



आईएफटीएम विश्वविद्यालय, मुरादाबाद, उत्तर प्रदेश  
**IFTM University, Moradabad, Uttar Pradesh**  
**NAAC ACCREDITED**

## E-Content

**IFTM University, Moradabad**

**PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATIONS ON *GLYCYRRHIZA GLABRAL.*, *ABRUS PRECATORIUS* L., AND *ZIZIPHUS MAURITIANA*, Lam.**

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# INTRODUCTION

- The concept of natural drugs and secondary metabolites as a therapeutic agent for diseases has come from ancient literature and believes. In modern era of science when these are evaluated for their efficacy by conducting various experiments their efficacy is revealed and it is quite astonishing that these contain various secondary metabolites. The structure elucidation of the metabolites confirm structure activity relationship with the pronounced activities of the plant. In a screening programme in CDRI initiated in 1964 with a spanning period of 25 years and covered around 2500 plants with biological activities. (Mukherjee 2002)
- Some health related disorders and symptoms which are widely cured by herbs and (use of synthetic drugs is not recommended due to lower benefit/ harm ratio) in the literature are:
  - 1) Alopecia
  - 2) Lice infestation
  - 3) Fungal infections
  - 4) Aphrodisiacs or male fertility enhancers

# RESEARCH ENVISAGED AND PLAN OF WORK

- Collection and identification of the herbs.
- Authentication and identification of the herbs.
- Defatting of the herbs by Petroleum ether (60-80)0C
- Extraction of plant material with 90% ethanol.
- *In Vivo* evaluation of herbs for hair growth promoting effects in female rats and antiandrogenic effect in male rats.
- Study of extracts for mechanism involved in androgenic alopecia.
- Evaluation of extracts for pharmacological properties.
- Phytochemical screening and chromatographic profiling of the extract using TLC and isolation of the major phytoconstituents by column chromatography.
- Spectral characterization of the isolates.

# Plant profile

- ***Abrus precatorius***
- **Vernacular Names**
- **Sanskrit – Gunga.**
  - **English-** Black-eyed Susan, Coral-bead plant, Crab's eyes, Indian Liquorice, Jamaican Liquorice, Jequirity, Jequirity bean, Jumbie beads, Jumble beads, Liquorice vine, Love bean, Love nut, Lucky bean, Minnie minnies, Prayer beads, Prayer beans, Precatory, Precatory bean, Red beadvine, Rosary pea, Weather plant, Weather vine, Wild liquorice.
  - **Hindi** Ghungchi
  - **Bengali** Kunch
  - **Tamil** Gundumani
  - **Telgu** Guriginja
  - **Malayalam** Kakani
  - **Kannad** Gungi
  - **German** Paternoster-Erbse.

- **Systemic Position**

- Kingdom Plantae
- Subkingdom Tracheobionta
- Superdivision Spermatophyta
- Division Magnoliophyta
- Class Magnoliopsida
- Subclass Rosidae
- Order Fabales
- Family Fabaceae
- Genus Abrus
- Species *precatorius*

# Glycyrrhiza glabra

- **Vernacular Names**

- Sans- Yasthi-madhu, Hind -Jethi-madh, Beng Jashtimadhu, Eng- Liquorice.

- **Systematic position**

- Kingdom Plantae
- Subkingdom Mangliophyta
- Class Magnoliopsida
- Order Fables
- Family Fabaceae
- Sub Family Faboidae
- Genus *Glycyrrhiza*
- Species *glabra*

# *Ziziphus mauritiana*

- **VERNACULAR NAMES:**
- **English:** Chinese apple, Chinese date, cottony jujube, Indian cherry, Indian jujube, Indian plum, jujube
- **Fijian:** baer
- **French:** jujubier, massonnier
- **Hindi:** baher, bahir
- **Spanish:** azufaifo africano
- **Kingdom:** Plantae
- **Division:** Magnoliophyta
- **Class:** Magnoliopsida
- **Order:** Rosales
- **Family:** Rhamnaceae
- **Genus:** Ziziphus
- **Species:** *Z. mauritiana*
- **Binomial name** *Ziziphus mauritiana*



# PHYTOCHEMICAL STUDIES

- The plant materials were procured in the month of June 2011 from Corbett National Park, Ramnagar (Uttarakhand, India) and identified by Dr. D.V. Amla, Scientist G at National Botanical Research Institute, Lucknow (Uttar Pradesh, India) under voucher specimen no NBRI-SOP-202 and a specimen was preserved there for further references.

# PHARMACOLOGICAL STUDIES

- Animal use in experiments based on the guidelines of the Institutional animal ethics committee of IFTM University, Moradabad India.

# Results and discussion

- The following extracts are obtained from extraction of the plant materials:-
- 1. Petroleum ether extract of *A. precatorius*
- 2. Petroleum ether extract of *G. glabra*
- 3. Ethanolic extract of *A. precatorius*
- 4. Petroleum ether extract of *Z. mauritiana*
- 5. Ethanolic extract of *Z. mauritiana*

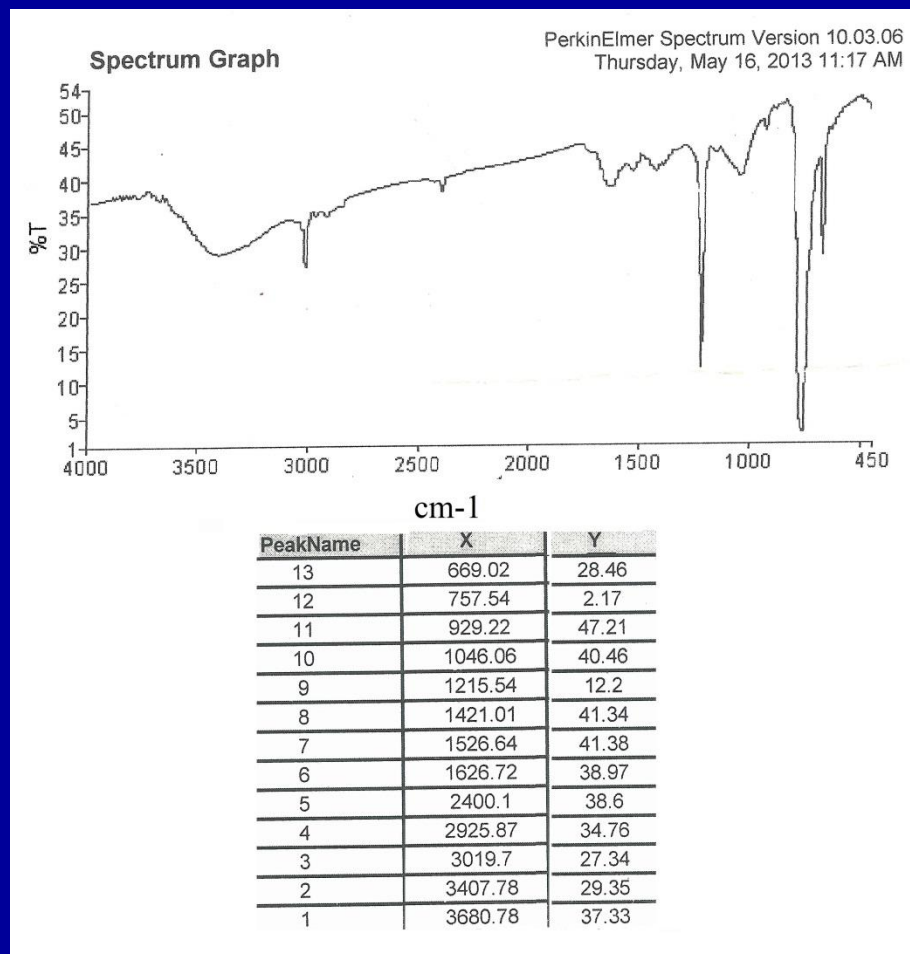
- - The petroleum ether extract of *A. precatorius* showed presence of steroids and ethanolic extract shows presence of flavonoids, glycosides and alkaloids.
- The petroleum ether extract of *G. glabra* shows presence of terpenoidal compounds while ethanolic extract showed presence of glycosides, carbohydrate, phenolics and flavonoids.
- The petroleum ether extract of *Z. mauritiana* showed presence of terpenoids while ethanolic extract shows presence of flavonoids, alkaloids and phenolics.
- Column chromatography was performed for isolation of the compounds.

