Chemical fingerprinting of a poisonous plant, *Cestrum diurnum using Liquid Chromatography-Electrospray-Tandem Mass Spectrometry (LC-ESI-MS/MS)*

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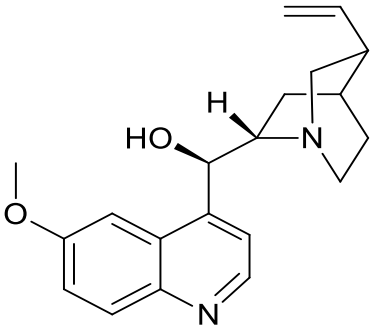
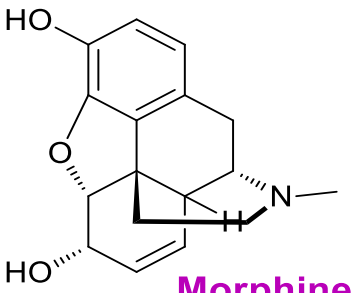
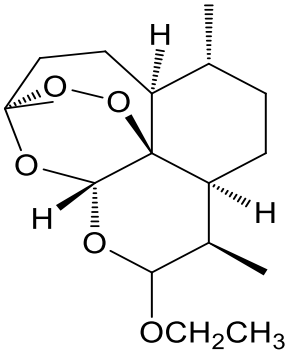
Historically Important Natural Products from Plants



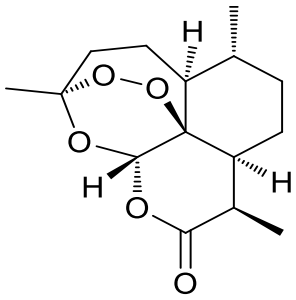
Quinine
Anti-malarial
Cinchona succirubra Pav.
Isolation: 1820, Pelletier *et* al.
Synthesis: 1944, Woodward



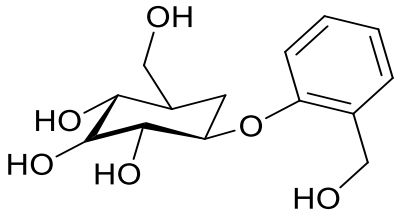
Morphine
Painkiller
Papaver somniferum L.
Isolation: 1806, Sertürner
Synthesis: 1954, Ginsberg



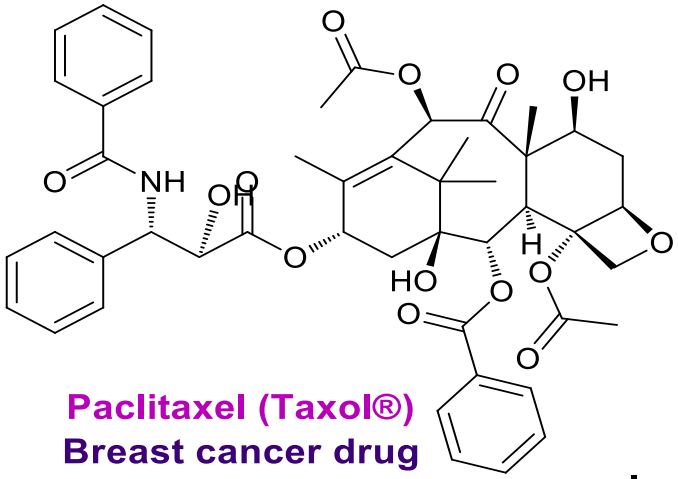
Arteether



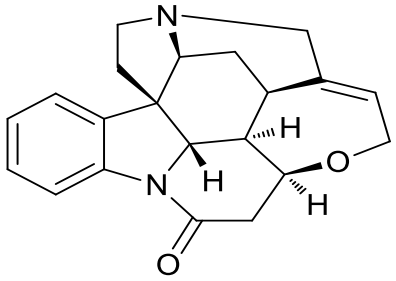
Artemisinin
Antimalarial drugs
Artemisia annua



Salcin
Salix alba L.
Structure and synthesis: 1906, Irvine

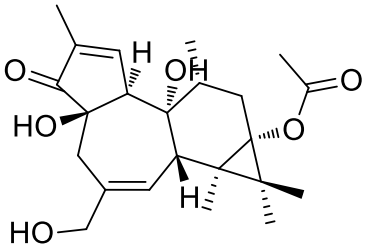


Paclitaxel (Taxol®)
Breast cancer drug
Taxus brevifolia
Isolation: 1971, Wani et al.



Strychnine
Pesticide
Strychnos nux-vomica
Isolation: 1818, Pelletier *et* al.
Synthesis: 1954, Woodward

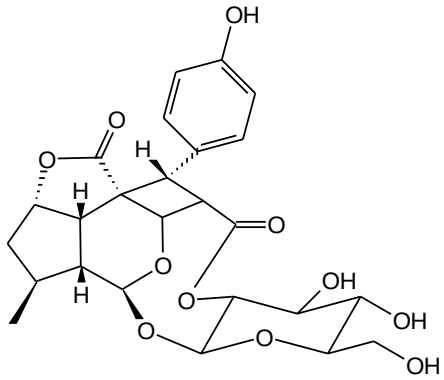
Historically Important Natural Products from Plants



Prostratin

Used for the treatment of (AIDS)

