropropagation and multiplication protocol was developed by leaf explant.

Effect of different concentration of 2,4-D, IAA and NAA callus induction in leaf and stem explants of *A. indica*

Two different explants (Leaf and Stem) of *A. indica* were used at different concentrations of 2, 4-D, IAA and NAA in MS for callus initiation. Combination of (0.5 + 0.5 + 1.0 - 5.0 + 4.0 + 3.5 mg/L) 2,4-D, IAA and NAA in MS medium to produced maximum number of callus (Figure1B-F)(Table-1). Low concentration of NAA and BAP to produced most significant effect of callus were produced in concentration of 0.5 + 2.0 - 2.0 + 3.5 mg/L NAA+BAP. (Table 2). In addition of BA and IAA at low concentration this concentration to produced maximum number of callus in stem and leaf explant. Specific phyotohormone concentration to produced 100 % callus initiation in leaf explant (1.5 + 0.5 and 4.0 + 1.0 mg/L) BA + NAA, (1.0 + 1.0 mg/L)BA + IAA, (5.0 + 1.0 mg/L) IPA + IAA, and 75% in 1.5 + 0.5 mg/L) BA + NAA,(5.0 + 1.0 mg/L) IPA + IAA and 100% (1.0 + 1.0 mg/L)BA + IAA, (4.0 + 1.0 mg/L) BA + NAA, in stem explant (Table -3).

Effect of growth regulators with coconut water induction leaf callus multiplication shoot initiation of *A. indica*

Callus multiplication medium containing Kn, BAP and 20% of coconut water (C.W) in the MS medium. The highest number of whitish green callus and Yellowish brown callus were obtained from the leaf callus, When the medium contained different concentration of hormone at Kn (1.0 -10)+BAP (1.0-5.0)+20% C.W. 100% of calls were obtained at the concentration of Kn + BAP + C.W% **(** 0.5 mg/L+ 1.0 mg/L+ 20% - 2.0 mg/L+ 2.0 mg/L+ 20% **).** Callus multiplication capacity reduced when increasing the concentration of BAP and Kn and callus color was change in to brown (Table – 4)

(Figure2. A). In another case, half strength of MS medium with different concentration of BAP (1.5 – 3.0 mg/l) increases the efficiency upto 75-95% of greenish shoot formation. Shoot multiplication capacity reduced when increasing the concentration of BAP (Table 5) (Figure2. B).

Ariostolochia indica

Table 1. Effect of different concentration of 2,4-D, IAA and NAA callus induction in leaf and stem explants of *A. indica*

S.No	MS medium with 2,4-D +IAA+NAA (mg/l)	% Leaf callus initiation	Leaf callus morphology	% Stem callus initiation	Stem callus morphology
1	0.5 + 0.5 + 1.0	100	White callus	75	White callus
2	1.0 + 1.0 + 1.5	100	White callus	100	White callus
3	2.0 + 1.5 + 2.0	100	White callus	50	White callus
4	3.0 + 2.0 + 2.5	100	Green callus	75	White callus
5	4.0 + 3.0 + 3.0	100	Green callus	75	White callus
6	5.0 + 4.0 + 3.5	100	Green callus	75	White callus
7	7.5 + 5.0 + 4.0	75	Green callus	50	White callus
8	10.0 + 7.5 + 4.5	75	Green callus	50	White callus
9	15.0 + 10.5 + 5.0	50	Brown callus	100	White callus
10	20.0 + 15.0 + 10.0	50	Brown callus	75	White callus

Table 2. MS medium with different concentration of growth regulator initiation the leaf and stem callus of *A. indica*

S.No	MS medium with NAA+BAP (mg/l)	Leaf callus initiation %	Stem callus initiation %
1	0.5 + 2.0	100	100
2	1.0 + 2.5	100	75
3	1.5 + 3.0	100	75
4	2.0 + 3.5	100	50
5	2.5 + 4.0	50	50
6	3.0 + 4.5	75	75
	MS medium with BA+BAP+IAA+NAA (mg/l)		
7	0.5 + 0.5 + 1.0 + 0.50	100	100
8	1.0 + 0.75 + 1.0 + 0.75	100	100
9	2.0 + 1 .0 + 1.0 + 0.90	75	100
10	3.0 + 1.5 + 1.0 + 1.0	75	100
11	4.0 + 2.0 + 1.0 + 1.25	75	75
12	4.0 + 2.0 + 1.0 + 1.25	50	75

Table 3. Effect of different concentration of growth regulators used in leaf and stem callus multiplication

S.No	MS medium with growth regulators (mg/l)	Leaf callus initiation %	Callus morphology	Stem callus initiation %	Callus morphology
1	**BA + NAA**				
	1.5 + 0.5	100	**G.C**	75	**G.C**
2	**BA + IAA**				
	1.0 + 1.0	100	**G.C**	100	**G.C**
3	**IPA + NAA**				
	5.0 + 1.0	100	**G.C**	75	**G.C**
4	**BA + NAA**				
	4.0 + 1.0	100	**G.C**	100	**G.C**

G.C - Green Callus

Table 4. Effect of growth regulators with coconut water induction leaf callus multiplication of *A. indica*

S.No	MS medium with Kn + BAP + C.W (mg/l)	Leaf callus initiation %	Callus morphology
1	0.5 + 1.0 + 20%	**100**	Whitish green callus
2	1. 0 + 1.5 + 20%	**100**	Whitish green callus
3	2.0 + 2.0 + 20%	**100**	Yellowish brown callus
4	3.0 + 2.5 + 20%	**75**	Yellowish brown callus
5	4.0 + 3.0 + 20%	**25**	Brown callus
6	5.0 + 4.0 + 20%	**25**	Brown callus
7	10.0 + 5.0 + 20%	**25**	Brown callus

Table 5. Effect of BAP induced the shoot initiation from leaf callus of *A. indica*

S.No	**MS medium with BAP (mg/l)**	Shoot initiation % of leaf callus	Developmental stage of Shoot morphology
1	1	**40**	Greenish shoot formation
2	1.5	**75**	Greenish shoot formation
3	2	**95**	Greenish shoot formation
4	2.5	**86**	Greenish shoot formation
5	3	**83**	Greenish shoot formation
6	4	**45**	Greenish shoot formation
7	5	**42**	Greenish shoot formation

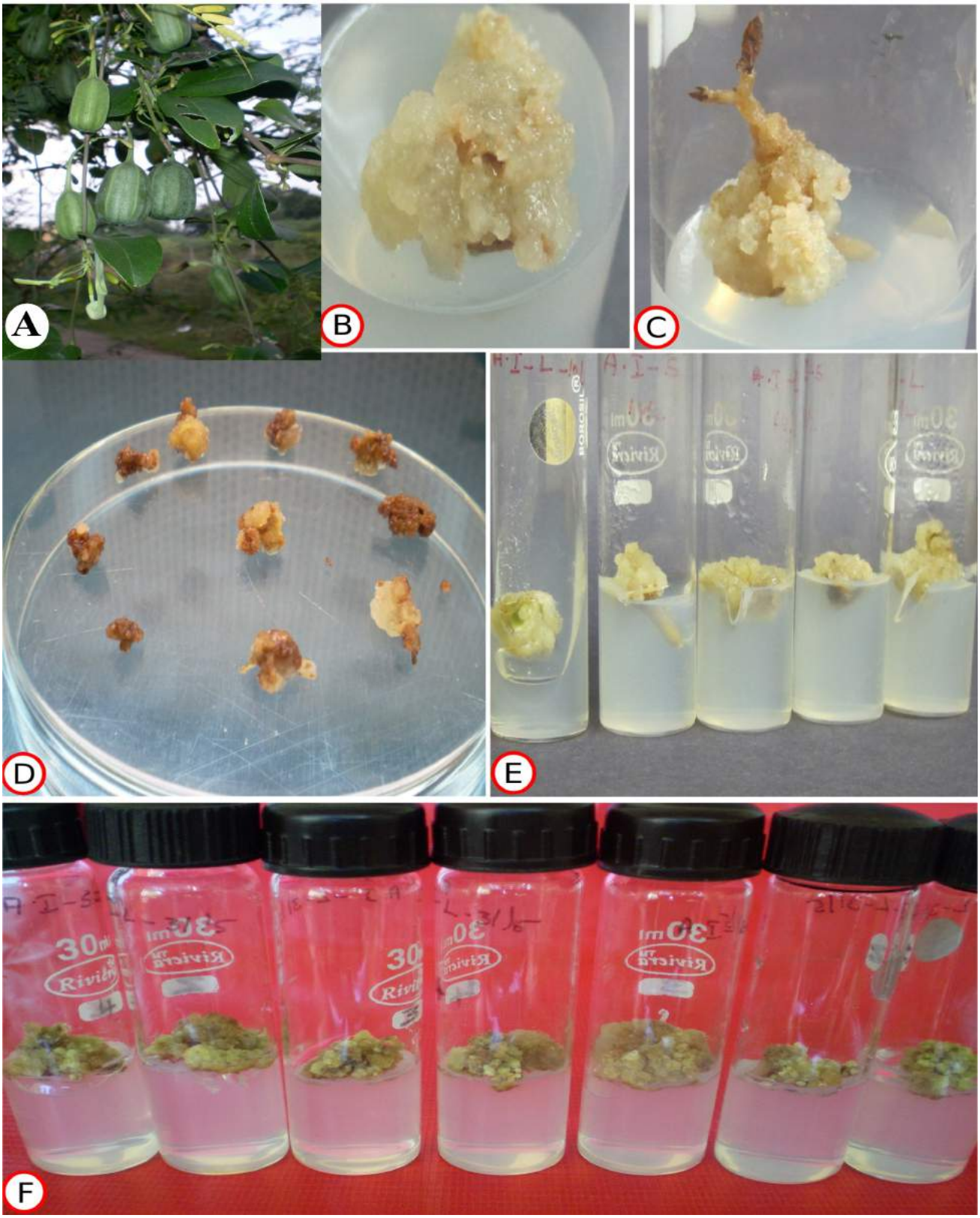


Figure 1: A, B – *Aristolochia indica* explant, **B,C,D,E,F –** Leaf callus initiation.

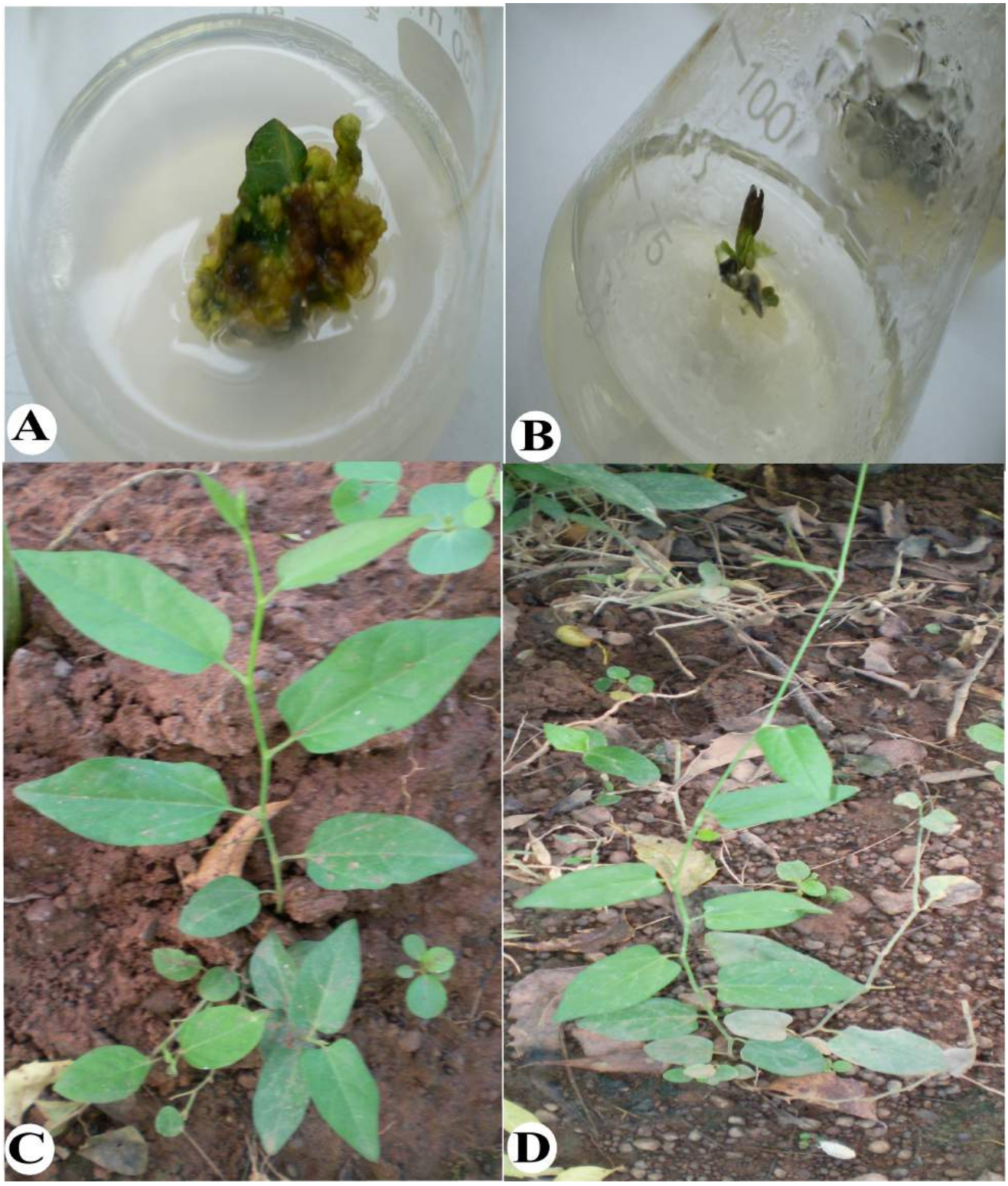
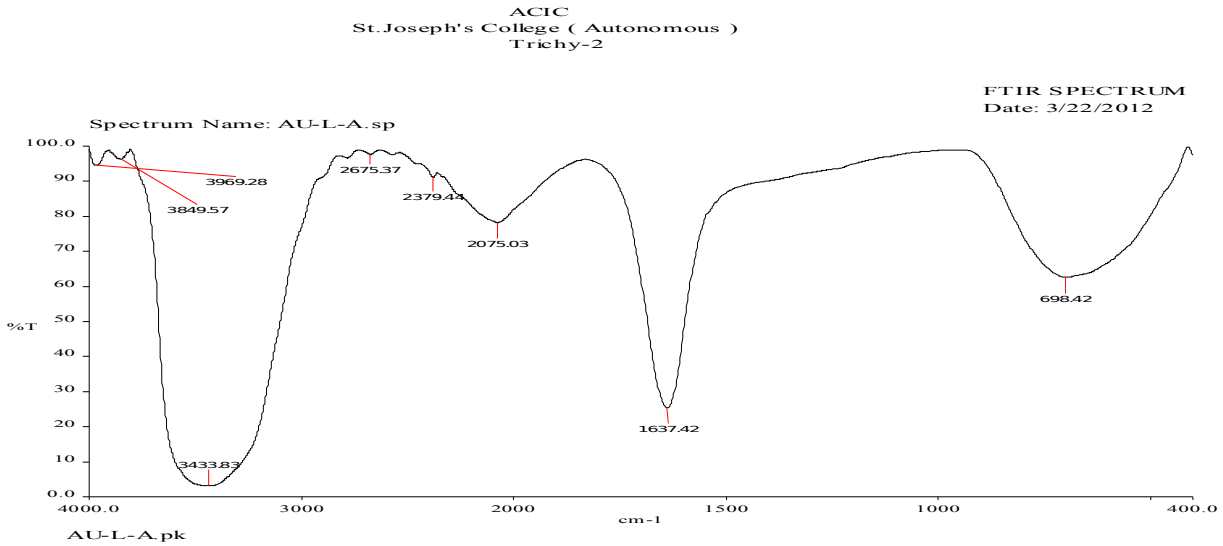


Figure 2: A – *Aristolochia indica* Leaf callus regeneration, **B** – Shoot let regeneration, **C,D-** Hardening for *in vivo* condition

FTIR – Result

Figure 2.5 *Aristolochia indica* Root - Petroleum ether extract

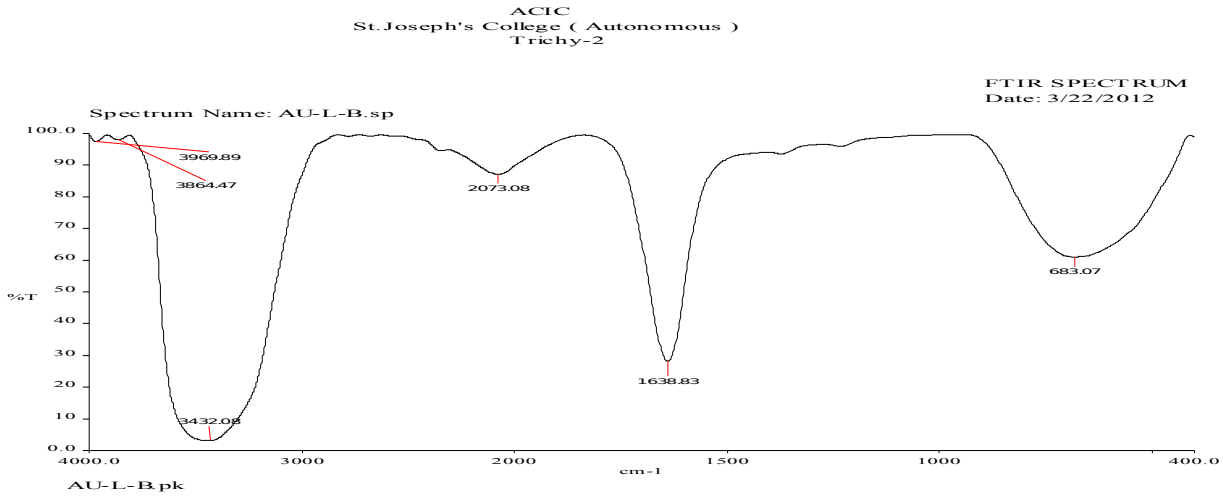


AU-L-A.pk

AU-L-A.sp 1801 4000.00 400.00 3.16 99.72 4.00 %T 15 1.00

REF 4000 99.20 2000 82.01 600
3969.28 94.52 3849.57 96.32 3433.83 3.16 2675.37 97.71 2379.44 91.15
2075.03 78.28 1637.42 25.36 698.42 62.62

Figure 2.6 *Aristolochia indica* Root - Methanol extract

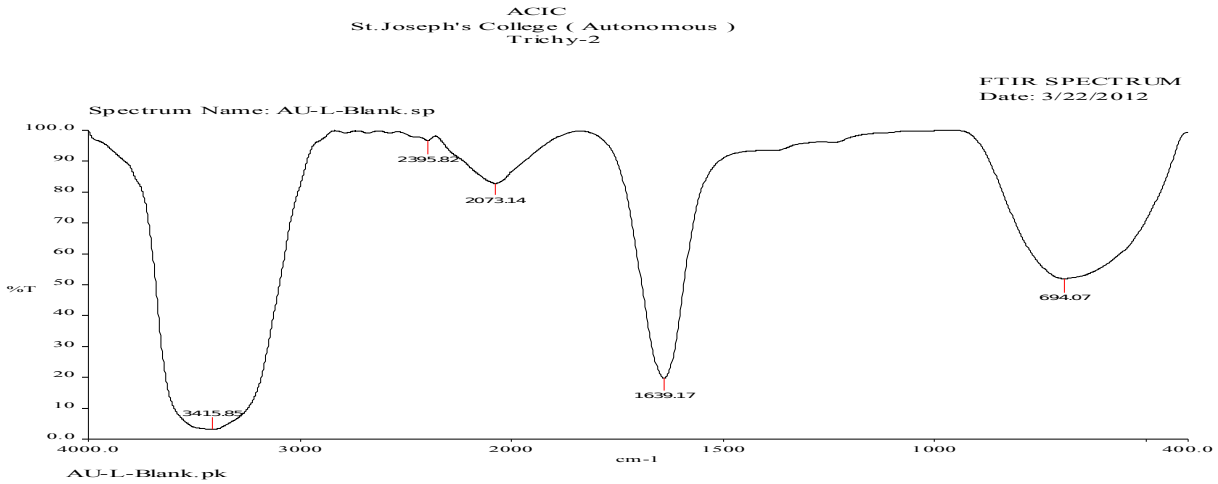


AU-L-B.pk

AU-L-B.sp 1801 4000.00 400.00 3.17 99.60 4.00 %T 15 1.00

REF 4000 99.60 2000 89.87 600
3969.89 97.43 3864.47 97.97 3432.08 3.17 2073.08 87.07 1638.83 28.13
683.07 61.03

Figure 2.7 *Aristolochia indica* Root - Chloroform extract

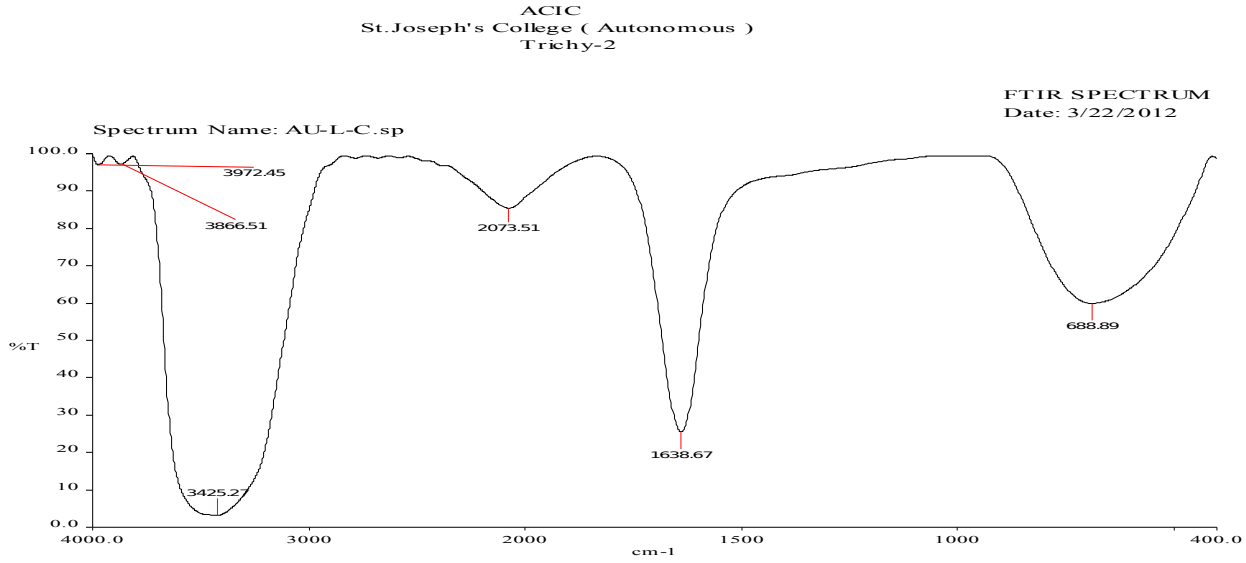


AU-L-Blank.pk

AU-L-B-1.SP 1801 4000.00 400.00 3.17 99.99 4.00 %T 15 1.00

REF 4000 99.99 2000 86.71 600
3415.85 3.17 2395.82 96.71 2073.14 82.82 1639.17 19.77 694.07 51.97

Figure 2.8 *Aristolochia indica* Root - Acetone extract

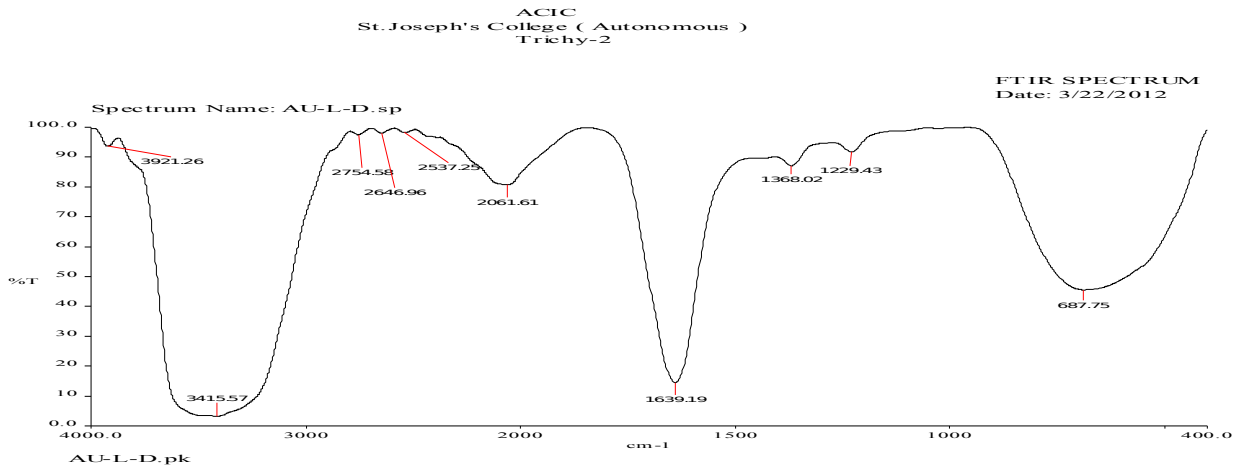


AU-L-C.pk

AU-L-C.sp 1801 4000.00 400.00 3.22 99.49 4.00 %T 15 1.00

REF 4000 99.49 2000 88.45 600
3972.45 96.95 3866.51 97.16 3425.27 3.22 2073.51 85.39 1638.67 25.58
688.89 59.96

Figure 2.9 *Aristolochia indica* Leaf - Petroleum ether extract

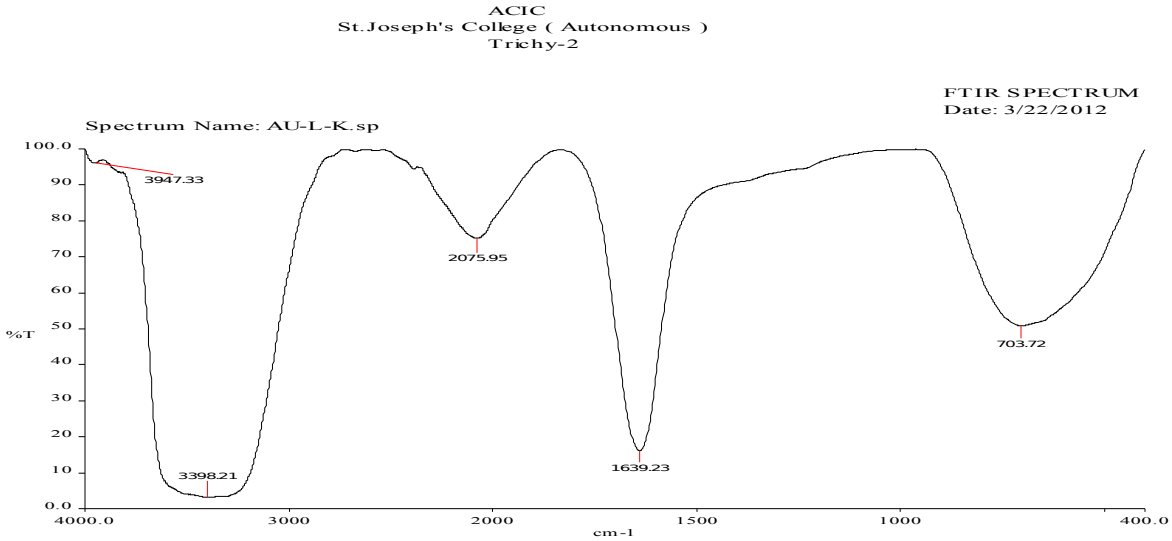


AU-L-D.pk

AU-L-D.sp 1801 4000.00 400.00 3.22 99.90 4.00 %T 15 1.00

REF 4000 99.48 2000 84.76 600
3921.26 93.69 3415.57 3.22 2754.58 97.48 2646.96 98.04 2537.25 98.18
2061.61 80.81 1639.19 14.46 1368.02 87.10 1229.43 91.75 687.75 45.56

Figure 2.10 *Aristolochia indica* Leaf - Methanol extract



AU-L-K.pk

AU-L-K.sp 1801 4000.00 400.00 3.16 100.00 4.00 %T 15 1.00

REF 4000 100.00 2000 80.31 600
3947.33 96.05 3398.21 3.16 2075.95 75.19 1639.23 16.11 703.72 50.88

Figure 2.11 *Aristolochia indica* Leaf - Chloroform extract

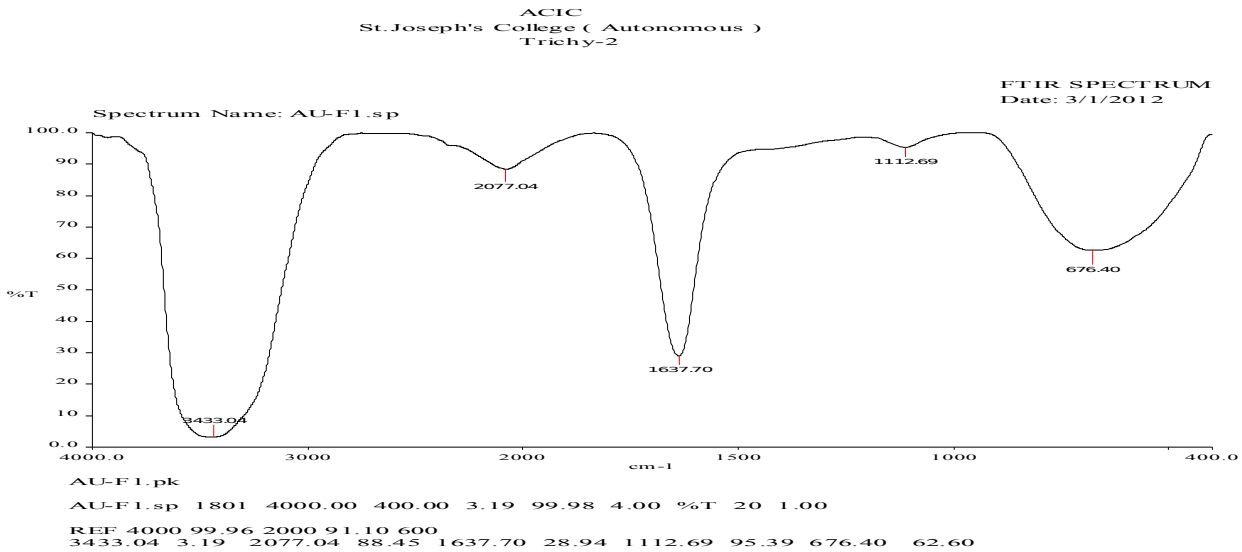


Figure 2.12 *Aristolochia indica* Leaf - Chloroform extract

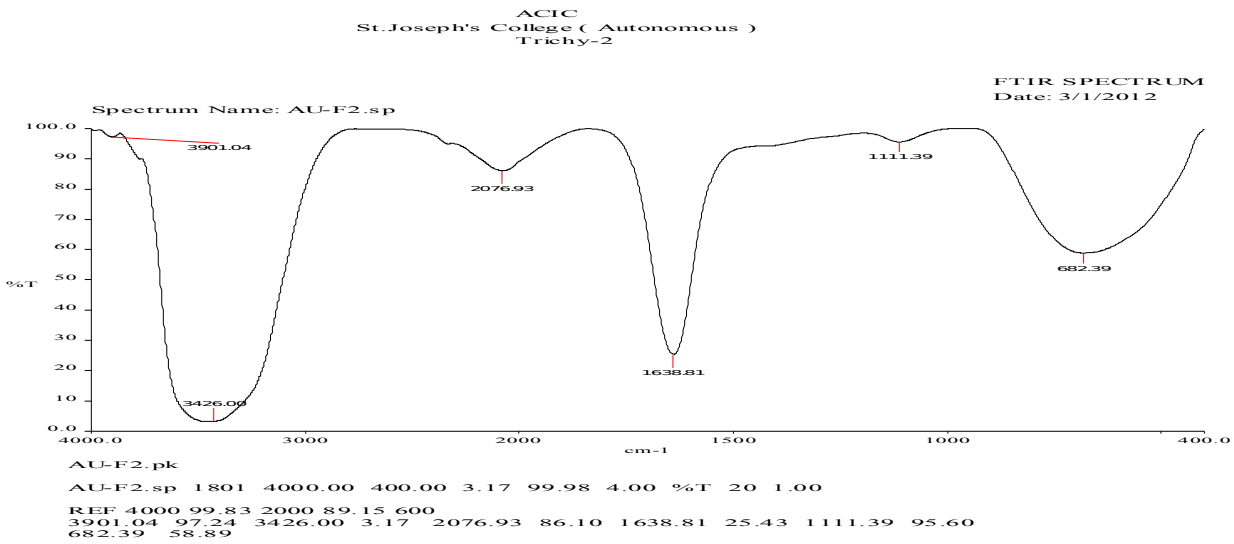


Figure 2.5-2.12: FTIR analysis shows that the *Aristolochia indica* root and leaf were extract with in four different solvent in methanol, acetone, chloroform and petroleum ether extract. The functional groups present in the samples were determined by FT-IR spectroscopy. The FT-IR spectrum confirmed the presence in Aristolochic acids, volatile oil and tannins of *Aristolochia indica*. The strong IR bands were observed at 3382, 2922, 2337, 1614, 1384, 1070 and 590 cm^{-1}. The bands appeared at 3382 and 2922 cm^{-1} are corresponds to -OH stretching and aliphatic -C-H stretching respectively. The bands at 2337 and 1613 cm^{-1} are due to the CO_2 and C=C stretching respectively. The IR bands observed at 1384 and 1070 cm^{-1} may be ascribed to -C-O and -C-O-C stretching modes respectively. The low band at 590 cm^{-1} corresponds to C-Cl stretching. Significant peaks were found at 2926 cm^{-1} corresponding to CH_2 group, 1646 cm^{-1} attributed to Carbonyl groups, and 1539 cm^{-1} corresponding to amino acid groups, all of which confirms the presence of alkaloids.

1. Plant Name : *Gloriosa superba* Linn.

2. Botanical Classification :

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Liliales
Family	Liliaceae
Genus	*Gloriosa*
Species	*superba*

3. Vernacular Names:
Climbing lily, Creeping lily, Flame lily, Glory lily, Gloriosa lily, Kallappai kilangu, Kandhal malar**,** Karthigai malar, Kalikari, Kanvali Veethai, Langli, Malabar glory lily, Nabhikkodi, Rothschild lily, Superb lily**,** Tiger claw.

4. Chemical Constituents:
Seed contain high level of colchicines. Cornigerine, 3-demethyl-N-formyl-N-deacetyl-b-lumicolchicine, 3-demethyl-g-lumicolchicine, 3-demethyl colchicines have been isolated from plant. b-sitosterol, its glucoside, a long chain fatty acid, b and g-lumiccolchicines from fresh tubers and luteolin, colchicines, N-formyldeacetylcolchicines and glucosides of 3-demethylcolchicine have been isolated from flowers.

5. Description Medicinal Property:
In Ayurveda and Yunani systems of medicine it is a reputed medicine. According to Ayurveda, tuber is pungent, bitter, acrid, heating, anthemirtic, laxative, alexiteric, abortifacient, and useful in ulcers, leprosy, piles, iflommations, abdominal pains, itching and thirst.

The roots are purgative, cholagogue, anthelmintic and used in rheumatic fever, leprosy, skin diseases, coil and snake bites, Tubers are abortifacient, Various plant parts are used in spleen complainst, tumours, erysipelas, sores and syphilis,

6. Useful parts:
Tubers, leaves and flowers.

7. Micropropagation aspects:
In our methods shows that mass propagation of *G.superba,* through tissue culture methods by using the corm bud explants.

8. Results

- The leaf, stem, root, rhizome and corm bud explant were used for callus initiation medium. But, only corm bud explant alone showed most significant effect of 99.40 % response in callus initiation at concentration of MS+2,4-D (1.0mg/l)+IAA (0.5 mg/l). Other explant did not showed any significant effect callus initiation.
- BAP, Kinetin and Coconut water combination was more suitable for 93.40% of shoot formation and BAP (2 mg/l) alone in Ms medium, it plays good role in greenish shoot formation for rooted callus.
- The regenerated rooted shootlets were successfully hardened and acclimatized, 87% of plantlets survived well under natural conditions after transplantation.
- The present investigation pertained the simple and an efficient protocol was developed the micropropagation of an endangered medicinal plant *G. superba* in under in vitro condition by using corm bud explants.
- This protocol provides a successful and rapid technique that can be used for ex-sit conservation. The application of this protocol can help minimize the pressure on wild populations and contribute to the conservation of the endangered flora of the southern India.

Five different explants (leaf, stem, root,rhizome and corm-bud) were inoculated in different concentration of 2,4-D (1.0-3.0 mg/l) and IAA (0.5-2.5 mg/l) in MS media (Table 1). Only the corm bud explants was response at 54.20±1.43to 99.40±0.40% of callus initiated in 25-30 days cultures (Figure1. A,B.). Increasing the hormone concentration of 2,4-D and IAA which reduced callus initiation in corm bud explant. Callus multiplication medium contain different concentration and combination of (0.5 -5.0+0.5-2.50+1.0+0.5-1.5 mg/l) BA+BAP+IAA+NAA the maximum number 97% of yellowish callus were obtained at (2.0 +1.00 +1.0 +0.90 and 3.0 +1.50 +1.0 +1.00 mg/l) concentration. In another case in addiction of 4.0 +1.0 +1.0 +1.5 +2.0 mg/l (GA_3 +Kn +IBA+BAP+IAA) to produced 94 % of yellow callus (Table 2). In another way to rapidly initiate we add activated charcoals (AC-2 g/ L) within the MS media, 16% induction of root initiation for best result was observed (Table 3). Corm bud callus explants highly regenerated up to 96.00±1.22% of roots formation obtained (Fig.) at the concentration of MS+BAP (8.0)+GA_3 (1.0)+Zen (0.5)+NAA (1.0)+2 g AC and (Figure 1. C,D,E) with out addition of activated charcoals in the MS medium at produced 80.30±1.86 to 91.40±0.75% rhizogenesis initiation (Table 3). The micro rhizome initiation root callus were obtained at the concentration and combination of 0.1 + 1.0 mg/l (Kn + BAP) micro rhizome initiation capacity reduced when increasing the concentration of Kn + BAP (Table 4). The 30 days old corm bud callus transferred to the half strength MS medium contains

different concentration and combination of cytokinins. The 92.80±2.35% of greenish shoot formation was obtained half strength of MS medium with BAP 2.0 mg/l lower concentration of BAP can be used for obtaining the desirable shoot regeneration. Shoot initiation was reduced, when increasing the concentration of BAP on the medium (Table 5). In another case shoot multiplication, the maximum number (93.40±1.89%) of dark greenish multiple shoots was obtained on half strength of MS medium with (Kn 1.0+BAP 1.5 mg/l+20% CW) (Table 5) (Figure 1. FGH)

Gloriosa superba Linn.