# **SPECTRAL ANALYSIS OF THE ISOLATES**

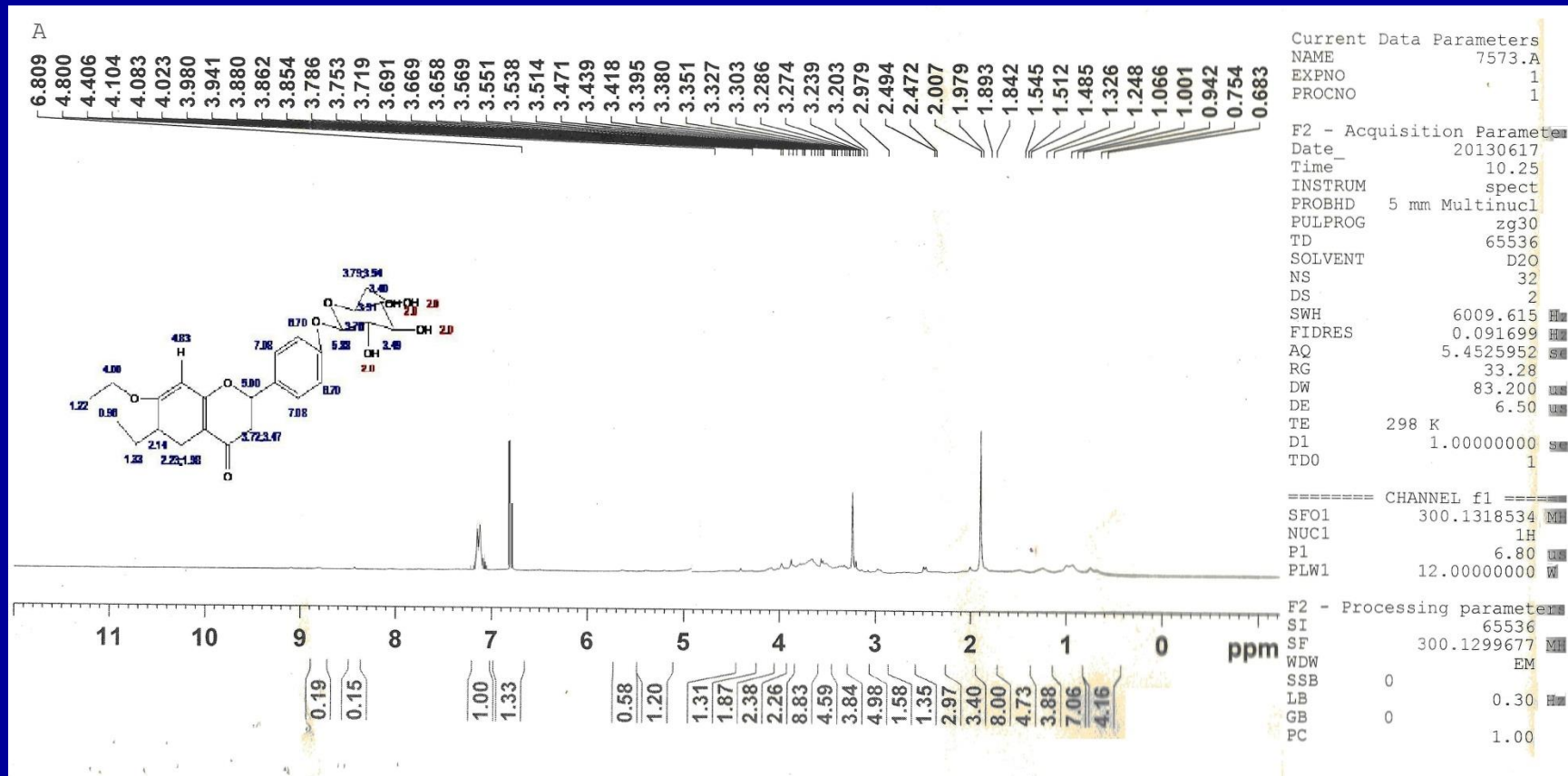
- **The analysis was performed by NMR and Mass fragmentation analysis and molecular weight analysis by software Chemdraw Ultra 8.0**

- **COMPOUND A**
- Isolated from *Glycyrrhiza glabra* roots
- Solvent of Elution Chloroform:Methanol in ratio 94:07
- R<sub>f</sub> 0.9 in solvent system nButanol :Ethanol:water 10:10:4.1
- Nature Flavanoid Glycoside confirmed by Shinoda test and Molish test
- Yield 20mg
- **Description**
- Yellow prisms; mp 156 °C; UV λ MeOH
- max nm (log ε): 325 (4.46), 277 (4.40); IR ν<sub>max</sub> (KBr) cm<sup>-1</sup>: 3680,3407,3019,2925
- , 1626, , 1526, , and 1421; <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ 3.0-3.5 , 3.71-3.78 , 3.98, 5.1 6.8,, 6.99,7.10 8.4 ESIMS m/z (%): 475 ([M-H].

# IR



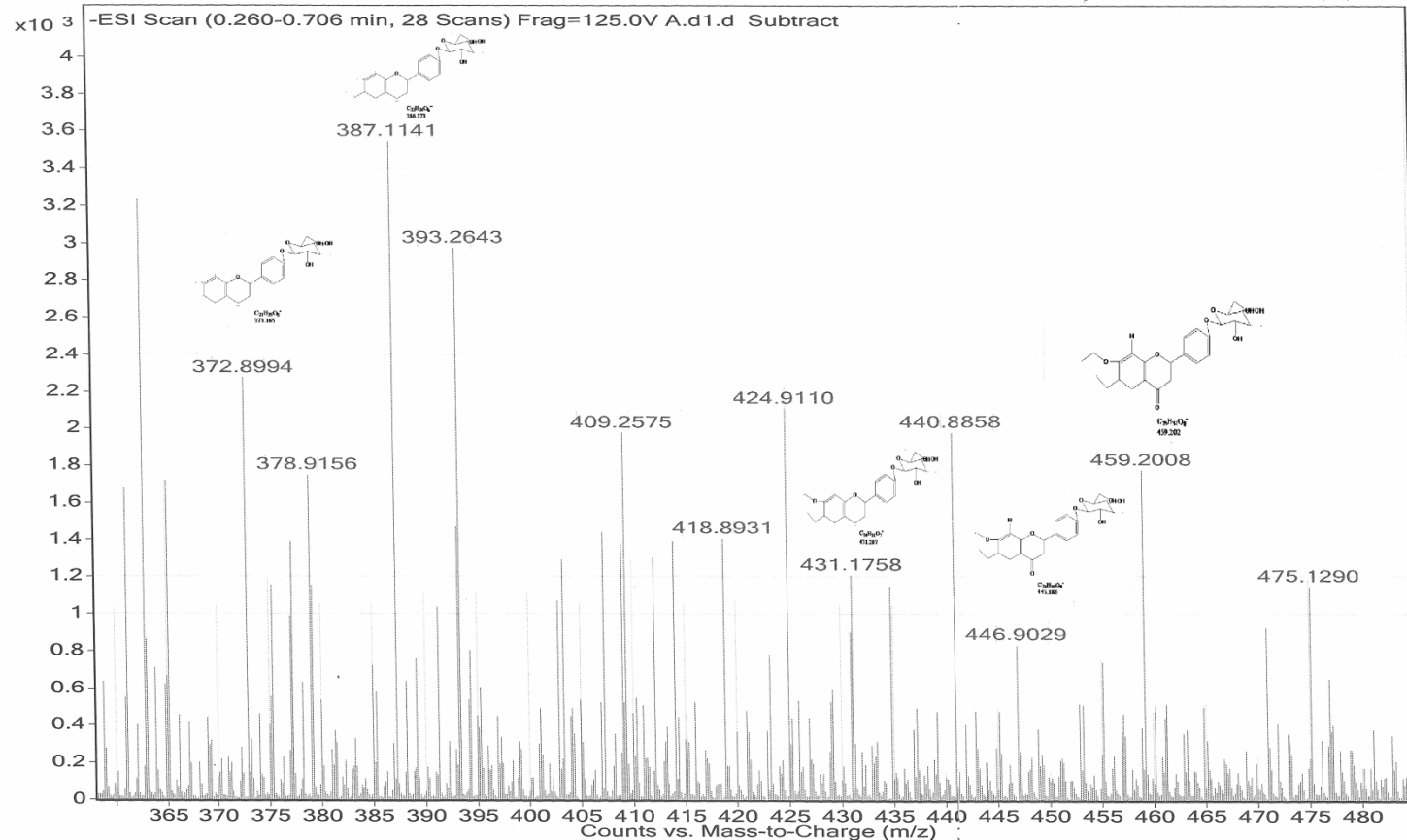
# NMR





# Mass spectrometry

<b>Sample Name</b>	SUKIRTI(7573)	<b>Position</b>	Vial 41	<b>Instrument Name</b>	Instrument 1	<b>User Name</b>	
<b>Inj Vol</b>	10	<b>InjPosition</b>		<b>SampleType</b>	Sample	<b>IRM Calibration Status</b>	Success
<b>Data Filename</b>	A.d1.d	<b>ACQ Method</b>	ISOCRATIC GENERAL NE	<b>Comment</b>		<b>Acquired Time</b>	5/20/2013 10:31:04 AM