Homalanthus nutans

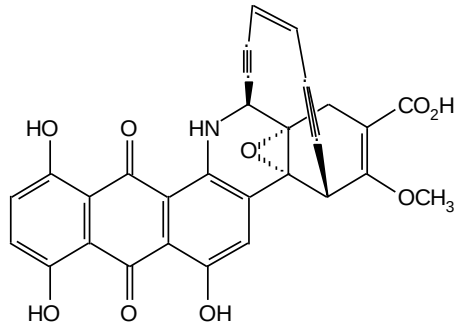


(-)-Littoralisone

Neurotrophic Growth Factor

Verbena littoralis L

Isolation and structure: 2001, Li
Synthesis: 2005, Mangion

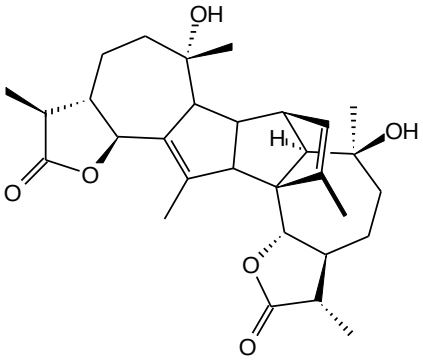


Dynemicin A

Antibiotic

Micromonospora chersina

Structure: 1989, Matsumoto *et* al
Synthesis: 1991, Nicolau

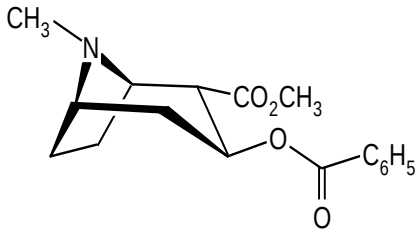


(+)-Absinthin

Anti-inflammatory Agent

Artemisia absinthium L

Isolation: 1953, Herout
Synthesis: 2004, Zhang

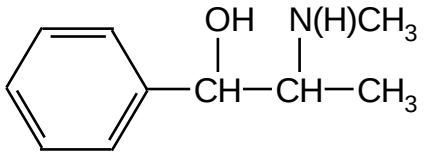


Cocaine

Appetite Supressant

Erythroxylon coca

Isolation: 1859, Niemann
Synthesis: 1923: Willstätter



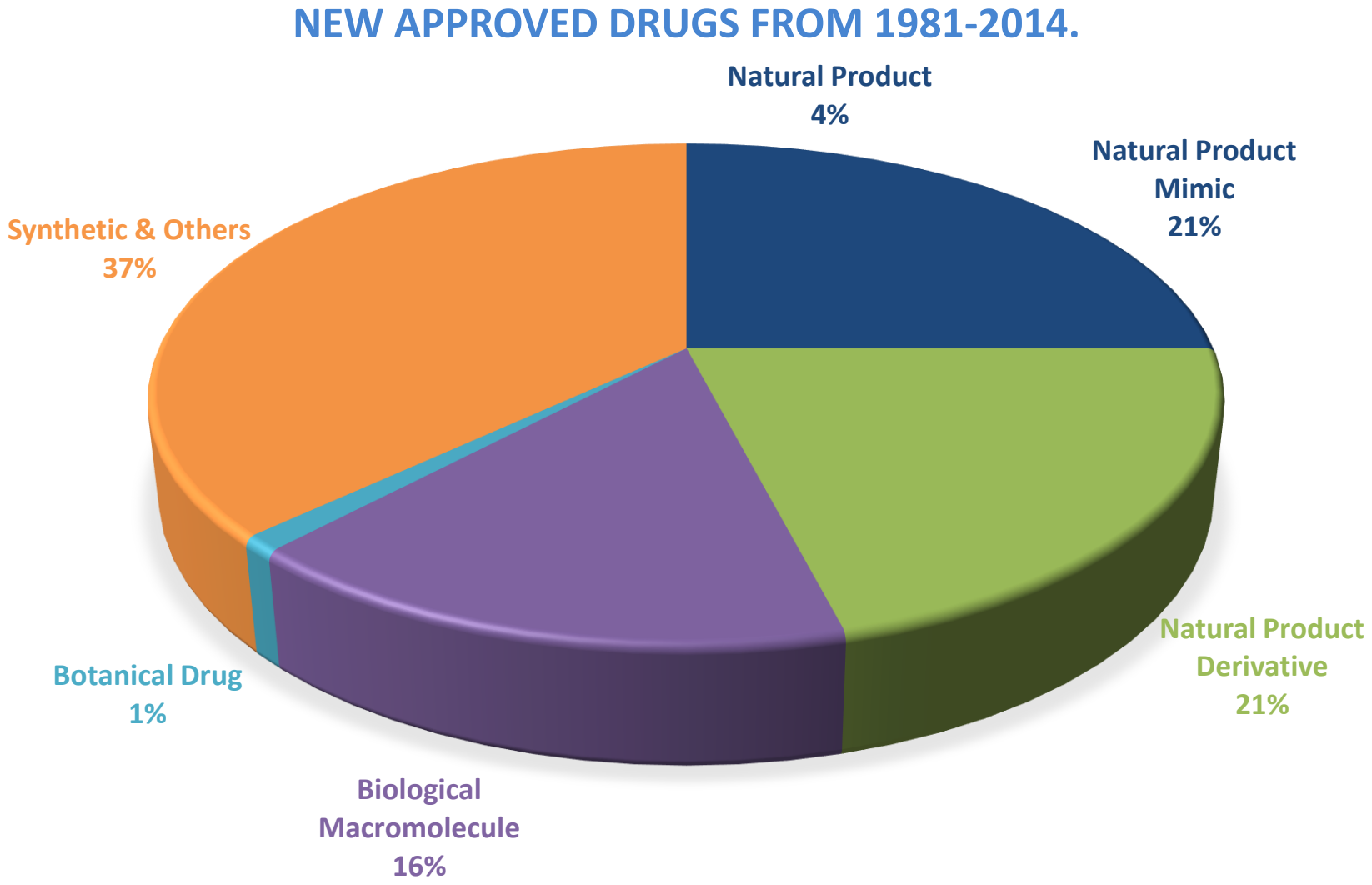
Ephedrin

Decongestant

Ephedra equisetrina

Structure and synthesis:
1920, Späth and Göring

Plants: A Well Accepted Remedy since Human Civilization



Newman, D. J., & Cragg, G. M. (2016). Natural Products as Sources of New Drugs from 1981 to 2014. *J Nat Prod, 79*(3), 629-661.

Some Important Toxic Plants

Caster seeds
Toxin: Ricin
Side effect: Deadly in small amount (Organ failure within 4 days)

Rosary Pea
Toxin: Abrin
Side effect: 3 microgram can kill an adult (Organ failure within 4 days)

Tobacco
Toxin: Nicotine, Anabasine
Side effect: Psychoactive and addictive. (more than 5 million deaths per year)