Table 1. Effect of different concentration of 2,4-D and IAA callus induction in different explants of *G.superba*

S.No	**MS medium with 2,4-D + IAA (mg/l)**	Leaf callus initiation %	Stem callus initiation %	Root callus initiation %	Rhizome callus initiation %	Corm-bud callus initiation %
1	**1.0 + 0.5**	-	-	-	-	**99.40 ± 0.40**
2	**1.5 + 1.0**	-	-	-	-	**98.50 ± 0.45**
3	**2.0 + 1.5**	-	-	-	-	**77.20 ± 0.86**
4	**2.5 + 2.0**	-	-	-	-	**74.60 ± 1.08**
5	**3.0 + 2.5**	-	-	-	-	**54.20 ± 1.43**

The combination of hormone to developed the yellowish callus initiation of corm bud explants

Table 2. Effect of callus multiplication and growth regulator with corm bud explants of *G.superba*

S.No	**MS medium with BA+BAP+IAA+NAA (mg/l)**	Corm bud explants produced callus initiation %	Callus morphology
1	0.5 +0.50 +1.0 +0.50	**72.40 ± 0.93**	Yellowish callus
2	1.0 +0.75 +1.0 +0.75	**98.90 ± 0.40**	Yellowish callus
3	2.0 +1.00 +1.0 +0.90	**97.00 ± 0.71**	Yellowish callus
4	3.0 +1.50 +1.0 +1.00	**97.00 ± 0.84**	Yellowish callus
5	4.0 +2.00 +1.0 +1.25	**76.00 ± 1.70**	Yellowish callus
6	5.0 +2.50 +1.0 +1.50	**73.60 ± 1.17**	Yellowish callus
	GA_3+Kn +IBA+BAP+IAA		
7	2.0 +1.0 +0.5 +1.5 +1.0	**50.00 ± 1.70**	Green callus
8	4.0 +1.0 +1.0 +1.5 +2.0	**94.00 ± 1.52**	Yellow callus
9	6.0 +1.0 +2.0 +1.5 +3.0	**73.00 ± 2.10**	Yellow callus
10	8.0 +1.0 +4.0 +1.5 +4.0	**93.40 ± 2.62**	Yellow callus
11	10.0 +1.0 +5.0 +1.5 +5.0	**91.60 ± 1.96**	Yellow callus

Table 3. Effect of activated charcoals induced the root initiation of corm bub explants of *G. superba*

S.No	**MS medium with BAP+GA_3+Zen+NAA (mg/l)**	Mean number of roots initiation for corm bud callus ± S.E. **Add to 2 g/l of AC**	Mean number of roots initiation for corm bud callus ± S.E. **With out AC**
1	8.0 +1.0+0.5+1.0	**96.00 ± 1.22**	**80.00 ± 1.81**
2	10.0+2.0+1.0+1.0	**94.40 ± 0.68**	**91.40 ± 0.75**
3	12.0+3.0+1.5+1.0	**94.80 ± 0.97**	**85.80 ± 1.39**
4	14.0+4.0+2.0+1.0	**92.00 ± 1.14**	**87.20 ± 1.66**
5	16.0+5.0+2.5+1.0	**90.60 ± 1.50**	**84.20 ± 1.46**

S.E -Standard Error.

Table 4. Effect of different concentration of Kn and BAP induction root callus of *G.superba*

S.No	**MS medium with Kn+BAP (mg/l)**	Rhizome initiation % of root callus	Developmental stage of Rhizome morphology
1	0.5 +1.0	**38.40 ± 2.66**	White color Rhizome initiation
2	1.0 +1.5	**32.20 ± 1.50**	White color Rhizome initiation
3	2.0 +2.0	**27.20 ± 1.02**	White color Rhizome initiation
4	3.0 +2.5	**24.80 ± 1.39**	White color Rhizome initiation
5	4.0 +3.0	**23.80 ± 1.36**	White color Rhizome initiation
6	5.0 +4.0	**20.60 ± 1.08**	White color Rhizome initiation
7	10.0 +5.0	**20.20 ± 1.85**	White color Rhizome initiation

Table 5. Shoots multiplication with combination growth regulators of *G.superba*

S.No	**½ MS medium with BAP (mg/l)**	Shoot initiation % of rooted callus	Developmental stage of Shoot morphology
1	1	**35.40 ± 1.86**	Greenish shoot formation
2	1.5	**26.00 ± 1.70**	Greenish shoot formation
3	2	**92.80 ± 2.35**	Greenish shoot formation
4	2.5	**87.80 ± 2.50**	Greenish shoot formation
5	3	**84.20 ± 1.69**	Greenish shoot formation
6	4	**23.40 ± 1.66**	Greenish shoot formation
7	5	**23.20 ± 2.89**	Greenish shoot formation
	Kn+BAP+CW		
8	0.5 +1.0 +20%	**49.40 ± 2.32**	Light greenish shoot formation
9	1.0 +1.5 +20%	**93.40 ± 1.89**	Dark greenish shoot formation
10	2.0 +2.0 +20%	**73.60 ± 2.11**	Light greenish shoot formation
11	3.0 +2.5 +20%	**71.40 ± 2.50**	Dark greenish shoot formation
12	4.0 +3.0 +20%	**52.80 ± 2.52**	Light greenish shoot formation
13	5.0 +4.0 +20%	**52.00 ± 1.41**	Light greenish shoot formation
14	10.0 +5.0+20%	**49.00 ± 1.73**	Dark greenish shoot formation

The result are the mean ± S.E of 5 replicates,

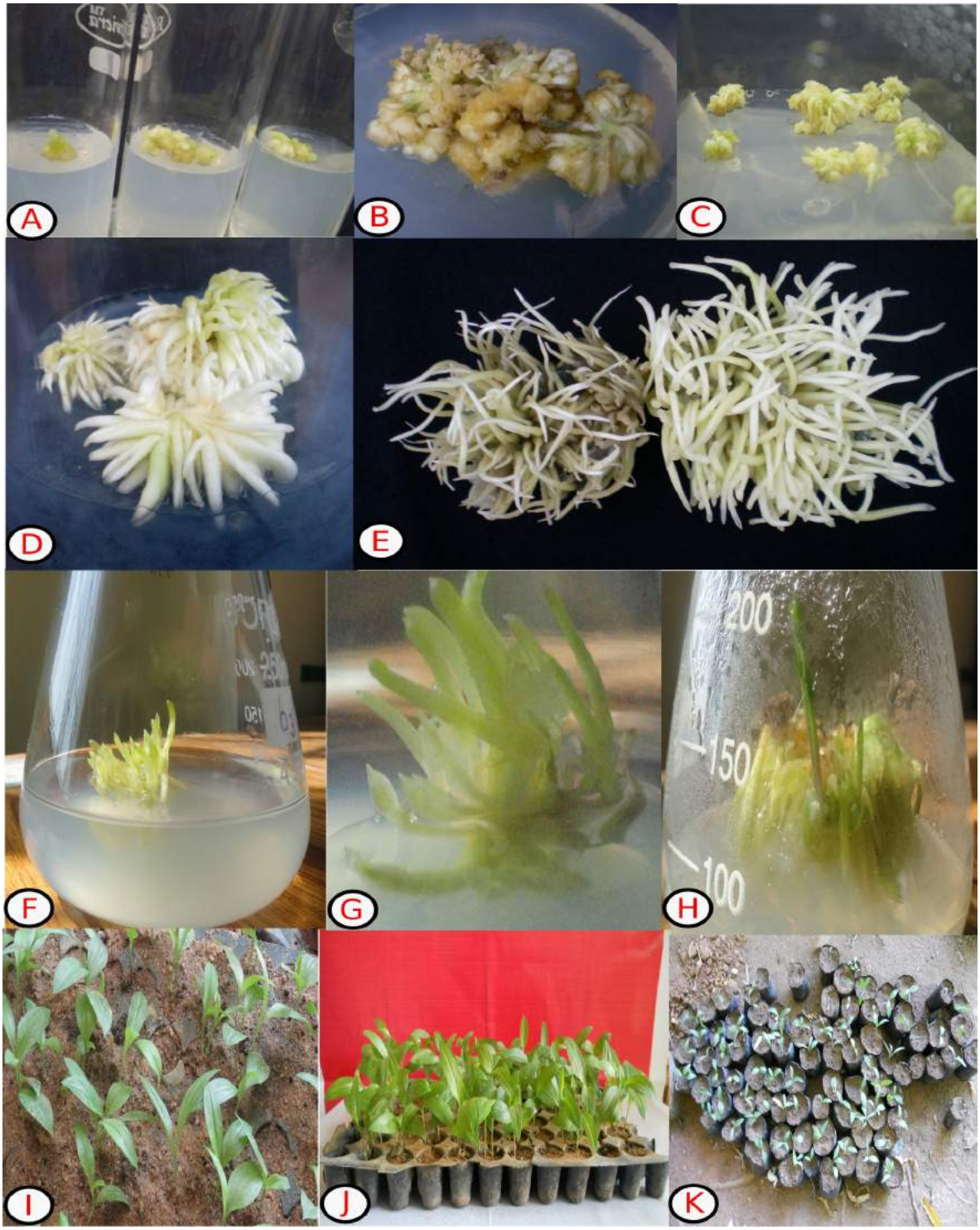


Figure 1: A, B – *Gloriosa superba* corm bud callus, **C,D,E**- Multiple roots induction, **F,G,H** - Multiple shoots induction. **I, J**- Plantlets were transplanted with plastic tray to acclimatization for 2 month in mist house, **K**- Plant hardening for *in vivo* condition.

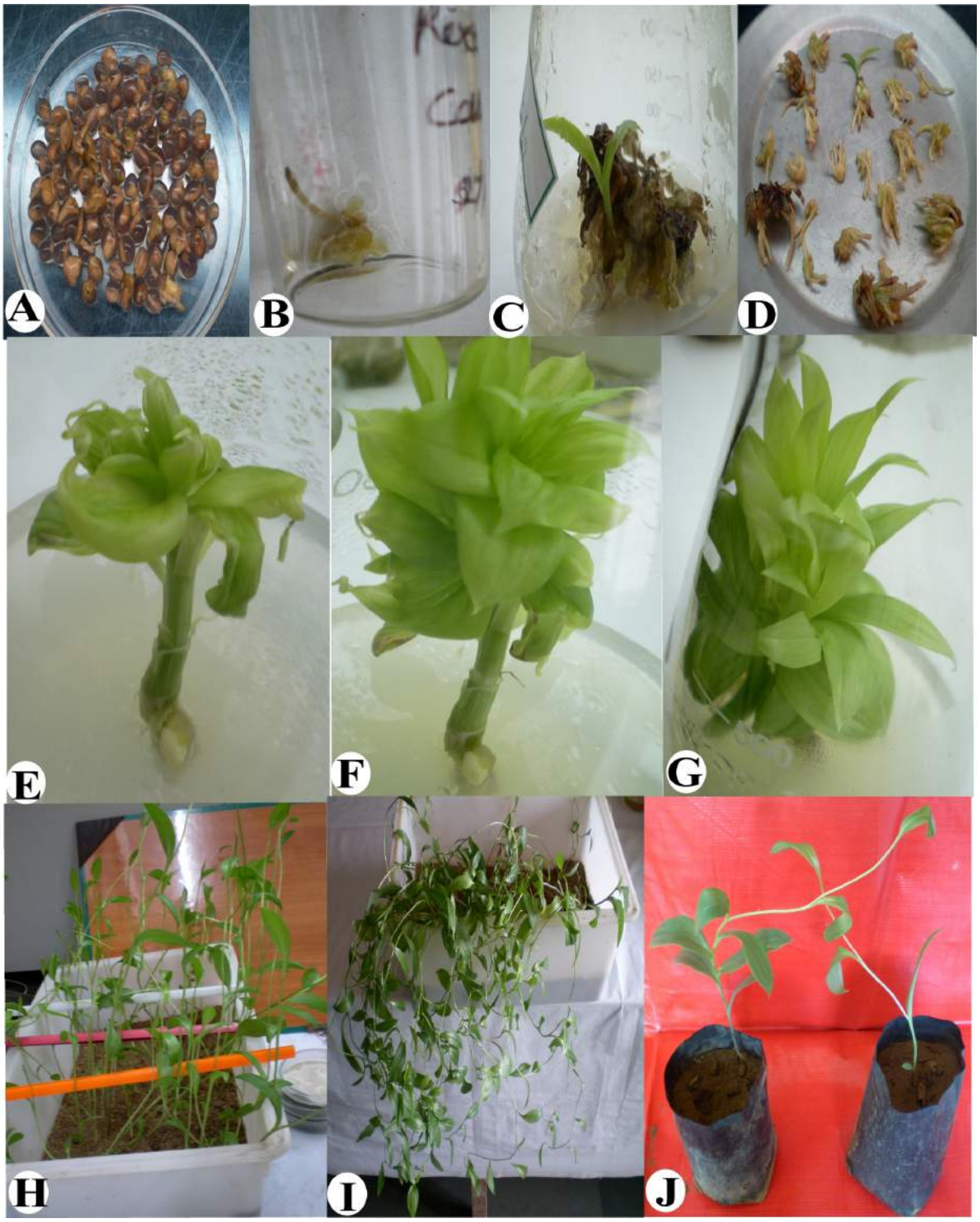
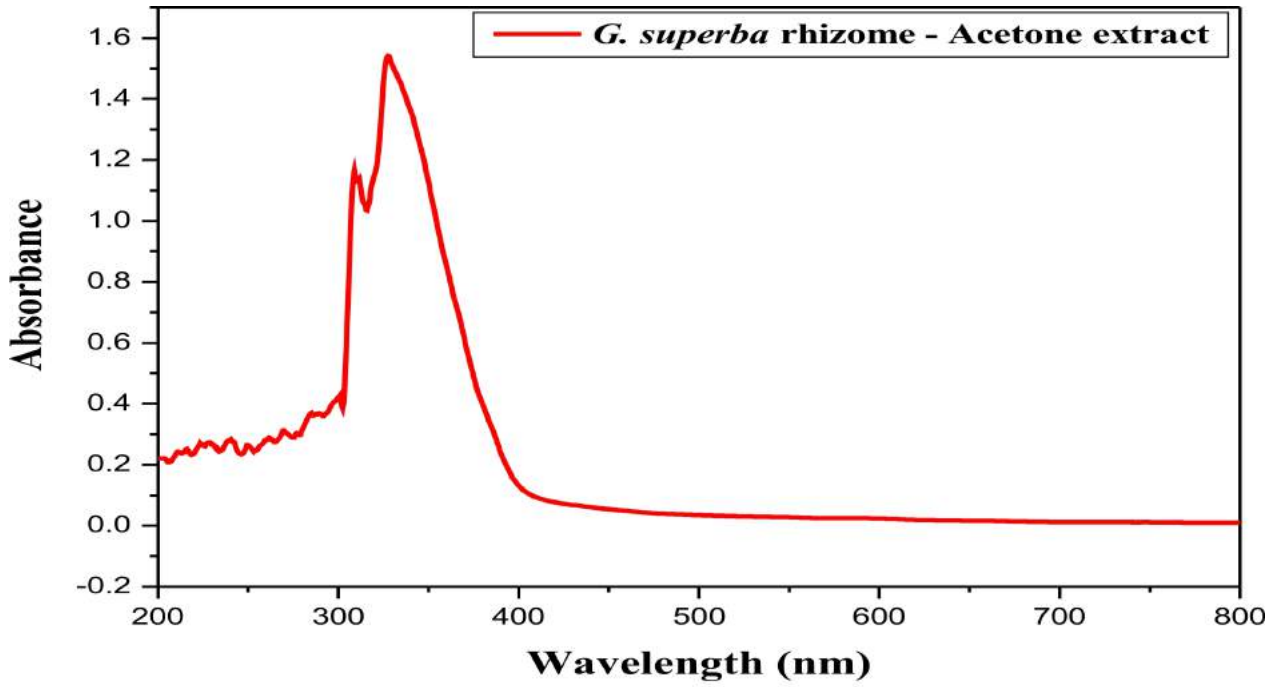


Figure 2 : A – *Gloriosa superba* immobilization of rooted callus, B- Root callus induction, C,D – Rooted shoot lets, E,F,G - Multiple shoots induction. H,I- Plantlets were transplanted with plastic tray to acclimatization for 2 month in mist house, J- Plant hardening for *in vivo* condition.

UV – Result

Figure 1.3



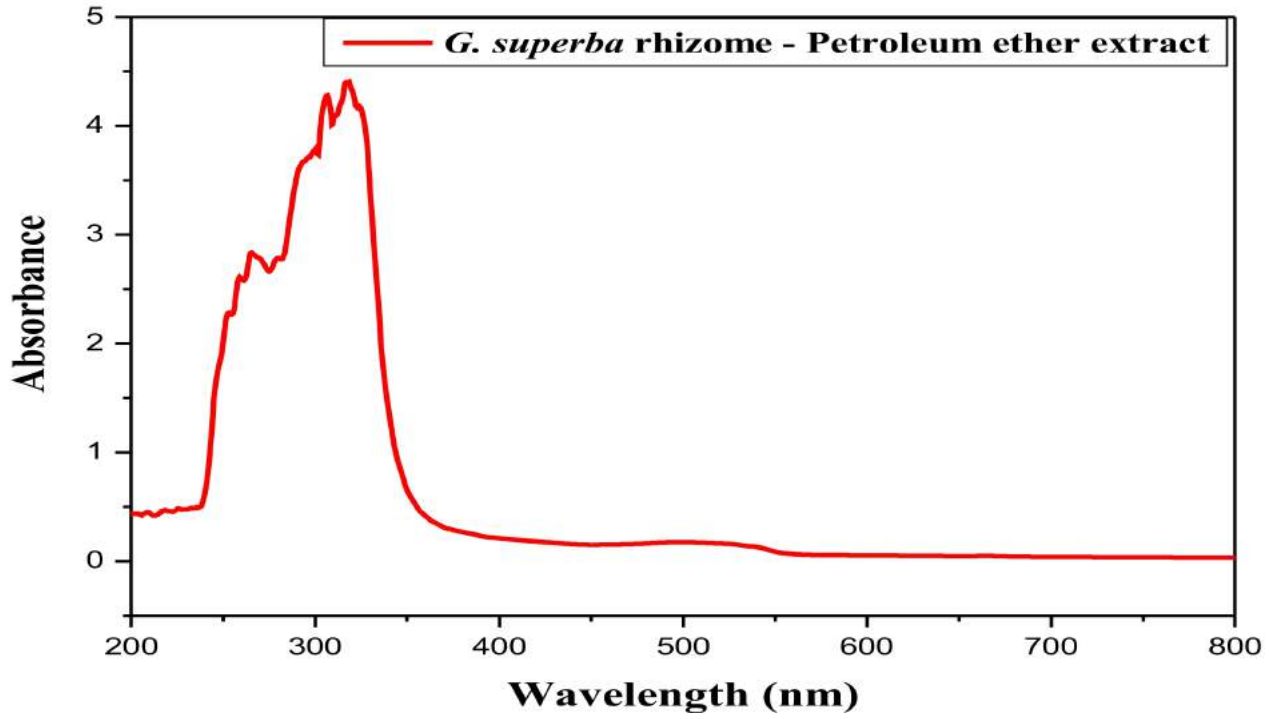


Figure 1.4

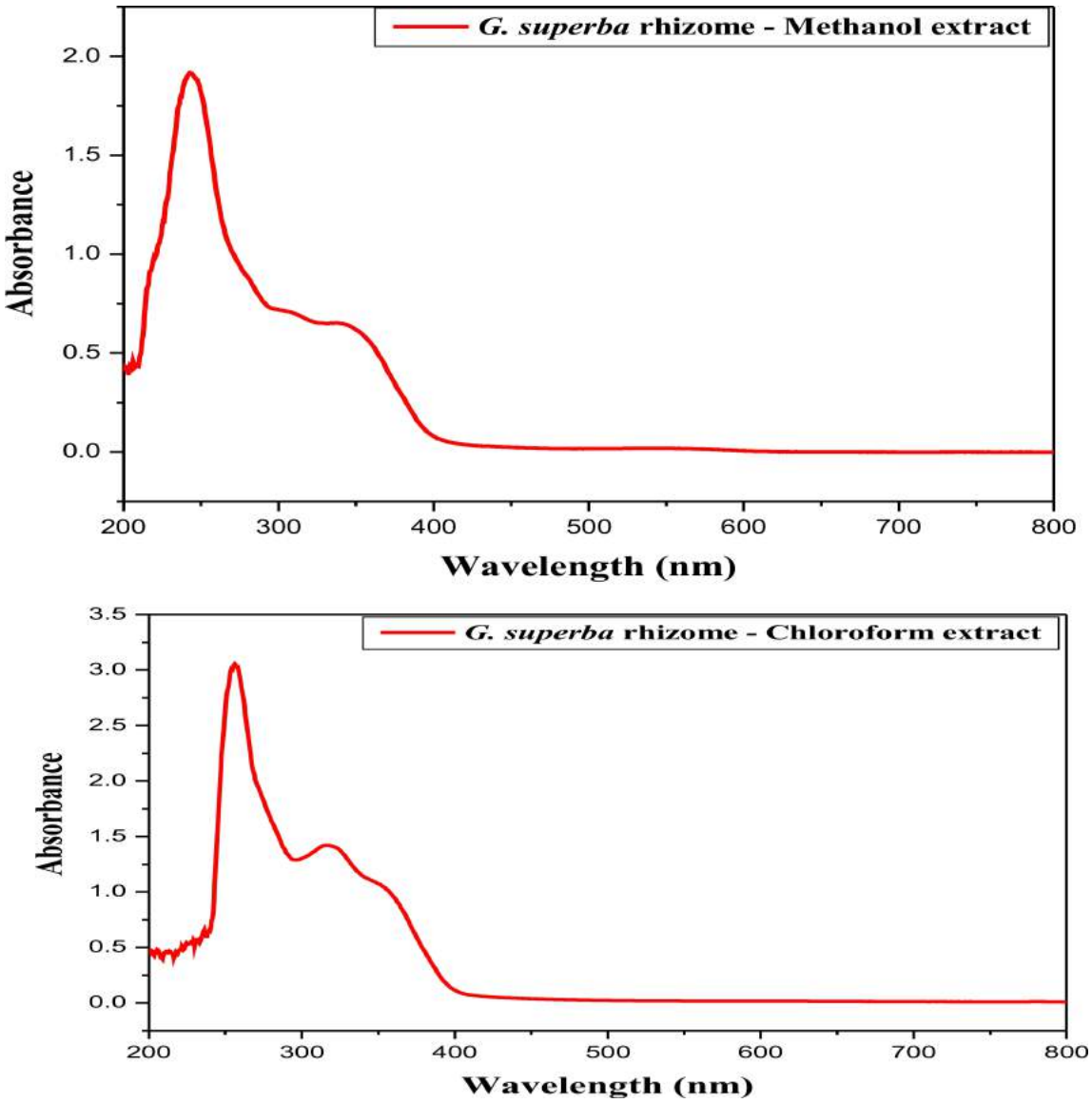


Fig 1.3 - 1.4 *Gloriosa superba* Rhizome extract in four different solvents were subjected to the near-ultraviolet absorption spectrum of colchicine from 290 to 420 nm has been examined in a variety of solvents and bound to tubulin by multiple differentiation of the absorption spectra. The absorption band is shown to be comprised of two transitions, which are τ–τ* in nature and of different excited-state character. The energies of the transitions do not correlate with solvent properties such as dipole moment, dielectric constant, or refractive index. The lower energy transition was found to shift to shorter wavelength in solvents capable of donating a hydrogen bond, while the higher energy transition was essentially invariant with solvent properties.

FTIR – Result

Figure 2.13 *Gloriosa superba* leaf - Methanol extract

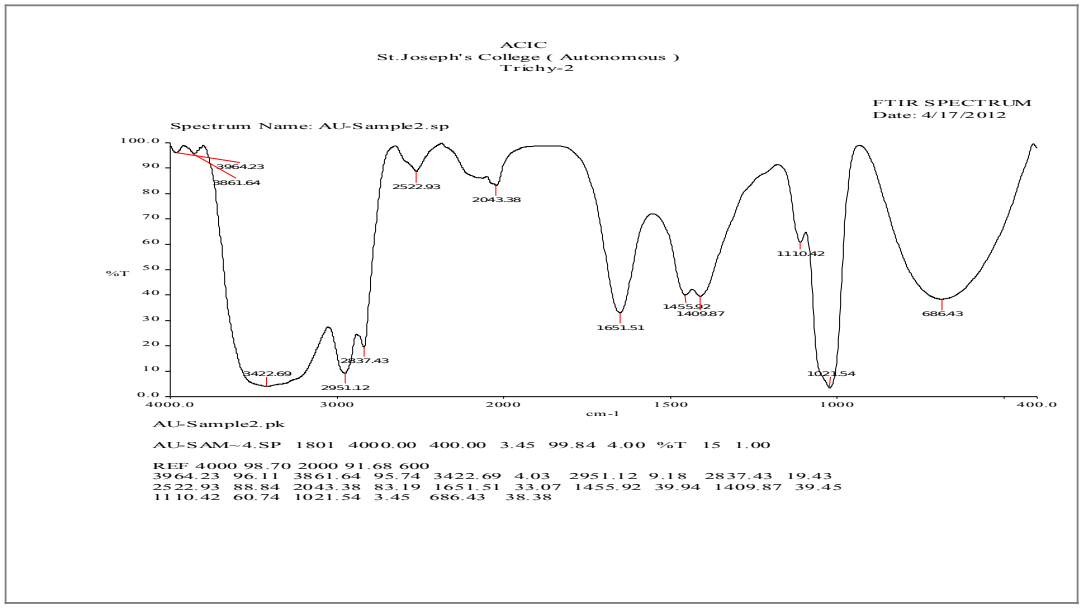


Figure 2.14 *Gloriosa superba* stem - Methanol extract

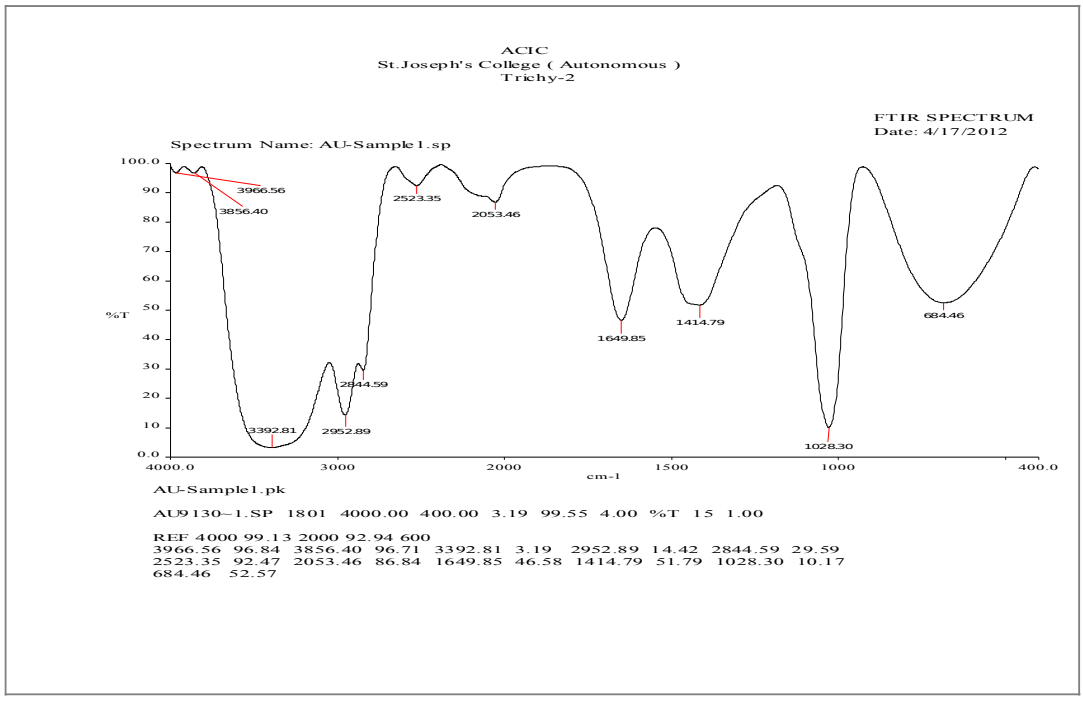


Figure 2.15 *Gloriosa superba* rhizome - Methanol extract

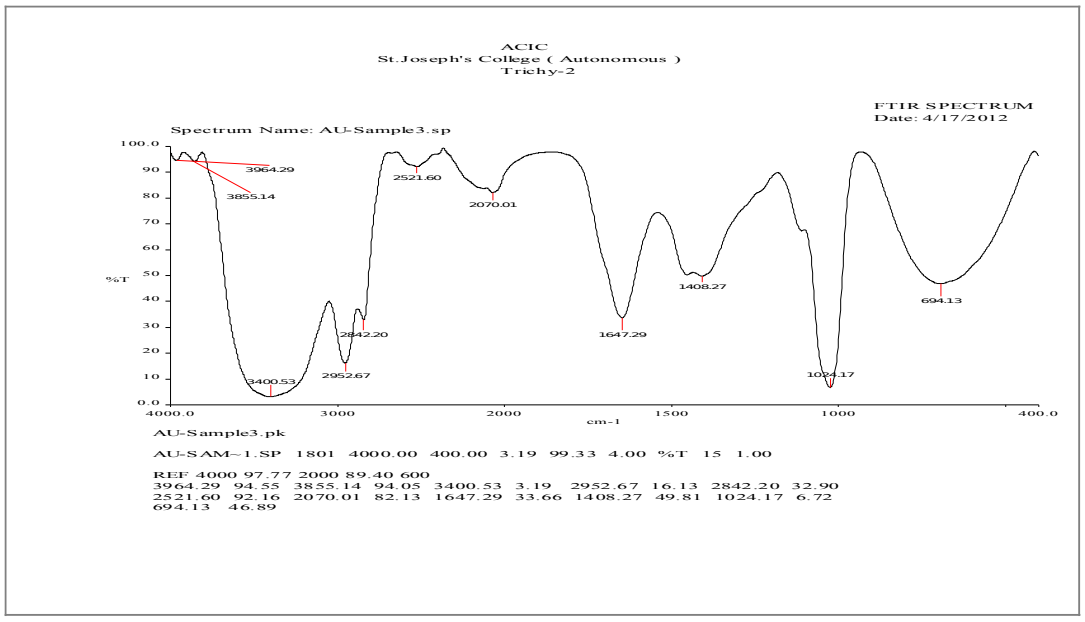


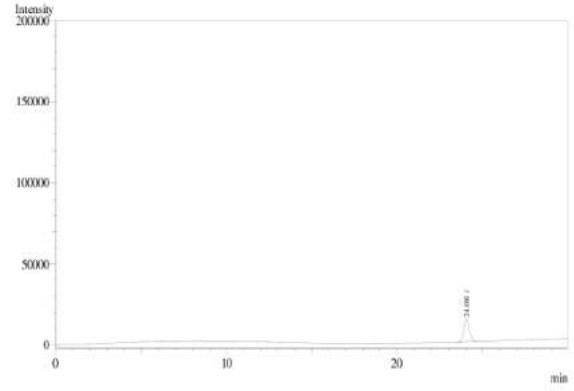
Fig 2.13 - 2.15 FTIR – analysis shows that the *Gloriosa superba* leaf, stem and tuber were extract with methanol extract. The functional groups present in the samples were determined by FT-IR spectroscopy. The FT-IR spectrum confirmed the presence of colchicines in *G. superba*, Significant peaks were found at 2926 cm^{-1} corresponding to CH_2 group, 1646 cm^{-1} attributed to Carbonyl groups, and 1539 cm^{-1} corresponding to amino acid groups, all of which confirms the presence of colchicines.

3. GC – Analysis

Figure 3.1

Analysis Date & Time : 1/31/2013 5:09:26 PM
User Name : Admin
Vial# : 8
Sample Name : GS leaf
Sample ID : GS leaf
Sample Type : Unknown
Injection Volume : 1.00
ISTD Amount :

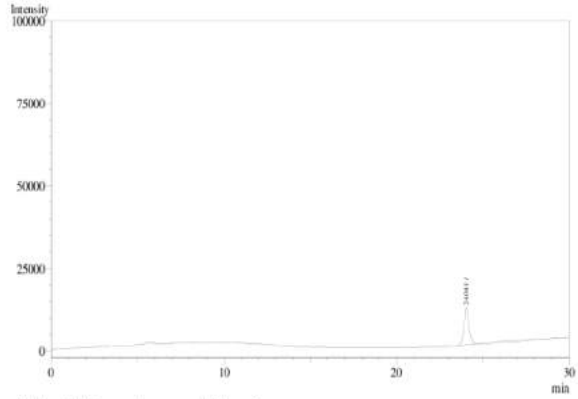
Data Name : E:\GC DATA\Batch\SElva JK NP 31.01. 13\8.gcd
Method Name : E:\GC DATA\METHODE\Selvakumaran\Selva JK NP.gcm



Peak#	Ret.Time	Area	Height	Conc.
1	24.081	297844	14143	100.000
Total		297844	14143	100.000

Analysis Date & Time : 1/31/2013 5:45:36 PM
User Name : Admin
Vial# : 9
Sample Name : GS stem
Sample ID : GS stem
Sample Type : Unknown
Injection Volume : 1.00
ISTD Amount :

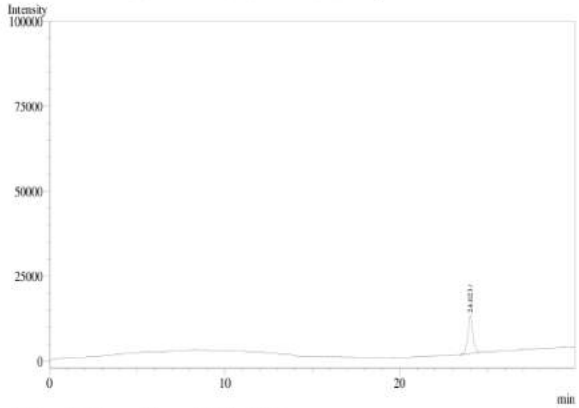
Data Name : E:\GC DATA\Batch\SElva JK NP 31.01. 13\9.gcd
Method Name : E:\GC DATA\METHODE\Selvakumaran\Selva JK NP.gcm



Peak#	Ret.Time	Area	Height	Conc.
1	24.041	230551	11306	100.000
Total		230551	11306	100.000

Analysis Date & Time : 1/31/2013 6:21:46 PM
User Name : Admin
Vial# : 10
Sample Name : GS rhizome
Sample ID : GS rhizome
Sample Type : Unknown
Injection Volume : 1.00
ISTD Amount :

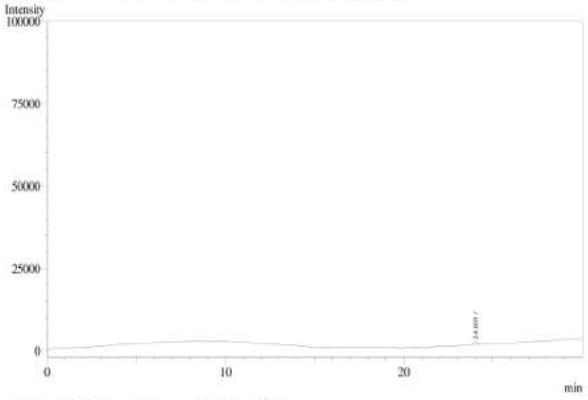
Data Name : E:\GC DATA\Batch\SElva JK NP 31.01. 13\10.gcd
Method Name : E:\GC DATA\METHODE\Selvakumaran\Selva JK NP.gcm



Peak#	Ret.Time	Area	Height	Conc.
1	24.023	228799	11570	100.000
Total		228799	11570	100.000

Analysis Date & Time : 1/31/2013 6:57:59 PM
User Name : Admin
Vial# : 11
Sample Name : GS seed
Sample ID : GS seed
Sample Type : Unknown
Injection Volume : 1.00
ISTD Amount :

Data Name : E:\GC DATA\Batch\SElva JK NP 31.01. 13\11.gcd
Method Name : E:\GC DATA\METHODE\Selvakumaran\Selva JK NP.gcm



Peak#	Ret.Time	Area	Height	Conc.
1	24.005	15174	984	100.000
Total		15174	984	100.000

Gas Chromatography

The methanolic extract of *in vivo* roots, stems, rhizomes and seeds of *Gloriosa superba* L was analyzed using GC, to know the bio-active compounds present in the extract.

Table 1: Activity of Phytocomponents identified in the roots, stems, rhizomes and seeds of *Gloriosa superba* through GC - analysis

S. No	Sample Code	R.T	Name of the compound	Molecular formula	Compound Nature	Activity
1	G. s - Root	24.08	1,2Benzenedicarboxylic acid,bis(2methylpropyl) ester	$C_{16}H_{22}O_4$	Plasticizer compound	Anti fouling agent Antimicrobial
2	G. s - Stem	24.04	1,2Benzenedicarboxylic acid,bis(2methylpropyl) ester	$C_{16}H_{22}O_4$	Plasticizer compound	Anti fouling agent Antimicrobial
3	G. s - Rhizome	24.02	1,2Benzenedicarboxylic acid,bis(2methylpropyl) ester	$C_{16}H_{22}O_4$	Plasticizer compound	Anti fouling agent Antimicrobial
4	G. s - Seed	24.01	1,2Benzenedicarboxylic acid,bis(2methylpropyl) ester	$C_{16}H_{22}O_4$	Plasticizer compound	Anti fouling agent Antimicrobial

The maximum amount of bioactive compound was found to be 1,2 Benzenedicarboxylic acid,bis (2methylpropyl) ester. (Fig 3.1: Table - 1)

Phytochemical synthesis of new sucrose crystal derived from the hot MeOH extract of the rhizome powder of *G. superba.*

Abstract

The title of the compound, $C_{12}H_{22}O_{11}$ was prepared by the *Gloriosa superba* rhizome powder with methanol extract at 70 ˚C. Sucrose crystal was grown by the slow evaporation method at room temperature. From the single crystal X-ray diffraction pattern showed that monoclinic phase and space group P21.