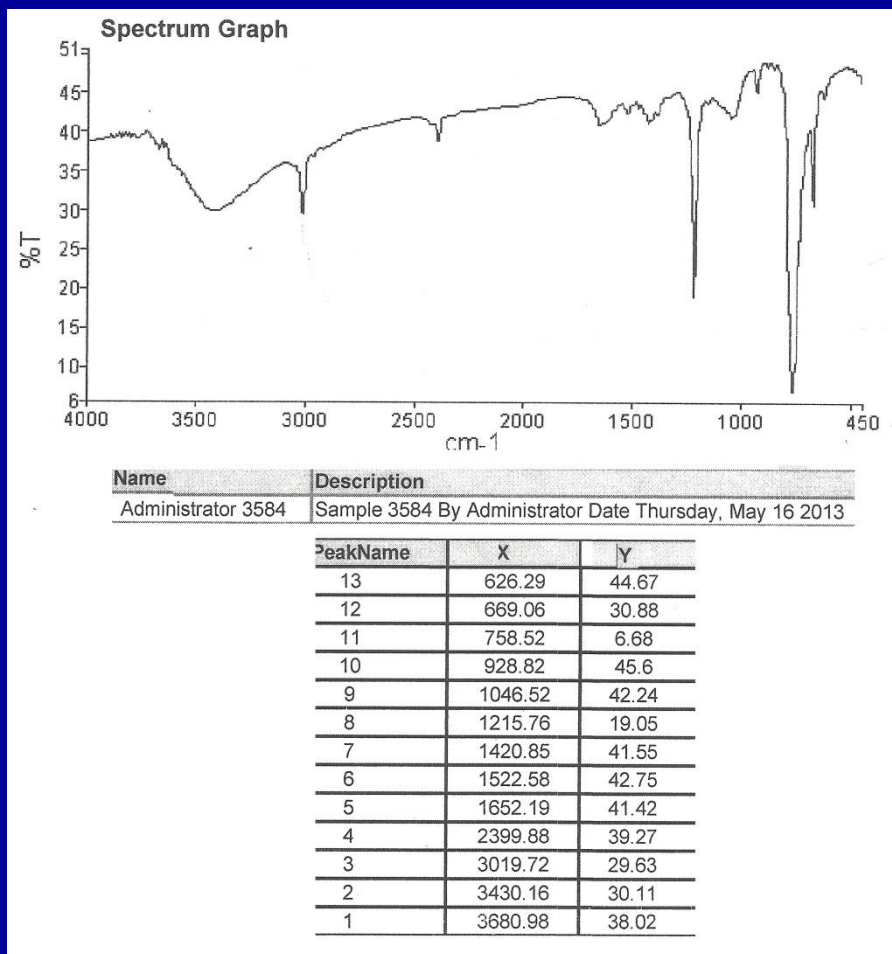


- The proton NMR of the compound A shows presence of two methyl groups, 5 methylene groups, 11 methine groups (belongs to benzene and tetrahydropyran) and 3 hydroxyl groups. Although C 13 NMR is not so clear but proton NMR strongly supports the proposed structure doublets at 7.1 ppm shows presence of aromatic structure. The peaks available at 2.0 or near indicates the presence of hydroxyl group and the multiplet at 3.0-3.5 is similar feature to flavonoid glycosides due to presence of glucose moiety. Although there are other peaks are also present but they are ignorable or impurity peak. The structure of compound A is slightly modified to structure of previously identified flavonoid glycoside cirsimarin. (Lin 2006)

# Compound B

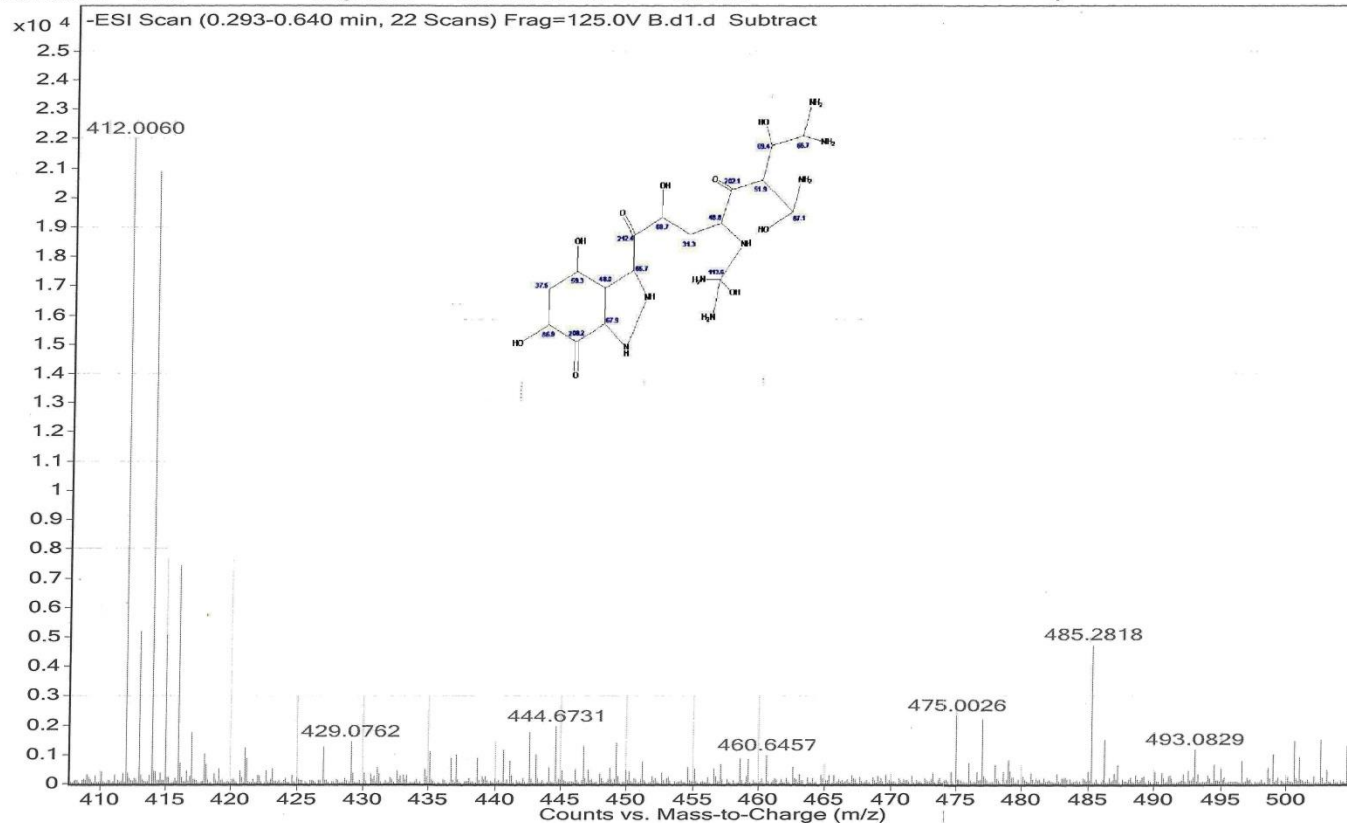
- Isolated from *Ziziphus mauritiana*
- Solvent of Elution Pet ether :chloroform in ratio 90:10
- Rf 0.33 in solvent system n Butanol: Acetic acid: water 5.5:3.0:1.5
- Nature Alkaloid confirmed by Mayer and Dragendroff's reagent
- Yield 48mg
- **Description**
- Brown amorphous; mp 220 °C; IR(KBr) cm<sup>-1</sup>: 3680,3430,3019,2399,1652,1522,1420,1215,1046,928,758,669,626.
- (br), 1661, 1603, 1566, 1519, and 1461; <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ 1.2-1.4 2.0,2.3-2.8,3.0,3.3-3.7,3.8-4.8 ESIMS *m/z* : 493 ([M-H].

# IR

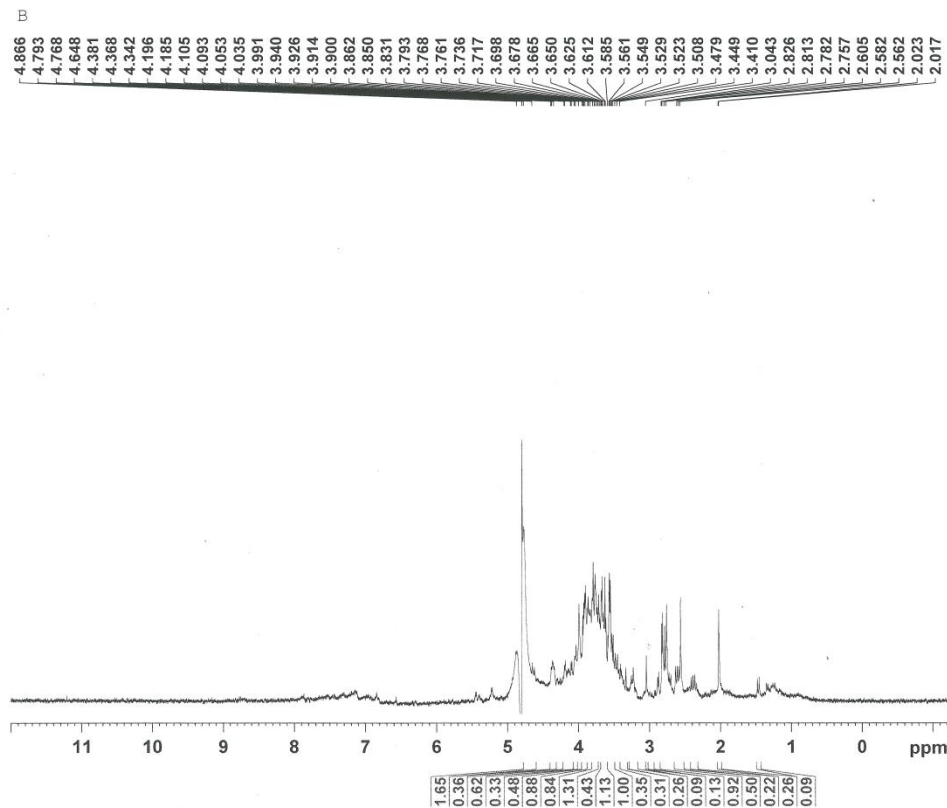


# Mass spectrometry

Sample Name	SUKIRTI(7573)	Position	Vial 42	Instrument Name	Instrument 1	User Name	
Inj Vol	10	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	B.d1.d	ACQ Method	ISOCRATIC GENERAL NE	Comment		Acquired Time	5/20/2013 10:37:46 AM