White Snakeroot
Toxin: Termatol
Side effect: Abnormal acidity of the blood, and death (poisoned by simply drinking the milk of a cow who had grazed on the plant)

Atropa Belladona
Toxin: Atropine, hyocyamine
Side effect: Adverse health issues (possible confusion with other berries)

Water Hemlock
Toxin: Cicutoxin
Side effect: Respiratory paralysis (confused with edible parsnips or celery)

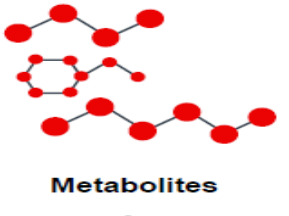
Musharraf et al, Am. Soc. Mass Spectrom.,
https://www.britannica.com/list/7-of-the-worlds-deadliest-plants

How to differentiate the medicinal plants from the toxic plants?

Two-stage Mass Spectrometry Chemical fingerprinting Approach for the Analysis of Plant Extract using LC-MS

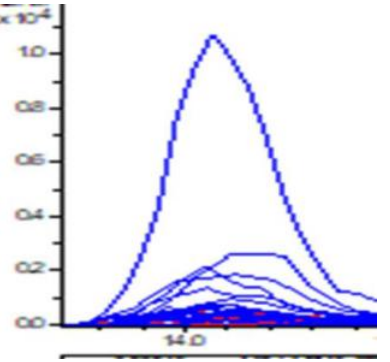
Stage I

UPLC-HR-ESI-MS/MS
(QToF)



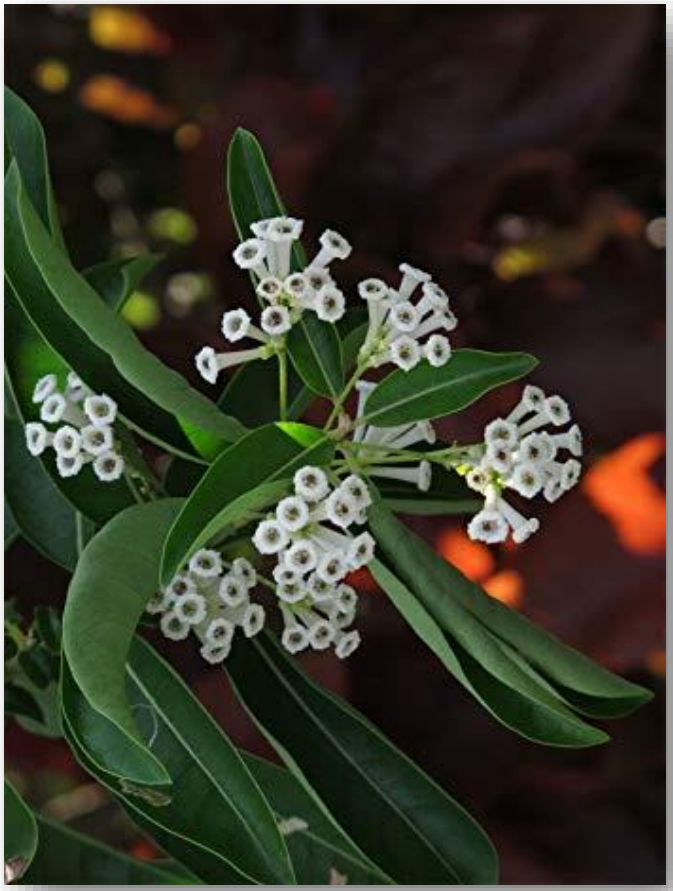
Stage II

UPLC-ESI-MS/MS
(QQQ, iontrap)



Musharraf *et al.*, RSC advances (2018) 8, 41023-41031

Cestrum diurnum
(king-of-the-day)



- Commonly known as Din-Ka-Raja.
- Tropical to subtropical plant.
- Cultivated widely as an ornamental in warmer parts.
- All parts of the plant are toxic.
- Clinical signs of toxicity are weight loss, stiffness and progressive lameness.
- Dystrophic calcinosis of the elastic tissues of the heart, arteries, tendons and ligaments.