1. Introduction

Sucrose is an organic compound with general chemical formula $C_{12}H_{22}O_{11}$ a disaccharide and non-reducing sugars. It is widely distributed in nature, have been found universally throughout the plant kingdom in fruits, seeds, roots and tubers. Melting temperature of sucrose crystal is 186 ˚C. Sucrose crystal is isolated from Plam juice (Palmyrah tree) (Kumaran & Moorthy Babu, 1999). Sucrose is widely used for food industry and bakery items. Recently, sucrose is used as a promising material for dosimetry (Nakajima & Otsuki, 1989; Hamzaoui *et al.,* 2009; Peimel-Stuglik, 2010), hologram (Ponce-Lee *et al.,* 2004), biological probe (Predoi *et al.,* 2010), X-ray osteodensitometry (Ryzhikov *et al.,* 2005), nonlinear optics (Kaminskii, 2003), phase transition (Son *et al.,* 2010) and Low-temperature electrolytic coloration (Hongen Gu *et al.,* 2012).

We have successfully isolated for the sucrose crystal derived from the hot MeOH extract of the rhizome powder of *G. superba*. This condensed extract has been used to grow single crystals from aqueous MeOH solvent. The grown crystal has been characterized with single crystal X-ray diffraction, X-ray diffraction, NMR spectroscopy, TG-DTA, Micro Raman, FTIR and UV-visible spectroscopy studies, To our knowledge, this is the first report for the phytochemical synthesis of new sucrose crystal derived from the hot MeOH extract of the rhizome powder of *G. superba.*

2. Experimental

2.1 Collection of rhizome

Gloriosa superba rhizome were collected from Siruvandal village, Ramanathapuram (DT), Tamil

Nadu, India

2.2 Sample preparation

First the *Gloriosa superba* rhizome were cleaned with tap water after that samples were cut into pieces of about 0.5 - 1 cm and shadow dry for one week. Dried samples were ground in a grinder with a 2 mm diameter mesh.

2.3 Soxhlet extraction

Dried and powdered sample 20g and 350 ml of methanol solvent were placed in a 500ml Soxhlet apparatus, boiled at 70 °C for 6 hours under the heating mantel. The extract has a yellow colour with the extraction solvent. The yellowish color turned in to pink color. The excess solvent was evaporated with the rotary evaporator. Final concentration 60 ml of solvent mixed compound were filtered through Whatman No. 1 filter paper and stored at vials. Sucrose crystals were successfully grown by the slow evaporation method at room temperature.

2.4 Characterization

UV – visible spectra of the phytosynthesized sucrose was obtained for the wavelength in the range of 350–800 nm using Shimadzu spectrophotometer (Model UV-1800) operating at a resolution of 1 nm. Fourier Transform Infrared Spectroscopy (FTIR) measurements were prepared by sucrose powder with KBr. This powder is then compressed into a thin pellet 12 mm in diameter and about 15 mg in weight. Spectrum was recorded in the 4000–400 cm^{-1} region. The micro raman analysis of our samples carried out in the instrument of princeton acton SP2500, Cs spectrometer 0.5 focal length triple grating monochromator excitation source, Ar^{+} Laser, 515.5 nm wavelength. Powder XRD - pattern was recorded by Cu Kα radiation with (λ= 1.54060 Å) nickel monochromator. The scanning was performed in the region of 2θ from 10° to 80°. The crystalline nature was calculated through the Scherrer's formula $D = 0.9\lambda / \beta\cos\theta$. The thermal behaviors of the sucrose crystals were studied with TGA and DTA in a air atmosphere, using 'Seiko SSC 5200 H' model analyser. NMR analyses were performed in CECRI at Karaikudi. Liquid state ^{1}H and ^{13}C NMR spectra were recorded on a BRUKER 400 MHz spectrometer. The proton spectra at 400 MHz were recorded at room temperature (33.2°C). Sample were prepared by dissolving about 0.25 g of the sample in 2.5 ml of DMSO. Proton spectrum has the following experimental parameters. Number of scans 15; spectral width 10000.00 Hz; acquisition time

1.63 s. The carbon (^{13}C) NMR spectra at 100 MHz were recorded at room temperature on the same instrument. The sample were prepared by dissolving about 0.25 g of the sample in 2.5 ml of DMSO. Proton spectrum following experimental parameter: number of scan 587; spectral width 29761.904 Mz; acquisition time 0.55 s. Single crystal X-ray intensity data sucrose was collected at room temperature (T=296 K) on a Bruker X8 KAPPA APEX-II CCD diffractometer equipped with graphite monochromated Mo Kα radiation. Initial unit cell parameters were obtained from SMART software. Data integration, correction for Lorentz and polarization effects and final cell refinement were performed by SAINTPLUS. An empirical absorption correction based on the multiple measurements of equivalent reflections was applied using SADABS program. Structure was obtained by a combination of the direct methods and difference Fourier syntheses and refined by full-matrix least-squares on F^2 using the SHELXTL (Sheldrick, 1997). All non hydrogen atoms were refined anisotropically. All hydrogen atoms were replaced in ideal positions and refined as riding atoms with relative isotropic displacement parameters.

3 Results and discussion

3.1 Single Crystal X-ray Diffraction

The sucrose crystal and morphology of sucrose crystal as shown in the Fig. 1(a-b) is subjected by single crystal X-ray diffraction studies, solved and refined. Tables 1 and 2 show the detailed report of the structure refinement (SMART V5.05 Software for CCD Detector System: AINTPLUS, V5.00 Software for the CCD Detector System)and hydrogen coordinates of crystal. Sucrose crystals are monoclinic space group as P21 with cell dimensions are a = 7.7540 Å, b = 8.7130 Å, c = 10. 8600Å and β = 103.0380 (SADABS. Program for absorption correction using SMART CCD based on the method of Blessing). The ORTEP diagram is show in Fig. 2. The atomic displacement parameters ($Å^2$) are given in Table 3. In sucrose crystal structures, two rings which consists of a glucopyran ring and glucofuran ring. The glucopyran ring C5-O4-C4 and glucofuran ring C7-O8-C10 the bond angles are 115.82˚ and 111.69˚. The interconnection of oxygen molecule binding with glucopyran ring and glucofuran ring C5-O6-C7 bond length are 113.72˚. The bond length, bond angle and Torsion angles are given in Table 4. The hydrogen bonds of the sucrose crystal are O-H…O highest >DHA values are

attributed to the O1-H (1A)…O9 for 174 ˚ and hydrogen bonds are given in Table 5. The molecular packing of sucrose crystal is shown in Fig. 3, packing diagram of a-axis, b-axis and c-axis.

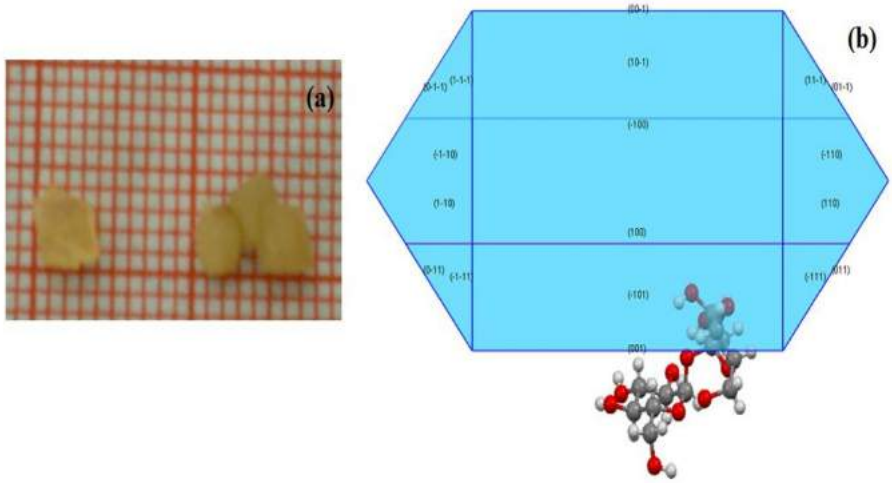


Fig. 1. The crystal diagram of (a) sucrose crystal and (b) Morphology of sucrose crystal using (Mercury 2.4 software).

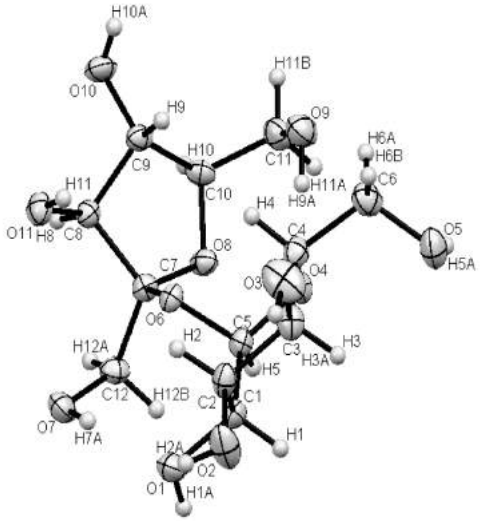


Fig. 2. ORTEP diagram of sucrose crystals

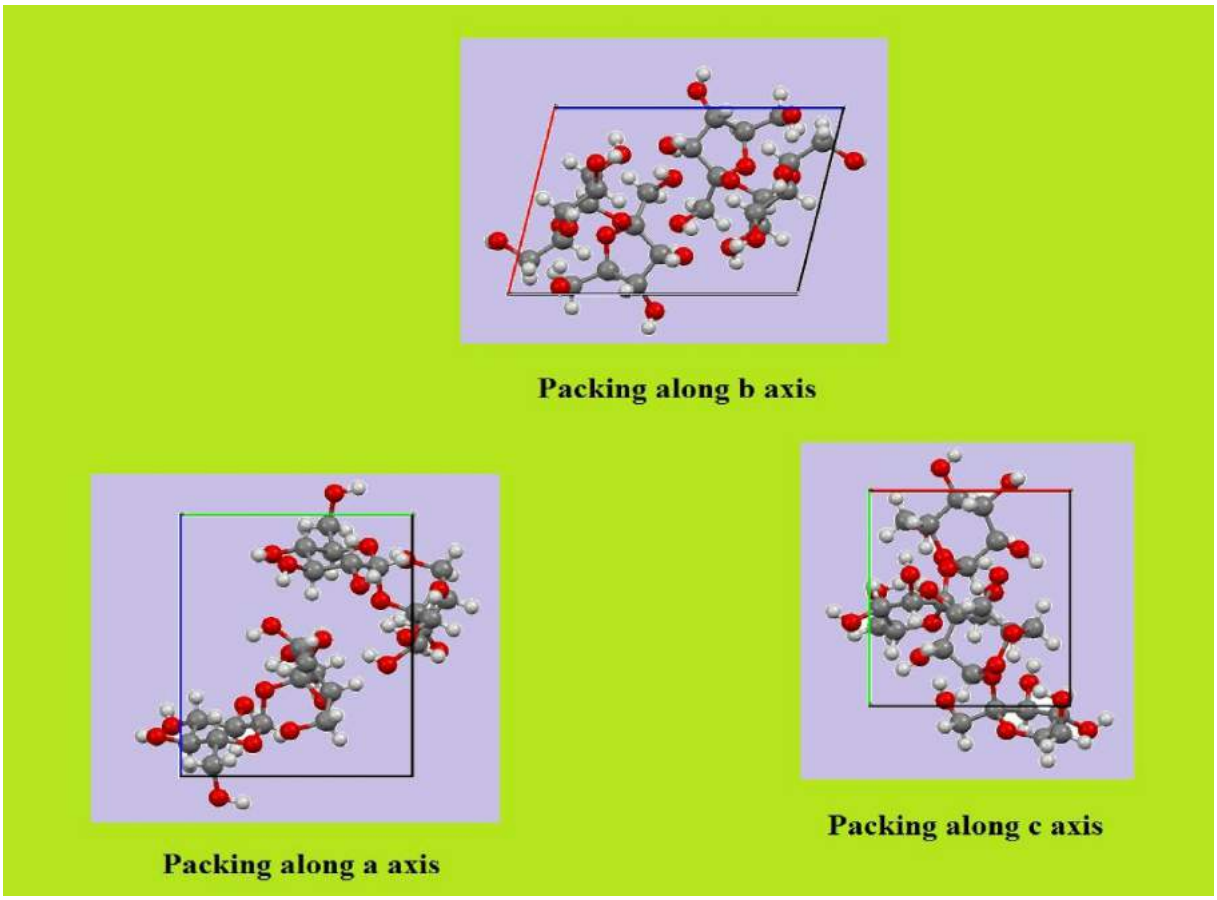


Fig. 3. The molecular packing of sucrose crystals packing along a, b and c axis diagram.

Table 1. Single crystal X-ray diffraction data of sucrose crystal.

Identification code	Sucrose crystal
Empirical formula	C_{12} H_{22} O_{11}
Formula weight	342.30
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P21
Unit cell dimensions a (Å)	7.7540(2)
b (Å)	8.7130(2)
c (Å)	10.86000(10)
α (º)	90
β (º)	103.0380(10)
γ (º)	90
Volume	714.79(3) $Å^3$
Z, Calculated density	2, 1.590 Mg/m^3
Absorption coefficient	0.142 mm^{-1}
F(000)	364
Crystal size	0.35 x 0.30 x 0.20 mm
Theta range for data collection	2.70 to 24.99 deg.
Limiting indices	-7<=h<=9, -10<=k<=10, -12<=l<=12
Reflections collected / unique	6820 / 2450 [R(int) = 0.0249]
Completeness to theta	= 24.99 99.9 %
	Semi-empirical from equivalents

	0.9865 and 0.9145
Absorption correction	
	Full-matrix least-squares on F^2
Max. and min. transmission	2450 / 1 / 240
Refinement method	1.077
Data / restraints / parameters	R1 = 0.0229, wR2 = 0.0589
	R1 = 0.0235, wR2 = 0.0593
Goodness-of-fit on F^2	
	-0.1(7)
Final R indices [I >2sigma(I)]	0.163 and -0.155 e.Å^3
R indices (all data)	
Absolute structure parameter	
Largest diff. peak and hole	

Table 2. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters (Å$^2 \times 10^3$)

Atom	X	Y	Z	U(eq)
C(1)	6355(2)	2343(2)	8127(1)	23(1)
C(2)	5642(2)	728(2)	7857(1)	25(1)
C(3)	4421(2)	382(2)	8739(2)	26(1)
C(4)	2955(2)	1572(2)	8591(1)	25(1)
C(5)	4854(2)	3513(2)	8001(1)	20(1)
C(6)	1840(2)	1383(2)	9569(2)	31(1)
C(7)	3698(2)	5165(2)	6246(1)	20(1)
C(8)	2146(2)	5184(2)	5073(1)	20(1)
C(9)	554(2)	5421(2)	5651(1)	22(1)
C(10)	1285(2)	6476(2)	6763(1)	23(1)
C(11)	471(2)	6272(2)	7890(	30(1)

			2)	
C(12)	5434(2)	5782(2)	6032(2)	25(1)
O(1)	7473(2)	2731(2)	7290(1)	29(1)
O(2)	7027(2)	-387(2)	8090(1)	39(1)
O(3)	3554(2)	-1047(2)	8486(2)	46(1)
O(4)	3686(1)	3104(1)	8774(1)	23(1)
O(5)	2859(2)	1634(2)	10816(1)	36(1)
O(6)	3911(1)	3623(1)	6713(1)	19(1)
O(7)	6208(1)	4737(2)	5299(1)	30(1)
O(8)	3163(1)	6146(1)	7122(1)	23(1)
O(9)	398(2)	4709(2)	8268(1)	35(1)
O(10)	-894(2)	6118(2)	4783(1)	30(1)
O(11)	2042(1)	3913(2)	4259(1)	27(1)

Table 3. Atomic displacement parameters ($Å^2$)

Atom	**U11**	**U22**	**U33**	**U23**	**U13**	**U12**
C(1)	22(1)	27(1)	18(1)	2(1)	2(1)	2(1)
C(2)	33(1)	23(1)	19(1)	2(1)	5(1)	5(1)
C(3)	34(1)	21(1)	24(1)	0(1)	8(1)	-2(1)
C(4)	27(1)	25(1)	22(1)	2(1)	4(1)	-4(1)
C(5)	22(1)	20(1)	17(1)	-1(1)	3(1)	0(1)
C(6)	31(1)	32(1)	33(1)	6(1)	11(1)	0(1)
C(7)	20(1)	17(1)	21(1)	0(1)	4(1)	0(1)
C(8)	20(1)	20(1)	20(1)	2(1)	3(1)	-2(1)
C(9)	18(1)	23(1)	23(1)	5(1)	3(1)	1(1)
C(10)	19(1)	21(1)	30(1)	0(1)	6(1)	3(1)
C(11)	28(1)	35(1)	31(1)	-5(1)	11(1)	2(1)
C(12)	20(1)	25(1)	30(1)	2(1)	6(1)	-2(1)

O(1)	22(1)	34(1)	31(1)	2(1)	8(1)	1(1)
O(2)	54(1)	31(1)	36(1)	10(1)	22(1)	20(1)
O(3)	51(1)	23(1)	68(1)	-7(1)	25(1)	-8(1)
O(4)	27(1)	22(1)	21(1)	-1(1)	9(1)	0(1)
O(5)	47(1)	38(1)	26(1)	5(1)	14(1)	7(1)
O(6)	22(1)	17(1)	17(1)	1(1)	2(1)	-1(1)
O(7)	26(1)	38(1)	30(1)	4(1)	13(1)	1(1)
O(8)	19(1)	25(1)	25(1)	-7(1)	3(1)	1(1)
O(9)	29(1)	42(1)	36(1)	8(1)	12(1)	2(1)
O(10)	18(1)	39(1)	32(1)	8(1)	1(1)	3(1)
O(11)	30(1)	27(1)	22(1)	-4(1)	5(1)	-3(1)

Table 4. Bond length **(Å), Bond angles (˚) and** Torsion angles **(˚) of sucrose crystals.**

Bond length	**Values (Å)**	**Bond angles**	**Values (˚)**	**Torsion angles**	**Values (˚)**
C(1)-O(1)	1.4316(19)	O(1)-C(1)-C(2)	110.06(13)	O(1)-C(1)-C(2)-O(2)	63.20(15)
C(1)-C(2)	1.516(2)	O(1)-C(1)-C(5)	110.08(12)	C(5)-C(1)-C(2)-O(2)	-174.41(12)
C(1)-C(5)	1.530(2)	C(2)-C(1)-C(5)	111.38(12)	O(1)-C(1)-C(2)-C(3)	-178.34(12)
C(1)-H(1)	0.98	O(1)-C(1)-H(1)	108.4	C(5)-C(1)-C(2)-C(3)	-55.95(15)
C(2)-O(2)	1.427(2)	C(2)-C(1)-H(1)	108.4	O(2)-C(2)-C(3)-O(3)	-64.55(18)
C(2)-C(3)	1.521(2)	C(5)-C(1)-H(1)	108.4	C(1)-C(2)-C(3)-O(3)	174.46(14)
C(2)-H(2)	0.98	O(2)-C(2)-C(1)	111.74(13)	O(2)-C(2)-C(3)-C(4)	177.26(13)
C(3)-O(3)	1.412(2)	O(2)-C(2)-C(3)	107.72(13)	C(1)-C(2)-C(3)-C(4)	56.28(16)
C(3)-C(4)	1.521(2)	C(1)-C(2)-C(3)	108.26(13)	O(3)-C(3)-C(4)-O(4)	-177.41(12)
C(3)-H(3)	0.98	O(2)-C(2)-H(2)	109.7	C(2)-C(3)-C(4)-O(4)	-54.89(16)
C(4)-O(4)	1.446(2)	C(1)-C(2)-H(2)	109.7	O(3)-C(3)-C(4)-	64.54(17)

				C(6)	
C(4)-C(6)	1.522(2)	C(3)-C(2)-H(2)	109.7	C(2)-C(3)-C(4)-C(6)	-172.93(13)
C(4)-H(4)	0.98	O(3)-C(3)-C(4)	105.59(14)	O(1)-C(1)-C(5)-O(4)	177.03(11)
C(5)-O(4)	1.4119(18)	O(3)-C(3)-C(2)	112.87(14)	C(2)-C(1)-C(5)-O(4)	54.65(16)
C(5)-O(6)	1.4283(17)	C(4)-C(3)-C(2)	110.81(13)	O(1)-C(1)-C(5)-O(6)	54.96(16)
C(5)-H(5)	0.98	O(3)-C(3)-H(3)	109.2	C(2)-C(1)-C(5)-O(6)	-67.41(15)
C(6)-O(5)	1.423(2)	C(4)-C(3)-H(3)	109.2	O(4)-C(4)-C(6)-O(5)	-56.95(17)
C(6)-H(6A)	0.97	C(2)-C(3)-H(3)	109.2	C(3)-C(4)-C(6)-O(5)	64.00(19)
C(6)-H(6B)	0.97	O(4)-C(4)-C(3)	110.73(12)	O(8)-C(7)-C(8)-O(11)	-157.67(12)
C(7)-O(8)	1.4088(18)	O(4)-C(4)-C(6)	105.75(13)	O(6)-C(7)-C(8)-O(11)	-39.21(16)
C(7)-O(6)	1.4328(18)	C(3)-C(4)-C(6)	112.43(13)	C(12)-C(7)-C(8)-O(11)	84.81(16)
C(7)-C(12)	1.5158(19)	O(4)-C(4)-H(4)	109.3	O(8)-C(7)-C(8)-C(9)	-31.27(15)
C(7)-C(8)	1.5430(19)	C(3)-C(4)-H(4)	109.3	O(6)-C(7)-C(8)-C(9)	87.20(13)
C(8)-O(11)	1.4076(18)	C(6)-C(4)-H(4)	109.3	C(12)-C(7)-C(8)-C(9)	-148.79(13)
C(8)-C(9)	1.520(2)	O(4)-C(5)-O(6)	110.11(11)	O(11)-C(8)-C(9)-O(10)	-78.57(17)
C(8)-H(8)	0.98	O(4)-C(5)-C(1)	110.85(12)	C(7)-C(8)-C(9)-O(10)	154.86(12)
C(9)-O(10)	1.4285(18)	O(6)-C(5)-C(1)	110.07(11)	O(11)-C(8)-C(9)-C(10)	161.93(12)
C(9)-C(10)	1.522(2)	O(4)-C(5)-H(5)	108.6	C(7)-C(8)-C(9)-C(10)	35.36(15)
C(9)-H(9)	0.98	O(6)-C(5)-H(5)	108.6	O(10)-C(9)-C(10)-O(8)	-147.54(12)
C(10)-O(8)	1.4493(17)	C(1)-C(5)-H(5)	108.6	C(8)-C(9)-C(10)-O(8)	-27.52(15)
C(10)-C(11)	1.509(2)	O(5)-C(6)-C(4)	111.50(13)	O(10)-C(9)-	91.44(16)

				C(10)-C(11)	
C(10)-H(10)	0.98	O(5)-C(6)-H(6A)	109.3	C(8)-C(9)-C(10)-C(11)	-148.54(13)
C(11)-O(9)	1.427(2)	C(4)-C(6)-H(6A)	109.3	O(8)-C(10)-C(11)-O(9)	-69.86(17)
C(11)-H(11A)	0.97	O(5)-C(6)-H(6B)	109.3	C(9)-C(10)-C(11)-O(9)	48.75(19)
C(11)-H(11B)	0.97	C(4)-C(6)-H(6B)	109.3	O(8)-C(7)-C(12)-O(7)	171.55(12)
C(12)-O(7)	1.428(2)	H(6A)-C(6)-H(6B)	108	O(6)-C(7)-C(12)-O(7)	50.46(16)
C(12)-H(12A)	0.97	O(8)-C(7)-O(6)	111.01(11)	C(8)-C(7)-C(12)-O(7)	-72.19(16)
C(12)-H(12B)	0.97	O(8)-C(7)-C(12)	107.16(12)	O(6)-C(5)-O(4)-C(4)	67.72(15)
O(1)-H(1A)	0.83(3)	O(6)-C(7)-C(12)	110.55(12)	C(1)-C(5)-O(4)-C(4)	-54.32(15)
O(2)-H(2A)	0.77(2)	O(8)-C(7)-C(8)	104.94(11)	C(3)-C(4)-O(4)-C(5)	54.90(15)
O(3)-H(3A)	0.76(3)	O(6)-C(7)-C(8)	107.98(11)	C(6)-C(4)-O(4)-C(5)	176.94(11)
O(5)-H(5A)	0.92(3)	C(12)-C(7)-C(8)	115.08(12)	O(4)-C(5)-O(6)-C(7)	108.30(13)
O(7)-H(7A)	0.85(3)	O(11)-C(8)-C(9)	115.42(12)	C(1)-C(5)-O(6)-C(7)	-129.20(12)
O(9)-H(9A)	0.90(3)	O(11)-C(8)-C(7)	115.68(12)	O(8)-C(7)-O(6)-C(5)	-45.29(15)
O(10)-H(10A)	0.84(2)	C(9)-C(8)-C(7)	102.40(11)	C(12)-C(7)-O(6)-C(5)	73.49(14)
O(11)-H(11)	0.81(3)	O(11)-C(8)-H(8)	107.6	C(8)-C(7)-O(6)-C(5)	-159.80(11)
		C(9)-C(8)-H(8)	107.6	O(6)-C(7)-O(8)-C(10)	-101.80(13)
		C(7)-C(8)-H(8)	107.6	C(12)-C(7)-O(8)-C(10)	137.39(12)
		O(10)-C(9)-C(8)	112.06(12)	C(8)-C(7)-O(8)-C(10)	14.60(15)
		O(10)-C(9)-C(10)	111.30(13)	C(11)-C(10)-O(8)-C(7)	132.59(13)
		C(8)-C(9)-	102.62(11)	C(9)-C(10)-	8.15(16)

C(10)	O(8)-C(7)	
	O(10)-C(9)-H(9)	110.2
	C(8)-C(9)-H(9)	110.2
	C(10)-C(9)-H(9)	110.2
	O(8)-C(10)-C(11)	109.74(12)
	O(8)-C(10)-C(9)	105.38(11)
	C(11)-C(10)-C(9)	115.07(13)
	O(8)-C(10)-H(10)	108.8
	C(11)-C(10)-H(10)	108.8
	C(9)-C(10)-H(10)	108.8
	O(9)-C(11)-C(10)	113.35(13)
	O(9)-C(11)-H(11A)	108.9
	C(10)-C(11)-H(11A)	108.9
	O(9)-C(11)-H(11B)	108.9
	C(10)-C(11)-H(11B)	108.9
	H(11A)-C(11)-H(11B)	107.7
	O(7)-C(12)-C(7)	111.02(13)
	O(7)-C(12)-H(12A)	109.4
	C(7)-C(12)-H(12A)	109.4
	O(7)-C(12)-H(12B)	109.4
	C(7)-C(12)-H(12B)	109.4
	H(12A)-C(12)-H(12B)	108
	C(1)-O(1)-H(1A)	112.7(17)
	C(2)-O(2)-H(2A)	113.8(17)
	C(3)-O(3)-H(3A)	108(2)

C(5)-O(4)-C(4)	115.82(11)
C(6)-O(5)-H(5A)	109.0(18)
C(5)-O(6)-C(7)	113.72(11)
C(12)-O(7)-H(7A)	105.8(16)
C(7)-O(8)-C(10)	111.69(11)
C(11)-O(9)-H(9A)	108.6(17)
C(9)-O(10)-H(10A)	104.2(16)
C(8)-O(11)-H(11)	110.8(16)

Table 5. Hydrogen bonding (Å and ˚)

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(11)-H(11)...O(10)#1	0.81(3)	2.06(3)	2.8675(19)	170(2)
O(1)-H(1A)...O(9)#2	0.83(3)	2.03(3)	2.8553(19)	174(2)
O(10)-H(10A)...O(7)#3	0.84(2)	1.89(2)	2.7167(17)	169(2)
O(9)-H(9A)...O(4)	0.90(3)	1.96(3)	2.8509(16)	169(3)
O(7)-H(7A)...O(1)	0.85(3)	1.97(3)	2.7809(18)	158(2)
O(2)-H(2A)...O(11)#4	0.77(2)	2.10(2)	2.8688(18)	171(2)
O(3)-H(3A)...O(8)#5	0.76(3)	2.44(3)	2.8399(18)	115(3)
O(3)-H(3A)...O(2)	0.76(3)	2.53(3)	2.880(2)	110(3)
O(5)-H(5A)...O(2)#6	0.92(3)	1.95(3)	2.848(2)	164(3)

Symmetry transformations used to generate equivalent atoms:
#1 -x,y-1/2,-z+1 #2 x+1,y,z #3 x-1,y,z #4 -x+1,y-1/2,-z+1
#5 x,y-1,z #6 -x+1,y+1/2,-z+2.

3.2 UV-VIS spectroscopy studies

The UV-Vis absorption spectrum of sucrose record in liquid phase using as a solvent methanol is shown in Fig. 4. Recorded in the range of 200-900nm. The cut off wavelength for this crystal is found to be 253nm are attributed to the overlapping of π-π^* transition. The absorption is very low and found to be steady from 253-900nm is an advantage is it is the key requirement for materials are using the good optical studies.

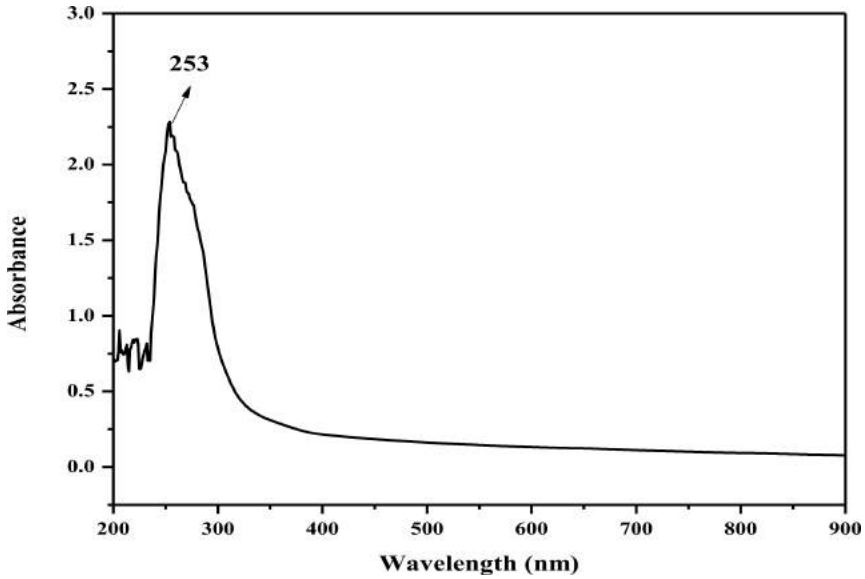


Fig. 4. UV-Vis absorption spectrum of sucrose samples

3.3 FT-IR and Micro-Raman Spectroscopy

The sucrose sample is characterized by FT-IR and Micro-Raman spectra in order to identify the functional groups and detect the vibration modes of molecules of the sample are shown in Fig. 5(a-b). The hydroxyl stretching vibration are generally [36] observed in the around 3500cm-1. The O-H group vibration are likely to be the most sensitive to the environment , so they show pronounced shift in the spectra of hydrogen bond species. The band due to the O-H stretching are broad band appears at 3335 cm^{-1} in IR spectrum and Strong band appeared in 3227 cm^{-1} for Raman spectrum. The FT-IR band observed 2923 cm^{-1} have been assigned to CH_2 symmetric stretching vibrations. The CH_2 bending modes follow in decreasing frequency, the general order CH_2 deformation > CH_2 Scis > CH_2 Wagg > CH_2 twist > CH_2 rock>. Since the bending modes involving the hydrogen atom attached to the central carbon atoms falls in 1440-1312cm^{-1} range, there is extensive vibrational coupling of these modes with CH_2 twist. It is some not that both ρ CH_2 and r CH_2 are sensitive to the molecular conformation. The

scissoring modes can be assigned to the medium IR & Raman bands at 1356 and 1312 cm^{-1}. The out-of-plane deformation modes (τOH and γOH) observed values are 1239 cm^{-1} and 1248 cm^{-1} for IR and Raman spectrum. The IR bands are observed at 1067 cm^{-1} are owing to the stretching of C-O-C [13, 14] likes in sucrose. The C-C stretching modes are strongly coupled with other modes and are associated with the observed 676, 917 and 1192 cm^{-1} for IR and Raman spectrum. The glucofuran ring deformation is clearly assigned to the IR band at 558 cm^{-1}.

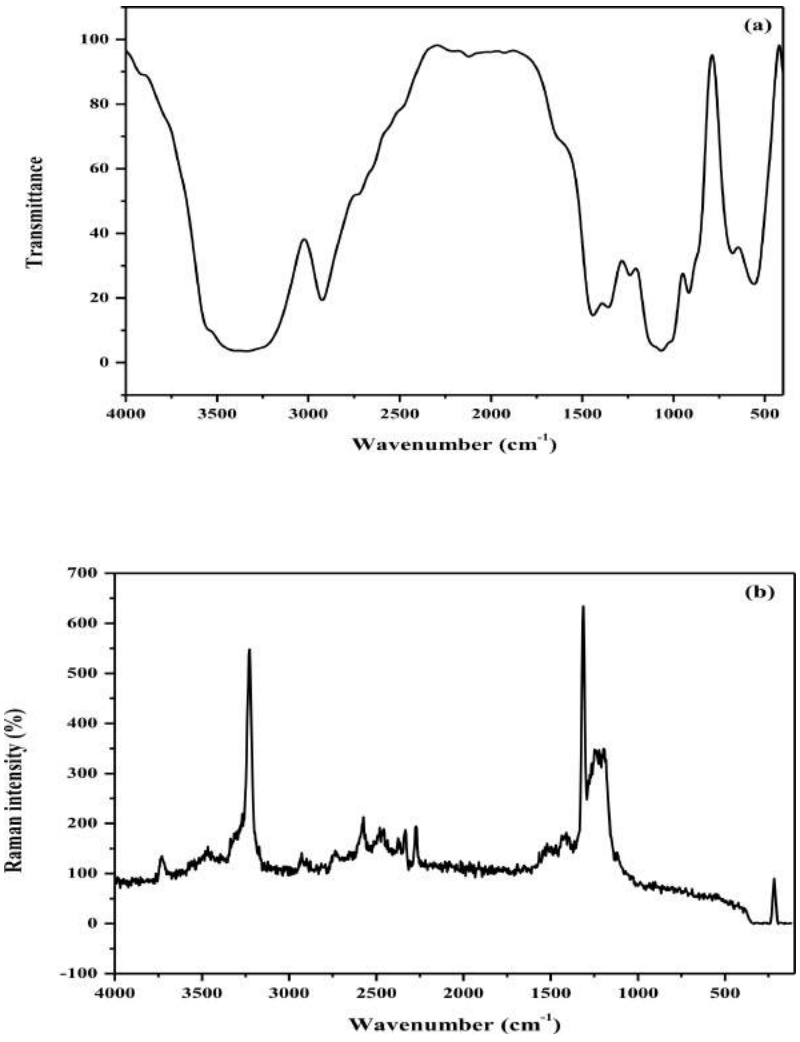


Fig. 5. (a) FT-IR spectrum and (b) FT-Raman spectrum of sucrose samples

3.4 Powder X-ray diffraction pattern

(Fig.6). XRD analysis showed seven distinct diffraction peaks at 11.61°, 13.10°, 18.77°, 19.54°, 24.73°, 24.73°, 25.19° and 38.28°, which can be indexed with the planes (001), (110), (111), (210), (211), (300), and (412) for the monoclinic sucrose. A number of Bragg reflections corresponding to the lattice planes are observed which may be indexed based on the primitive monoclinic sucrose and the values are matched with standard database values (JCPDS No. 24-1977).

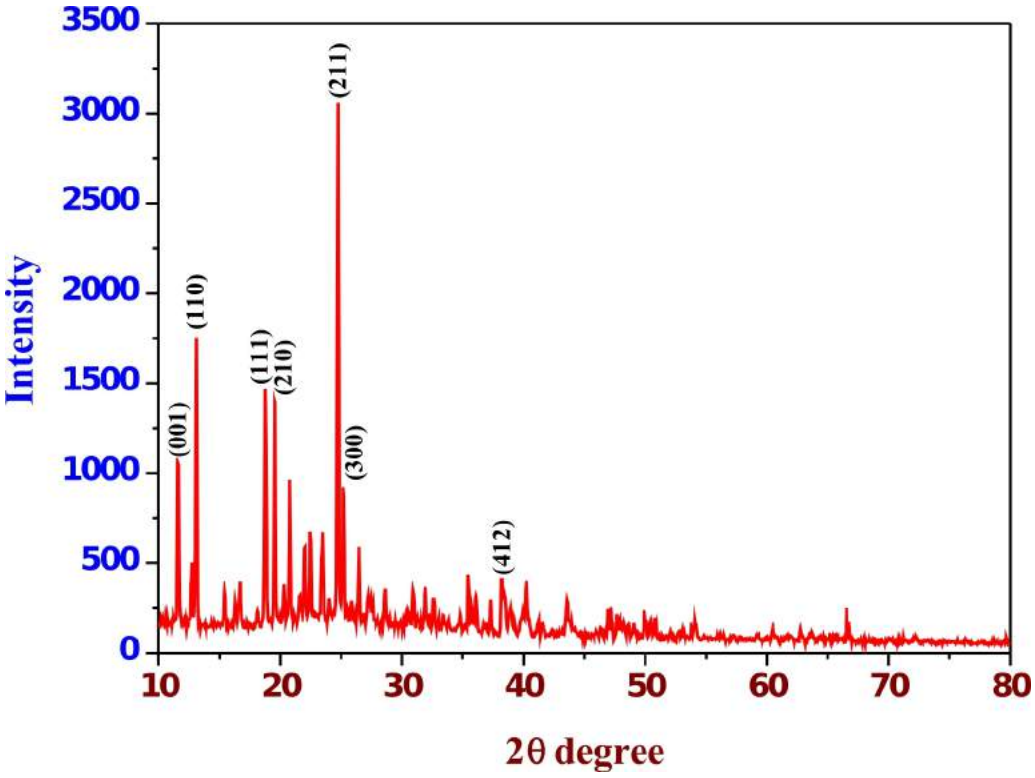


Fig. 6 XRD analysis of sucrose crystal

3.5 H^1 NMR, DMSO – D6 -300 MHz

(Fig . 7) 3.138 – 3.107 (m, 24), 3.181 – 3.155 (m, 2H), 3.396 (s, 2H), 3.560 (s, 4H), 3.643 (q, j = 11.7 Hz1 1H) 3.767 (Q, J = 15Hz, 1H), 3.758 (d, J = 9.9 Hz, 1H) 4.372 (d, J = 4.5 Hz, 2H) 4.501 (d, J = 6Hz, 1H) 4.797 (t, J = 8.7 Hz, 3H), 5.7173 (s, 1H), 5.184 (s1, 2H).