# NMR



Current Data Parameters  
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 EXPNO 3  
 PROCNO 1

F2 - Acquisition Parameters  
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 Time\_ 9.51  
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 PROBHD 5 mm Multinucl  
 PULPROG zgpr  
 TD 65536  
 SOLVENT D2O  
 NS 64  
 DS 2  
 SWH 6009.615 Hz  
 FIDRES 0.091699 Hz  
 AQ 5.4525952 sec  
 RG 211.41  
 DW 83.200 usec  
 DE 6.50 usec  
 TE 298 K  
 D1 1.00000000 sec  
 D12 0.00002000 sec  
 TDO 1

===== CHANNEL f1 =====  
 SFO1 300.1314091 MHz  
 NUC1 1H  
 P1 6.80 usec  
 PLW1 12.00000000 W  
 PLW9 0.00002219 W

F2 - Processing parameters  
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 WDW EM  
 SSB 0  
 LB 0.30 Hz  
 GB 0  
 PC 1.00

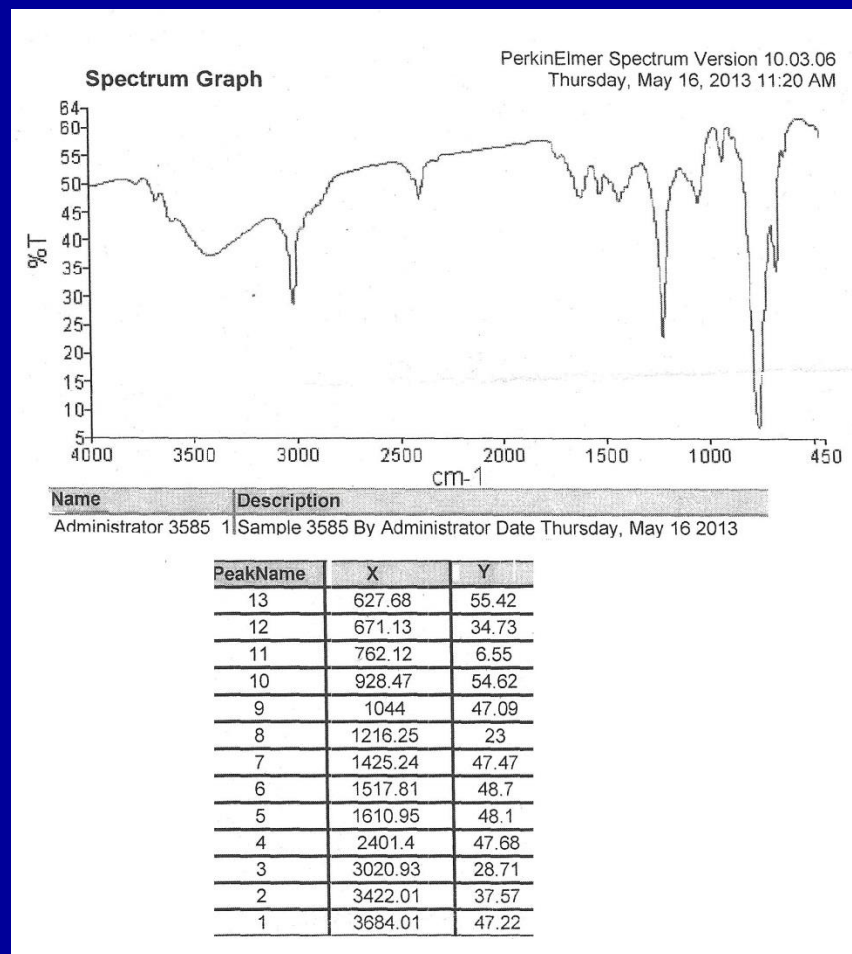




- Although there are not many signals are obtained by C 13 NMR but the drawn structure supports the computational structure. The signals obtained from proton NMR and Mass spectrometry and also mass fragmentation supports the proposed structure. The molecular formula corresponds to  $C_{17}H_{34}N_8O_9$ ,  $m/e=494.24$ . The basis for structure is proton NMR while the arrangement of this structure may vary in further research as the compound B is a new alkaloid which is different from previously reported macrocyclic alkaloids of *Z. mauritiana*. The probable structure of compound B is on the basis of proton NMR and Mass spectrometry. (Zhou and Tan, 1986)

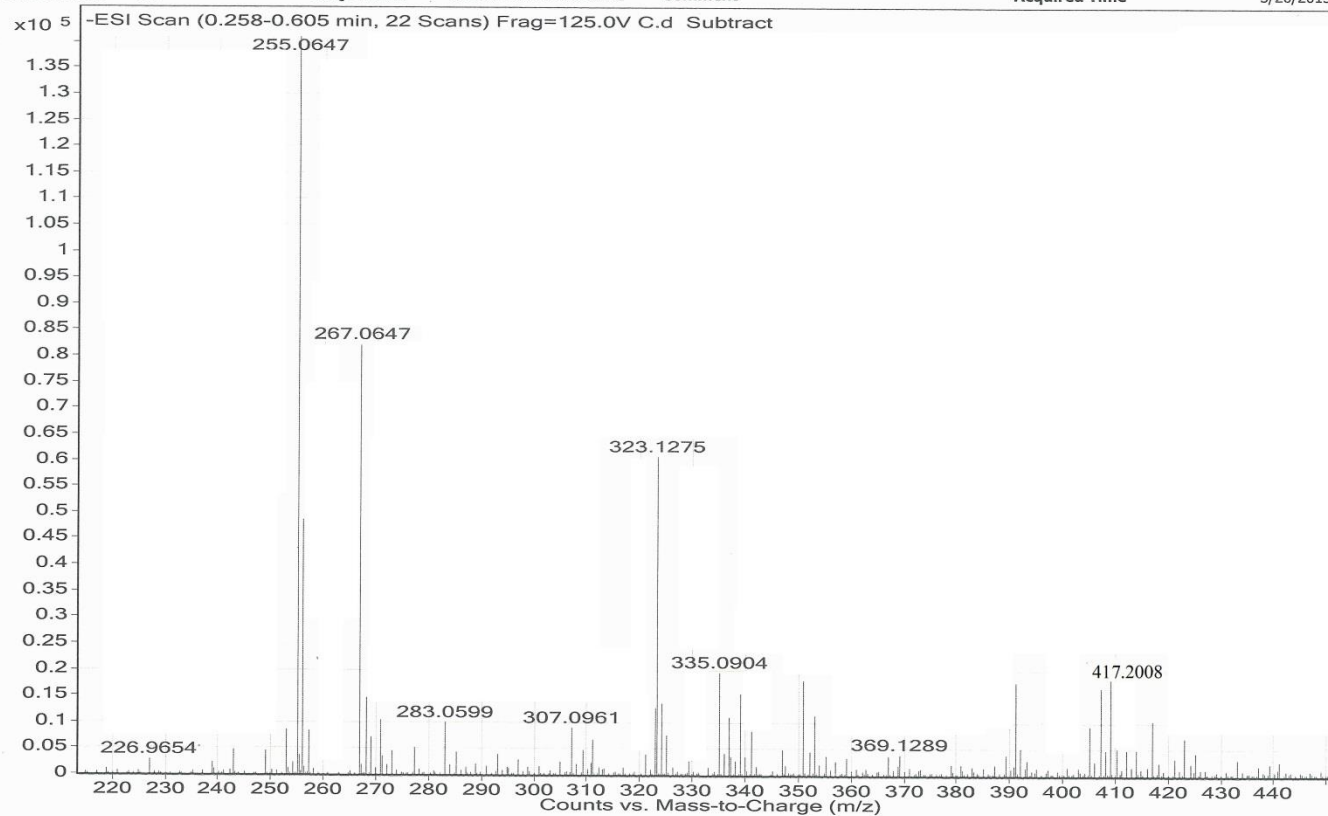
- **COMPOUND C**
- Isolated from *Glycyrrhiza glabra*
- Solvent of Elution Chloroform:Methanol in ratio 95:5
- Rf 0.83 in solvent system nButanol:Ethanol:water 10:10:4.1
- Nature Flavanoid confirmed by Shinoda test, Flavonoid glycoside
- Yield 35mg
- **Description**
- Yellow,crystalline; mp 210 °C;  
3684,3422,3020,2401,1610,1517,1425,1216,1044,928,762,671,627.
- <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ2.0,2.6 3.2-3.5,3.6-3.9,4.0,4.1,4.6,4.7,4.8,4.9,5.2 ESIMS ([M-H]417.