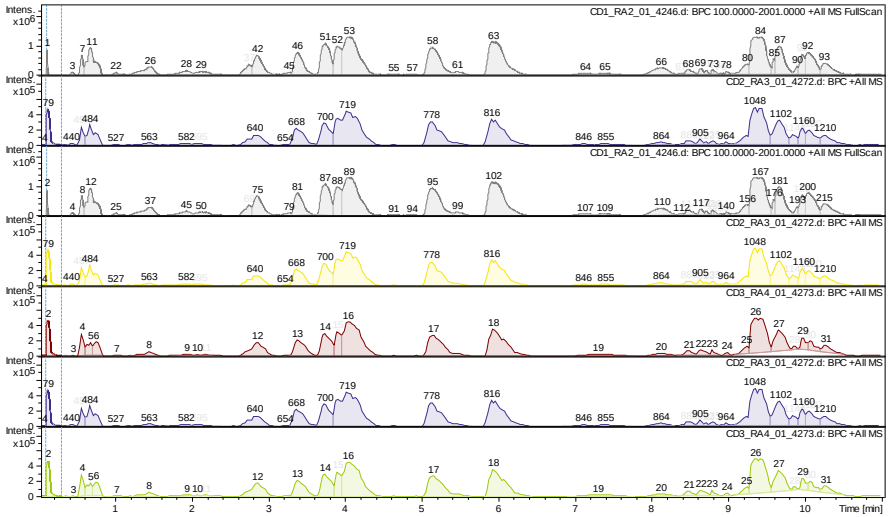
https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/cestrum-diurnum

Musharraf et al, Am. Soc. Mass Spectrom., (Accepted)

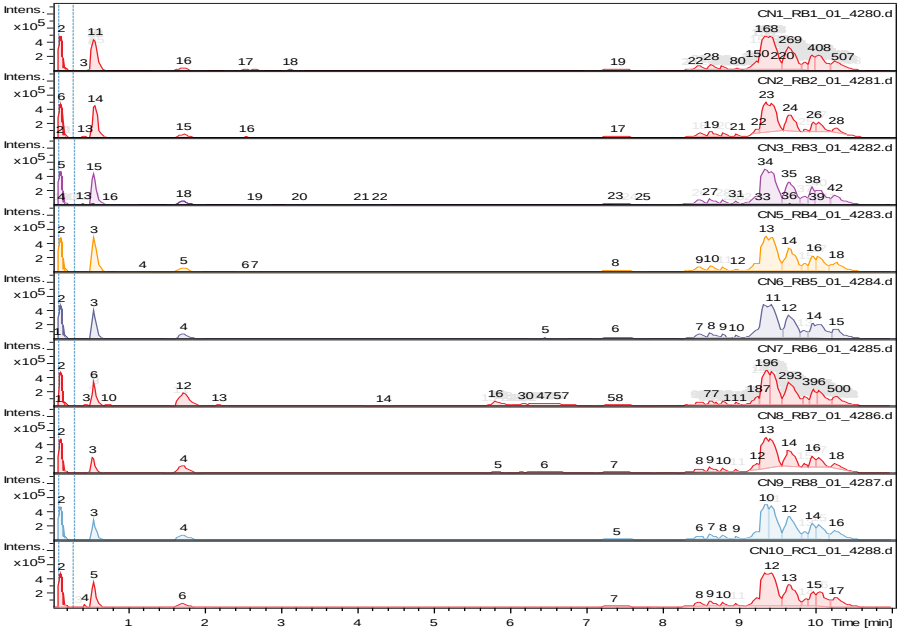
Species of *Cestrum*

Botanic name	*Cestrum diurnum*	*Cestrum nocturnum*	*Cestrum parqui*
Common name	Day blooming jasmine Din ka Raja	Night-blooming jasmine Raatki rani	Green cestrum Mortal nightshade
Plant family	Solanaceae.	Solanaceae.	Solanaceae
Visible identity			
Distribution	Subtropics and tropical	Subtropical regions	Subtropics and tropical
Medicinal uses	Thrombolytic Cytotoxic	Antioxidant Antibacterial analgesic Hepatoprotective Antifungal Anti-HIV	Anti-spasmodic Anti-epilepsy
Medicinal rating	+	+ + + +	+ +
Toxic parts	Leaves	Fruits	All parts
Toxic Compounds	Vitamin D3, 25 hydroxyvitamin D3, 1,25-dihydroxy-vitaminD-glycoside	Atropine like anticholinergic	Glycoalkaloids Atropine like alkaloids

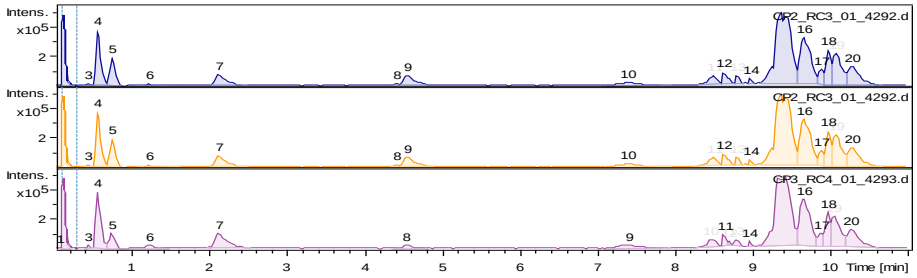
Comparative LC-MS/MS Fingerprint Chromatograms of 20 Accessions of *Cestrum* Species.