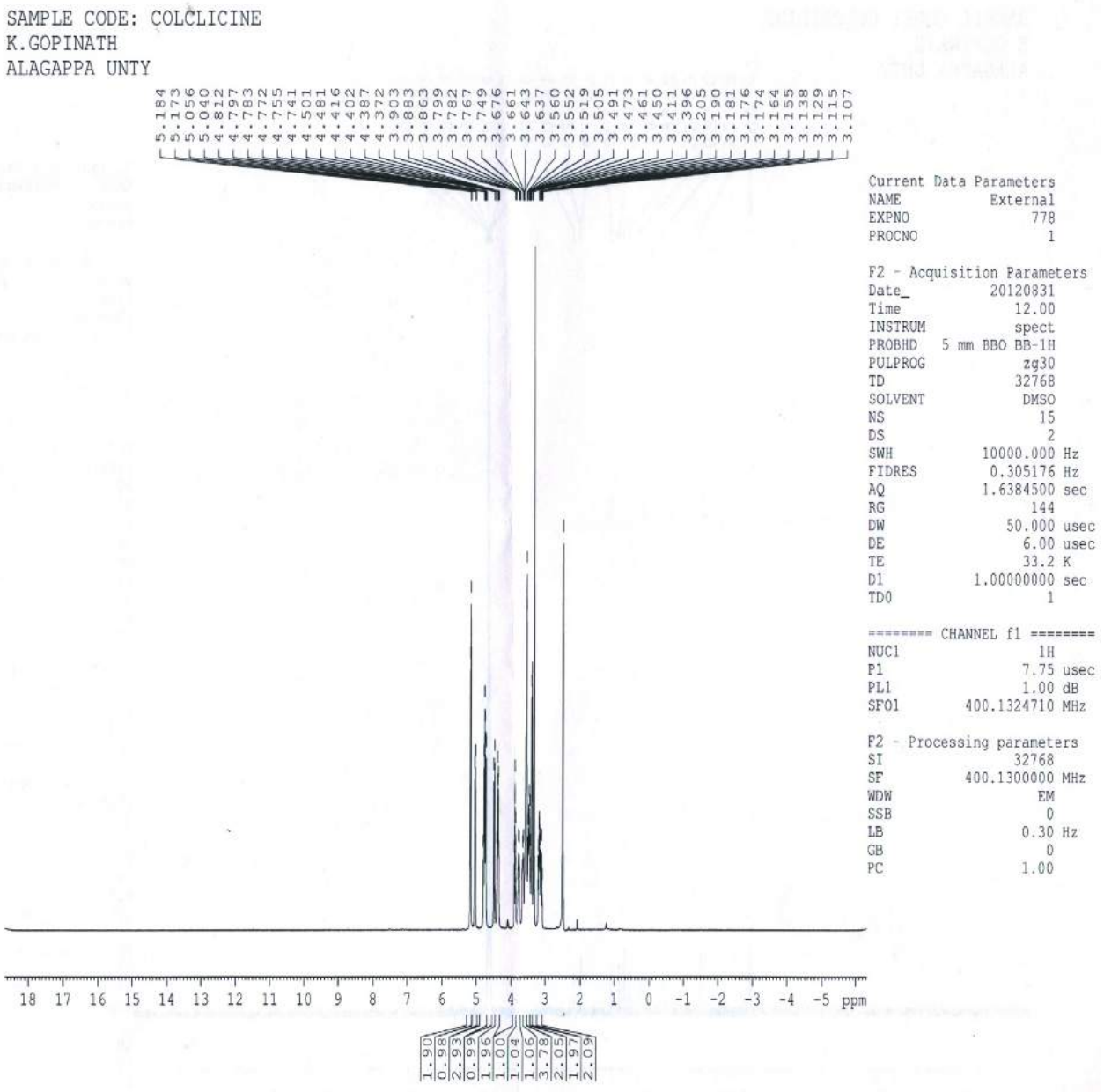


Fig. 7: H^1 **NMR analysis of sucrose**

3.5.1 C^{13} NMR

(Fig. 8) C1 – 62.57, C2 – 70.27, C3 – 72.07 C4 – 73.26, C5 – 74.70, C6- 77.43, C7 – 83.00, C8 – 92.18, C9 – 104.17.

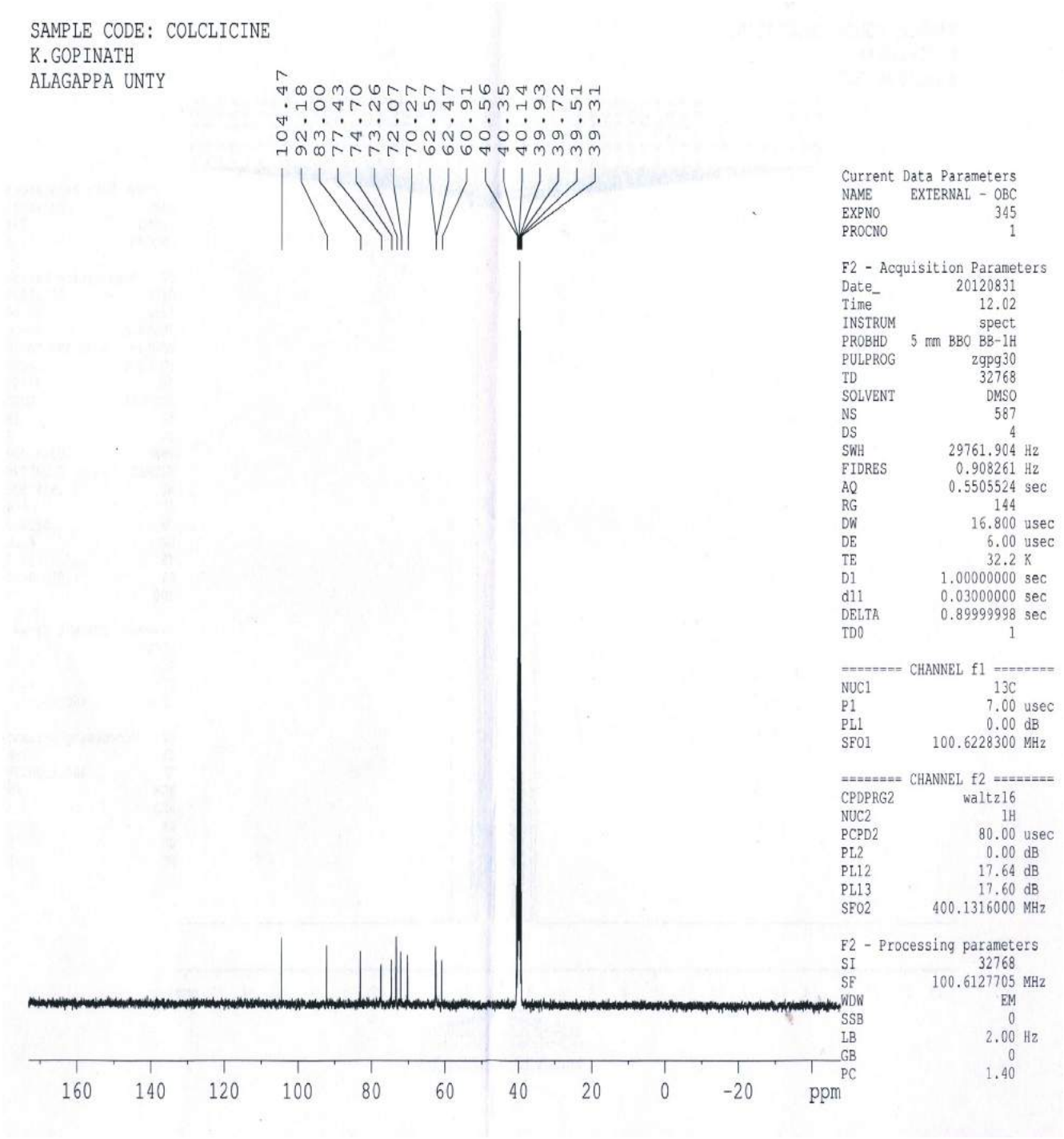


Fig. 8: C^{13} NMR analysis of sucrose

3.6. **Thermal studies**

Thermo gravimetric/ differential thermal analysis (TG/DTA) measurement is used to examine the thermal stability of the crystalline sample. The sucrose sample recorded in the temperature range 30-800 ˚Cat a heating rate of 20 ˚C/min in nitrogen atmosphere is shown in Fig. 9. The TG analysis sample exhibits three stages of decomposition takes place at 209 ˚C, 322 ˚C and 558˚C, respectively. In TGA curve shows the up to 209 ˚C their weight is 100% (no weight loss) for sucrose sample. The sucrose sample shows the major weight loss 48.6648 % in the temperature range between 209 ˚C to 322 ˚C. This can be attributed to decomposition of might involve dehydration of –OH groups which are not hydrogen bonded, in addition to expulsion of –CO, H_2 and CO_2. The TGA analysis shows the 100 % weight loss not involving the temperature between the 322˚C to 558˚C, this stage there is a degradation leading to the formation of a thermally stable species maintaining stability up to 800 ˚C.

The DTA analysis has three endothermic peaks, at around 195 ˚C, 226 ˚C and 400 ˚C, respectively. The form of heat induced decomposition. Since all the decomposition peaks are endothermic, there is no oxidation reaction when the sucrose is subjected to heat changes. There froe, the three stages of weight loss is shown in TGA are owing to endothermic peaks.

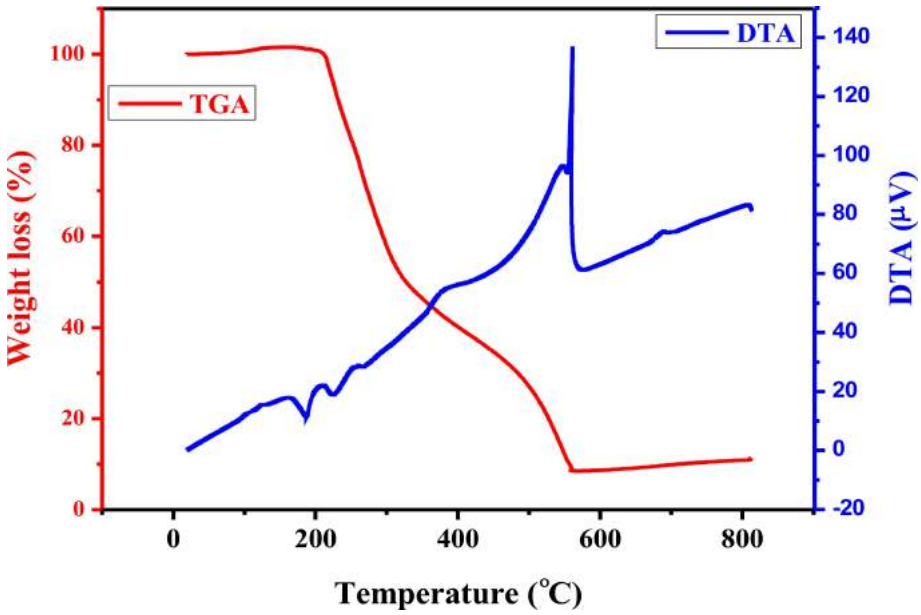


Fig. 9. TG-DTA curves of thermal decomposition of the single crystal sucrose.

3.7 Homo-lumo analysis

The electronic absorption corresponding to the transition from the ground state to the first excited state is mainly described by one electron excitation from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) (Kavitha *et al.,* 2010: Prasad *et al.,* 2010). HOMO represents the ability to donate an electron whereas LUMO represents the ability to obtain an electron. The HOMO locates on the glucofuran ring. The LUMO is located on the glucopyran ring. Consequently, the HOMO-LUMO transition implies an electron density transfer from, the orbitals are localized on the rings, both orbitals are π-antibonding-type orbitals.

The frontier molecular orbital's of DAST is shown in Fig. 10. The energy of HOMO, LUMO and HOMO-LUMO gap are -6.7152 eV, 1.4536 eV, and - 8.1688 eV respectively.

The calculated Self Consistent Field (SCF) energy of sucrose is -1297.5558 a.u. at B3LYP/6-311 G(d,p). The HOMO and LUMO energy gap explains the fact that eventual charge transfer interaction is taking place within the molecule.

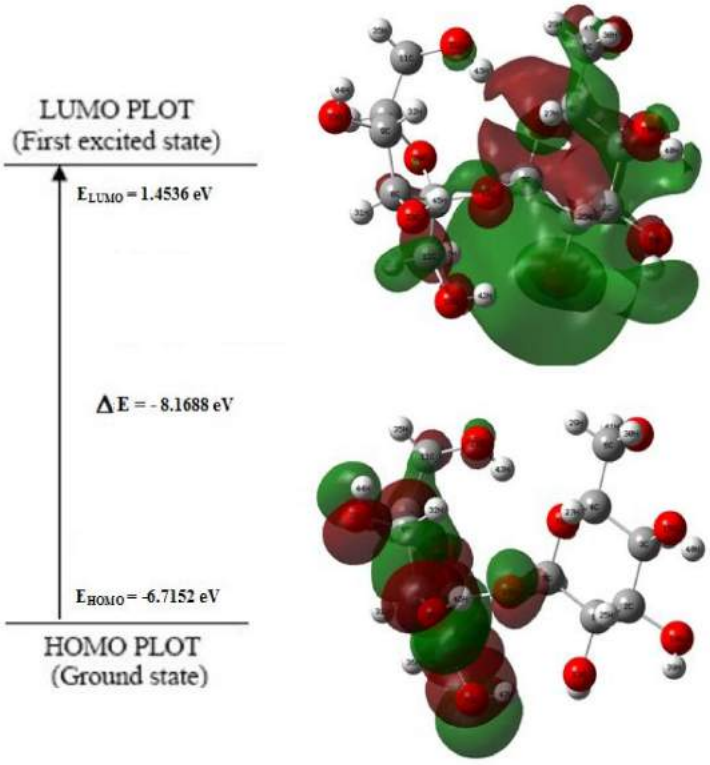


Fig. 10: Homo-lumo analysis of sucrose crystal

3.8 Molecular electrostatic potential (MEP)

The 3D plots of molecular electrostatic potential (MEP) of sucrose molecule is illustrated in Fig. 11. The MEP is a plot of electrostatic potential mapped onto the constant electron density surface. The MEP surface super-imposed on top of the total energy density. The MEP is a useful property to study reactivity given that an approaching electrophile will be attracted to negative region (where the electron distribution effect is dominant). In the majority of the MEPs, while the maximum negative region which preferred site of for electrophilic attack indication as red colour, the maximum positive region which preferred site for nucleophilic attack symptoms as blue colour. The importance of MEP lies in the fact that it simultaneous displays molecular size, shape as well as positive, nagative and neutral elelctrostatic potential regions in terms of colour grading (Fig. 11) and is very useful in research of molecular structure with its physiochemical property relationship (Murry and Sen, 1998: Scrocco *et al.,* 1978). The resulting surface simultaneously displays molecular size and shape and electrostatic potential value.

The different values of the electrostatic potential at the surface are represented by different colours. The potential increases in the order red < orange < yellow < green< blue. The colour code of these maps is the range between the – 0.0738 a.u. (Deepest red) to 0.0738 a.u. (Deepest blue) in sucrose molecules. Whereas blue colour indicates the strongest attraction and red colour indicates the strongest repulsion. The regions of negative V(r) are usually associated with the lone pair of electro native atoms. As can be seen from the MEP map of the sucrose molecule, while regions having the negative potential are over the electronegative atom (oxygen atoms), the regions having the positive potential are over the hydrogen atoms. The contour map of electrostatic potential of the sucrose molecule has been constructed by the B3LYP/ 6-311 G(d,p) basis set is shown in Fig. 11 also confirms the different negative and positive potential sites of the molecules in accordance with the total electron density surface.

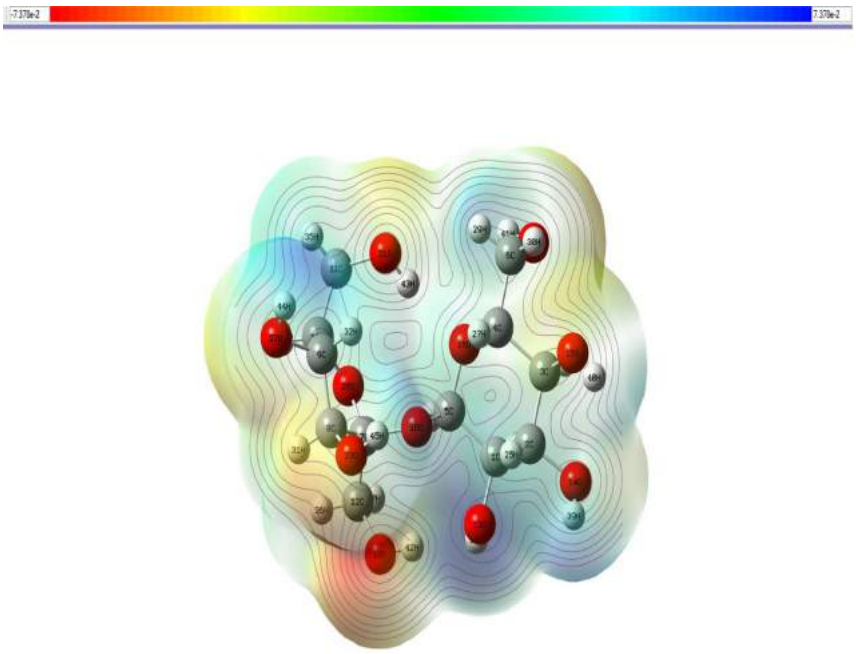


Fig. 11. The atomic orbital compositions of the frontier molecular orbital of DAST.

3.9 Thermodynamic properties

Several calculated thermodynamic parameters are presented in Table 6. Scale factors have been recommended (Palafox. 2000) for an accurate prediction in determining the zero-point vibration energies (ZPVE), and the entropy, Svib(T). The variations in the ZPVE's seem to be insignificant. The total energies and the change in the total entropy of sucrose at room temperature at different methods are also presented.

Table 6. Theoretically computed Total energy, Zero-Point vibrational energy, Rotational constant, Rotational temperature, Vibrational temperature, and Thermal energy, Molar capacity at constant volume, Entropy for Sucrose crystal.

Table 6. Theoretically computed Total energy, Zero-Point vibrational energy, Rotational constant, Rotational temperature, Vibrational temperature, and Thermal energy, Molar capacity at constant volume, Entropy for Sucrose crystal.

Parameters	**B3LYP /6-311G (d,p)**
Zero-point vibrational energy (Kcal/Mol)	243.37578
Rotational constants (GHz) A B C	 0.34923 0.21848 0.16320
Rotational temperature(K) A B C	 0.01676 0.01049 0.00783
Thermal energy (KCal/Mol) Total Translation Rotational Vibrational	 251.201 0.889 0.889 249.423
Molar capacity at constant volume (Cal/Mol-Kelvin) Total Translation Rotational Vibrational	 57.312 2.981 2.981 51.351
Entropy(Cal/Mol-Kelvin) Total Translation Rotational Vibrational	110.078 43.384 34.511 32.183

3.10 Non-linear optical properties

The mean polarizability (α_{tot}), anisotropy of polarizability ($\Delta\alpha$) and average value of the first order hyperpolarizability (β_{tot}) of sucrose are calculated using B3LYP/6-311 G(d,p) basis set, based on the finite-field approach. In the presence of an applied electric field, the energy of a system is a function of the electric field. First order hyperpolarizalbility is a third rank tensor that can be described by 3x3x3 matrices. The 27 components of the 3D matrix can be reduced to 10 components due to the Kleinman symmetry (Kleinman,1962). It can be given in the lower tetrahedral format. It is obvious that lower part of the 3x3x3 matrices is a tetrahedral. The components of β are defined as the coefficients in the Taylor series expansion of the energy in the external electric field. When the external electric field is weak and homogeneous, this expansion becomes:

$$E = E^0 - \mu_\alpha F_\alpha - 1/2\mu_{\alpha\beta} F_\alpha F_\beta - 1/\mu_{\alpha\beta\gamma} F_\alpha F_\beta F_\gamma + \ldots\ldots \quad (1)$$

Where E^0 is the energy of the unperturbed molecules, F_α in the field at the region, μ_α, $\mu_{\alpha\beta}$ and $\mu_{\alpha\beta\gamma}$ are the components of dipole moments, polarizability and the first order hyperpolarizability, respectively. The polarizability (μ_{xx}, μ_{xy}, μ_{yy}, μ_{xz}, μ_{yz}, μ_{zz}) and first order hyperpolarizability (β_{xxx}, β_{xxy}, β_{xyy} ,β_{yyy}, β_{xxz}, β_{xyz}, β_{yyz}, β_{xzz}, β_{yzz}, β_{zzz}) tensor can be obtained the output file of Gaussian 09W. However, α and β values of Gaussian output are in atomic units (a.u.) therefore they have been converted into electronic units (esu) (for α_{tot}, 1 a.u. = 0.1482 x 10^{-24} esu and for β_{tot}, 1 a.u. = 8.6393x10^{-33} esu). The mean polarizability (α_{tot}), anisotropy of polarizability ($\Delta\alpha$) and average value of the first order hyperpolarizability (β_{tot}) can be calculated using the (2)-(4), respectively.

$$A_{tot} = 1/3\ (\alpha_{xx}+\alpha_{yy}+\alpha_{zz}) \quad (2)$$

$$\Delta\alpha = 1/\sqrt{2}\ [(\alpha_{xx} - \alpha_{yy})^2 + (\alpha_{yy} - \alpha_{zz})^2 + (\alpha_{zz} - \alpha_{xx})^2 + 6\alpha^2_{xz} + 6\alpha^2_{xy} + 6\alpha^2_{yz}]^{1/2} \quad (3)$$

$$\beta_{tot} = [(\beta_{xxx} + \beta_{xyy} + \beta_{xzz})^2 + (\beta_{yyy} + \beta_{yzz} + \beta_{yxx})^2 + (\beta_{zzz} + \beta_{zxx} + \beta_{zyy})^2\ ]^{1/2} \quad (4)$$

The calculated parameters as described above are shown in Fig. 12(a-b) for sucrose crystal. The calculate dipole moment is equal to 2.3544 Debye (D). Total polarizability (α_{tot}) is calculated as 21.1191 x 10^{-24} esu for sucrose crystal using B3LYP/6-311 G(d, p) basis set values. The first order hyperpolarizability is determined by the electron excitations that involve both the ground and excited states. Besides transition energy and transition dipole moment, the dipole moment difference between the ground and excited states is also important in the calculation of β_{tot}. The first order

hyperpolarizability values (β_{tot}) of the sucrose crystal are found to be 1533.639 x 10^{-33} esu. The first order hyperpolarizability β_{tot} dominated by the longitudinal components β_{zzz}. Domination of particular component indicates on a substantial delocalization of charges in the direction.

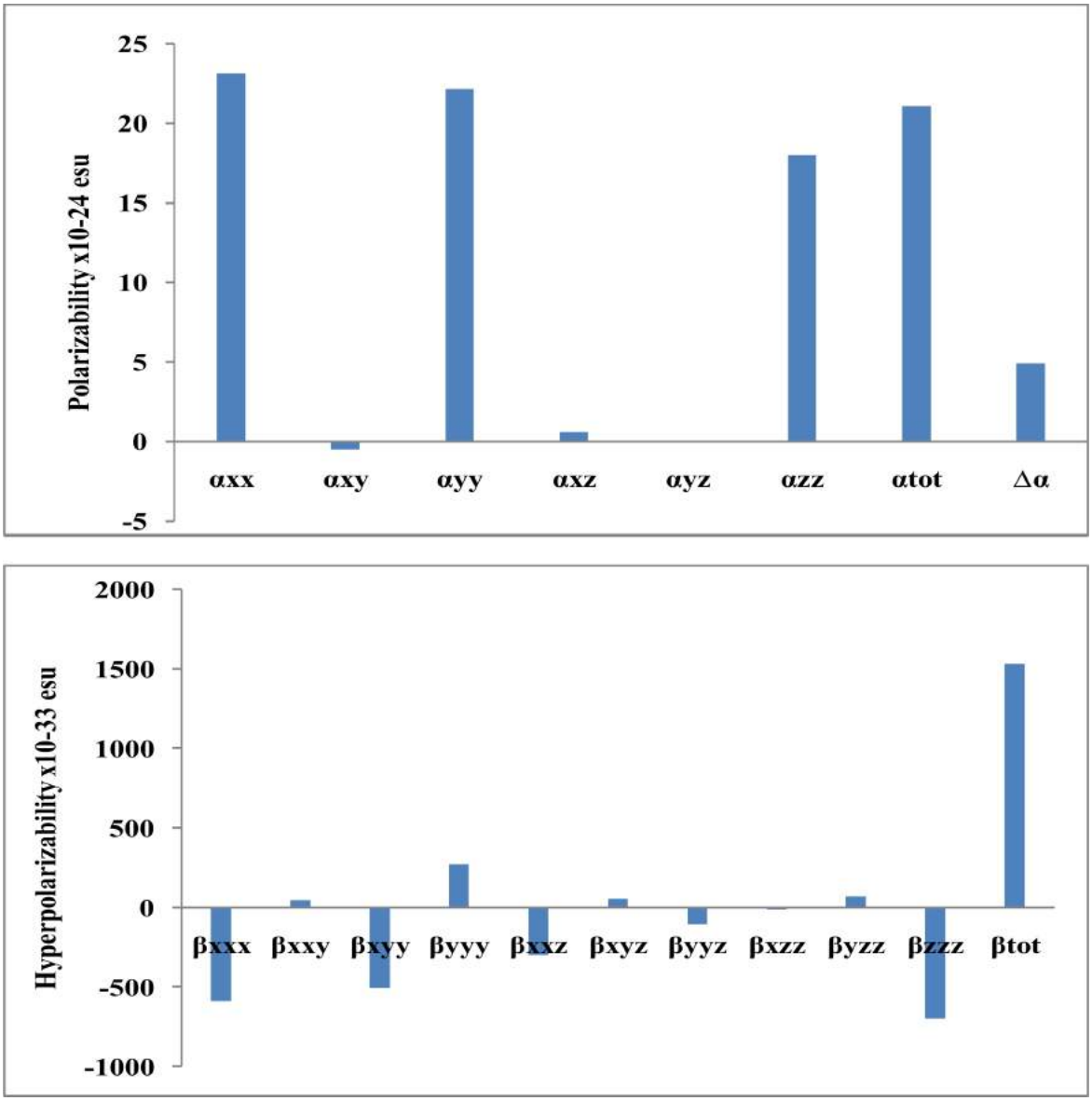


Fig. 12. B3LYP/6-311 G(d,p) of DAST molecules (a) polarizabilty and (b) first order hyperpolarizability.

4.Conclusions

Sucrose crystals were grown using the isolated from the hot MeOH extract of the rhizome powder of *G. superba.* X-ray powder diffraction and X- ray single crystal structural refinement indicate monoclinic structure and good crystalline quality. Differential thermal analysis and thermal gravimetric analysis clearly showed thermal decomposition of sucrose was considered to start at 186°C. IR and Raman studies on the grown crystals reveal the presence of various -OH, -CH2, etc. groups as the respective vibration frequency peaks were assigned. The UV-Vis spectra confirms that the crystal has sharp and strong absorbance peak at 253 nm.

5.Supporting information

Crystallographic data for the structure have been deposited to the Cambridge Crystallographic Data Centre, under reference number CCDC 913655. The data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Tel: +44-(0) 1223 762911) or e-mail: deposit@ccdc.cam.ac.uk).

6.Acknowledgements

We wish to thank, the Sophisticated Analytical Instrument Facility at the Indian Institute of Technology, Madras for X-ray data collection using the Bruker X8 KAPPA APEX-II CCD diffractometer and also thank to the University Grants Commission (UGC), New Delhi, for providing the fund for major research project (37-14/2009 - SR).

References

[1] R. Kumaresan, S. Moorthy Babu Materials Chemistry and Physics 49 (1997) 83-86

[2] T. Nakajima, T. Otsuki, Appl. Radiat. Isotopes 41 (1989) 359–365.

[3] A.H. Hamzaoui, K. Farah, K. Marzougui, S. Horchani, N. Ben Nessib, A. M'Nif, Radiat. Meas. 44 (2009) 374–377.

[4] Z. Peimel-Stuglik, Appl. Radiat. Isotopes 68 (2010) 654–657.

[5] E.L. Ponce-Lee, A. Olivares-Pérez, I. Fuentes-Tapia, Opt. Mater. 26 (2004) 5–10.

[6] D. Predoi, Dig. J. Nanomater. Biostruct. 5 (2010) 373–377.

[7] V.D. Ryzhikov, O.D. Opolonin, S.V. Naidenov, D.N. Kozin, E.K. Lisetskaya, V.L. Danilenko, Biomed. Eng. 39 (2005) 65–68.

[8] A.A. Kaminskii, Crystallogr. Rep. 48 (2003) 295–299.

[9] P.V. Son, T. Fujita, K. Ohshima, J. Phys. Soc. Jpn. 79 (2010) 034603.1–034603.3.

[10] H.E. Gu, C.Y. Song, L. Han, J. Lumin. 117 (2006) 123–128.

[11] G. M. Sheldrick, SHELXTL, V6.1; Bruker Analytical X-ray systems, Inc.: madison, WI, 1997.

[12] SMART V5.05 Software for CCD Detector System; Bruker Analytical X-ray Systems, madison, WI, 1998.

[13] SAINTPLUS, V5.00 Software for the CCD Detector System; Bruker Analytical X-ray System, Inc.: Madison, WI, 1998.

[14] SADABS. Program for absorption correction using SMART CCD based on the method of Blessing, R. H. Acta Crystallogr. *A51* (1995) 33.

[15] E. Kavitha, N. Sundaraganesan, S. Sebatain, Indian. J. Pure and Applied Physics 48 (2010) 20-30.

[16] O. Prasad, L. Sinha, N. Kumar, J. Atom mol. Sci. 1 (2010) 201-214.

[17-] J. S. Murry, K. Sen, Molecular Electrostatic Potential, Concepts and Application, Elsevier, Amsterdam, 1996.

[18] E. Scrocco, J. Tomasi in: P. Lowdin (Ed.), Advances in Quantum Chemistry, Academic press, New York, 1978.

[19] M. A. Palafox, Int. J. Quant. Chem. 77 (2000) 661.

[20] D. A. Kleinman, Phys. Rev. 128 (1962) 1761-1775.

1. Plant Name : *Cassia alata* Linn.

2. Botanical classification :

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Sub Family	Caesalpinioideae
Genus	*Cassia*
Species	*alata*

3. Vernacular Names:

Seemaiagathi, Vantu-kolli, Herpetic alata, Candle bush, Candlestick senna, Candlesticks, Christmas-candle, Ringworm senna, Ringworm bush, Ringworm shrub, Seven-golden-candlesticks, Fleur palmiste, Fleur dartre, Wild senna, Ringworm cassia, Guajava, Ketepeng badak, Flor del Secreto, Tarantana, Akapulko, Man-slabriki, Akapulco, Gelenggang, Candelabra bush.

4. Chemical Constituents:

Cassia alata leaf contains free agylcones(rhein, emodin, aloe-emodin, crysophanol, and isochrysophanol), glycosides rhein, of aloe-emodin and physcione. It also contains kaempferol, β-sitosterol and sennoside A, B, C and D.

5. Description Medicinal Property:

Leaf used in diseases like herpes, blotch, mycosis (washerman's itch). Dried leaves in leprosy. A strong decoction is used for ringworm, eczema and herpes.

Leaves:

Leaves are used a purgative. Young pods contain rhein, emodin and aloe-emodin, The antibactrial activity of the leaves is reported to be due to rhein.

Roots:

The roots contain anthraqunione. Emodin, aloe-emodin and anthraquninone contribute to the purgative activity of the leaves and roots are rubbed on to the skin to cure ring worm and to control *Tinea imbricata* a skin fungus.

6. Useful parts.

Leaves and bark

7. Micropropagation aspects:

In our methods shows that *Cassia alata* micropropagation and mass propagation by using the leaf explants.

8.Results

* The leaf and stem explant showed that most significant effect of 87 and 100% response in callus initiation at MS+2,4-D (1.0mg/l)+NAA (0.5mg/l).
* In addiction, stem explant showed 100% of greenish callus formation observed in the combination BA and NAA.
* The 100 % of yellowish rooted shootlets initiation from the leaf callus at the combination of BAP (2 & 2.5 mg/l) + IPA (1 mg/l).
* The regenerated plantlets were successfully hardened and acclimatized, 95% of plantlets survived well under natural conditions after transplantation.
* In our study, observed a quick, reliable and reproducible capacity for *in vitro* clonal propagation of *C. alata* coupled with a high morphogenetic regeneration capacity of leaf explants with multiple shoot and root formation from the leaf callus on MS medium.
* This protocol can be used as an efficient tool for rapid multiplication, conservation and maintenance of germplasm of this important medicinal species.

1. Effect of different concentration of growth regulators used callus induction from leaf and stem explants of *Cassia alata*

Two different explants (leaf and stem) of *cassia alata* were used at different concentrations of 2, 4-D and NAA in MS for callus initiation. The maximum callus were observed at low concentration of 1.0 + 0.5 mg/l (2, 4-D + NAA) leaf 87% and stem 100% callus initiation. Different combination and concentration of BA+BAP+IAA+NAA (0.5-5.0 +0.5-2.5+ 1+ 0.5-1.5mg/l) in MS medium to produced maximum number 75% of callus (Table-1). In addition, BAP+BA+Kn+IAA (1.5+3.0+1.0+1.0 and 2.0+2.0+1.0+1.0mg/l) to produced 100% of callus in both explants, In another case IAA+BA+Kn+BAP (2.0+2.0+1.0+0.5 mg/l) to produced 100% of callus in stem and leaf explants (Table 1.) (Figure1 B-F)

2. Effect of different concentration of growth regulators used in stem callus multiplication of *C. alata*

The multiple stem callus were observed in different concentration and combination of BA+NAA with MS medium (0.5-5.0 + 0.5-5.0 mg/l) at the different concentration to produced the 100% of green stem callus as well as BAP+NAA+GA_3+C.W (0.5-2.5 + 0.5+1.0-5.0+ 20%) all of concentration to produce 100% of green and greenish brown callus derived from stem explant. In addition different concentration and combination of BAP+NAA+IAA (0.5-5.0 +1.0+1.0-10mg/l) stem explant to produced 56-88% callus formation. In another case NAA+BA+GA_3+A.C (0.5-1.0 + 2.0-20.0 +1+ 2g) to developed the 75 -100 callus initiation (Table -2) (Figure1 G-I)

3.Effect of BAP+IPA induced the root with shoot initiation from leaf callus of *Cassia alata*

Leaf callus transferred in to shoot initiation medium contain different concentration and combination of BAP+IPA (1.0-5.0+1mg/l) maximum number of rooted shootlets were observed at the concentration of 2-2.5 mg/l BAP + 1 mg/l IPA (Figure 2-A, B). All of the concentration to produced Yellow root with greenish shoot formation. Shoot multiplication capacity reduced when increasing the concentration of BAP (Table 3).