# IR

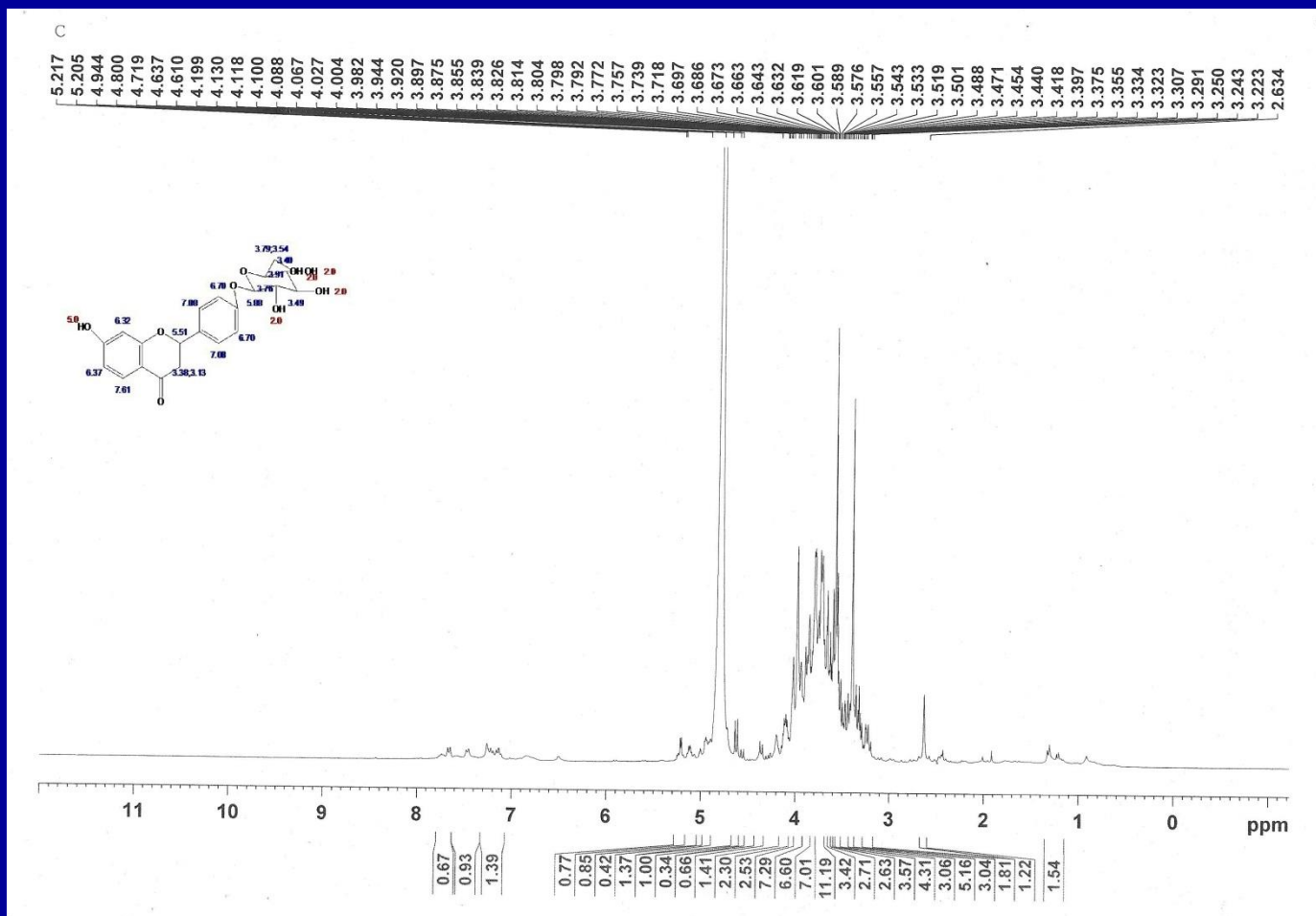


# Mass spectrometry

Sample Name	SUKIRTI(7573)	Position	Vial 43	Instrument Name	Instrument 1	User Name	
Inj Vol	5	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	C.d	ACQ Method	ISOCRATIC GENERAL NE	Comment		Acquired Time	5/20/2013 10:04:21 AM



# NMR



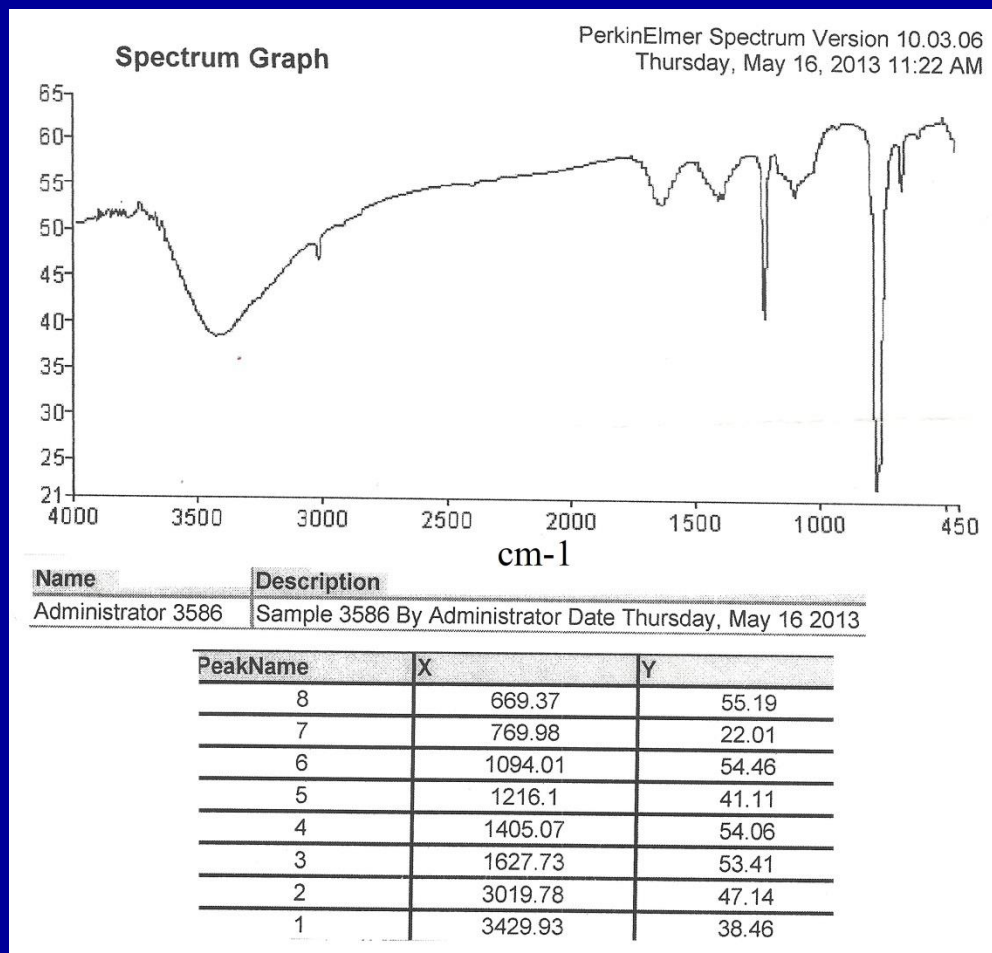


- Although not many signals are obtained by C13 NMR but the available signals supports the structure obtained by chemdraw. The proton NMR chemical shifts and mass spectrometry supports the structure of liquiritigenin and mass fragment of 255 obtained from negative ESI mass also supports this because that is exactly same as that of liquiritigenin( The flavonoid of liquiritin).(Lee 2009)

- **COMPOUND D**
- 
- Isolated from *Glycyrrhiza glabra*
- Colour Dark brown flakes
- Nature Crystalline
- MP 110°C
- Solvent of Elution Chloroform;Methanol in ratio 95:5
- Rf 0.95 in solvent system nButanol:Ethanol:water 10:10:4.1
- Nature saponin confirmed by foam test
- Yield 124mg
- **Description**
- Dark brown flakes; mp °C; IR  $\nu_{\text{max}}$  (KBr)  $\text{cm}^{-1}$ : 3429,3019,1627,1405,1216,1094,769,669.
- $^1\text{H}$ NMR(300)MHz, D2O)0.7,1.0,1.3,1.4,1.5,1.7,2.0,3.0,3.1,3.2,3.3,3.4,3.5,3.6,3.7,3.8,3.9,4.0,4.6,4.86.7, 6.8,7.1;ESIMS  $m/z$  (%): 469 [M-H].