Cestrum diurnum
7 Samples



Cestrum nocturnum
10 samples



Cestrum parqui
3 samples

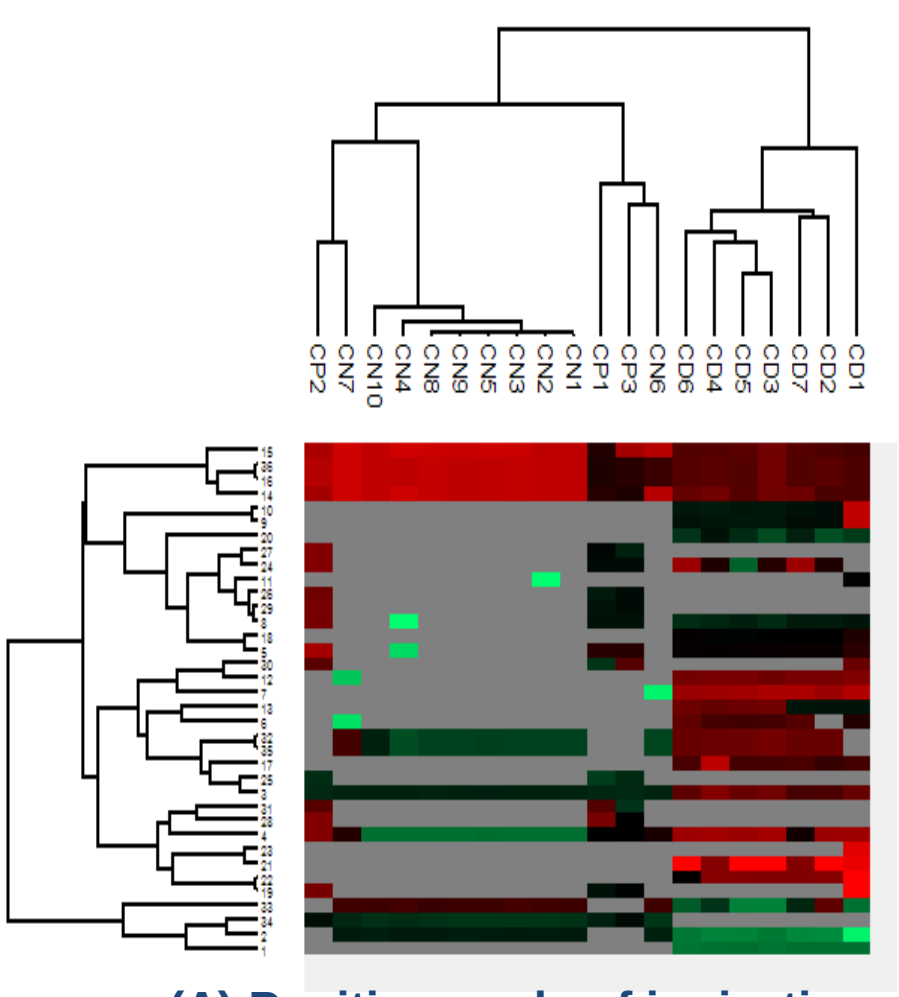
Metabolites Identified in *Cestrum* Species Positive mode of Ionization

Codes	Compound names	Codes	Compound names	Codes	Compound names
1	Pentalenolactone	14	Maltotriose	28	Tributyl phosphate
2	N-Acetyl-D-galactosaminitol	15	Sucrose	29	L-Tryptophan
3	Glycan 3*α*-Galactobiose	16	2*α*-Mannobiose	30	Tryptophan
4	Lamiide	17	D-Turanose	31	Terbutaline
5	(2S,3S,4S,5R,6S)-3,4,5-Trihydroxy-6-[5-hydroxy-2-(4-hydroxyphenyl)-6-methoxy-4-oxochromen-7-yl]oxyoxane-2-carboxylic acid	18	Methyl (1S,4*α*S,6*R*,7R,7*α*R)-1-(*β*-D-glucopyranosyloxy)-5,6-dihydroxy-7-methyl-1,4*α*,5,6,7,7α-hexahydrocyclopenta[c]pyran-4-carboxylate	32	Magnoloside D
6	Genipin	19	Sinapinic acid	33	Dioctyl phthalate
7	5-hydroxy-2-[2-hydroxy-3-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]-7,8-dimethoxychromen-4-one	20	Nornicotine	34	24*S*-Hydroxycholesterol
8	Loliolid	21	*Acteoside*	35	Dihydrotachysterol
9	7-Hydroxy-4-(methoxymethyl)coumarin	22	isorhamnetin-3-O-glucoside	36	Bis(2-ethylhexyl) phthalate
10	Scoparone	23	Ladroside	37	*β*-D-Glucose
11	5-hydroxy-2-[4-hydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] oxyphenyl]-7-methoxychromen-4-one	24	Magnoloside D		
12	Mulberroside A	25	Trinexapac		
13	Coumarin-6-carboxaldehyde	26	1-O-*β*-D-Glucopyranosyl sinapate		