Table 1. Effect of different concentration of growth regulators used callus induction from leaf and stem explants of *Cassia alata*

S.No	MS medium with 2,4-D + NAA (mg/l)	Leaf callus initiation %	Stem callus initiation %
1	1.0 + 0.5	87	100
2	1.5 + 1.0	87	25
3	3.0 + 2.5	87	25
4	4.0 + 3.5	38	25
5	5.0 + 4.5	38	38
	BA+BAP+IAA+NAA		
6	0.5 + 0.50 + 1.0 + 0.50	75	75
7	1.0 + 0.75 + 1.0 + 0.75	25	25
8	2.0 + 1.0 + 1.0 + 0.90	75	75
9	3.0 + 1.5 + 1.0 + 1.0	75	75
10	4.0 + 2.0 + 1.0 + 1.25	25	25
11	5.0 + 2.5 + 1.0 + 1.50	25	25
	BAP+BA+Kn+IAA		
12	1.5+3.0+1.0+1.0	100	100
13	2.0+2.0+1.0+1.0	100	100
	IAA+BA+Kn+BAP		
14	2.0+2.0+1.0+0.5	100	100

Table 2. Effect of different concentration of growth regulators used in stem callus multiplication of *C. alata*

S.No	**MS medium with BA+NAA (mg/l)**	Stem callus initiation %	Callus morphology
	BA+NAA		
1	0.5+0.5	100	Green callus
2	1.0+0.5	100	Green callus
3	2.0+0.5	100	Green callus
4	3.0+0.5	100	Green callus
5	4.0+0.5	100	Green callus
6	5.0+0.5	100	Green callus
	BAP+NAA+GA_3+C.W		
7	0.5 + 0.5 + 1.0 + 20%	100	Green callus
8	1.0 + 0.5 + 2.0 + 20%	100	Green callus
9	1.5 + 0.5 + 3.0 + 20%	100	Greenish brown callus
10	2.0 + 0.5 + 4.0 + 20%	100	Greenish brown callus
11	2.5 + 0.5 + 5.0 + 20%	100	Green callus
	BAP+NAA+IAA		
12	0.5 + 1.0 + 1.0	75	Green callus
13	1.0 + 1.0 + 1.5	88	Green callus
14	1.5 + 1.0 + 2.0	75	Green callus
15	2.0 + 1.0 + 2.5	68	Green callus
16	2.5 + 1.0 + 5.0	68	Green callus
17	5.0 + 1.0 + 10.0	56	Green callus
	NAA+BA+GA_3+A.C		
18	0.0 + 2.0 + 1.0 + 2g	100	Green callus
19	0.0 + 4.0 + 1.0 + 2g	75	Green callus
20	0.5 + 6.0 + 1.0 + 2g	100	Green callus
21	0.5 + 8.0 + 1.0 + 2g	75	Brownish green callus
22	1.0 + 10.0 + 1.0 + 2g	75	Brownish green callus
24	1.0 + 15.0 + 1.0 + 2g	75	Green callus
25	1.0 + 20.0 + 1.0 + 2g	50	Green callus

Table 3. Effect of BAP+IPA induced the root with shoot initiation from leaf callus of *Cassia alata*

S.No	**MS medium with BAP + IPA (mg/l)**	Shoot initiation % of leaf callus	Developmental stage of Shoot morphology
1	1+1	**85**	Yellow root with greenish shoot formation
2	1.5+1	**90**	Yellow root with greenish shoot formation
3	2+1	**100**	Yellow root with greenish shoot formation
4	2.5+1	**100**	Yellow root with greenish shoot formation
5	3+1	**95**	Yellow root with greenish shoot formation
6	4+1	**82**	Yellow root with greenish shoot formation
7	5+1	**78**	Yellow root with greenish shoot formation

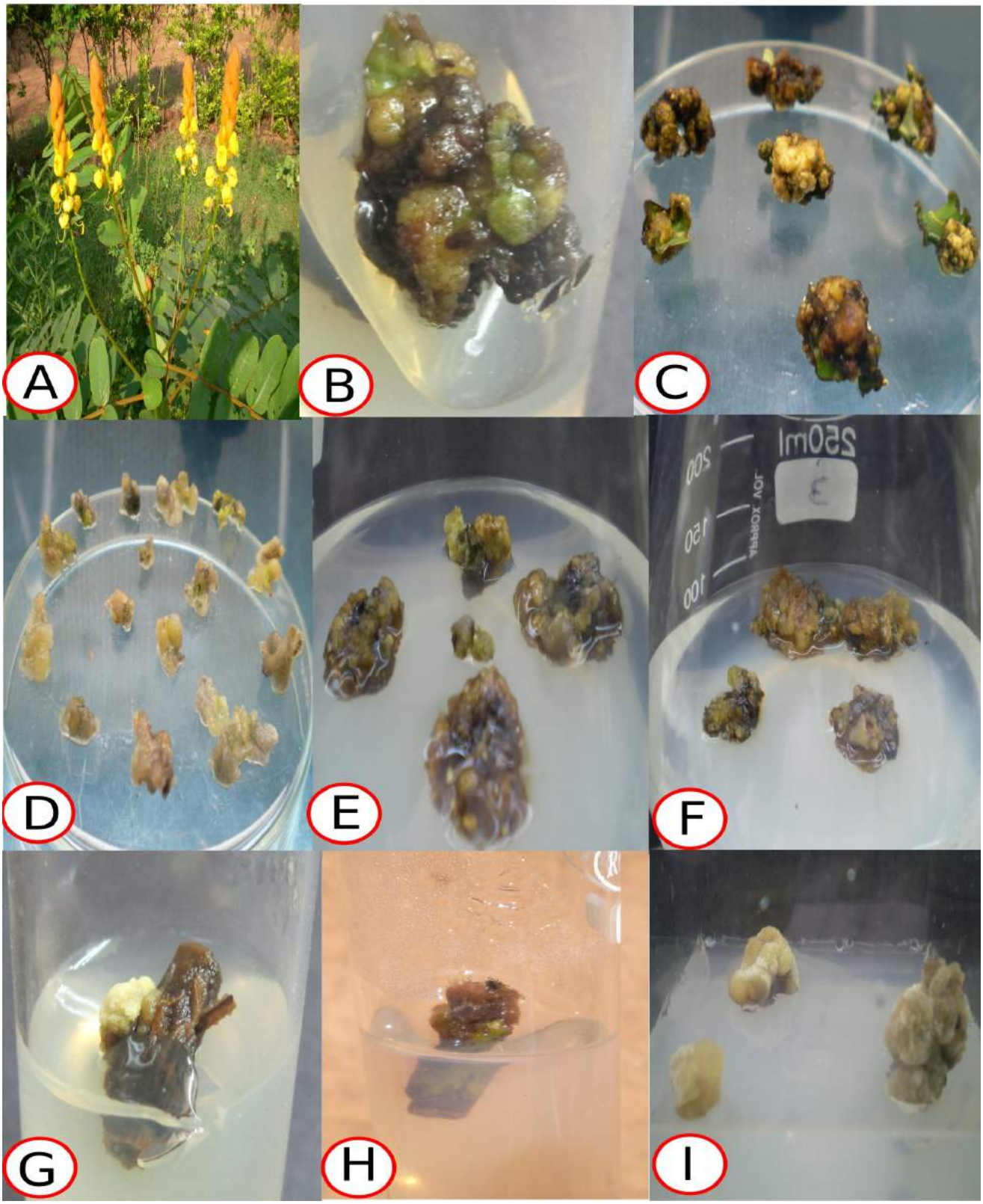


Figure 1: A – *Cassia alata* explant **B,C,D,E,F –** Leaf callus regeneration, **G,H,I** - Stem callus regeneration

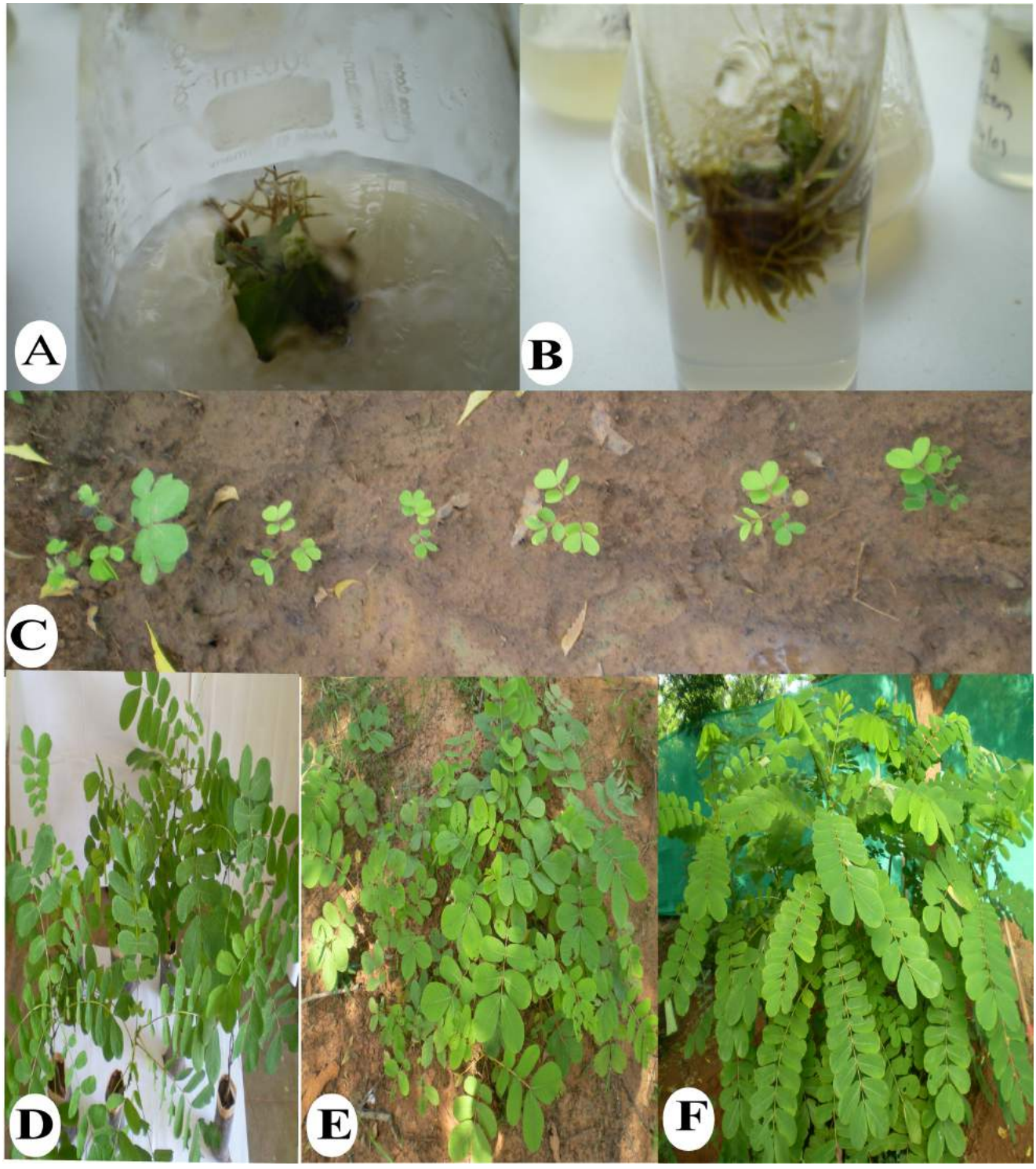


Figure 2 : A – *Cassia alata* leaf explant root initiation **B –** Leaf callus root with shoot regeneration, **C,D,E,F** - Hardening for *in vivo* condition

Figure 2.16 *Cassia alata* leaf - Methanol extract

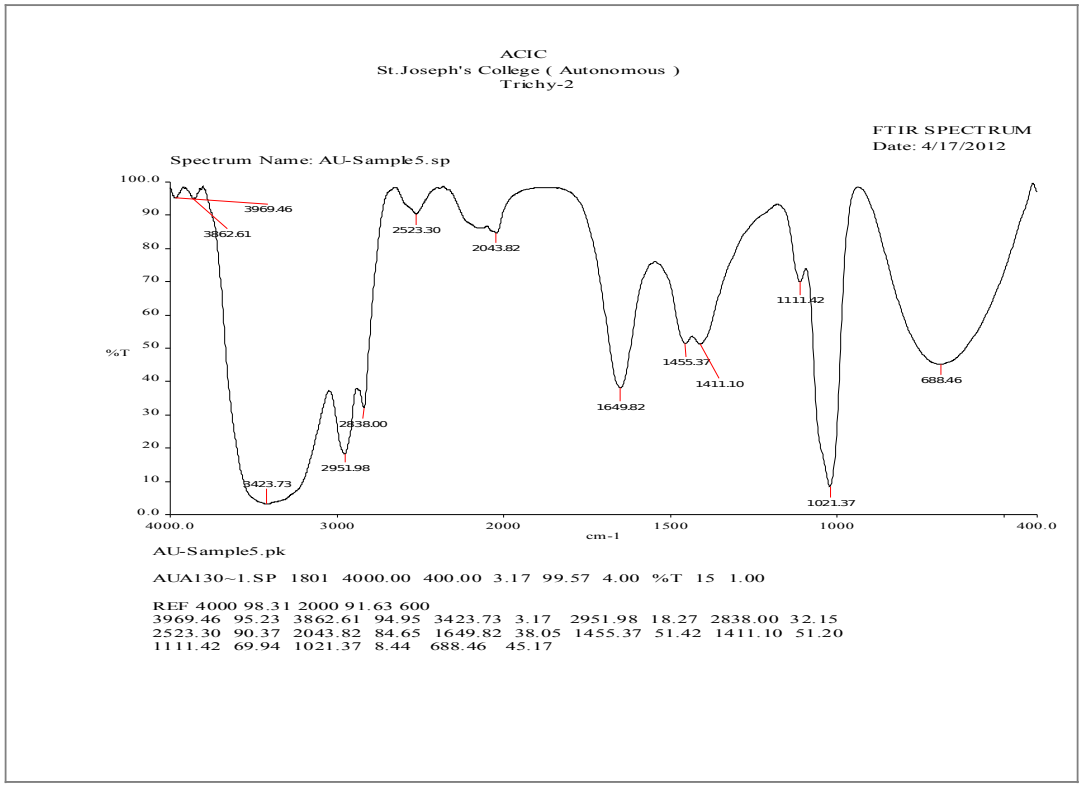


Figure 2.17 *Cassia alata* stem - Methanol extract

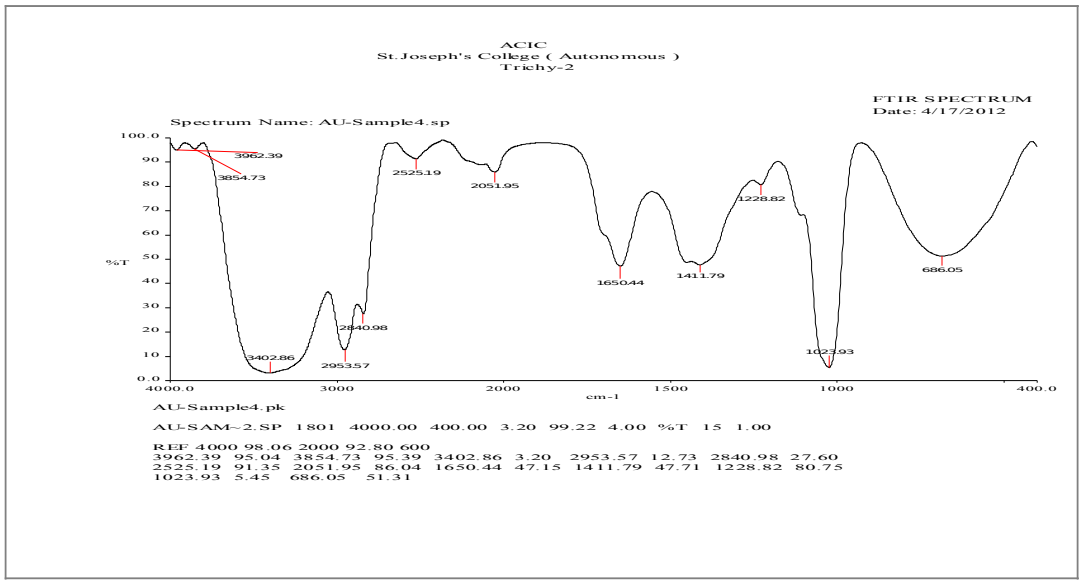


Figure 2.18 *Cassia alata* root - Methanol extract

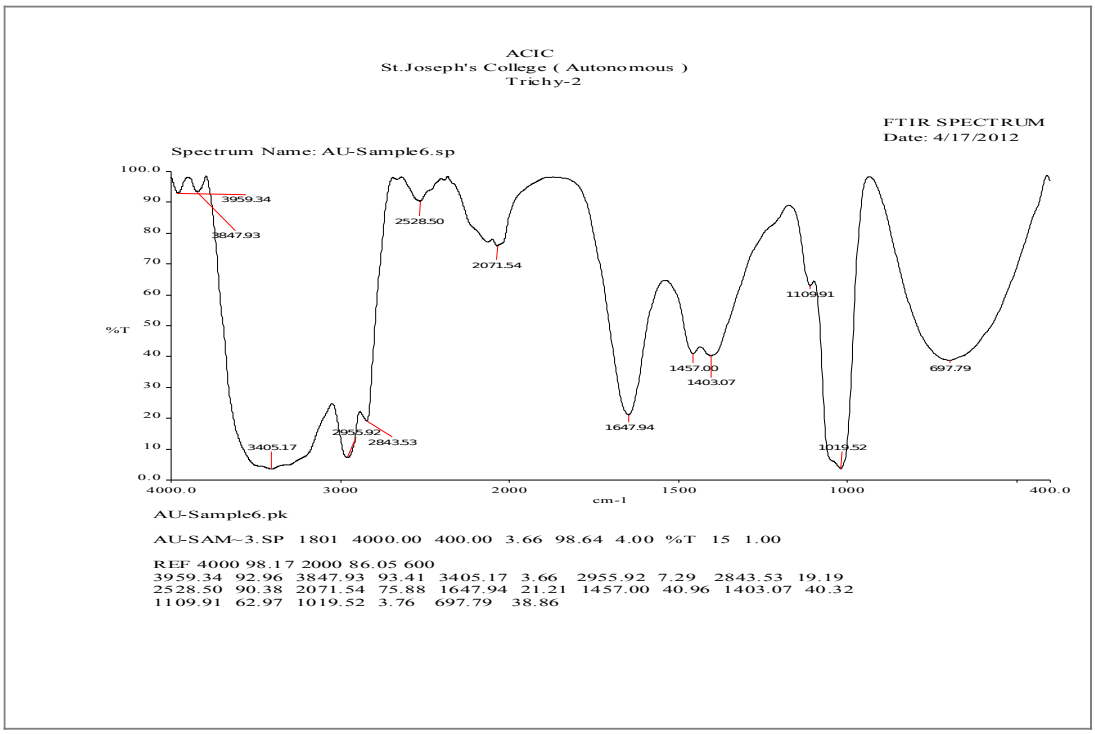
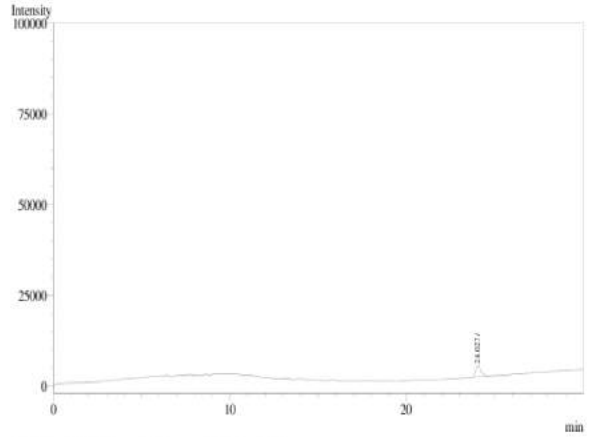


Fig 2.6 - 2.118 FTIR analysis shows that the *Cassia alata* leaf, stem and root were extract with methanol extract. The functional groups present in the samples were determined by FT-IR spectroscopy. The FT-IR spectrum confirmed the presence of Squalene *Cassia alata.* FTIR absorption peaks located at about 3,426; 2,077; 1,637; 1,112 and 676 cm^{-1} in the region 400–4,000cm^{-1}. The FTIR spectra revealed the presence of different functional groups such as free NH_2-stretching, C–N stretching, NH-bending, C–Ostretching and Cl-stretching. *Cassia alata* methanol extract resulted a strong band at 3,426 cm^{-1} corresponding to N–H stretching vibration of primary amines, the band at 2,077 cm-1 corresponding to C–N stretching of any R–N=C=S, the medium band at 1,638 cm^{-1} corresponding to similar conjugation effects to N–H bending frequency, the low band 676 cm^{-1} corresponding to Cl stretching.

Figure 3.2

Analysis Date & Time : 1/31/2013 4:33:15 PM
User Name : Admin
Vial# : 7
Sample Name : CA stem
Sample ID : CA stem
Sample Type : Unknown
Injection Volume : 1.00
ISTD Amount :

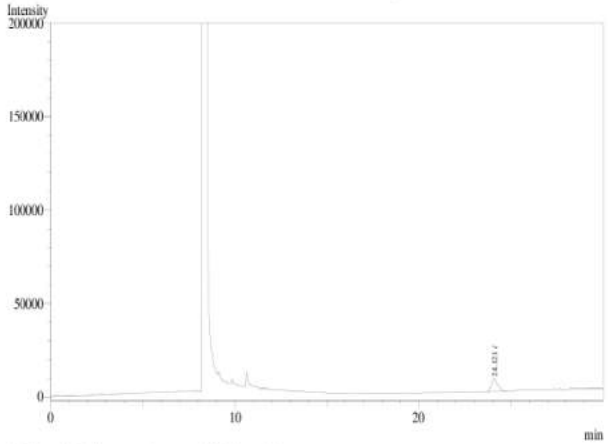
Data Name : E:\GC DATA\Batch\SElva JK NP 31.01. 13\7.gcd
Method Name : E:\GC DATA\METHODE\Selvakumaran\Selva JK NP.gcm



Peak#	Ret.Time	Area	Height	Conc.
1	24.027	65307	3205	100.000
Total		65307	3205	100.000

Analysis Date & Time : 1/31/2013 3:57:05 PM
User Name : Admin
Vial# : 6
Sample Name : CA root
Sample ID : CA root
Sample Type : Unknown
Injection Volume : 1.00
ISTD Amount :

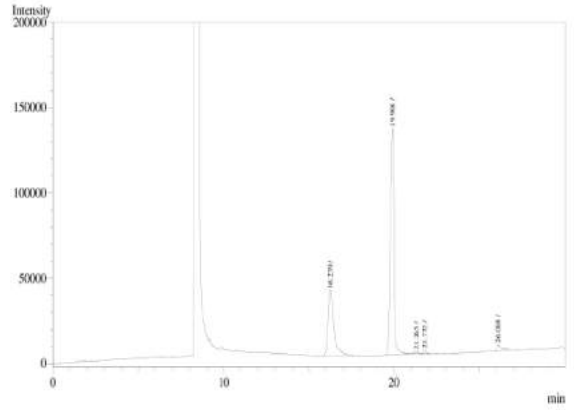
Data Name : E:\GC DATA\Batch\SElva JK NP 31.01. 13\6.gcd
Method Name : E:\GC DATA\METHODE\Selvakumaran\Selva JK NP.gcm



Peak#	Ret.Time	Area	Height	Conc.
1	24.121	156007	6775	100.000
Total		156007	6775	100.000

Analysis Date & Time : 1/31/2013 12:56:40 PM
User Name : Admin
Vial# : 1
Sample Name : RT stem
Sample ID : RT stem
Sample Type : Unknown
Injection Volume : 1.00
ISTD Amount :

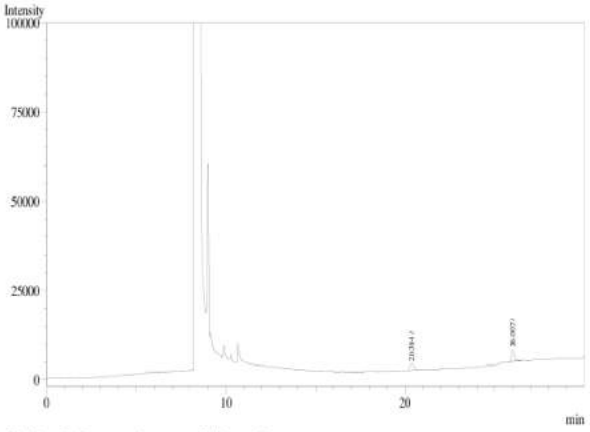
Data Name : E:\GC DATA\DATA\Selvakumaran\31.01.2013\1.gcd
Method Name : E:\GC DATA\METHODE\Selvakumaran\Selva JK NP.gcm



Peak#	Ret.Time	Area	Height	Conc.
1	16.239	885926	38348	30.144
2	19.901	1946557	132742	66.233
3	21.265	20351	1208	0.692
4	21.772	21983	2024	0.748
5	26.068	64137	3498	2.182
Total		2938954	177820	100.000

Analysis Date & Time : 1/31/2013 1:32:42 PM
User Name : Admin
Vial# : 2
Sample Name : RT leaf
Sample ID : RT leaf
Sample Type : Unknown
Injection Volume : 1.00
ISTD Amount :

Data Name : E:\GC DATA\Batch\SElva JK NP 31.01. 13\2.gcd
Method Name : E:\GC DATA\METHODE\Selvakumaran\Selva JK NP.gcm



Peak#	Ret.Time	Area	Height	Conc.
1	20.384	30693	2020	44.997
2	26.007	37517	3304	55.003
Total		68210	5324	100.000

Table 2: Activity of Phytocomponents identified in the stems and roots of *Cassia alata* through GC - analysis

S. No	Sample Code	R.T	Name of the compound	Molecular formula	Compound Nature	Activity
1	C.a - Stem	24.02	Squalene	$C_{30}H_{50}$	Polyunsaturated lipids	Antioxidant effect
2	C.a - Root	24.12	Squalene	$C_{30}H_{50}$	Polyunsaturated lipids	Antioxidant effect

Squalene

Squalene is a triterpene and an intermediate in the biosynthesis of sterols in the plant and animal world. The richest known source of squalene is shark liver oil. In vegetable oils, squalene is found over broad ranges. For example, in flaxseed, grape seed, and soybean oils it is not detected, but is quite prominent in peanut (1.28 g/kg), pumpkin (3.53 g/kg), and olive oils (5.99 g/kg). Squalene is the main component of skin surface polyunsaturated lipids and shows some advantages for the skin as an emollient and antitumor compound. The tri-terpene has also been found to have protective activity against several carcinogens (Fig 3.2: Table 2)

1. Plant Name : *Plumbago zeylanica* L.

2. Botanical Classification :

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Plumbaginales
Family	Plumbaginaceae
Genus	*Plumbago*
Species	*zeylanica*

3. Vernacular Names:
White Leadwort, Chitrak, Chitra, Agnimatha, Agnisikha, Bilay-chitramula, Ceylonische Bleiwurz, Ceylon Ieadwort, Chita, Chitaro, Chitawa, Chiti, Chittira, Chittiramulam, Chitrak, Chitraka, Chitramula, Chitra-mulam, Chitruk, Dentelaire de Ceylon, Vellakoduveli, Vellakotuveri.

4. Chemical Constituents:
The root bark contain a napthaquinone, plumbagin (0.91-1%), sitosterol, tannins, 3-biplumbagin, chloroplumbagin, chitranone and elliptone.

5. Description Medicinal Property
The roots are highly valued for their curative action on leuccoderma and other skin diseases. They are digestive stimulant, diuretic, germicidal, vesicant and abortifacient. Chitrak is also used for haemorrhoidal inflammation of anus, diabetes, diarrhoea and elephantiasis. Chitrak is used in treating intestinal troubles, dysentery, leucoderma, inflammation, piles, bronchitis, itching, diseases of the liver, and consumption. The leaves of this herb work well for treating laryngitis, rheumatism, diseases of the spleen, ring worm, scabies, and it acts as an aphrodisiac. A tincture of the root bark is used as an anti-periodic. Chitrak root helps improve digestion and it stimulates the appetite. Chitrak root is also an acro-narcotic poison that can cause an abortion.

6. Useful parts.
The whole plant, the roots, powder of the roots.

7. Micropropagation aspects:
In our study the result shows *Plumbago zeylanica* micropropagation were enable the mass propagation protocol was developed by using the stem explant

8. Results

* The leaf and stem explant showed that most significant effect of 100% and 80-100% of response in callus initiation at MS+2,4-D (1.0 – 4.5 mg/l)+NAA (0.5- 4.0 mg/l).

* 2,4-D, IAA and 15% of Coconut water combination was produced minimum number of callus initiation for leaf and stem explant.

* Stem explant showed 80% of direct regeneration for dark greenish shoot formation observed in the combination MS with BAP,NAA and IAA (2.0+1.0+2.5 mg/l).

* The direct regenerated plantlets were successfully hardened and acclimatized, 90% of plantlets survived well under natural conditions after transplantation.

* In our study results showed that *P. zeylanica* micropropagation were enable the mass propagation protocol was developed by using the stem explant.

Effect of different concentration of 2,4-D, IAA and NAA callus induction in leaf and stem explants of *P. zeylanica.*

Two different explants (Stem and Leaf) of *P. zeylanica* were used at different concentrations and combination of 2, 4-D, IAA and NAA in MS medium for callus initiation (Figure 1-B-H). Combination of (1.0 -10 mg/L) 2,4-D and (1.0- 5.0mg/l) IAA in MS medium produced maximum number 100 % of light greenish callus derived from leaf explant and 80-100% of brownish green callus derived from stem explant (1.0 -4.5 mg/l 2, 4-D,) (Table-1). In addition of NAA to inhibited the callus formation only minimum number of calls were produced leaf and stem explant 50-75% (Table – 1). The multiple callus were observed at MS medium with combination of (2.0 + 1.0 + 1.0 + 0.90 mg/L) BA+BAP+IAA+NAA (Table -2).

Direct regeneration and shoots multiplication with growth regulators used in stem explant of *P. zeylanica*

Direct regeneration of *P. zeylanica* stem explant were used at different concentrations and combination of BAP+NAA+IAA (0.5-2.5 + 1 + 1.0-5.0 mg/l). The maximum number 80% of Dark greenish shootlets were produced at concentration of (2.0 + 1.0 + 2.5). In another way the multiple shoot were observed at MS medium with combination of BAP+GA_3+Zen+NAA+A.C (8-16 + 1.0-5.0 + 0.5-2.5 + 2g) (Table 3) (Figure 1. I).

Plumbago zeylanica L.

Table 1. Effect of different concentration of 2,4-D, IAA and NAA callus induction in leaf and stem explants of *P. zeylanica*

S.No	MS medium with 2,4-D + IAA (mg/l)	Leaf Callus %	Leaf callus morphology	Stem Callus %	Stem callus morphology
1	1.0 + 0.5	100	Green callus	80	Green callus
2	1.5 + 1.0	100	Light green callus	80	Green callus
3	2.0 + 1.5	100	Green callus	80	Green callus
4	2.5 + 2.0	100	Green callus	100	Brownish green callus
5	3.0 + 2.5	100	Dark green callus	100	Brownish green callus
6	3.5 + 3.0	100	Green callus	100	Brownish green callus
7	4.0 + 3.5	100	Light green callus	100	Brownish green callus
8	4.5 + 4.0	100	Light green callus	100	Brownish green callus
9	5.0 + 4.5	80	Whitish callus	50	Brownish callus
10	10.0 + 5.0	50	Whitish callus	25	Brownish callus
	2,4-D + IAA + NAA				
11	0.5 + 0.5 + 1.0	75	Green callus	75	Green callus
12	1.0 + 1.0 + 1.5	75	Green callus	75	Green callus
13	2.0 + 1.5 + 2.0	75	Green callus	75	Green callus
14	3.0 + 2.0 + 2.5	75	Green callus	75	Green callus
15	4.0 + 3.0 + 3.0	75	Green callus	50	Green callus
16	5.0 + 4.0 + 3.5	75	Green callus	75	Green callus
17	7.5 + 5.0 + 4.0	75	Green callus	75	Green callus
18	10.0 + 7.5 + 4.5	75	Green callus	50	Light green callus
19	15.0 + 10.5 + 5.0	50	Green callus	50	Light green callus
20	20.0 + 15.0 + 10.0	50	Green callus	50	Light green callus

Table 2. Effect of different concentration of growth regulators used in leaf and stem callus multiplication.

S.No	MS medium with 2,4-D+IAA+C.W (mg/l)	Leaf callus initiation %	Stem callus initiation %
1	1.0 +0.5 +15%	87	62
2	1.5 +1.0 +15%	75	63
3	2.0 +1.5 +15%	62	75
4	3.0 +2.5 +15%	75	38
5	4.0 +3.5 +15%	75	38
6	10.0 +5.0 +15%	75	50
	BA+BAP+IAA+NAA		
7	0.5 + 0.50 + 1.0 + 0.50	75	75
8	1.0 + 0.75 + 1.0 + 0.75	50	75
8	2.0 + 1.0 + 1.0 + 0.90	100	75
9	3.0 + 1.50 + 1.0 + 1.0	75	75
10	4.0 + 2.0 + 1.0 + 1.25	50	75
11	5.0 + 2.5 + 1.0 + 1.50	75	50

Table 3. Direct regeneration and shoots multiplication with growth regulators used in stem explant of *P. zeylanica*

S.No	MS medium with BAP+NAA+IAA (mg/l)	Stem explant s Direct regeneration	Shoot initiation morphology
1	0.5 + 1.0 + 1.0	50	Greenish shoot formation
2	1.0 + 1.0 + 1.5	25	Greenish shoot formation
3	1.5 + 1.0 + 2.0	25	Greenish shoot formation
4	2.0 + 1.0 + 2.5	80	Dark greenish shoot formation
5	2.5 + 1.0 + 5.0	75	Dark greenish shoot formation
	MS+BAP+GA_3+Zen+NAA+A.C		
6	8.0 + 1.0+ 0.5+1 0 + 2g	70	Dark greenish shoot formation
7	10.0 + 2.0+ 1.0 +1.0 + 2g	20	Greenish shoot formation
8	12.0 + 3.0+ 1.5+ 1.0 + 2g	70	Dark greenish shoot formation
9	14.0 + 4.0+ 2.0+ 1.0 + 2g	60	Greenish shoot formation
10	16.0 + 5.0+ 2.5+ 1.0 + 2g	55	Greenish shoot formation

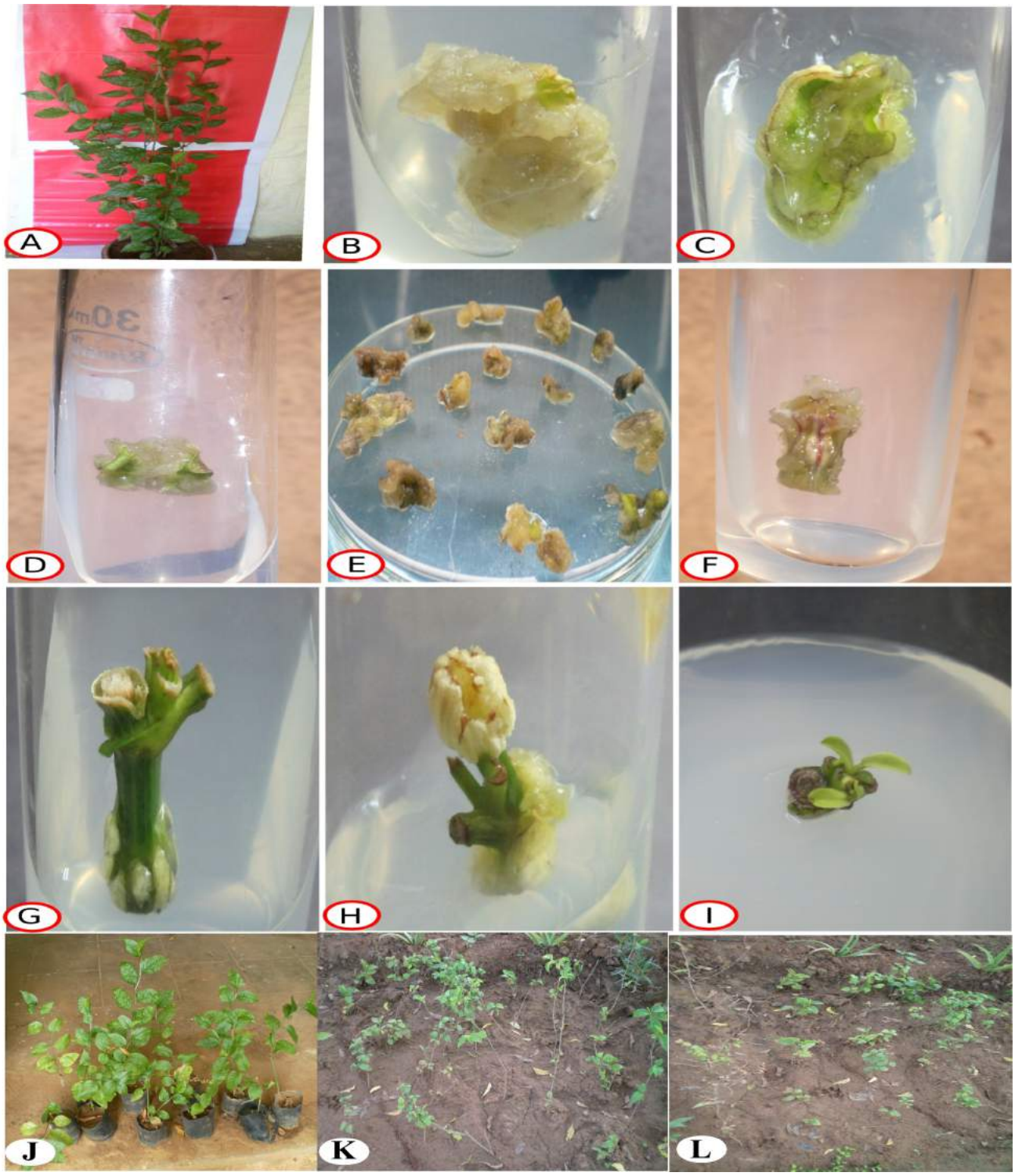


Figure 1: A – ***Plumbago zeylanica*** explant, **B,C,D,E, –** Leaf callus induction, **F,G,H** – Stem callus induction, **I –** Direct regeneration stem explant, **J -** Plantlets were transplanted with polythene bags to acclimatization for 2 month in mist house, **K,L -** Plant hardening for *in vivo* condition.