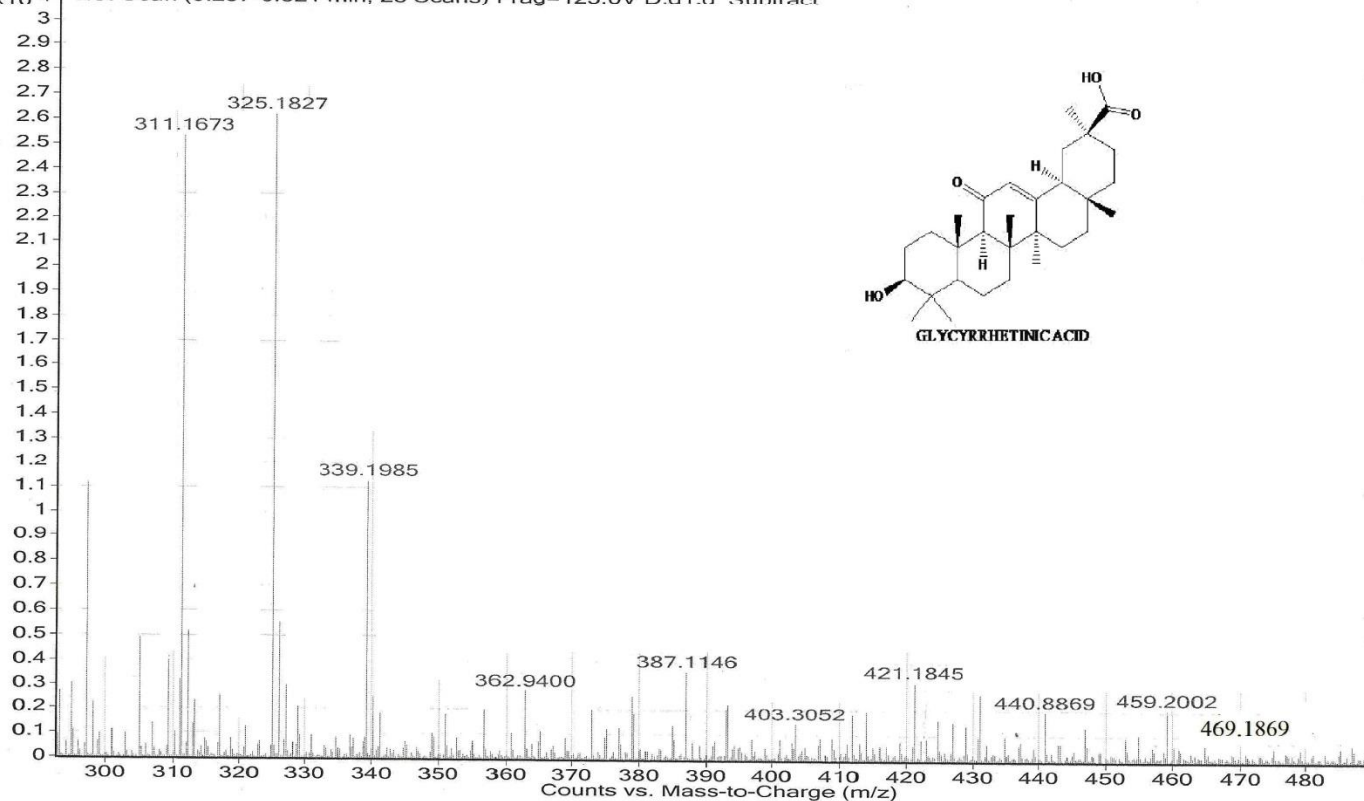
# IR



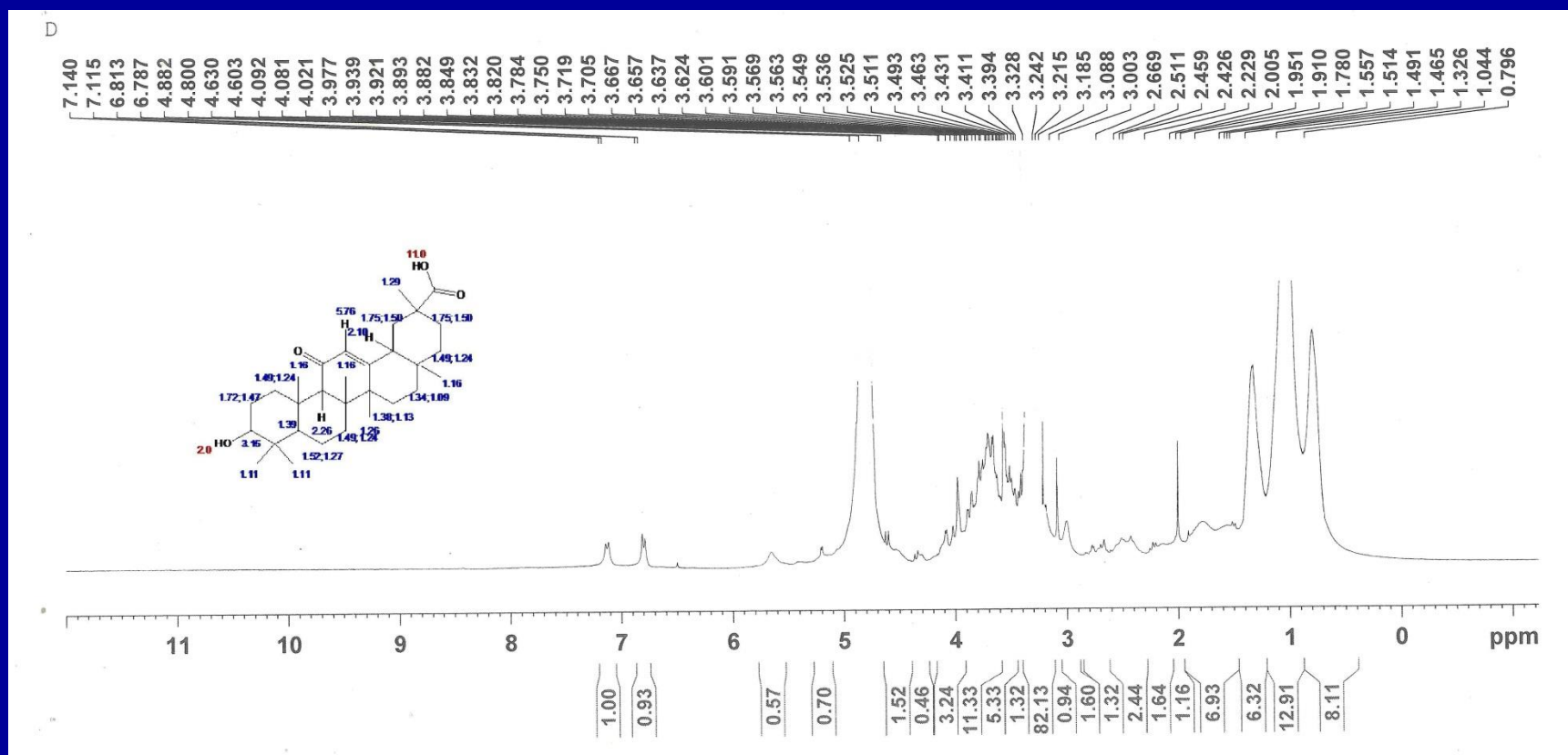
# Mass spectrometry

Sample Name	SUKIRTI(7573)	Position	Vial 44	Instrument Name	Instrument 1	User Name	Success
Inj Vol	10	InjPosition		SampleType	Sample	IRM Calibration Status	5/20/2013 10:44:28 AM
Data Filename	D.d1.d	ACQ Method	ISOCRATIC GENERAL NE	Comment		Acquired Time	

x10<sup>4</sup> -ESI Scan (0.257-0.621 min, 23 Scans) Frag=125.0V D.d1.d Subtract



# NMR

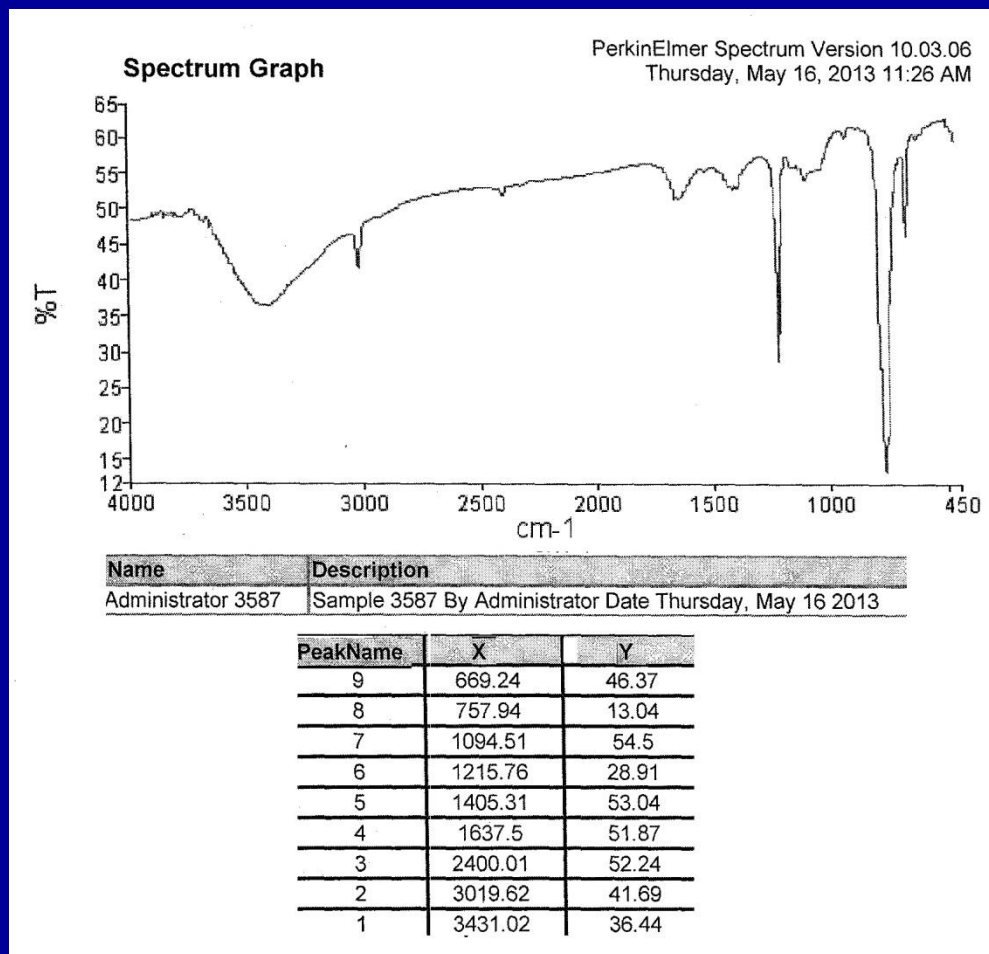


- The proton NMR, MASS and NMR data of compound D are similar to triterpenoidal saponin Glycyrrhetic acid. **(Li et al 2012,)**