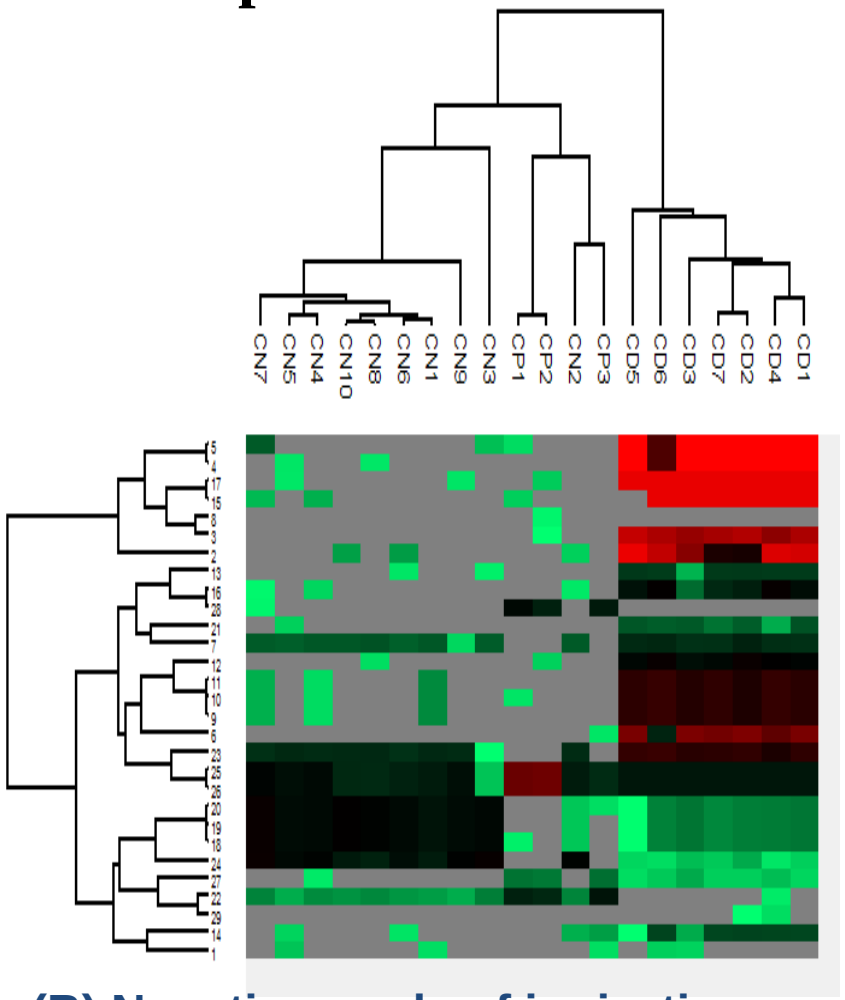
Metabolites Identified in *Cestrum* Species
Negative mode of Ionization