FTIR – Results

Figure 2.19 *Plambago zeylanica* leaf - Methanol extract

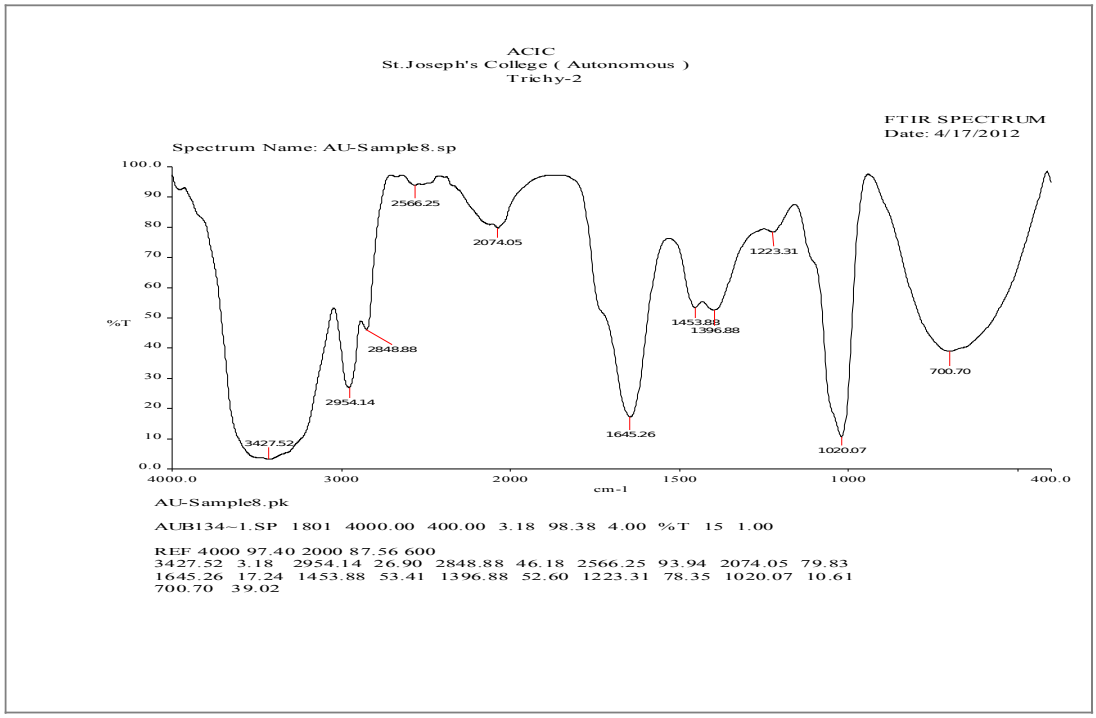


Figure 2.20 *Plambago zeylanica* stem - Methanol extract

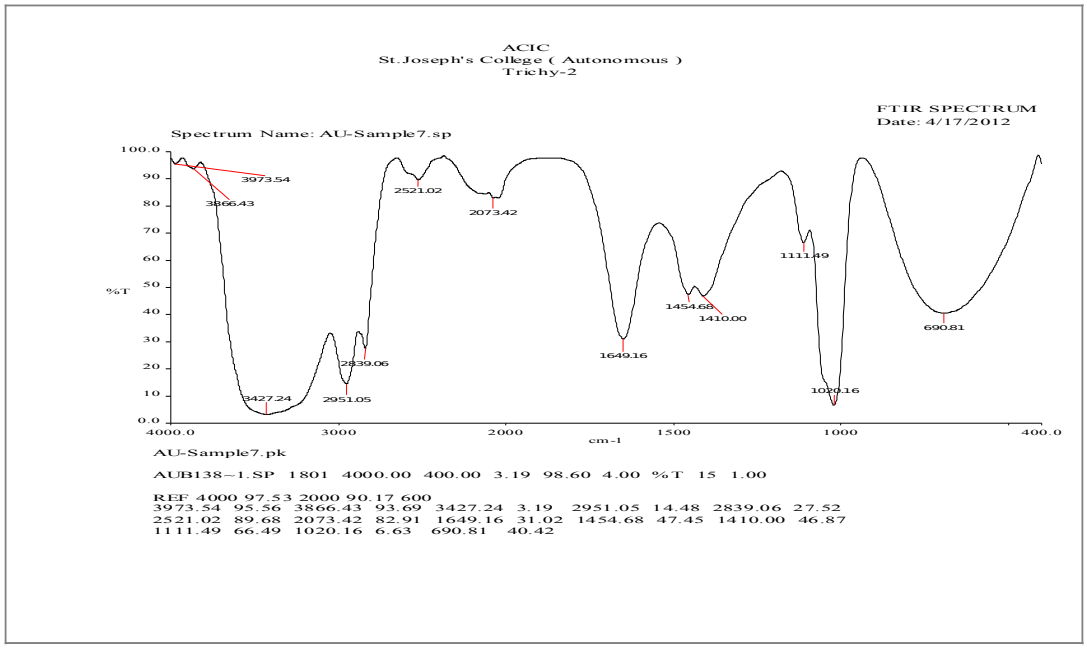


Figure 2.21 *Plambago zeylanica* root - Methanol extract

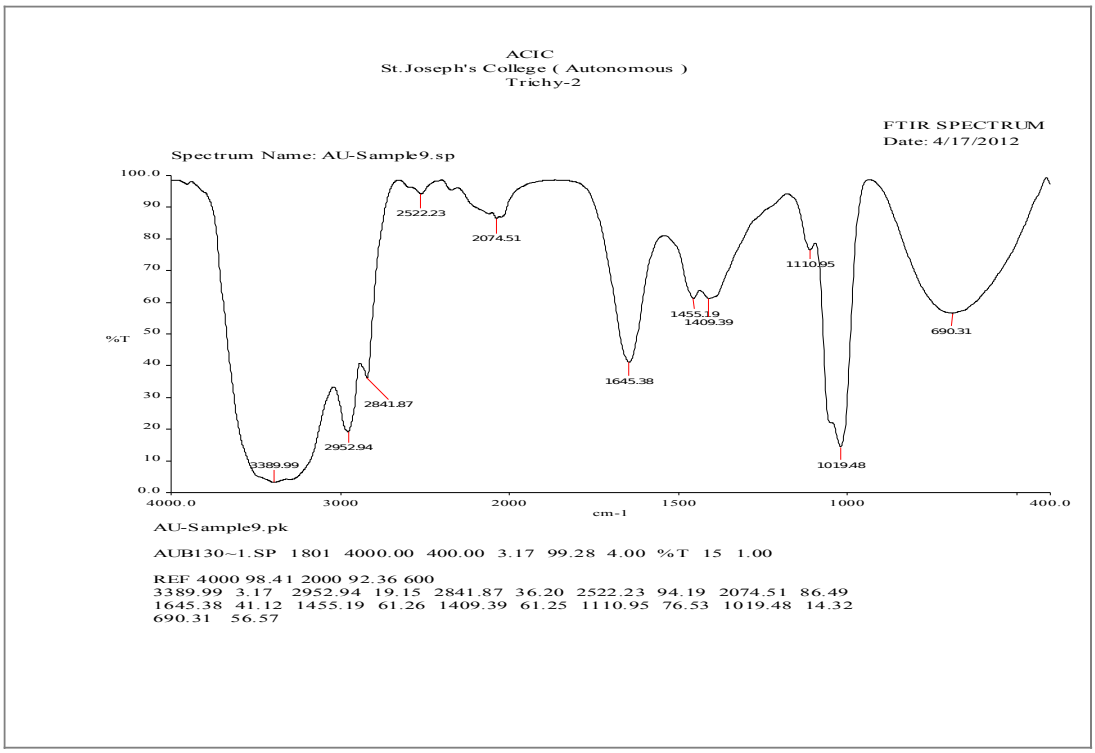


Fig 2.19 - 2.21 FTIR analysis shows that the *Plumbago zeylanica* leaf, stem and root were extract with methanol extract. The strong IR bands were observed at 3382, 2922, 2337, 1614, 1384, 1070 and 590 cm^{-1}. The bands appeared at 3382 and 2922 cm^{-1} are corresponds to -OH stretching and aliphatic -C-H stretching respectively. The bands at 2337 and 1613 cm^{-1} are due to the CO_2 and C=C stretching respectively. The IR bands observed at 1384 and 1070 cm^{-1} may be ascribed to -C-O and -C-O-C stretching modes respectively. The low band at 590 cm^{-1} corresponds to C-Cl stretching.

1. Plant Name **:** *Rauvolfia tetraphylla* L.

2. Botanical Classification :

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Gentianales
Family	Apocynaceae
Genus	*Rauvolfia*
Species	*tetraphylla*

3. Vernacular Names

Rauvolfia tetraphylla, Be still tree, Devil-pepper, Chevanamalpodi, Sarpagandha, Chandra, Bara Chadar, Wild Snake Root, American serpentwood, Devil root, Milkbush, Barachandrika, Chandrabhaga, Pampukaalaachchedi, Pampumkolli, Kattamalpori, Papataku, Dodda chandrike, Bar chandrika, Gandhanakuli , Patalagarudi, Vanasarpagandha, Sarpanasini.

4. Major Alkaloids:

The root contains ophioxylin (an alkaloid having orange colored crystalline principle), resin, starch and wax. The total alkaloid yield is 0.8%. Four crystalline alkaloids isolated are ajmaline, ajmalicine, serpentine, serpentinine (plant) and five alkaloids are Canescine, rauvollscine, reserpine, recanescine, yohimbine isolated from roots.

5. Medicinal Use:

The roots are hypotensive and sedative. An extract of the plant mixed with castor oil is prescribed as a liniment to treat certain chronic and refractory skin ailments. Roots are sedative, tonic and febrifuge. It is a valuable remedy in high blood pressure; used for the treatment of insomnia, madness, painful affections of the bowels, hypochondria and irritative conditions of the central nervous system. Roots have been employed for centuries for relief of various nervous disorders like anxiety, excitement, schizophrenia, insanity, insomnia and epilepsy. Root extracts are also valued in diarrhoea, dysentery, cholera, colic and fever. Decoction is employed to increase uterine contractions and promotes expulsion of the foetus. The dry root bark crushed and administered in small doses in body pain and fever in Jointiapur of Sylhet. Root paste along with orange peel is used against fever by the tribal in Madhupur.

6. Useful Parts:

Roots and leaves.

7. Micropropagation aspects:

In our methods shows that *Rauvolfia tetraphylla* micropropagation and mass propagation by using the stem explants.

8. Results

* The leaf and stem explant were used for callus initiation medium. But, only stem explant alone showed most significant effect of 83 % response in brownish callus initiation at concentration of BAP+BA+GA_3+Kn (1.5+2.0+2.0+1.0 mg/l).
* Stem explant showed 90% of direct regeneration for greenish shoot formation observed in the combination of MS with BAP, GA_3, Zen, NAA and activated charcoal (10.0mg/l+2.0mg/l+1.0mg/l+1.0mg/l+ 2.0 g).
* The direct regenerated plantlets were successfully hardened and acclimatized, 85% of plantlets survived well under natural conditions after transplantation.
* In our method showed that *R. tetraphylla* micropropagation and mass propagation protocol was developed by using the stem explant.

Effect of different concentration of growth regeneration callus induction in leaf and stem explants of *R. tetraphylla*

Two different explants (leaf and stem) of *Rauvolfia tetraphylla* were inoculated in different concentration of BAP (1.0-4.0 mg/l), BA (1.0-4.0 mg/l), GA_3 (1.0-4.0 mg/l) and Kn (1.0-mg/l) in MS media (Table 1). The leaf explants was response at 60% of Brownish callus initiated for higher concentration (4.0+4.0+4.0+1.0mg/l) in 25-30 days cultures (Figure1.,B.). Callus multiplication medium contain different concentration and combination of (1.5+2.0-3.0+1.0-2.0+1.0 mg/l) BAP+BA+ Kn+IAA did not show more significant effect of the callus initiation (Table 1)

Direct regeneration and shoots multiplication with growth regulators used in stem explant of *Rauvolfia tetraphylla*

Direct regeneration of *Rauvolfia tetraphylla* leaf and stem explant were used at different concentrations and combination of BAP+GA_3+Zen+NAA+ A.C (mg/l) (8.0-16.0 + 1.0-5.0 + 0.5-2.5+1 mg/l+2g). The maximum number 90% of greenish shootlets were produced at concentration of (10.0 + 2.0+ 1.0 +1.0 + 2g) stem explant (Table - 2). (Figure 1 C,D)

Table 1. Effect of different concentration of growth regeneration callus induction in leaf and stem explants of *R. tetraphylla*

S.No	MS medium with BAP+BA+GA_3+Kn (mg/l)	Leaf callus initiation %	Leaf callus morphology	Stem Callus initiation %	Stem callus morphology
1	1.0+1.0+1.0+1.0	20	B.C	66	B.C
2	1.5+2.0+2.0+1.0	30	B.C	83	B.C
3	2.0+2.5+3.0+1.0	25	B.C	33	B.C
4	4.0+4.0+4.0+1.0	60	B.C	-	-
	BAP+BA+Kn+IAA				
5	1.5 +3.0 +1.0 +1.0	25	B.C	40	B.C
6	1.5 +2.0 +2.0 +1.0	35	B.C	50	B.C

B.C - Brownish Callus, - - Not responses

Table 2. Effect of different concentration of growth regulators used in leaf callus multiplication and stem explats direct regeneration of *R. tetraphylla*

S.No	MS medium with BAP+GA_3+Zen+NAA+ A.C (mg/l)	Leaf explant callus initiation %	Leaf callus morphology	Stem explants shoots initiation %	Stem explant Direct shoots morphology
1	8.0 + 1.0+ 0.5+1 0 + 2g	75	G.C	40	Whitish green shoots
2	10.0 + 2.0+ 1.0 +1.0 + 2g	75	G.C	90	Green shoot
3	12.0 + 3.0+ 1.5+ 1.0 + 2g	45	G.C	75	Whitish green shoots
4	14.0 + 4.0+ 2.0+ 1.0 + 2g	72	G.C	75	Whitish green shoots
5	16.0 + 5.0+ 2.5+ 1.0 + 2g	73	G.C	50	Whitish green shoots

G.C – Green Callus,

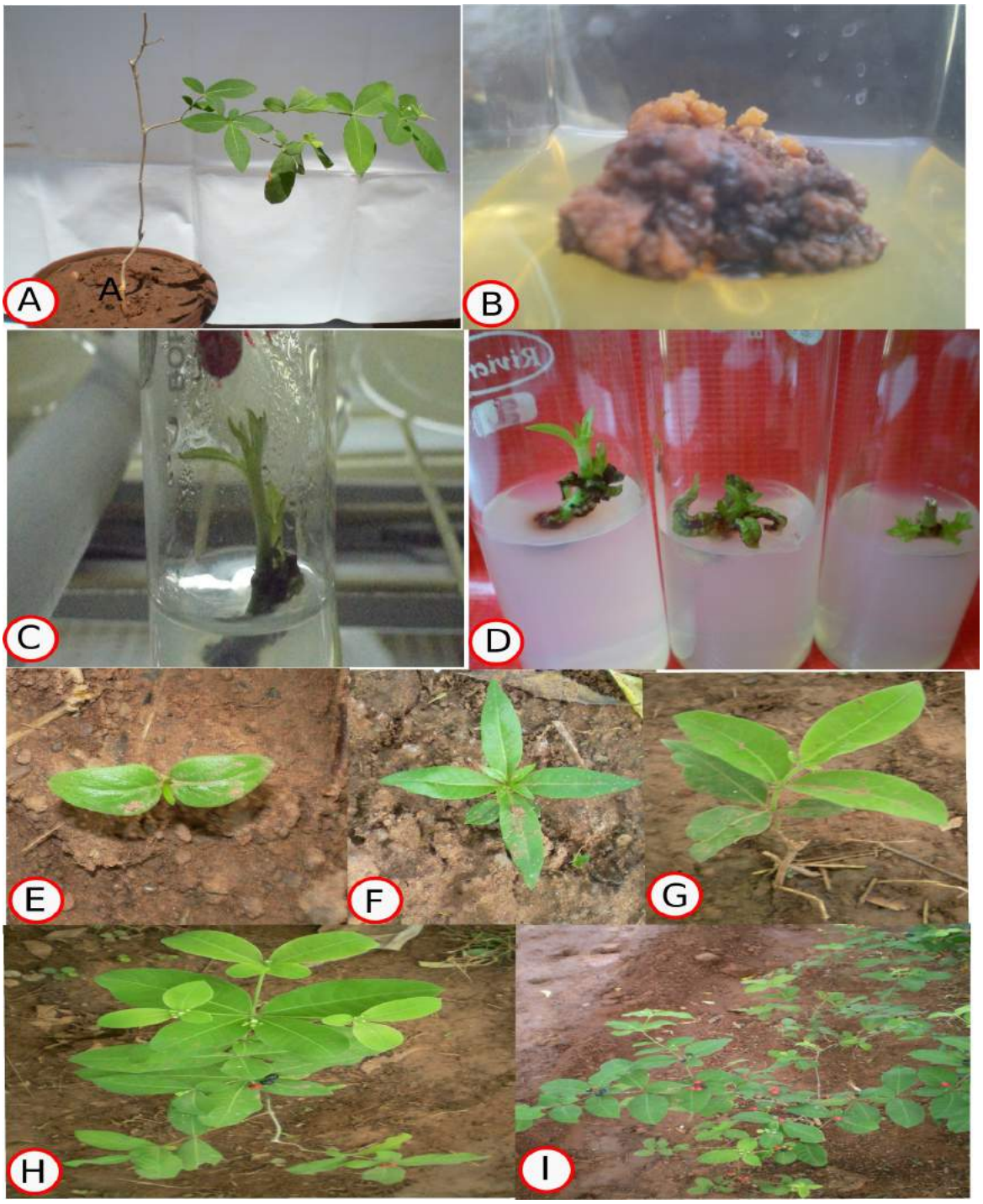
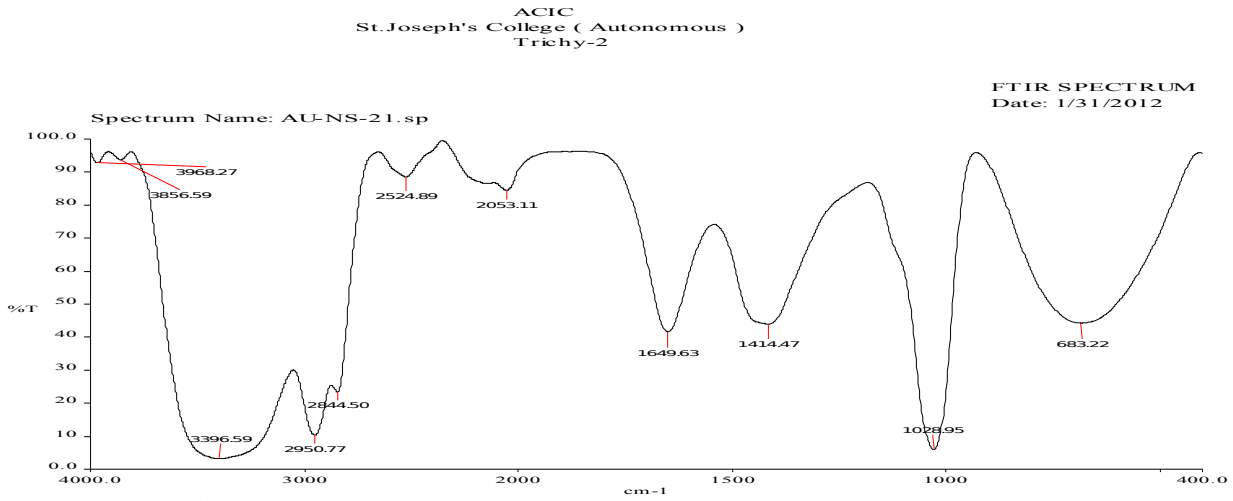


Figure 1: A – *Rauvolfia tetraphylla* explant, **B-** Leaf callus induction, **C,D** – Direct regeneration of stem explant, **E,F,G,H,I -** Plant hardening for *in vivo* condition.

FTIR - Result

Figure 2.22 *Rauvolfia tetraphylla* leaf - Methanol extract

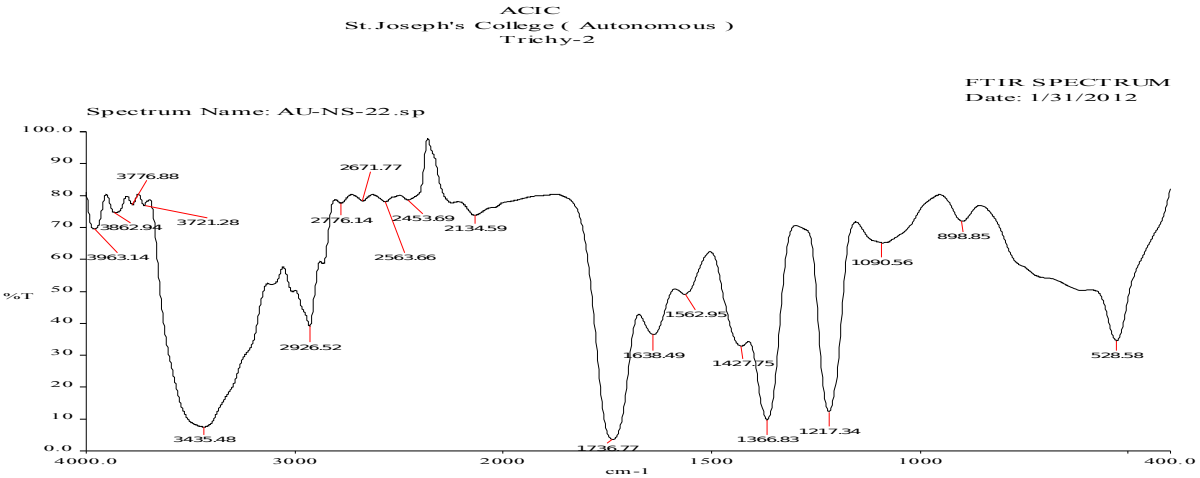


AU-NS-21.pk

AU-NS-21.sp 1801 4000.00 400.00 3.19 99.56 4.00 %T 18 1.00

REF 4000 96.25 2000 90.75 600
3968.27 92.79 3856.59 93.63 3396.59 3.19 2950.77 10.35 2844.50 23.47
2524.89 88.52 2053.11 84.48 1649.63 41.69 1414.47 43.94 1028.95 5.97
683.22 44.28

Figure 2.23 *Rauvolfia tetraphylla* leaf - Acetone extract

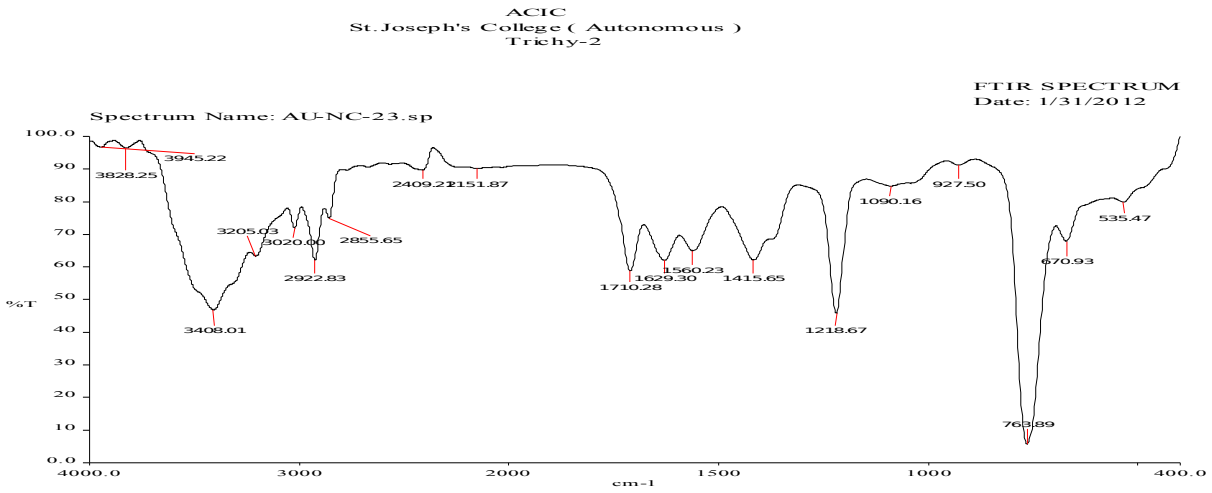


AU-NS-22.pk

AU-NS-22.sp 1801 4000.00 400.00 3.62 97.95 4.00 %T 18 1.00

REF 4000 81.15 2000 77.76 600
3963.14 69.51 3862.94 74.67 3776.88 77.14 3721.28 76.90 3435.48 7.55
2926.52 39.19 2776.14 77.57 2671.77 78.26 2563.66 78.01 2453.69 78.58
2134.59 73.85 1736.77 3.61 1638.49 36.45 1562.95 49.08 1427.75 32.87
1366.83 9.80 1217.34 12.26 1090.56 65.17 898.85 71.92 528.58 34.57

Figure 2.24 *Rauvolfia tetraphylla* leaf- Chloroform extract



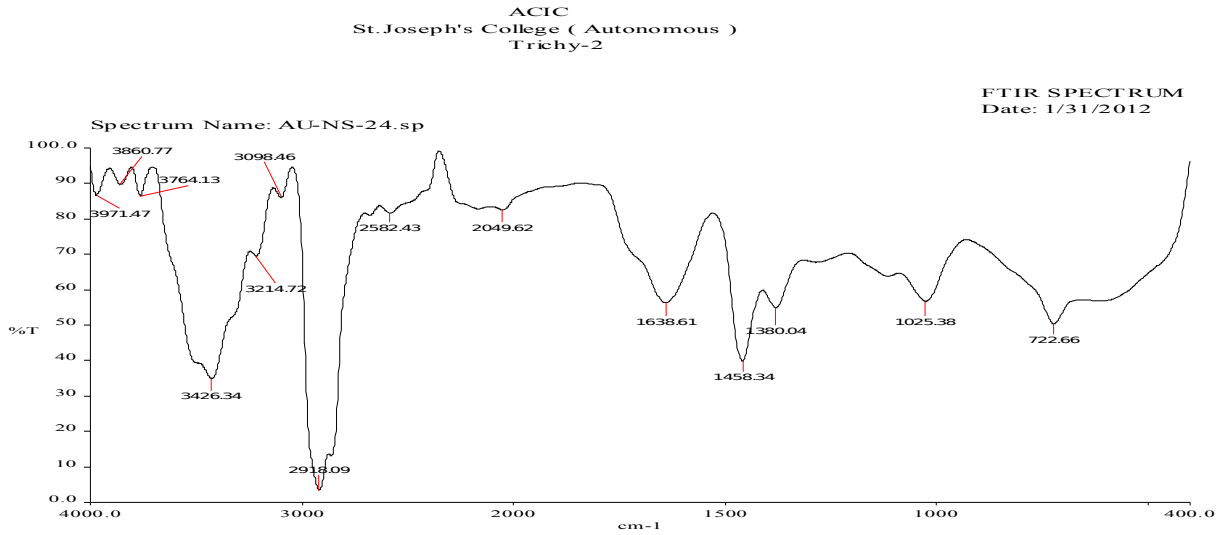
AU-NC-23.pk

AU-NC-23.sp 1801 4000.00 400.00 5.66 100.00 4.00 %T 18 1.00

REF 4000 98.49 2000 90.85 600

3945.22	96.67	3828.25	96.45	3408.01	46.94	3205.03	63.35	3020.00	71.95
2922.83	61.97	2855.65	74.89	2409.21	89.74	2151.87	90.18	1710.28	58.77
1629.30	61.99	1560.23	64.98	1415.65	62.16	1218.67	45.87	1090.16	84.82
927.50	91.24	763.89	5.66	670.93	67.83	535.47	79.92		

Figure 2.25 *Rauvolfia tetraphylla* leaf - Petroleum ether extract



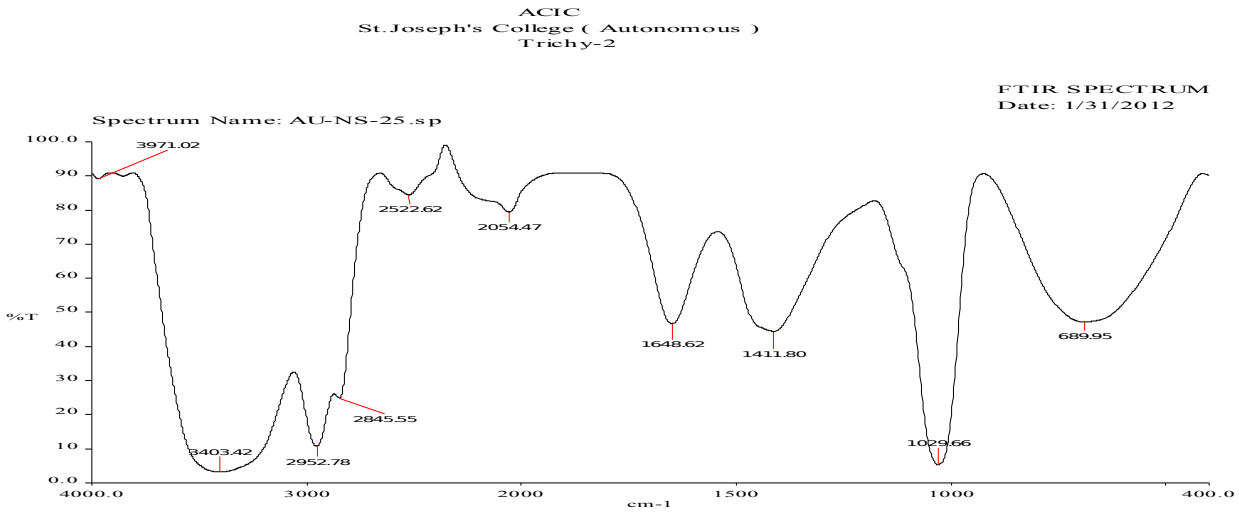
AU-NS-24.pk

AU-NS-24.sp 1801 4000.00 400.00 3.53 99.20 4.00 %T 18 1.00

REF 4000 94.80 2000 85.63 600

3971.47	86.60	3860.77	89.74	3764.13	86.45	3426.34	34.92	3214.72	69.49
3098.46	85.95	2918.09	3.53	2582.43	81.71	2049.62	82.50	1638.61	56.38
1458.34	39.87	1380.04	54.96	1025.38	56.75	722.66	50.36		

Figure 2.26 *Rauvolfia tetraphylla* stem - Methanol extract

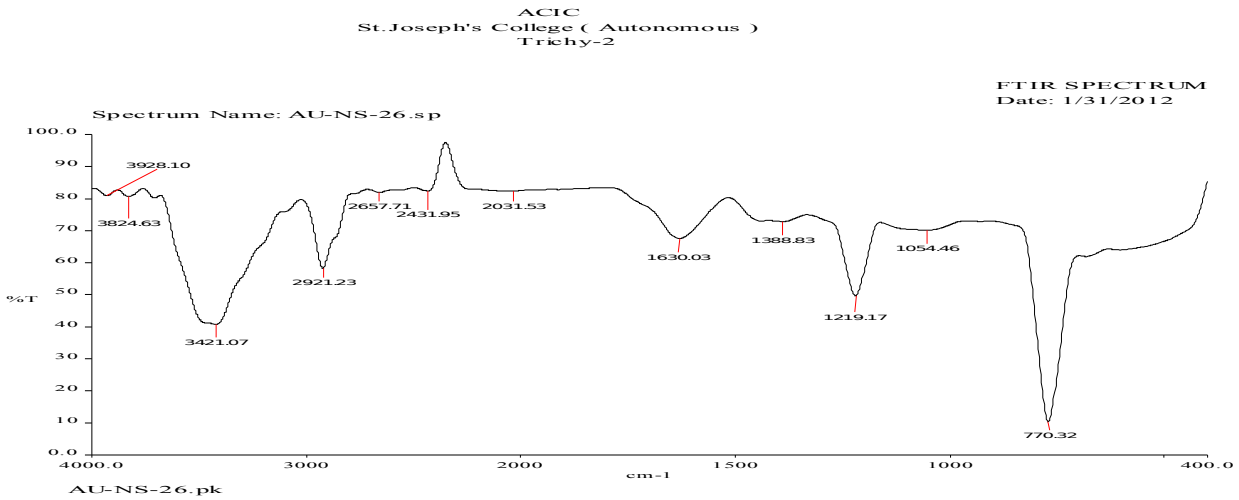


AU-NS-25.pk

AU-NS-25.sp 1801 4000.00 400.00 3.18 99.01 4.00 %T 18 1.00

REF 4000 90.73 2000 85.51 600
3971.02 89.26 3403.42 3.18 2952.78 10.74 2845.55 24.84 2522.62 84.45
2054.47 79.40 1648.62 46.71 1411.80 44.42 1029.66 5.29 689.95 47.21

Figure 2.27 *Rauvolfia tetraphylla* stem - Chloroform extract

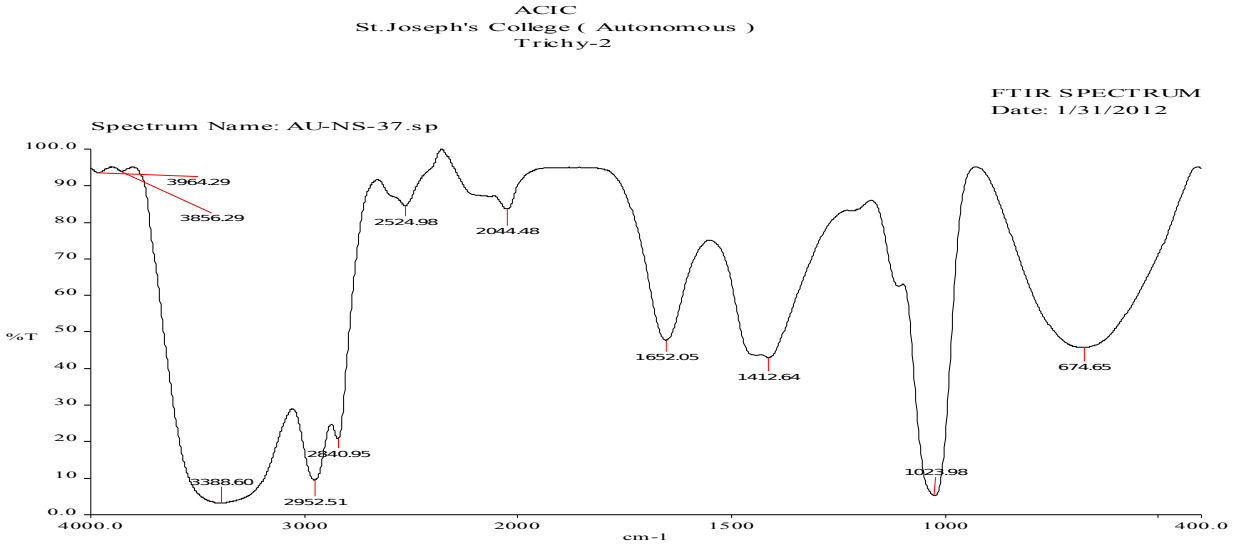


AU-NS-26.pk

AU-NS-26.sp 1801 4000.00 400.00 10.37 97.49 4.00 %T 18 1.00

REF 4000 83.19 2000 82.54 600
3928.10 80.91 3824.63 80.65 3421.07 40.79 2921.23 58.15 2657.71 81.94
2431.95 82.41 2031.53 82.29 1630.03 67.56 1388.83 72.85 1219.17 49.73
1054.46 70.15 770.32 10.36

Figure 2.28 *Rauvolfia tetraphylla* stem - Petroleum ether extract

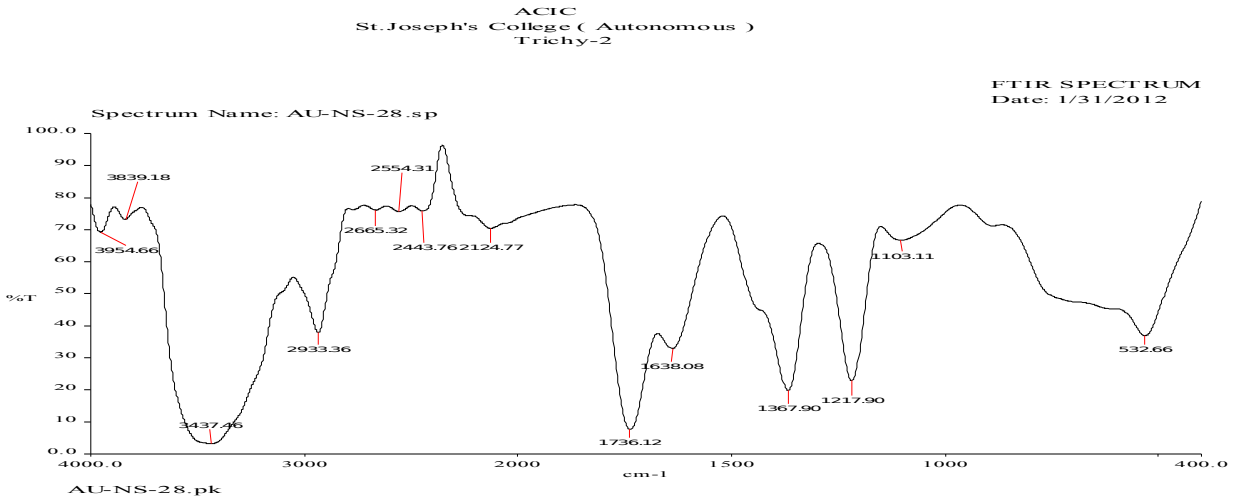


AU-NS-37.pk

AU-NS-37.sp 1801 4000.00 400.00 3.16 99.95 4.00 %T 18 1.00

REF 4000 94.86 2000 90.28 600
3964.29 93.43 3856.29 93.92 3388.60 3.16 2952.51 9.49 2840.95 20.83
2524.98 84.57 2044.48 83.69 1652.05 47.74 1412.64 43.00 1023.98 5.29
674.65 45.82

Figure 2.29 *Rauvolfia tetraphylla* stem - Acetone extract



AU-NS-28.pk

AU-NS-28.sp 1801 4000.00 400.00 3.23 96.39 4.00 %T 18 1.00

REF 4000 77.93 2000 73.56 600
3954.66 69.21 3839.18 73.18 3437.46 3.23 2933.36 37.88 2665.32 76.14
2554.31 75.64 2443.76 75.84 2124.77 70.40 1736.12 7.72 1638.08 32.93
1367.90 19.79 1217.90 22.83 1103.11 66.69 532.66 36.86

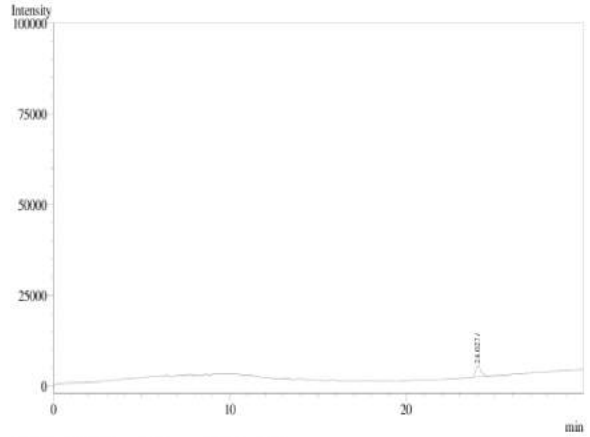
FTIR – Result

Fig 2.22 - 2.29 FTIR analysis shows that the *Rauvolfia tetraphyla* leaf, stem and root were extract with in four different solvent in methanol, acetone, chloroform and petroleum ether extract. The functional groups present in the samples were determined by FT-IR spectroscopy. The FT-IR spectrum confirmed the presence in 18-Hydroxy-yohimbine, Hetero-yohimbine, Sarpagine, Ajmaline and Spirobenzylisoquiniline of *Rauvolfia tetraphyla.* Significant peaks were found at 2926 cm^{-1} corresponding to CH_2 group, 1646 cm^{-1} attributed to Carbonyl groups, and 1539 cm^{-1} corresponding to amino acid groups, all of which confirms the presence of alkaloids.