# Compound E

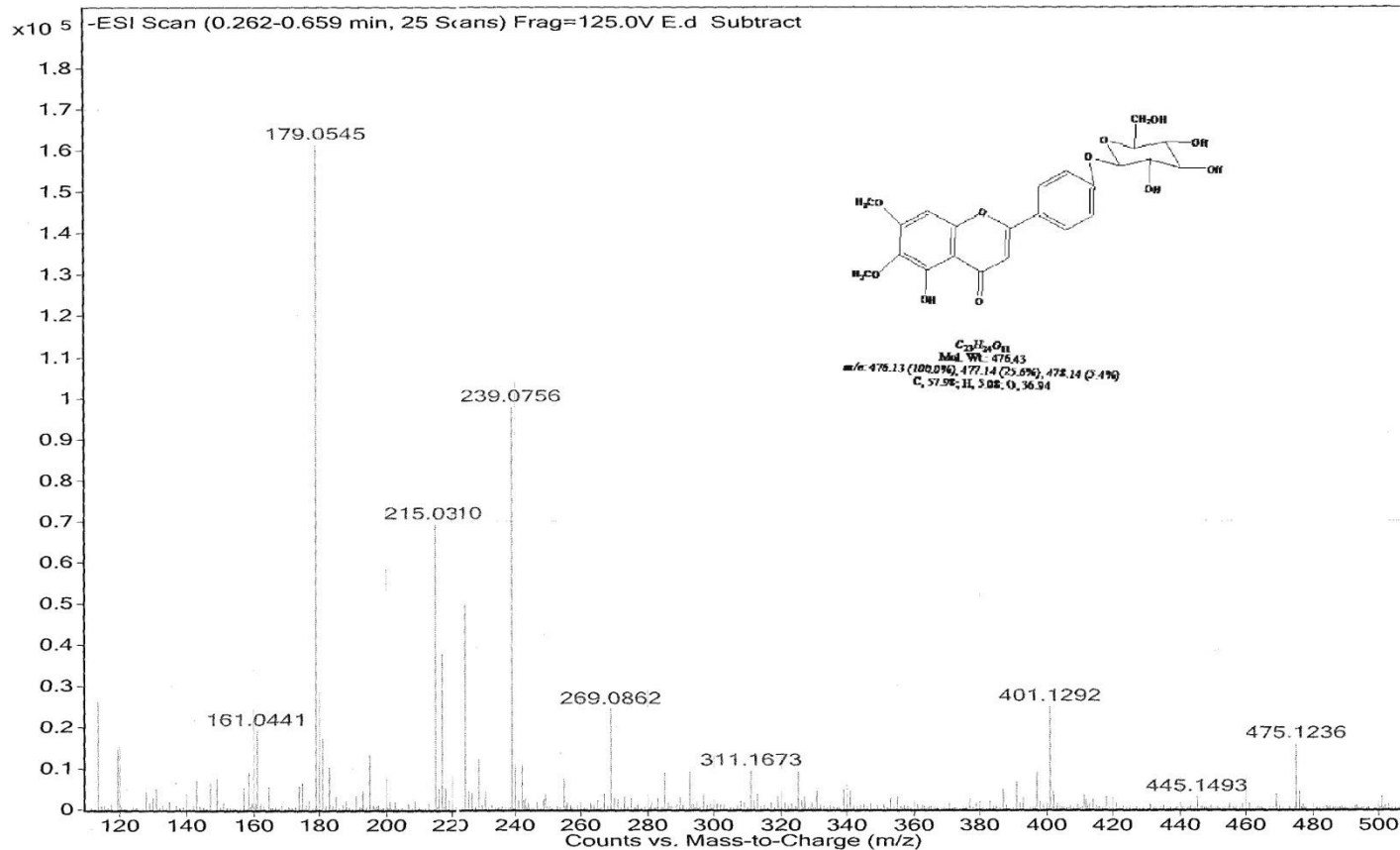
- Isolated from *Abrus precatorius*
- Colour Yellowish orange
- Nature Crystalline
- MP 158-60 °C
- Solvent of Elution Chloroform:Methanol in ratio 97:03
- R<sub>f</sub> 0.9 in solvent system nButanol:Ethanol:water 10:10:4.1
- Nature Flavanoid glycoside, confirmed by Shinoda test, Molish test
- Yield 94mg.
- **Description**
- White prisms; mp 158-160 °C; UV λ MeOH
- max nm (log ε): 325 (4.45), 277 (4.44); IR ν<sub>max</sub> (KBr) cm<sup>-1</sup>: 3200-3400
- (br), 1661, 1603, 1566, 1519, and 1461; <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O):  
0.8, 1.2, 1.3, 1.8, 2.4, 2.7, 2.8, 3.1, 3.2, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.7, 4.8, 5.3, 5.4, 5.5,  
6.3, 6.5, 6.8, 6.9, 7.1, 7.4, 7.5, 7.6, 7.7 ESIMS *m/z*: 475 ([M-H]<sup>-</sup>)