Codes	Compound names	Codes	Compound names	Codes	Compound names
1	Methyl (1*S*,4*αR*,6S,7R,7*αS*)-1-(*β*-D-glucopyranosyloxy)-4*α*,6,7-trihydroxy-7-methyl-1,4*α*,5,6,7,7*α*-hexahydrocyclopenta[c]pyran-4-carboxylate	10	5,7-dihydroxy-2-[4-hydroxy-3-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]-3-methoxychromen-4-one	19	Galactinol
				20	Vernolic Acid
2	Methyl (1S,4*α*S,6*R*,7*R*,7*αR*)-1-(*β*-D-glucopyranosyloxy)-5,6-dihydroxy-7-methyl-1,4*α*,5,6,7,7*α*-hexahydrocyclopenta[c]pyran-4-carboxylate	11	3-[(3*S*,5*S*,8*R*,10*S*,13*R*,14*S*,17*R*)-3-[4,5-dihydroxy-6-(hydroxymethyl)-3-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxan-2-yl]oxy-14-hydroxy-10,13-dimethyl1,2,3,4,5,6,7,8,9,11,12,15,16,17tetradecahydrocyclopenta[a]phenanthren-17-yl]-2H-furan-5-one	21	Sucrose
				22	Peonidin-3-O-glucoside
3	Chrysoeriol 6,8-di-C-glucoside	12	Cyclopenta[c]pyran-4-carboxylic acid, 1-(*β*-D-glucopyranosyloxy)-1,4*α*,5,6,7,7*a*-hexahydro-5-hydroxy-7-methyl-6-[[(2*E*)-1-oxo-3-phenyl-2-propen-1-yl]oxy]-, methyl ester, (1*S*,4*αS*,5*S*,6*R*,7*R*,7*αR*)-	23	2-(3,4-dihydroxyphenyl)ethyl (1*S*,4*αR*,7*αR*)-4a-hydroxy-7-methyl-5-oxo-1-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-1,6,7,7*α*-tetrahydrocyclopenta[c]pyran-4-carboxylate
4	[(2*R*,3*S*,4*R*,5*R*,6*R*)-6-[2-(3,4-dihydroxyphenyl)ethoxy]-4,5-dihydroxy-2-[[(2*R*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-3-yl] (*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoate	13	Cyclopenta[c]pyran-4-carboxylic acid, 6-(acetyloxy)-5-(benzoyloxy)-1-(hexopyranosyloxy)-1,4*α*,5,6,7,7*α*-hexahydro-7-methyl-, methyl ester	24	Chlorogenoquinone
5	(2*R*,3*R*,4*S*,5*R*,6*R*)-6-[2-(3,4-dihydroxyphenyl)ethoxy]-3,5-dihydroxy-4-[(2*R*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxyoxan-2-yl]methyl (*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoate	14	Cyclopenta[c]pyran-4-carboxylic acid, 1-(*β*-D-glucopyranosyloxy)-1,4*α*,5,6,7,7*α*-hexahydro-5-hydroxy-7-methyl-6-[[(2E)-1-oxo-3-phenyl-2-propen-1-yl]oxy]-(1*S*,4*αS*,5*S*,6*R*,7*R*,7*αR*)- methyl ester	25	(2E)-4-Hydroxy-3,4-dimethyl-2-penten-1-yl 6-O-[(2*S*,3*R*,4*R*)-3,4-dihydroxy-4-(hydroxymethyl)tetrahydro-2-furanyl]-*β*-D-glucopyranoside
6	3-{(*E*)-2-[4-(*β*-D-Glucopyranosyloxy)-2-hydroxyphenyl]vinyl}-5-hydroxyphenyl *β*-D-glucopyranoside	15	5,7-dihydroxy-2-(4-hydroxyphenyl)-3,6-dimethoxy-4H-chromen-4-one	26	9*S*-hydroxy-10*E*,12*Z*,15*Z*-octadecatrienoic acid
7	Isorhamnetin-3-O-glucoside	16	Tricin	27	Caffeoyl quinic acid
8	*β*-D-Glucopyranoside, 5-(4-hydroxyphenyl)-1-[2-(4-hydroxyphenyl)ethyl]pentyl 6-*O*-(6-deoxy-alpha-L-mannopyranosyl)-	17	5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-6-methoxy-7-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	28	Quercetin 3,7-dimethyl ether
9	Malvidin-3-O-*β*-D-galactoside	18	Benzoic acid	29	Oenin

Hierarchical Clustering of Identified Metabolites in *Cestrum* Species



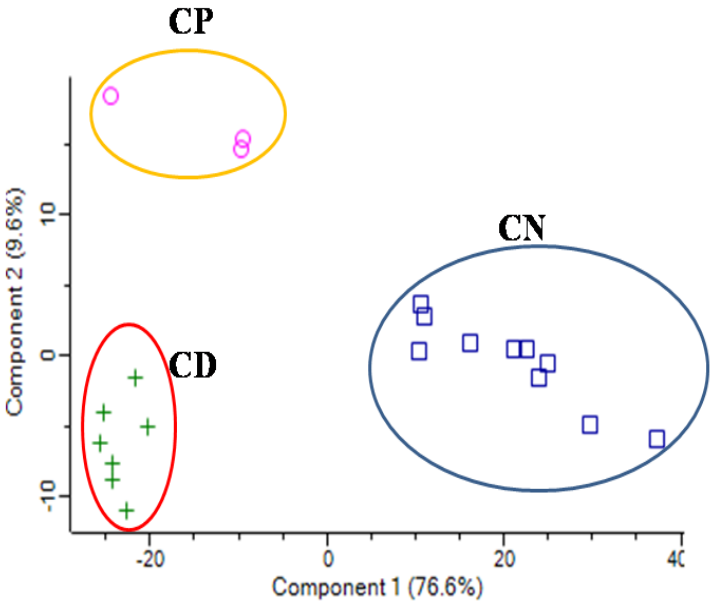
(A) Positive mode of ionization.



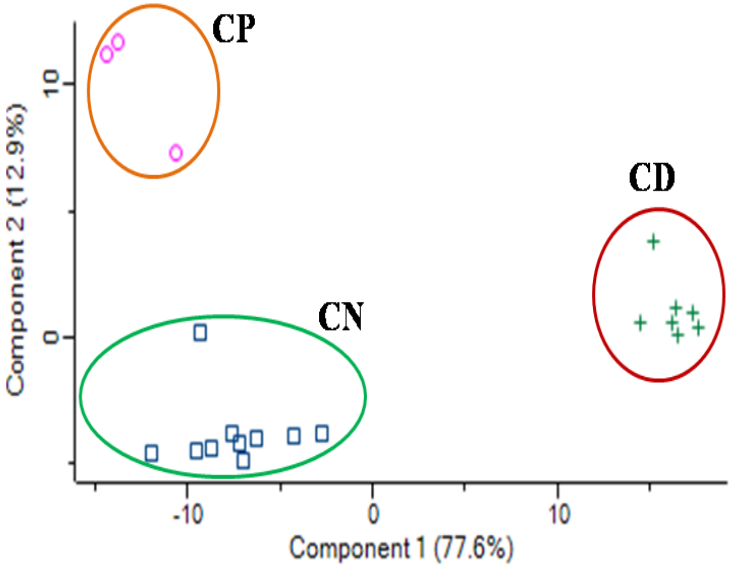
(B) Negative mode of ionization.