Figure 3.2

Analysis Date & Time : 1/31/2013 4:33:15 PM
User Name : Admin
Vial# : 7
Sample Name : CA stem
Sample ID : CA stem
Sample Type : Unknown
Injection Volume : 1.00
ISTD Amount :

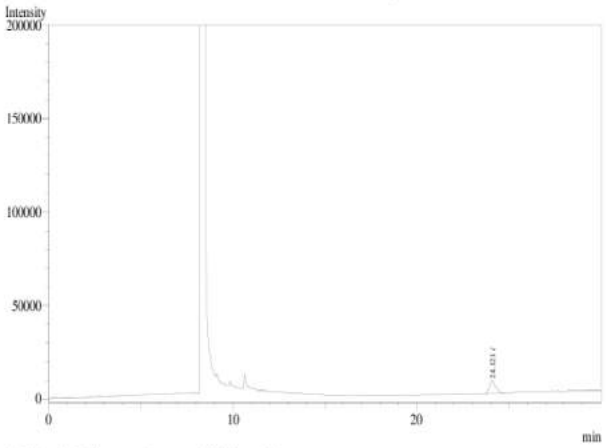
Data Name : E:\GC DATA\Batch\SElva JK NP 31.01. 13\7.gcd
Method Name : E:\GC DATA\METHODE\Selvakumaran\Selva JK NP.gcm



Peak#	Ret.Time	Area	Height	Conc.
1	24.027	65307	3205	100.000
Total		65307	3205	100.000

Analysis Date & Time : 1/31/2013 3:57:05 PM
User Name : Admin
Vial# : 6
Sample Name : CA root
Sample ID : CA root
Sample Type : Unknown
Injection Volume : 1.00
ISTD Amount :

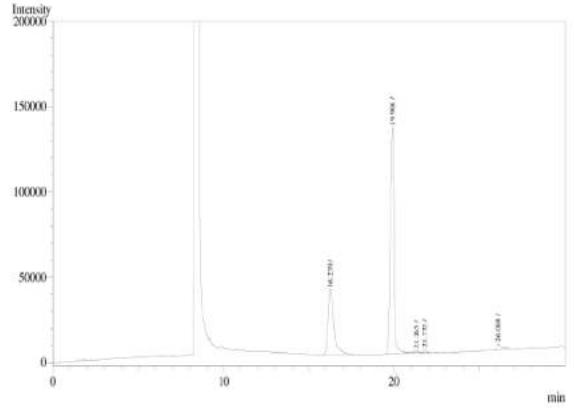
Data Name : E:\GC DATA\Batch\SElva JK NP 31.01. 13\6.gcd
Method Name : E:\GC DATA\METHODE\Selvakumaran\Selva JK NP.gcm



Peak#	Ret.Time	Area	Height	Conc.
1	24.121	156007	6775	100.000
Total		156007	6775	100.000

Analysis Date & Time : 1/31/2013 12:56:40 PM
User Name : Admin
Vial# : 1
Sample Name : RT stem
Sample ID : RT stem
Sample Type : Unknown
Injection Volume : 1.00
ISTD Amount :

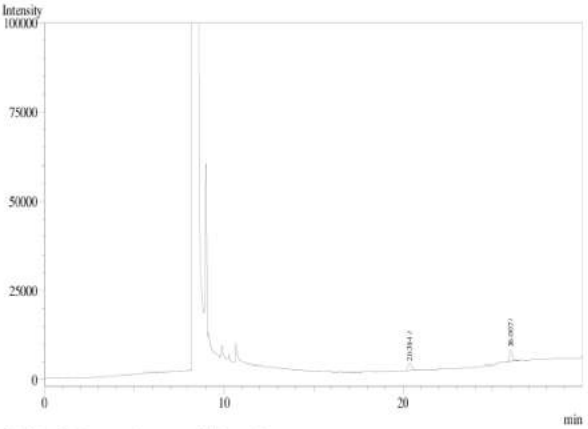
Data Name : E:\GC DATA\DATA\Selvakumaran\31.01.2013\1.gcd
Method Name : E:\GC DATA\METHODE\Selvakumaran\Selva JK NP.gcm



Peak#	Ret.Time	Area	Height	Conc.
1	16.239	883926	38348	30.144
2	19.901	1946557	132742	66.233
3	21.265	20351	1208	0.692
4	21.772	21983	2024	0.748
5	26.068	64137	3498	2.182
Total		2938954	177820	100.000

Analysis Date & Time : 1/31/2013 1:32:42 PM
User Name : Admin
Vial# : 2
Sample Name : RT leaf
Sample ID : RT leaf
Sample Type : Unknown
Injection Volume : 1.00
ISTD Amount :

Data Name : E:\GC DATA\Batch\SElva JK NP 31.01. 13\2.gcd
Method Name : E:\GC DATA\METHODE\Selvakumaran\Selva JK NP.gcm



Peak#	Ret.Time	Area	Height	Conc.
1	20.384	30693	2020	44.997
2	26.007	37517	3304	55.003
Total		68210	5324	100.000

Table 3: Activity of Phytocomponents identified in the stem and leaf of *Rauvolfia tetraphylla* through GC - analysis

S. No	Sample Code	R.T	Name of the compound	Molecular formula	Alkaloids
1	R.t - Stem	16.24	18-Hydroxy-yohimbine	$C_{32}H_{40}N_2O_9$ $C_{32}H_{42}N_2O_9$	Reserpine , Rescinnamine
	R.t - Stem	19.9	Hetero-yohimbine	$C_{22}H_{26}N_2O_1$ $C_{23}H_{28}N_2O_3$ $C_{22}H_{26}N_2O_1$	Isoreserpinine Reserpiline Reserpinine
	R.t - Stem	21.26	Sarpagine	$C_{19}H_{22}N_2O_2$	Sarpagine
	R.t - Stem	21.77	Ajmaline	$C_{20}H_{26}N_2O_2$	Ajmaline
	R.t - Stem	26.06	Spirobenzylisoquiniline	$C_{20}H_{15}NO_6$	Densiflorine
2	R.t - Leaf	20.38	Hetero-yohimbine	$C_{22}H_{26}N_2O_1$ $C_{23}H_{28}N_2O_3$ $C_{22}H_{26}N_2O_1$	Isoreserpinine Reserpiline Reserpinine
	R.t - Leaf	26	Spirobenzylisoquiniline	$C_{20}H_{15}NO_6$	Densiflorine

A phytochemical investigation of *Rauvolfia tetraphylla* has revealed the presence of several alkaloids of which the reserpine, ajmaline and densiflorine are the most important and major alkaloids. The alkaloids are concentrated mostly in the bark of the stem, the quantity being much less in the leaf; the stem is reported to yield about 90 per cent of the total alkaloidal content. The various alkaloids isolated from *Rauvolfia tetraphylla* are given in table 3 (Fig 3.2 : Table 3)

1. Plant Name : *Tinospora cordifolia* (Wild.) Mires ex Hook.f. & Thoms

2. Botanical Classification :

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Ranunculaes
Family	Menispermaceae
Genus	*Tinospora*
Species	*cordifolia*

3. Vernacular Names:
Guduchi, Amrita, Giloya, Tinospora, Gulancha, Shindil Kodi, Giloya, Tippaatigo, Gulavel, Galo, Amrita balli, Ambrithu, Gilo, Chinnaruha, Vatsadaani, Tantrika, Kundalini, Chakralakshanika.

4. Chemical Constituents.
Main chemical components are tinocordifolin, tinocordifolioside, tinosponone, tinocordioside, cordioside, picroretine, colombine and columbin. The plant Contains alkaloid – Berberine and a glucosoid – Giloin.

5. Description Medicinal Property
Tinospora is very helpful in building up natural protection against various diseases. It is often used to fight against general debility, dyspepsia, and some other gastrointestinal diseases. This herbal medication is stomachic and diuretic in nature which effectively stimulates the secretion of bile juice, relieves thirst, burning sensation, vomiting tendencies, and jaundice.

6. Useful parts
All parts of the plant are useful.

7. Micropropagation aspects:
In our study the result shows *Tinospora cordifolia* micropropagation mass propagation protocol was developed by using the leaf explants.

8. Result

* The leaf and stem explant were used for callus initiation medium. But, only leaf explant alone showed most significant effect of 100 % response in brownish callus initiation at concentration of BAP+BA+GA_3+Kn (1.0+1.0+1.0+1.0 mg/l). In addiction 92 % of callus initiation were observed in combination of BAP+BA+Kn+IAA (2.0+3.0+2.5+1.0 mg/l)

* Stem explant showed 80% of direct regeneration for greenish shoot formation observed in the combination of MS with BAP,NAA and IAA (2.0+1.0+2.5 mg/l).

* The direct regenerated plantlets were successfully hardened and acclimatized, 95 of plantlets survived well under natural conditions after transplantation.

* In our study results showed that *T. cordifolia* micropropagation were enable the mass propagation protocol was developed by using the stem explant.

Effect of different concentration of growth regeneration callus induction in leaf and stem explants of *Tinospora cordifolia*

Two different explants (leaf and stem) of *Tinospora cordifolia* were inoculated in different concentration of BAP (1.0-4.0 mg/l), BA (1.0-4.0 mg/l), GA_3 (1.0-4.0 mg/l) and Kn (1.0-mg/l) in MS media (Table 1). The leaf explants was response at 100% of brownish callus initiated for low concentration of (1.0 +1.0 +1.0 +1.0 mg/l) in 25-30 days cultures (Figure1. C,D.). In addition callus multiplication medium contain different concentration and combination of (2.0 + 3.0 + 2.5 +1.0 mg/l) BAP+BA+ Kn+IAA showed most significant effect of the callus initiation for leaf 92% and stem 56% (Table 1)

Direct regeneration and shoots multiplication with growth regulators used in stem explant of *Tinospora cordifolia*

Direct regeneration of *Rauvolfia tetraphylla* stem explant was used at different concentrations and combination of (BAP+NAA+ IAA mg/l) (0.5-2.5 + 1.0 + 1.0-5.0). The maximum number 80% of dark greenish shootlets were produced at concentration of (2.0 + 1.0 + 2.5) stem explant (Table - 2). In another way 70% of the multiple shoot were observed at MS medium with combination of BAP+GA_3+Zen+NAA+A.C (8.0 + 1.0+ 0.5+1 0 + 2g and 12.0 + 3.0+ 1.5+ 1.0 + 2g) stem explant (Table 2).()Figure 1-E)

Tinospora cordifoila (Wild.) Mires ex Hook.f. & Thoms

Table 1. Effect of different concentration of growth regeneration callus induction in leaf and stem explants of *T. cordifoila.*

S.No	MS medium with BAP+BA+GA_3+Kn (mg/l)	Leaf callus initiation %	Leaf callus morphology	Stem Callus initiation %	Stem callus morphology
1	1.0 +1.0 +1.0 +1.0	100	B.C	30	B.C
2	1.5 +2.0 +2.0 +1.0	30	B.C	45	B.C
3	2.0 +2.5 +3.0 +1.0	30	B.C	25	B.C
4	4.0 +4.0 +4.0 +1.0	40	B.C	55	B.C
5	5.0 +5.0 +5.0 +1.0	25	B.C	47	B.C
	BAP+BA+Kn+IAA				
6	2.0 + 3.0 + 2.5 +1.0	92	B.C	56	B.C

B.C - Brownish Callus,

Table 2 Effect of different concentration of growth regulators used in leaf callus multiplication and stem explats direct regeneration of *T. cordifoila.*

S.No	MS medium with BAP+NAA+IAA (mg/l)	Stem explant s Direct regeneration	Shoot initiation morphology
1	0.5 + 1.0 + 1.0	50	Greenish shoot formation
2	1.0 + 1.0 + 1.5	25	Greenish shoot formation
3	1.5 + 1.0 + 2.0	25	Greenish shoot formation
4	2.0 + 1.0 + 2.5	80	Dark greenish shoot formation
5	2.5 + 1.0 + 5.0	75	Dark greenish shoot formation
	MS+BAP+GA_3+Zen+NAA+A.C		
6	8.0 + 1.0+ 0.5+1 0 + 2g	70	Dark greenish shoot formation
7	10.0 + 2.0+ 1.0 +1.0 + 2g	20	Greenish shoot formation
8	12.0 + 3.0+ 1.5+ 1.0 + 2g	70	Dark greenish shoot formation
9	14.0 + 4.0+ 2.0+ 1.0 + 2g	60	Greenish shoot formation
10	16.0 + 5.0+ 2.5+ 1.0 + 2g	55	Greenish shoot formation

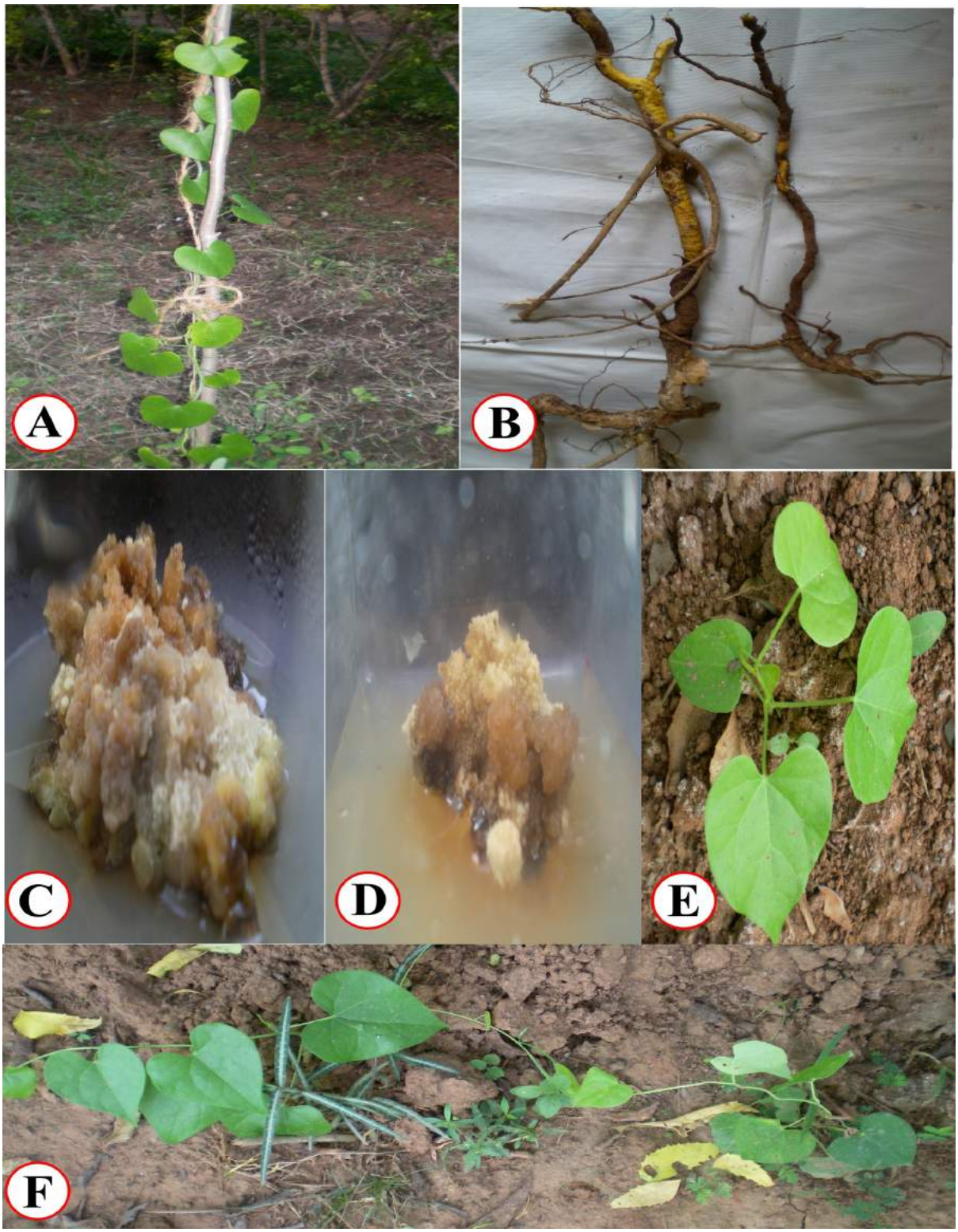
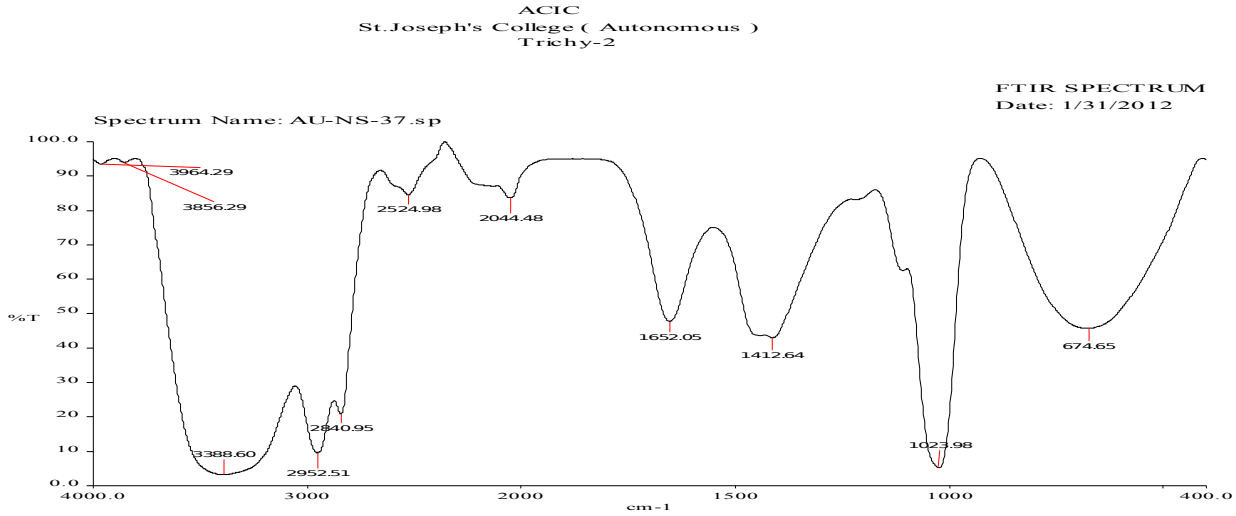


Figure: A, B– *Tinospora cordifolia* **explant, C,D** – Leaf callus **E**-Direct regeneration of stem explant, **F -** Plant hardening for *in vivo* condition.

FTIR - Result

Figure 2.30 *Tinospora cordifolia* leaf - Methanol extract

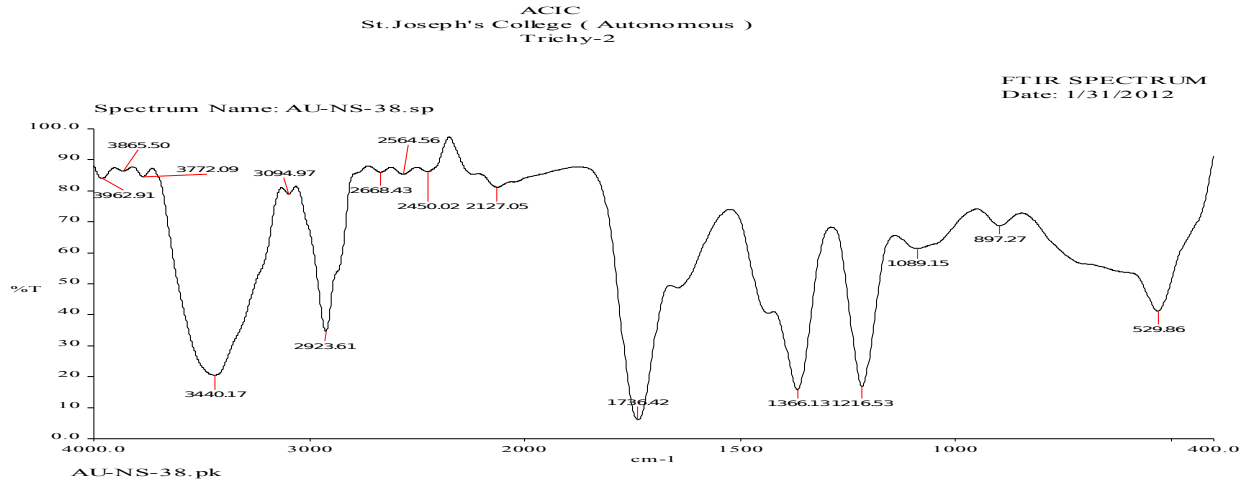


AU-NS-37.pk

AU-NS-37.sp 1801 4000.00 400.00 3.16 99.95 4.00 %T 18 1.00

REF 4000 94.86 2000 90.28 600
3964.29 93.43 3856.29 93.92 3388.60 3.16 2952.51 9.49 2840.95 20.83
2524.98 84.57 2044.48 83.69 1652.05 47.74 1412.64 43.00 1023.98 5.29
674.65 45.82

Figure 2.31 *Tinospora cordifolia* leaf - Acetone extract

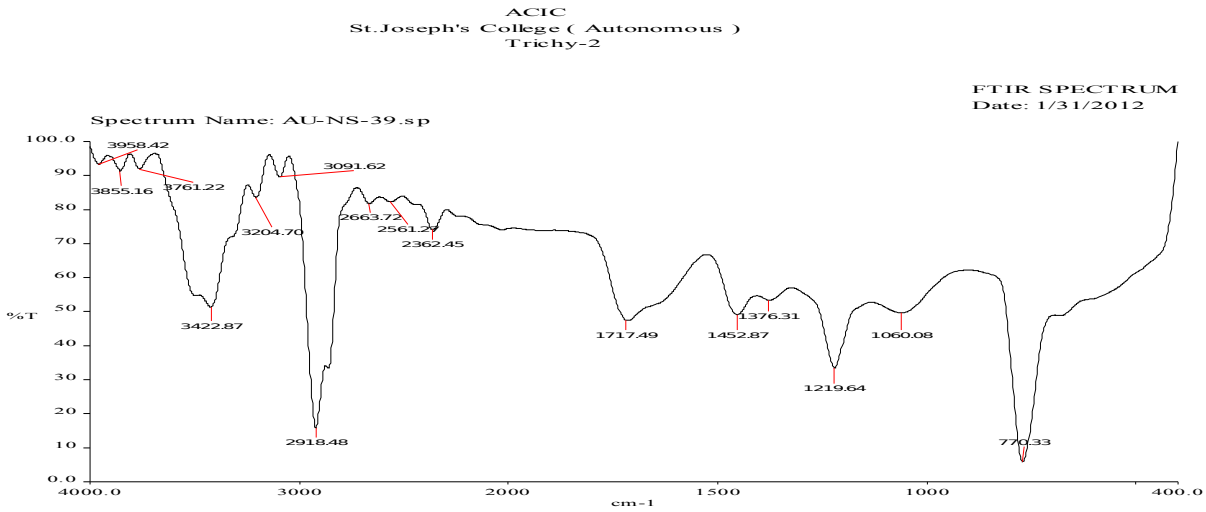


AU-NS-38.pk

AU-NS-38.sp 1801 4000.00 400.00 6.07 97.36 4.00 %T 18 1.00

REF 4000 88.07 2000 84.14 600
3962.91 83.95 3865.50 86.34 3772.09 84.53 3440.17 20.47 3094.97 78.94
2923.61 34.71 2668.43 86.01 2564.56 85.30 2450.02 86.15 2127.05 81.12
1736.42 6.06 1366.13 15.78 1216.53 16.81 1089.15 61.35 897.27 68.73
529.86 41.06

Figure 2.32 ***Tinospora cordifolia*** **leaf - Chloroform extract**



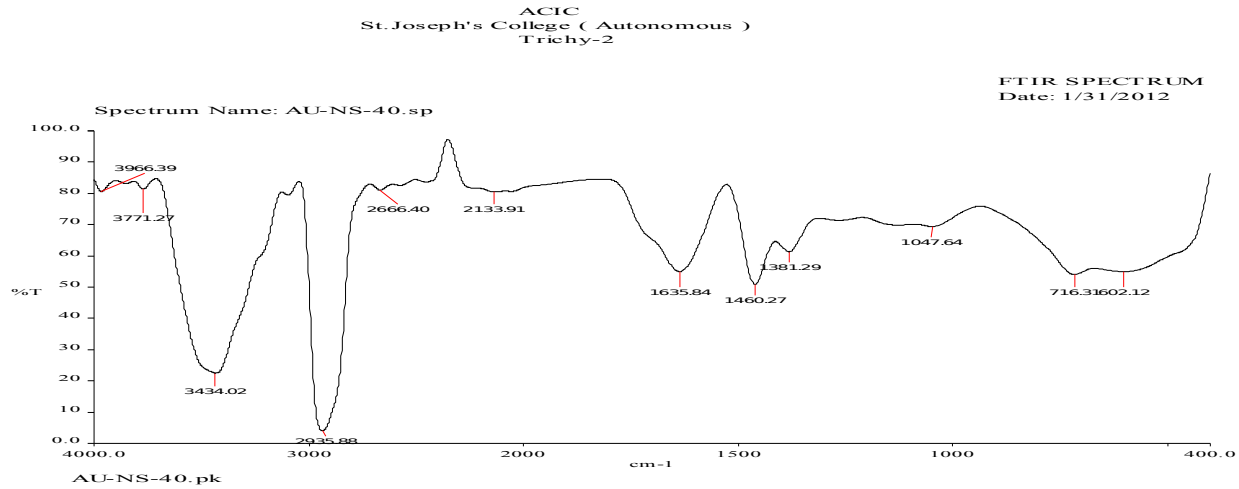
AU-NS-39.pk

AU-NS-39.sp 1801 4000.00 400.00 5.87 100.00 4.00 %T 18 1.00

REF 4000 98.48 2000 74.44 600

3958.42	93.34	3855.16	91.36	3761.22	91.98	3422.87	51.41	3204.70	83.62
3091.62	89.56	2918.48	15.72	2663.72	81.77	2561.27	82.25	2362.45	74.17
1717.49	47.39	1452.87	49.17	1376.31	53.32	1219.64	33.42	1060.08	49.64
770.33	5.86								

Figure 2.34 ***Tinospora cordifolia*** **leaf - Petroleum ether extract**



AU-NS-40.pk

AU-NS-40.sp 1801 4000.00 400.00 4.01 97.23 4.00 %T 18 1.00

REF 4000 84.50 2000 81.92 600

3966.39	80.68	3771.27	81.42	3434.02	22.52	2935.88	4.01	2666.40	81.00
2133.91	80.56	1635.84	54.96	1460.27	50.87	1381.29	61.31	1047.64	69.43
716.31	54.09	602.12	55.00						

Figure 2.35 *Tinospora cordifolia* stem - Methanol extract

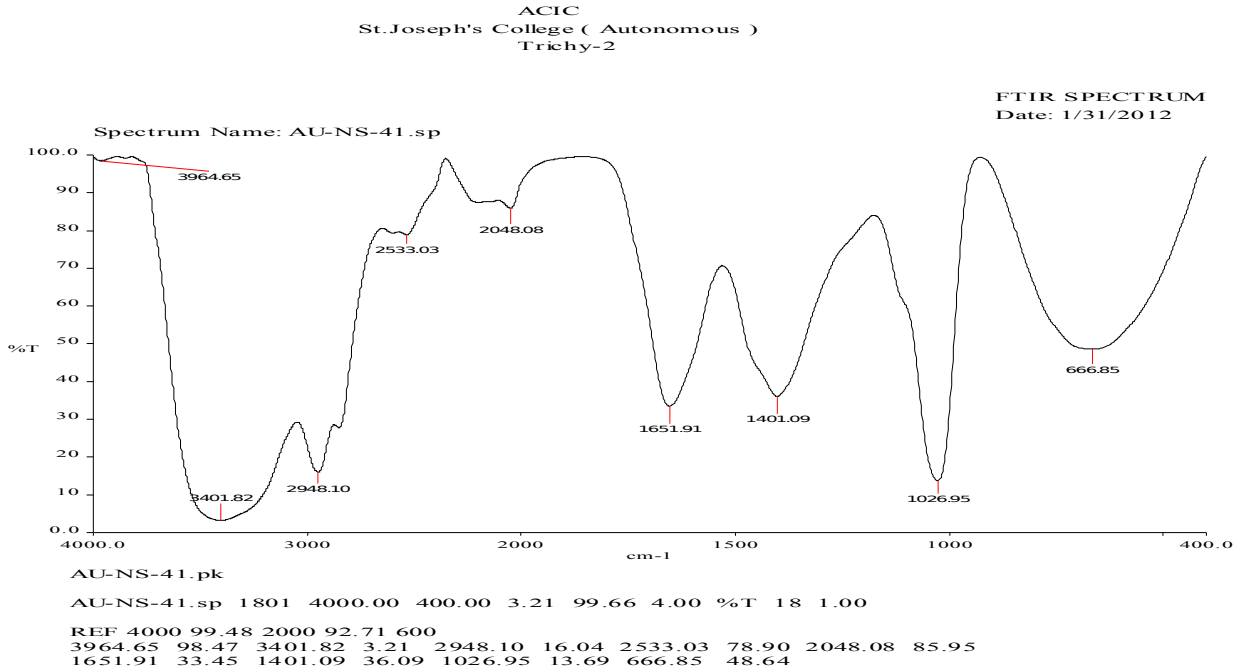


Figure 2.36 *Tinospora cordifolia* stem - Acetone extract

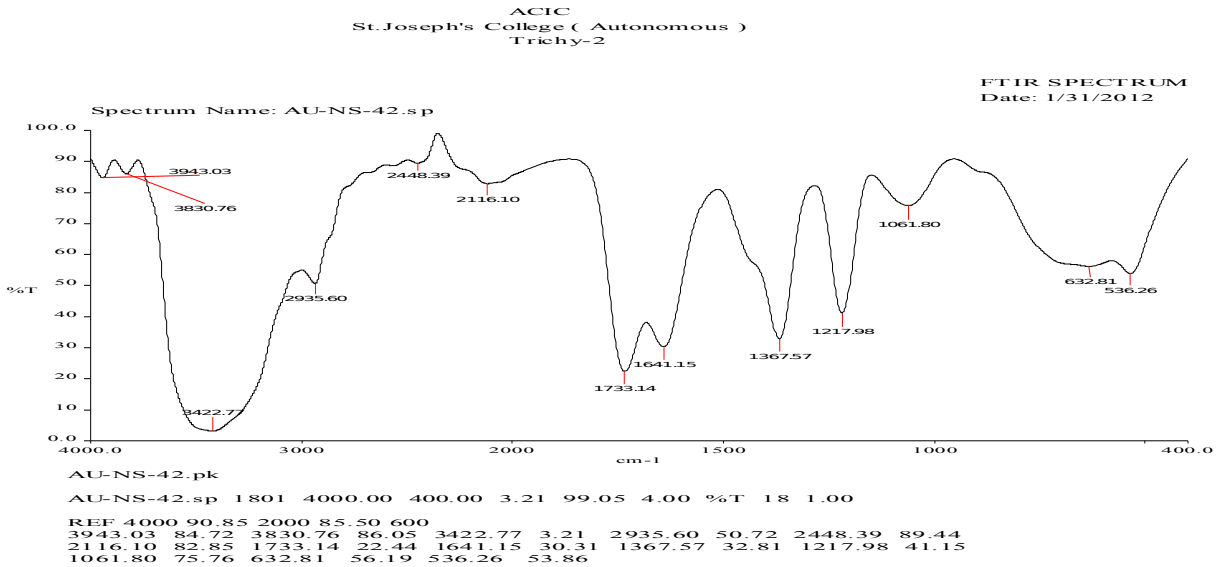
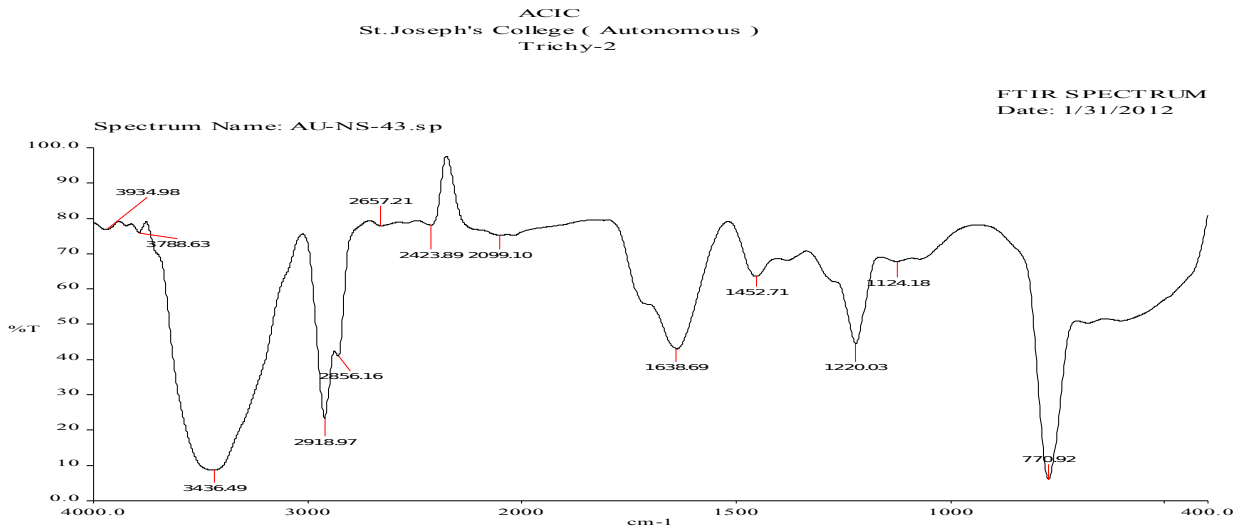


Figure 2.37 *Tinospora cordifolia* stem - Chloroform extract

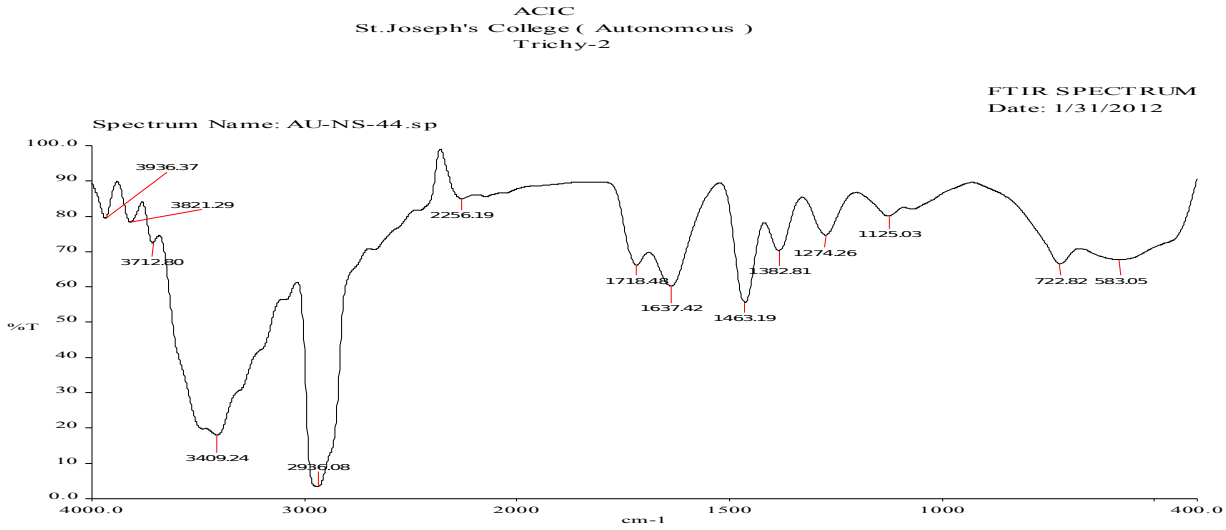


AU-NS-43.pk

AU-NS-43.sp 1801 4000.00 400.00 6.01 97.62 4.00 %T 18 1.00

REF 4000 78.87 2000 76.14 600
3934.98 76.78 3788.63 75.90 3436.49 8.66 2918.97 23.39 2856.16 40.99
2657.21 77.93 2423.89 78.11 2099.10 75.22 1638.69 43.03 1452.71 63.60
1220.03 44.43 1124.18 67.73 770.92 5.97

Figure 2.38 *Tinospora cordifolia* stem - Petroleum ether extract



AU-NS-44.pk

AU-NS-44.sp 1801 4000.00 400.00 3.44 99.05 4.00 %T 18 1.00

REF 4000 89.42 2000 87.77 600
3936.37 79.32 3821.29 78.38 3712.80 72.36 3409.24 18.08 2936.08 3.44
2256.19 85.00 1718.48 66.12 1637.42 60.19 1463.19 55.51 1382.81 70.27
1274.26 74.68 1125.03 80.01 722.82 66.48 583.05 67.66

FTIR – Result

Fig 2.30 – 2.38 FTIR analysis shows that the *Tinospora cordifolia* leaf and stem were extract with in four different solvent in methanol, acetone, chloroform and petroleum ether extract. The FT-IR spectrum confirmed the presence in Amino-furanose of *Tinospora cordifolia.* The FTIR spectra revealed the presence of different functional groups such as free NH_2-stretching, C–N stretching, NH-bending, C–O stretching and Cl-stretching. *Tinospora cordifolia* methanol extract resulted a strong band at 3,426 cm^{-1} corresponding to N–H stretching vibration of primary amines, the band at 2,077 cm-1 corresponding to C–N stretching of any R–N=C=S, the medium band at 1,638 cm^{-1} corresponding to similar conjugation effects to N–H bending frequency, the low band 676 cm^{-1} corresponding to Cl stretching.