# IR

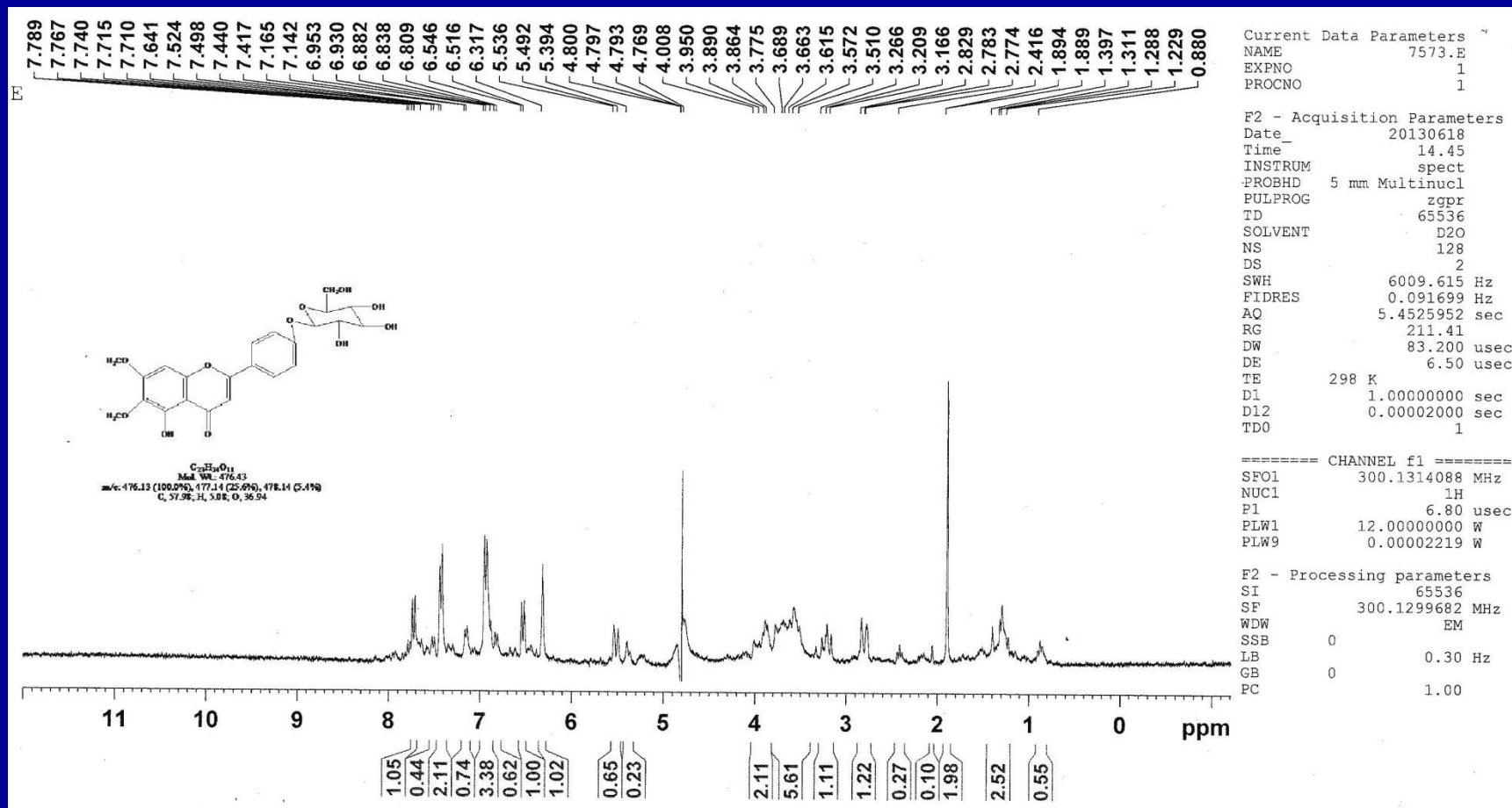


# Mass spectrometry

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<b>Inj Vol</b>	5	<b>InjPosition</b>		<b>SampleType</b>	Sample	<b>IRM Calibration Status</b>	Success
<b>Data Filename</b>	E.d	<b>ACQ Method</b>	ISOCRATIC GENERAL NE	<b>Comment</b>		<b>Acquired Time</b>	5/20/2013 10:17:41 AM



# NMR







- **Discussion (Ma 1998)**
- Although not many signals are obtained by C13 NMR but the available signals supports the structure obtained by chemdraw. The proposed structure of compound E shows exact similarity with compound Cirsimarin. The basis of the structure recognition is proton NMR and mass fragmentation analysis

# Hair growth promotant activity of petroleum ether and ethanolic extracts in female wistar albino Rats

- **Discussion**

- The petroleum ether extract of *G. glabra* and *A. precatorius* showed excellent hair growth promoting activity as compared to control and minoxidil treated animals

The ethanolic extract of *A. precatorius* also showed excellent hair growth promoting activity

- **5  $\alpha$  reductase activity**
- The 5  $\alpha$  reductase activity is relevant with inhibition of conversion of testosterone in 5 DHT and the 5DHT is responsible for causing androgenic alopecia so this activity of the extracts was evaluated by inhibitory concentration

- The IC<sub>50</sub> values calculated for petroleum ether extract of *A.precatorius* and *G. glabra* and ethanolic extract of *A. precatorius*, and finasteride were 1.78 mg, 1.88mg, 1.70 mg and 0.72 µg showed enzyme inhibitory activity if these compounds.
- While the Ethanolic extract of *G.glabra*, Petroleum ether extract of *Z mauritiana* and ethanolic extract of *Z mauritiana* do not shows 5 alpha reductase inhibitory activity.

## Effect of petroleum ether and ethanol fractions of extracts on androgenic alopecia

- In the negative control group baldness start to visualize from Day 15 on dorsal side of skin, comparatively the group of animals on (T+finasteride) do not show alopecia, and the Pet ether extract of *Glycyrrhiza* root, Abrus seed and ethanolic extract of Abrus seed and treated group showed best results as they do not develop the sign of alopecia and hair growth remain almost similar as positive control while pet ether extract of *Ziziphus* and ethanolic extract of *Ziziphus* and *Glycyrrhiza glabra* treated animals become bald

# Antilice activity

- The use of *A.precatorius* extract for controlling lice infestations has been authenticated from the excellent results obtained after screening for potential anti-lice and ovicidal activity of the extract.

# Antifungal activity

- The antifungal activity of the various extracts was evaluated in fungus *C.albicans* and *A.niger*. The antifungal effect was evaluated by zone of inhibition method
- The antifungal activity of petroleum ether and ethanolic extract of *A.precatorius* and *G.glabra* was excellent as the results were well compared with standard drug Itraconazole



## Effect of ethanolic extract of *Ziziphus mauritiana* on fertility and sexual behavior of male wistar albino rats

- **Discussion**
- There was a significant increase ( $p < 0.01$ ) in serum levels of testosterone of the male rats treated with extract as well as alkaloidal fraction of *Z. mauritiana* roots. When compared with the control group. Elevation in serum testosterone level might be accounted for enhanced secretion of androgen resulted from adequate sperm production in both testicles and epididymis. It might be a probable cause for testicular and epididymal function as a result of androgen availability. Hence, sperm production in both organs were affected in treated male rats. This study showed that both ethanol extract and alkaloidal fraction of *Z. mauritiana* root induced dose dependent increase in functional fertility and enhanced sexual activity in male wistar albino rats. The potential lies in alkaloidal extract and ten alkaloids are present in the fraction. Out of these two alkaloids are present in major quantity and one alkaloid is isolated and its probable structure is elucidated.

# future scope

- The of the research is that it provides hair growth enhancing agent for both male and female separately as it is an hormonal phenomenon also. The estrogen is responsible for prolongation of anagen phase or long hair of females while testosterone is responsible for causing baldness in males. As the research deals with androgenic alopecia and it also evaluate hair growth promotion in females and the results shows that petroleum ether extract of *A. precatorius* and *G. glabra* shows very good results, although the mechanism involve is 5 alpha reductase inhibition and it is compared with antiandrogenic drug finasteride. So in future these extracts can also evaluate for androgen related disorders such as prostate enlargement, prostate cancer, polycystic ovary syndrome, acne etc.
- And the mechanism for hair growth promotion in female rats can be explored in future research.
- *Ziziphus mauritiana* extract as well as alkaloidal fraction shows effective male fertility enhancing and has positive effect in male sexual behavior also and it is also devoid of 5 alpha reductase inhibition so its androgenic effect is further investigated for development of effective drug for male fertility related problems.
- The excellent antilice activity of *A. precatorius* is boon to resistance developing lice.
- The potent antifungal activity of *G. glabra* and *A. precatorius* is of use in development of herbal antifungal agent.
- As from extract of *G. glabra* one new flavonoid is isolated. So there is scope of antioxidants in antiageing etc. So this flavanoid will be further studied for its properties. Aqueous methnolic extract of *A. precatorius* shows presence of cirsimarin so it become a new source for this compound.
- One new alkaloid is isolated from *Ziziphus mauritiana*. As alkaloidal fraction of this herb shows positive impact on male fertility and sexual behaviour so this alkaloid must be evaluated in future for enhancing male fertility and sexual behaviour.

# List of Publication

- **Total Publications: 08**
- **Upadhyay Sukirti, Ghosh A K, Singh Vijender (2011)** Antilice activity of *Abrus precatorius*. *Egyptian Dermatology Online Journal*, Vol 7, Issue 2:4, pp.1-6.
- **Upadhyay Sukirti, Upadhyay Prashant, Ghosh A K, Singh Vijender (2012)** *Ziziphus mauritiana*: a review on pharmacological potential of this underutilized plant. *International journal of current research and review*, Vol 4, Issue 3, pp. 141-44.
- **Upadhyay sukirti, Ghosh A K, Singh Vijender and Dixit V K (2012)** Effect of petroleum ether and ethanol fractions of *Abrus precatorius* on androgenic alopecia. *Revista Brasileira de Farmacognosia, Brazilian Journal of Pharmacognosy*, Vol 22, Issue 2, pp. 359-363. [Impact factor 0.673]
- **Sukirti Upadhyay, Ashoke K Ghosh and Vijender Singh (2012)** Hair Growth promotant Activity of Petroleum Ether Root Extract of *Glycyrrhiza Glabra* L. (Fabaceae) in Female Rats. *Tropical Journal of Pharmaceutical Research*, Vol 11, Issue 5, pp. 753-758. [Impact factor 0.51]
- **Upadhyay Sukirti, Upadhyay Prashant, Ghosh AK, Singh Vijender (2013)** Effect of ethanol extract and alkaloidal fraction of *Ziziphus mauritiana* (Fam-Rhamnaceae) roots on fertility and sexual behavior of male wistar albino rats. *Pharmacologyonline*, Vol 2, pp. 12-18. [Impact factor 0.2]
- **Upadhyay Sukirti, Singh Vijender (2013)** Potentiality of petroleum ether (60-80) °C extract of *Glycyrrhiza glabra* on androgenic alopecia. *Asian J Pharm Clin Res*, Vol 6, Suppl 3, pp. 52-55.[Impact factor 0.5]
- **Upadhyay Sukirti (2013)** Efficacy of *Abrus Precatorius* (Gunja) seeds oil as a Hair Growth Promoter (Keshya Rasayana) in Female Wistar Albino Rats. *Annals of Ayurvedic Medicine* ,Vol 2, Issue 4, pp.156-159.
- **Upadhyay Sukirti, Ghosh AK, Singh Vijender (2014)** Inefficiency of ethanolic extract of *G.glabra* and *Z. mauritiana* on androgenic alopecia. Accepted to published on *Journal of negative Pharmaceutical Results*. [Impact factor 0.16]

# Acknowledgements

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- Prof. A K Ghosh
- Prof. R M Dubey
- Prof. R Somvanshi, IVRI
- CIF, NBRI
- SAIF, CDRI
- RDC members

Thanks.....