Musharraf et al, Am. Soc. Mass Spectrom., (Accepted)

PCA Scattered Plot



(A) Positive mode of ionization.



(B) Negative mode of ionization.

Adulteration Studies by Using LC-ESI–QTOF-MS/MS

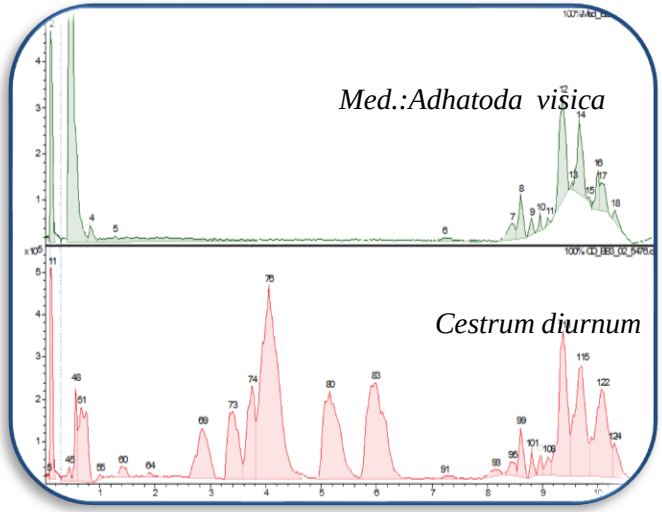
C. Diurnum (Poisonous plant)

Vasica .A (Medicinal plant)

physically resembling medicinal plants

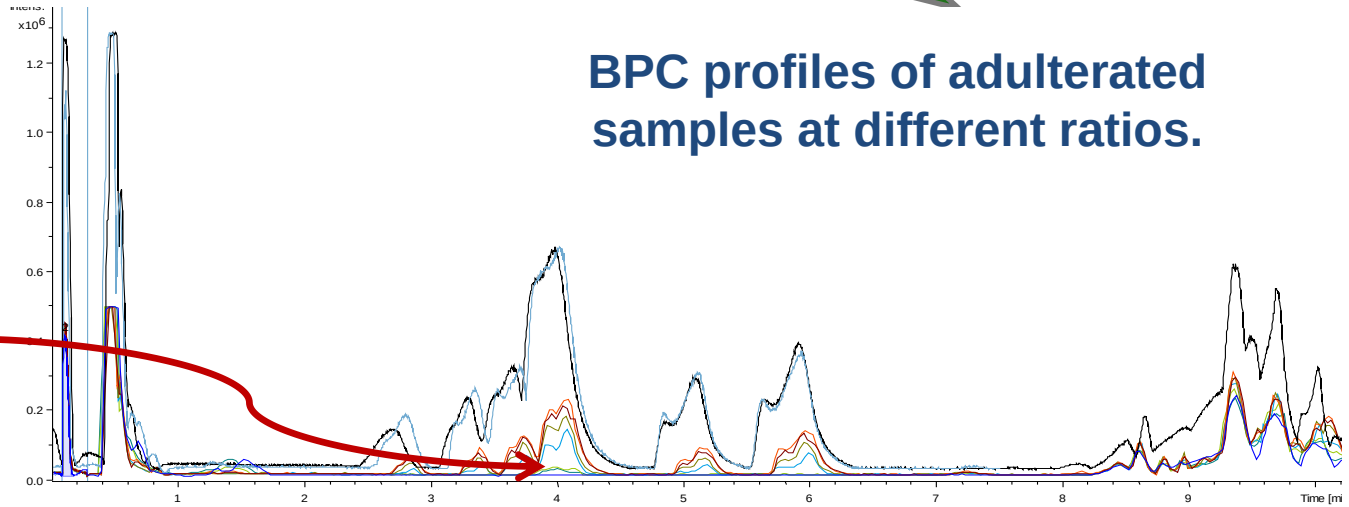
Mixed at Different Ratios

C. diurnum %	*A. Vasica %*
1	99
2	98
5	95
10	90
20	80
30	70
40	60
50	50
60	40



Comparing BPC profiles of *C. diurnum* and *A. vasica.*

BPC profiles of adulterated samples at different ratios.



We were able to see upto 5% adulteration.

Conclusion

A rapid method is developed for the authentic identification of a toxic plant based on LC-ESI-MS/MS approach.

Adulterated samples up to 5% can also be identified.

ACKNOWLEDGMENTS

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Some Relevant Publications

S. G. Musharraf, *et al.*, *Steroids* **100**, 5-10, (**2015)**.
S. G. Musharraf, *et al.*, *J. Am. Soc. Mass Spectrom.* 25, 532 (**2014)**.
S. G. Musharraf, *et al.*, *Rapid*. *Commun*. *Mass Spectrom*. **25**, 104 (**2011**).
S. G. Musharraf, *et al.*, *Steroids*, **77**, 138 (**2012**).
S. G. Musharraf, *et al.*, *Rapid Commun. Mass Spectrom.*, **27**, 169, (**2013**).
S. G. Musharraf, *et al.*, *I. J. Mass Spectrom.* **310**, 77 (**2012**).
S. G. Musharraf, *et al.*, *Chem. Cent. J.*, **6**, 120 (**2012**).