Figure 3.3

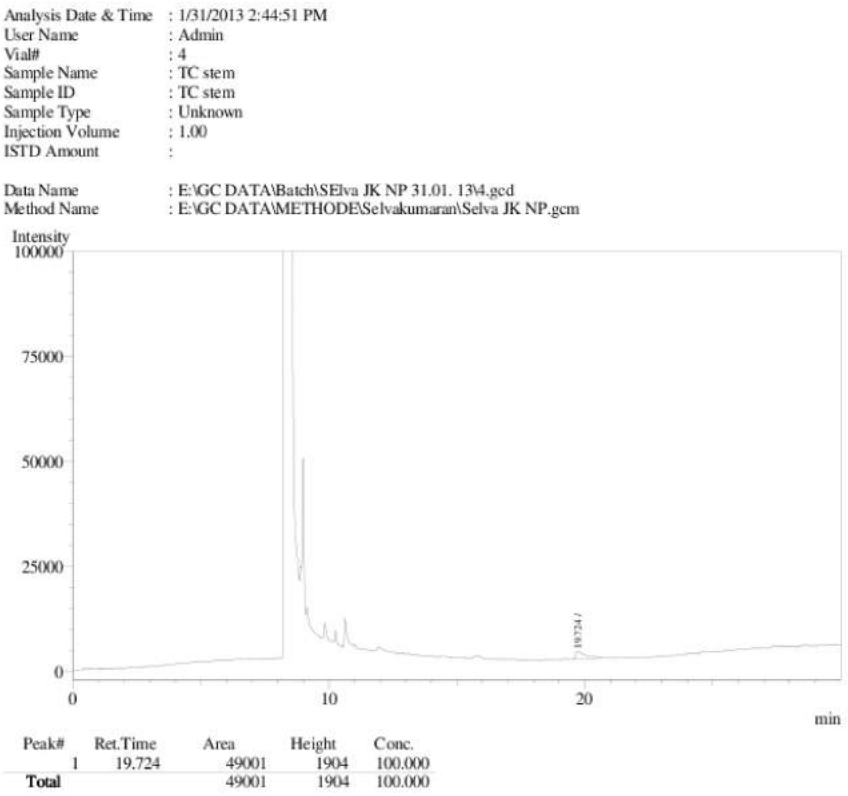


Table 4 Activity of Phytocomponents identified in the stem of *Tinospora cordiflia* through GC - analysis

S. No	Sample Code	R.T	Name of the compound	Molecular formula	Compound Nature
1	T.c - Stem	19.72	Amino-furanose	$C_{20}H_{32}N_2O_4$	Sugar derivative

The maximum amount of bioactive compound was found to be Amino-furanose sugar derivative. (Fig 3.3: Table - 4)

Proof of Publications

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Volume: 2: Issue-3: July-Sept -2011 IJABPT *ISSN 0976-4550*

MICRO PROPAGATION AND TISSUE CULTURE OF THE ENDANGERED MEDICINAL PLANT *WITHANIA SOMNIFERA* BY THE DIRECT SHOOT AND ROOT INITIATION METHOD

A. Arumugam*, K. Gopinath,

Division of Bio-Nanotechnology, Department of Nanoscience & Technology,

Alagappa University, Karaikudi-630003, Tamilnadu, India

*Corresponding Author: E-mail: sixmuga@yahoo.com

ABSTRACT: Leaf and Cotyledon explants of *Withania somnifera* (L). Dunal were used to evaluate the effect of different growth regulators on the in vitro direct shoot and root initiation methods. Four different explants were used to establish callus shoot and root direct regeneration. In the first experiment leaf segments were cultured on MS basal supplemented with 2,4 – Dichlorophenoxyacetic acid (2,4 – D, 0.1-20.0 mg/L), with combination of Naphthalene acetic acid (NAA 0.1-20 mg/L) and Benzylaminopurin (0.1-20 mg/L). This new protocol was standardized for easy mass propagation of *W. somnifera* medicinal plant. Callus initiation was observed best in MS media with (2,4- D 1.0-5.0 mg/L) after 16-20 days (93%). Highest maximum number of multiple shoots was obtained on MS medium (BAP 3.0 – 5.0 mg/L). The shoots were seaperated from the multiple-shoots, transferred to MS medium supplemented with 1.5 – 20 mg/L NAA favored roots formation occurred in most of the shoot let 88% were successfully achieved in the MS media. The rooted plantlets were transferred to polythene bags which was containing vermi compost, sand and red soil in the ratio of 1:2:2 and kept in a mist house. After acclimatization in the mist house for 2-months, it transferred to greenhouse. The plantlets were successfully planted in the field.

Key Words: *Withania somnifera*, **2,4**-Dichlorophenoxy acetic acid, α- Naphthalene acetic acid, Benzylaminopurine, **MS** -Murashige and Skoog Medium.

INTRODUCTION

Withania somnifera (L.) Dunal, is an erect, evergreen, perennial shrub and member of Solanaceae family is a widely used medicinal plant useful in the treatment of inflammatory, anti-tumour agent (Naidu et al., 2003). it is well known for years as an important drug in Ayurvedic literature. Root of the plant W*ithania somnifera* (Ashwagandha) reportedly exhibit antioxidant, immunomodulatory and haematopoietic properties (Mishra et al., 2000).

Ashwagandha roots used in Ayurveda and Unani medicines. Roots are prescribed as medicines for hiccups, several female disorders, bronchitis, rheumatism, dropsy, stomach and lung inflammation, and skin diseases. The ingredient in medicines prescribed for curing disability and sexual weakness in males (Joshi et al., 2010). According to red list of threatened species, 44 plant species are critically endangered, 113 endangered and 87 vulnerable. *W. Somnifera* proved to be 99.75% of the endangered medicinal plant (Siddique et al., 2005; Rahman, 2001). As over harvesting of *W. somnifera* that plant root is going to be endangered condition in the Southern India (Manickam et al., 2000). The active pharmacological components of *Withania somnifera* constituents are withanolides (Steroidal lactones with ergostane skeleton) and alkaloids (Elsakka et al., 1990). The active cintent of Indian *Withania somnifera* are withaterin-A and withanolide-D, both are present leaves and roots of the plant are used as a source of drugs. Total alkaloid content in the root of the Indian type has been reported to be between 0.13 to 0.31% of this plant showed antitumor and radio sensitizing effects in animal models (Sharma et al., 2009). It also possesses anti-stress, immunomodulatory, anti-oxidant and anti-bacterial activity (Kupchan et al., 1965 ; Devi et al., 1992; Devi et al., 1993).

Asian Journal of Biotechnology 4 (3): 120-128, 2012
ISSN 1996-0700 / DOI: 10.3923/ajbkr.2012.120.128

In vitro Micropropagation using Corm Bud Explants: An Endangered Medicinal Plant of *Gloriosa superba* L.

A. Arumugam and K. Gopinath
Division of Bio-Nanotechnology, Department of Nanoscience and Technology, Alagappa University, Karaikudi-630003, Tamil Nadu, India

Corresponding Author: A. Arumugam, Division of Bio-Nanotechnology, Department of Nanoscience and Technology, Alagappa University, Karaikudi-630003, Tamil Nadu, India

ABSTRACT

An efficient protocol was developed *in vitro* micropropagation of *Gloriosa superba* by using corm bud explant. MS basal medium supplemented with different concentration and combination of 2,4-D (2,4-dichlorophenoxy acetic acid), IAA (Indole-3-acetic acid), BAP (6-benzyl aminopurine), GA_3 (Gibberellic acid), Zen (Zeatin), NAA (α-naphthaleneacetic acid), AC (Activated Charcoals) and CW (Coconut Water) were used. The 98.30±0.84% of yellowish Callus initiation was observed in MS media with (2, 4-D 1.0 mg L^{-1}, IAA 0.5 mg L^{-1}) after 4 week culture. This corm bud callus transferred to the shoots initiation medium. The maximum number (94.00±2.92) of multiple shoots was obtained on half strength of MS medium with (Kn (Kinetin) 1.0+BAP1.5+20% CW). The shoot lets transferred to the roots initiation medium. The 96.20±2.59% of multiple roots was obtained at the concentration of MS medium with BAP(8.0)+GA_3(1.0)+Zen(0.5)+NAA(1.0)+2 g L^{-1} AC. In other case without addition of activated charcoal in the MS medium, only 92.20±1.92% of root initiation was occurred, 16% of root induction depends on the addition of activated charcoal present in the MS medium. The rooted plantlets were transferred into small plastic nursery tray which was containing vermi compost, sand and red soil in the ratio of 1:2:2 and kept in a mist house. After acclimatization in the mist house for 2-months, the regenerated plantlets were hardened in the greenhouse and successfully transferred into soil which shows 90% survival rate. This new protocol was standardized for easy mass propagation of such an endangered medicinal plant *G. superba* by using corm bud explants.

Key words: *Gloriosa superba*, micropropagation, callus regeneration, corm bud, endanger medicinal plant

INTRODUCTION

Gloriosa superba Linn. (Liliaceae) is a climber, tuberous and monocot plant. It is native to the tropical and southern part of Africa. *G. superba* is national flower of Zimbabwe, Tamil Eelam and state flower of Tamil Nadu. The altitudinal range of this species is above 1500-2530 m from the surface of the sea level. In India, it is a rare and endangered medicinal plant on southern part of Andhra Pradesh, Karnataka, Kerala and Tamil Nadu (Chopra *et al*., 1956; Chandel *et al*., 1996). Its vernacular name is Kanvali poo, Kaandal, Kalappai kizhangu, Karthigai poo in Tamil. Traditionally since 2000 BC, it is used as a medicinal plant by the tribes (Ade and Rai, 2009). All the parts of *G. superba* are used as medicinal purpose in Siddha, Ayurveda and Yunani system of medicine. The tuber is effective against paralysis rheumatism, snake bite, insect bites, intermittent

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading doi:

Micropropagation and *in vitro* micro-rhizome initiation of *Gloriosa superba* L. (an endangered medicinal plant).

Kasi Gopinath, Ayyakannu Arumugam*

Division of Bio-Nanotechnology, Department of Nanoscience & Technology,Alagappa University, Karaikudi-630 004, Tamilnadu, India

ARTICLE INFO

Article history:
Received 15 April 2012
Received in revised form
Accepted
Available online
Online First

Keywords:
Gloriosa superba
Micro-rhizome imitation
Rhizome callus
Rhizogenesis
Micro-propagation
Endangered medicinal plant.

ABSTRACT

Objective: A standard protocol for Micro-rhizome initiation from rhizome explant and micro-propagation of *Gloriosa superba* L. were developed in the present investigation. **Methods:** MS basal medium supplemented with Phytohormones like 2,4-D, IAA, BAP, GA_3, Kn, NAA and IBA in different combination and concentration. **Results:** The yellowish callus (99.40±0.40) was obtained in the concentration of (1.0 mg/L^{-1}) and (0.5 mg/L^{-1}) for 2,4-D and IAA respectively. The maximum (96.00±1.22) root initiation was obtained from rhizome callus on Ms medium with BAP (8.0 mg/L^{-1})+GA3 (1.0 mg/L^{-1})+NAA (1.0 mg/L^{-1})+2 g/L-1 AC. Rooted callus transfer to the MS medium containing Kn (0.5 mg/L^{-1}) and BAP (1.0 mg/L-1) to produce (38.40±2.66) of micro rhizome initiation. The rooted micro-rhizomes were transferred into half strength MS medium containing Kn (1.0 mg/L^{-1})+BAP (1.5 mg/L^{-1})+20% CW to produce (93.40±1.89) of multiple shoot formation. The rooted shoots were transferred into the small plastic tray, which contains vermi compost, sand and red soil in the ratio of 1:2:2 and kept in a mist house. After acclimatization in the mist house the regenerated plant lets were hardened in the greenhouse and transferred into soil, which shows 90% survival rate. **Conclusion:** This is the first report on media standardized for mass propagation of *G. superba*. by using rhizome explants. Histological studies were carried out to using confocal microscope to study the developmental stages of corm bud and the initiations of micro-rhizome. The morphological and anatomical structure of *in vitro* raised rhizome resembled those of *in vivo* plants.

1. Introduction

Gloriosa superba L., (Liliaceae) is a perennial, greenish, climbing herb native of tropical Africa. Since 2000 B.C, it is being used as a traditional medicine by the tribes[1]. Every part of the plant was used in Siddha, Ayurveda and Yunani system of medicine. The tuber powder was effectively used against paralysis, rheumatism, snake bite, insect bites, against lice, intermittent fevers, wounds, anti-fertility, gonorrhea, leprosy, piles, debility, dyspepsia, flatulence, haemorrhoids, helminthiasis and inflammations[2,3].

G. superba contained various active principle of, which the major alkaloids is Colchicine ($C_{22}H_{25}NO_6$) and Colchicosides ($C_{27}H_{33}O_{11}N$). The seeds consisted of Colchicines, which is 2-5 times higher than that of the tubers. Colchicine has an inhibitory effect on the growth of certain tumors in animals. It inhibits spindle formation by arresting the polymerization of β - tubulin proteins and thereby checks karyokinesis stages. Colchicine is occasionally used in cytological and plant breeding research and it is used for the cancer treatment. Due to its medicinal value this plant was collected from the wild and used, as raw material for large-scale medicinal industry, leading to over exploitation and it becomes an endangered plant species in India and also included in the Red data book. Seed takes four or five vegetative cycles to complete a reproductive phase[4]. Thus mass clonal multiplication through tissue culture method is an urgently needed for conversion of this *Gloriosa superba* species. Some of the reports are available on *in vitro* callus induction and regeneration of the plant using different explant in MS and B5 media[5].

The present investigation was carried out to develop a simple and efficient protocol for rapid micro-propagation

*Corresponding author: Division of Bio-Nanotechnology, Department of Nanoscience & Technology,Alagappa University, Karaikudi-630 004, Tamilnadu, India.
Tel: +914565- 228080 - 228095
Fax: +914565- 225202 - 225525
E-mail: sixmuga@yahoo.com

Effect of Temperature and pH on the *Gloriosa superba* L. pollen fertility

Gopinath. K and Arumugam .A*

Department of Nanoscience and Technology, Alagappa University, Karaikudi, Tamil Nadu - 630 004., India.

Recieved : November 2012
Accepted : November 2012
Published : December 2012

Abstract

Gloriosa superba L. is an endangered and important medicinal plant of Southern India. The seeds and tubers contains high quantity of colchicine and colchicoside alkaloids. it is used in disease like gout, snake bites, leprosy and piles. In the present investigation we studied the male sterility of *G. superba*. Two types of germplasm accessions of *G. superba*, one representing wild variety and other one is cultivated variety. These plant are grown in two different temperatures, low temperature 15-30°C and high temperature 31-37°C and four different pH of soil, the 6.0,6.5,7.0 and 7.5. It produced two types of pollen grains. One is infertile pollen grains and another one is fertile pollen grains. The mature pollen grains were stained and characterized using con-focal microscopic images and the cytological identification of male sterility has been find out. The increase in temperature an environmental condition above 31-37°C produced 92-95% of infertile pollens occurred in wild variety and only 25-40% occurred in cultivated variety. The alkaline pH of the soil, produced 8-40% infertility pollen grains in wild and cultivated variety. It's the major reason which inhibited the fruit formation and it will not survive in the environmental conditions and it becomes in the endanger plant list. pH and temperature two major factors to inhibited the inheritance of paternal characters and formation infertile pollen grains of *G. superba*.

Key Words: *Gloriosa superba*, Male sterility, pH, Temperature, Infertile Pollen Grains, Confocal micrography.

Introduction

Gloriosa superba Linn. (Liliaceae) is a climbing herbs. It's native is tropical and southern part of Africa. *G. superba* is a national flower of Zimbabwe, Tamil Eelam and a state flower of Tamil Nadu. To the altitudinal range of this species above 7,000 ft. from the surface of the sea level in southern India, it is an rare and endangered medicinal plants on southern part of Karnataka, Kerala, and Tamil Nadu. Local names as Kanvali poo, Kaandal, Kalappai kizhangu, Karthigai poo are Tamil. Traditionally since 2000 B.C, it is used as a medical plant by the tribes (Ravindar ade and Rai, 2009). All the parts of *Gloriosa* are used as medicinal purpose in Siddha, Ayurveda and Yunani system of medicine. The tuber are poisonous and used for effective against paralysis rheumatism, snake bite, insect bites, against lice, intermittent fevers, wounds, anti- fertility, gonorrhea, leprosy, piles. debility, dyspepsia, flatulence, haemorrhoids, helminthiasis, inflammations (Pulliah, 2002: Warrier *et al.*, 1995: Ambasta, 1986). The anthelmintic activity in promoting labor pain and expulsion of the placenta (Nadkarni, 1996), Colchicine is used for the cancer treatment (Chopra *et al.*, 1956). Most of the colchicines obtained from *Colchicum autumnale* and *Gloriosa superba* plant, Colchicine is used as a mitosis-arrest agent and in cancer therapy, It is used in plant breeding to induce polyploidy (Sayeed Hassan and Roy, 2005).

According to red list of threatened species, 44 plant species are critically endangered, 113 are endangered and 87 are vulnerable. Due to the medicinal value, these plants are collected from the wild and used as raw material for large-scale medicinal industry, leading to over exploitation and it becomes an endangered plant species. One of the main problems for commercial cultivation is that the seed viability is poor (Mamtha *et al.*, 1993). It takes four or five vegetative cycles to complete a reproductive phase (Samarajeewa *et al.*, 1993). These plants are commercially propagated through asexual reproduction by using tubers (Sivakumar *et al.*, 2003b). The mass multiplication of this plants through the tissue method, plant breeding hybridization and horticulture techniques were needed. These methods are used for not only to conserve this taxon but also to meet the demand for its need in medicinal field. It is a matter of great concern to conserve this plant otherwise we will be loosing it by 2020 (Ravindar ade and Rai, 2009).

Botanical description of *G. superba*, climbing herbs, stems leafy, rootstock tuberous, naked. Leaves alternate, opposite or ternately whorled, midrib prominent, tip elongate, spiral, functioning as a tendril. Flowers large, axillary, usually solitary, pedicels reflexed near the tip. Perianth petaloid, persistent, segments-6, free, spreading or reflexed, narrow, margins usually undulate. Stamens-6, hypogynous, filaments filiform, anthers linear, dorsifixed, versatile, extrorse. Ovary 3-celled, ovules many in each cell, style filiform, sharply deflexed, apex 3- fid, segments subulate, stigmatose within, Fruit a large, coriaceous, septicidal capsule, Seeds subglobose, testa spongy, embryo cylindric (Gamble, 1915) (Fig. 1).

Pollination was the peculiar structures of the large flowers with six perianth lobes bent backwards, six radiating anthers and the style bent almost 90° at the point of attachment to the ovary does not make them suitable for pollination by small insects. The possibility of cross-pollination is determined by wind and also large insects like bumble bees, wasps, some of the butterflies (Entomophily) and sun-birds (Ornithophily) like *Nectarinia zeylonica* and *Nectarinia asiatica* with help of long beaks (Subramanya *et al.*, 1992). Hand pollination is required due to peculiar position of stigma and anthers. Muslin cloth or

*Corresponding Author
Email : : sixmuga@yahoo.com

Plant Tissue Cult. & Biotech. **23**(1): 79-85, 2013 (June)

PTC&B

In vitro Regeneration of an Endangered Medicinal Plant *Withania somnifera* using Four Different Explants

A. Arumugam* and K. Gopinath

Division of Bio-Nanotechnology, Department of Nanoscience & Technology, Alagappa University, Karaikudi-630 004, Tamilnadu, India

Key words: Withania somnifera, Leaf callus, Cotyledons, Hypocotyls, Epicotyls

Abstract

An efficient protocol was developed for highly regenerative capacity from leaf explant of *Withania somnifera* (L.) Dunal - an endangered medicinal plant. Calli were regenerated from four different explants like leaves, cotyledons, hypocotyls and epicotyls. MS supplemented with different concentrations of 2,4-D, BAP and NAA were used. The calli (94.33 ± 1.20%) were obtained from the leaf explant in 2,4-D 3.0 mg/l. The highest number of multiple shoots (85.67 ± 0.88%) were obtained from the leaf callus at 4.0 mg/l BAP. Shootlets forming calli were transferred to the rooting medium containing 10.0 mg/l NAA to produce multiple roots (89.33 ± 0.88%). The regenerated rooted shootlets were transferred to small polythene bags, which contain a sterilized cow-dung, sand and red soil (1 : 2 : 3) and kept in a mist house. After acclimation in the mist house the regenerated plantlets were hardened in the greenhouse and transferred to soil, which showed 85% survival rate. This new protocol was standardized for easy mass propagation of *W. somnifera* using leaf explant.

Introduction

Withania somnifera (L.) Dunal, a member of Solanaceae is widely used in the treatment of inflammatory, anti-tumour agent (Chopra et al. 1958). Is short shrub, growing to a height of 30 - 150 cm, greyish-yellow long rhizomes. It is well-known for years as an important drug in Ayurvedic literature. Root of the *Withania somnifera* (Ashwagandha) plant reportedly exhibit antioxidant, immunomodulatory and haematopoietic properties (Mishra et al. 2000).

Ashwagandha leaves, roots and seeds are used in Ayurveda and Unani system of medicines. Roots were prescribed as medicines for hiccups, several female disorders, bronchitis, rheumatism, dropsy, stomach and lung inflammation, and skin diseases. It is also one of the ingredient in medicines prescribed for

*Author for correspondence: <sixmuga@yahoo.com>.

Industrial Crops and Products 50 (2013) 737–742

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Green synthesis of gold nanoparticles from leaf extract of *Terminalia arjuna*, for the enhanced mitotic cell division and pollen germination activity

K. Gopinath [a], K.S. Venkatesh [a], R. Ilangovan [a], K. Sankaranarayanan [b], A. Arumugam [a,*]

[a] Department of Nanoscience and Technology, Alagappa University, Karaikudi 630 004, Tamil Nadu, India
[b] Department of Physics, Alagappa University, Karaikudi 630 004, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 13 May 2013
Received in revised form 1 August 2013
Accepted 17 August 2013

Keywords:
Terminalia arjuna
Gold nanoparticles
Green synthesis
Mitotic cell division
Pollen germination

ABSTRACT

This present work reports an ecofriendly approach for the synthesis of spherical gold nanoparticles (Au NPs) using aqueous leaf extract of *Terminalia arjuna*. *T. arjuna* contains arjunetin, leucoanthocyanidins and hydrolyzable tannins, which are found to be responsible for the bio-reduction of Au NPs. The formed Au NPs were characterized by UV–vis, FTIR, XRD, AFM and TEM analysis. UV–visible spectra of the aqueous medium containing gold nanoparticles showed a surface plasmon resonance peak at 530 nm. FT-IR analysis was performed to analyze the biomolecules responsible for the reduction of Au NPs. XRD results confirmed the presence of gold nanoparticles with face centered cubic structure. The calculated crystallite sizes are in the range of 20 to 50 nm and the spherical nature of the Au NPs was ascertained by transmission electron microscopy. The efficacy of the synthesized Au NPs was tested for the mitotic cell division and pollen germination. It is suggested that Au NPs induces the mitotic cell division and pollen germination. There was no cytotoxic effect of *Allium cepa* root tip cells and *Gloriosa superba* pollen grains.

1. Introduction

Nanostructured materials have been attracting a keen attention owing to their unique properties. It finds application in many fields such as nanocomputers (Tseng and Ellenbogen, 2001), catalysis (Kim et al., 2003), optical devices (Kamat, 2002), cell labeling (Wu et al., 2003), cell tracking (Parak et al., 2002), *in vivo* imaging (Dubertret et al., 2002), DNA detection (Taylor et al., 2000), antimicrobial activity (Krishnaraj et al., 2012) and so on. Nanoparticle synthesis is usually carried out by various physical and chemical methods such as chemical vapour deposition (Dillon et al., 2006), sol–gel technique (Sobhani et al., 2008), aerosol technology (Magnusson et al., 1999), sono chemical method (Kenji et al., 2005), photochemical reduction (McGilvray et al., 2006) and so on. The chemicals used for these syntheses are often toxic, costly and non-ecofriendly. However, green synthesis approach for producing Au NPs is an alternative source of conventional methods and possesses excellent anti-fungal activity (Jayaseelan et al., 2013). Recently, the synthesis of Au NPs have been reported using extraction of plants such as *Anacardium occidentale* (Sheny et al., 2011), *Cassia auriculata* (Ganesh Kumar et al., 2011), *Centella asiatica* (Kumar Das et al., 2010), *Chenopodium album* (Dwivedi and Gopal, 2010), *Coleus amboinicus* (Narayanan and Sakthivel, 2010), *Crocus sativus* (Vijayakumar et al., 2011), *Macrotyloma uniflorum* (Aswathy Aromal et al., 2012), *Terminalia chebula* (Kumar et al., 2012), *Trigonella foenum-graecum* (Aswathy Aromal and Philip, 2012) and *Murraya koenigii* (Philip et al., 2011).

Metal nanoparticles are used for crop production. An et al. (2008) have reported an increase in ascorbate and chlorophyll contents in leaves of *Asparagus* treated with silver nanoparticles (Ag NPs). Arora et al. (2012) have reported that 10 ppm of gold nanoparticles treated of *B. juncea* seedlings which enhance the net productivity of seed yield. In another study, *Brassica juncea* plants treated with Ag NPs did not seem to accumulate Ag in any form (Haverkamp and Marshall, 2009). Lettuce and cucumber seeds were treated with different concentration of Au NPs. The results were observed in most significant effect of lettuce seed than cucumber seeds and did not showed any significant effect (Barrena et al., 2009). *Arabidopsis thaliana* seeds were treated with 10 μg/ml of Au NPs (24 nm) which enhance the seed yield. Consequently, 80 μg/ml dose of Au NPs was recommended for other vegetative crops productivity (Kumar et al., 2013).

Terminalia arjuna belongs to Combretaceae family and it is a large evergreen tree with spreading crown, drooping branches (Chopra and Ghosh, 1929; Caius et al., 1930). *T. arjuna* bark has been used for the treatment of coronary artery disease (Dwivedi and

* Corresponding author: Tel.: +91 4565 225630; fax: +91 4565 225202.
E-mail addresses: sixmuga@yahoo.com, kgnanee@gmail.com (A. Arumugam).

0926-6690/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.indcrop.2013.08.060

Research Article

THE SCITECH JOURNAL ISSN 2347-7318 ISSN 2348-2311 Online

In vitro Morphogenetic Regeneration from Root Explant of *Gloriosa superba* L. for Enhanced Crop Production

Gopinath K., Karthika V., Gowri S. and *Arumugam A.

Department of Nanoscience and Technology, Alagappa University, Karaikudi - 630 004 Tamil Nadu, India

Abstract

A standard protocol for the mass propagation from root explants of *Gloriosa superba* L. was developed. Murashige and Skoog (MS) basal medium have been supplemented with different concentration and combination of phytohormones viz. 2,4-Dichlorophenoxy acetic acid (2,4-D), Indole- 3-acetic acid (IAA), 6- Benzyl aminopurine (BAP), Gibberellic acid (GA_3), Indole-3-propionic acid (IPA), α-napthalene acetic acid (NAA) and Indole-3-butyric acid (IBA). The maximum percentage of yellowish callus (94.40%) were obtained from the root explants on MS medium supplemented with concentration of (2.0 mg/L), (1.0 mg/L), (0.75 mg/L) for 2, 4-D, IAA and NAA, respectively. The development of roots from the callus were highly regenerated (93.80%) on MS medium with combination of GA_3 (8.0 mg/L), IAA (4.0 mg/L) and BAP (2.0 mg/L). Rooted callus were transferred into half strength of MS medium containing BAP (3.0 mg/L), IBA (1.0 mg/L) and IPA (0.75 mg/L) to produce dark greenish shoots (90.60%). The rooted shoots were transferred into small polythene bags which contain a sterilized cow dung powder, sand and red soil in the ratio of 1:2:3 and kept in a mist house. After acclimatization in the mist house, the regenerated plantlets were hardened in the greenhouse and transferred into soil, which showed 80% survival rate.

Keywords : *Gloriosa superba*, Root explant, Callus, Micro-propagation, Endangered medicinal plant..

Introduction

Gloriosa superba L., (Colchicaceae) is a perennial, greenish, climbing herb and native of South Africa. It grows naturally in many countries of tropical South-Eastern Asia such as Bangladesh, India, Sri Lanka, Malaysia and Myanmar. It is widely cultivated throughout the world as an ornamental plant. Its flower is a national flower of Zimbabwe, Tamil Eelam and state flower of Tamil Nadu. It is acknowledged that the image of this flower was printed as a postal stamp in India, Malaysia, US and Zimbabwe. It is an endangered medicinal plant on southern part of Karnataka, Kerala, and Tamil Nadu (Sivakumar and Krishnamurthy, 2002). Since 2000 B.C, it is being used as a traditional medicine by the tribes (Ade and Rai, 2009). Every part of the plant was used in Siddha, Ayurveda and Unani system of medicine. *G. superba* is a tuberous plant with L or V-shaped cylindrical tubers that are pure white when it is young. The tuber powder was effectively used against paralysis, rheumatism, snake bite, insect bites, against lice, intermittent fevers, wounds, anti-fertility, gonorrhea, leprosy, piles, debility, dyspepsia, flatulence, haemorrhoids, helminthiasis and inflammations (Jana and Shekhawat, 2011). Very recently, *G. superba* leaf extract has been used for metal nanoparticle synthesis (Gopinath *et al.,* 2014a).

G.superba contains two major alkaloids namely Colchicines ($C_{12}H_{25}NO_6$) and Colchicosides ($C_{27}H_{33}O_{11}N$). The seeds consist of colchicines, which are 2-5 times higher than the tubers. Colchicine has an inhibitory effect on the growth of certain tumors in animals. It inhibits spindle formation by arresting the polymerization of β - tubulin proteins and thereby it checks karyokinesis stages (Andreu *et al.,* 1998). Colchicine is occasionally used in cytological and plant breeding research and it is used for the cancer treatment (Sayeed Hassan and Shyamal Roy, 2005). Due to the medicinal value, this plant is collected from the wild and it belongs to rare plant species in India. It has been included in the Red data book. The major problems in commercial cultivation of this plant is an infestation of caterpillars, *Curvularia lunata* fungal blight disease of leaves and tuber-rot caused by *Sclerotium* species (Mrudul *et al.,* 2001), inhibition of pollen fertility by factors like temperature and pH of the soil (Gopinath and Arumugam, 2012), low propagation rate in seeds and 50% percentage of seed viability (Gopinath *et al.,* 2014b). It takes four or five vegetative cycles to complete a reproductive phase (Mamtha *et al.,* 1993; Samarajeewa *et al.,* 1993). These conventional propagation methods are less efficient than micropropagation which produces a uniform plant, virus-free germplasm and also it reduces time and space consumption. Thus, mass clonal multiplication through tissue culture method is urgently needed for conversion of this taxon. *In vitro* callus induction and regeneration of the plant through shoot tip explant (Sivakumar and Krishnamurthy, 2000) and shoot formation from corm bud explant (Somani *et al.,* 1989; Arumugam and Gopinath, 2012) was also reported.

The present investigation was carried out to develop a simple and efficient protocol for rapid micro-propagation of *G. superba* using root explant. This is an alternative and cost effective method for improving the crop production of an endangered medicinal plant.

Material and Methods

G. superba plants and seeds were collected from Glorious Endangered Medicinal Plant Conservation Centre, Science Campus, Alagappa University, Karaikudi, Tamil Nadu, India (Fig.1 A-C). Taxonomic identifications have been done by Dr. S. John Britto, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. The voucher specimen was numbered (KG-001) and preserved in the Department of Nanoscience and Technology, Alagappa University, Karaikudi. Collected seeds were washed in running tap water for about 15-20 min to remove microbes and soil particles, then they were cleaned with liquid detergent Tween 20 (1% v/v) for 5-10 min and rinsed with sterile double distilled water. They were

Recieved: June 2014
Accepted: July 2014

*Corresponding Author
E mail: sixmuga@yahoo.com

Research Journal of Phytochemistry 9 (4): 144-160, 2015
ISSN 1819-3471 / DOI: 10.3923/rjphyto.2015.144.160

Phytochemical Synthesis and Crystallization of Sucrose from the Extract of *Gloriosa superba*

[1]K. Gopinath, [2]C. Karthikeyan, [2]A.S. Haja Hameed, [3]K. Arunkumar and [1]A. Arumugam
[1]Department of Nanoscience and Technology, Alagappa University, Karaikudi, 630003, Tamil Nadu, India
[2]PG and Research Department of Physics, Jamal Mohamed College, Tiruchirappalli, 620020, Tamil Nadu, India
[3]PG and Research Department of Botany, Alagappa Government Arts and Science Collage, Alagappa Univesity, Karakudi, 630003, Tamil Nadu, India

Corresponding Author: A. Arumugam, Department of Nanoscience and Technology, Alagappa University, Karaikudi, 630003, Tamil Nadu, India Tel: +91 4565 225630 Fax: +91 4565 225202

ABSTRACT

Sucrose is a major commodity in worldwide. In half a century the progress towards the understanding of the chemistry, structure, physical and functional properties of the sucrose molecule have been rapid. It was crystallized from the hot methanol extract of the rhizome powder of *Gloriosa superba* by using slow evaporation method at room temperature. The synthesized sucrose crystal lattice parameters were characterized by single crystal X-ray diffraction analysis. The presences of organic functional groups in the grown crystal were identified by FT-IR and Micro Raman analyses. The optical parameter of the grown single crystal was analyzed by UV-visible spectroscopy and maximum absorption was occurred at 253 nm. In addition, HOMO, LUMO, Molecular Electrostatic Potential (MESP), non-linear optical and several thermodynamic properties were analyzed by the DFT calculations. The thermal degradation of the sucrose crystal was tested by Thermo Gravimetric/Differential Thermal Analysis (TG/DTA).

Key words: Single crystal X-ray diffraction, FT-IR, Micro Raman, HOMO-LUMO, DFT

INTRODUCTION

Sucrose ($C_{12}H_{22}O_{11}$) is an organic compound, a nonreducing disaccharide composed of glucose and fructose linked their anomeric carbons. The molecular structure of sucrose ((2-[3,4-dihydroxy-2,5-bis(hydroxymethyl) oxolan-2-yl] oxy-6-(hydroxymethyl) oxane-3,4,5-triol) has been determined by X-ray reflections (Hynes and Page, 1991; Hanson *et al*., 1973). It was widely distributed and has been found universally throughout the plant kingdom in fruits, seeds, flowers, roots and tubers. It is obtained commercially from sugarcane (*Saccharum officinarum*), sugar beet (*Beta vulgaris*), palm (*Borassus flabellifer*) and soyben (*Glycine max*) (Kumaresan and Babu, 1997; Kraybill *et al*., 1937). Sucrose is widely used in food industry, bakery products and act to inhibit the growth of microorganisms due to the higher concentration gradient of sucrose molecule. For long periods, a considerable number of experimental studies related to sucrose have been published due to the physical and chemical properties. Recently, sucrose is used as a promising material for dosimetry (Nakajima and Otsuki, 1990; Hamzaoui *et al*., 2009; Peimel-Stuglik, 2010), hologram (Ponce-Lee *et al*., 2004), biological probe (Predoi, 2010), X-ray osteodensitometry (Ryzhikov *et al*., 2005), nonlinear optics (Kaminskii, 2003), phase transition (Son *et al*., 2010), Low-temperature electrolytic coloration (Gu *et al*., 2012) and cryoprotectant (Luzardo *et al*., 2000).

Peer Reviewed /ISSN: 2663-1040 *AJ Life Sci. 2020, 3 (2): 48-65* **Review Article**

Abasyn Journal of Life Sciences

Open Access

DOI: 10.34091/AJLS.3.2.5

Gloriosa superba L: A critical Review of Recent Advances

Saraswathi Umavathi[1], Kasi Gopinath[2,4†]*, M.S. Manjula[1], Chinnasamy Balalakshmi[3], Ayyakannu Arumugam[4†]*

[1]*Adhiyaman Arts and Science College for Women, Uthangarai, Tamil Nadu 635207, India.*
[2]*School of Materials and Energy, Southwest University in Chongqing, Beibei, China.*
[3]*Department of Nanoscience and Technology, Alagappa University, Karaikudi, Tamil Nadu 630 003, India*
[4]*Department of Botany, Alagappa University, Karaikudi, Tamil Nadu 630 003, India*

Abstract

Gloriosa superba is a flowering plant belongs to Colchicaceae. It refers to full of glory', and the *superba* refers 'superb' referring to the outstanding red and yellow flowers. *G. superba* is a highly toxic plant, due to the presence of an alkaloid, 'Colchicine'. Adequate ingestion of this alkaloid could cause fatalities to humans and animals. Despite its toxicity, *G. superba* considered as a medicinal plant because of its medicinal constituents and properties such as colchicine, gloriosine, pungent, bitter, acrid, heating, anthelmintic, laxative, alexiteric, and abortifacient. *G. superba* utilized for the treatment of snakebite, scorpion stings, parasitic skin disease, urological pains, colic, chronic ulcers, piles, gonorrhoea, gout, infertility, wounds, arthritis, cholera, kidney problems, itching, leprosy, cancer, sexually transmitted disease, and countless other diseases. This plant listed under the threatened category due to its indiscreet reaping from the wild as medicinal industries widely use it for its colchicine content. Field study and secondary sources were used to abridge the information on the recent advancement study of *G. superba*. In the present review, the information regarding the occurrence, botanical description, propagation, medicinal application pharmacological, ethnomedicinal, biological, and toxicological studies of *G. superba* and its recent advancement has been summarized. The detailed study of *G. superba* with current trends helps the researcher to focus on the future development needed for its conservation.

Keywords: *Gloriosa superba*, pharmacognosy, ethnomedicine, toxicity.

Article Info:
Received:
May 14, 2020
Received Revised:
December 10, 2020
Accepted:
December 25, 2020
Available online:
December 31, 2020

**Corresponding Author:*
gopiscientist@gmail.com;
ayyakannuarumugam@gmail.com

How to cite:
Umavathi S, Gopinath K, Manjula MS, Balalakshi C, Arumugam A. *Gloriosa superba* L: A critical Review of Recent Advances. *Abasyn Journal of Life Sciences 2020; 3(2): 48-65.*

CONTENTS