HPLC and GC-MS: Identification and Quantification of Strychnine and Brucine in Strychnos nux-vomica leaves

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Abstract: In this study, we have aimed to analyze the phytochemical composition of this plant and the concentration of strychnine and brucine. The identification of bioactive compounds was done by GC-MS with NIST Library. Strychnine and Brucine were quantified using HPLC. Twenty one medicinal bio active compounds were identified from the Strychnos nux-vomica leaf ethanolic extract. Strychnine is showing 28.43% purity and brucine was not detected in GCMS analysis. Quantified the concentration of strychnine (0.6 mg in 500mg of extract) and brucine (1.6 mg in 500mg of extract) was done by HPLC against Strychnine and Brucine standard. These compounds are having natural properties of Anti-inflammatory, Hypocholesterole, Cancer preventive, Hepatoprotective, Antimicrobial, Antioxidant, Cardio protective, Antiaging, Antialzheimeran, Antidermatitic, Immunostimulant, Anthepatotoxic, biosynthesis of steroid hormones, Nematicide, Antiandrogenic, 5-alpha reductase inhibitor, antipsychotic, analgesic, apoptotic effect, antidepressant, antidote for snake poisoning and diabetic activity.

Keywords: Strychnos nux-vomica, GCMS, HPLC, identification, quantification

INTRODUCTION

Medicinal plants have antimicrobial action and reduces stress (Srivastava *et al*., 2011; Meshram and N. Srivastava, 2014) and are the sources of bioflavonoids, alkaloids, polyhydroxy phenols, amphipathic glycosides and steroids (Mehta *et al*., 2013; Gotti *et al*., 2006; Kumar *et al*., 2009). Strychnos nux-vomica contains carbohydrate, alkaloid, tannin, steroid, triterpenoid and glycoside (Dineshkumar *et al*., 2012). Medicinal plants are the source for the production of therapeutic combinations (Velmurugan *et al*., 2010). Strychnos nux-vomica is distributed in Asia and Northern America. Seeds of Nux-vomica are used for antitumor, antimicrobial, anticonvulsant, anti-amnesic and immunomodulatory effects (Arunkumar *et al*., 2012). In India roughly 2,500 plants are identified for several medical applications (Natarajan *et al*., 2010; Alagesaboopathy, 2011). In Ayurveda Strychnos Nux vomica Linn is used in the treatment of several sicknesses, chickenpox, snake bite, etc. (Warrier *et al*., 1996; Murthy *et al*., 1986; Sheik *et al*., 2009).

MATERIALS AND METHODS

Plant Collection & authentication of Plant

Leaves of *Strychnos nux-vomica* was used for investigation obtained from Nellore district, Andhra Pradesh, India. The plant was authenticated by Botanical survey of India, Coimbatore.

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Extraction procedure and preparation for GCMS and HPLC

Leaves of Strychnos nux-vomica is washed with distilled water, shade dried, powered and used for the solvent extraction process. The crude extract was obtained by extracting 50 grams of dried plant powder in 200ml of 50% Ethanol in a water shaker for 72 hrs. Repeatedly solvent extraction was done with the same solvent till colour less solvent obtained. The hydro ethanolic plant extract was further concentrated by using Rota evaporator at 45-50 °C. After concentration, the residue occurred was dissolved in methanol and analysis is carried out by using GC-MS, 500mg of plant extract taken into a 10ml of volumetric flask ,dissolved in methanol and used for HPLC analysis.

Mobile phase and standard preparation for HPLC

The Composition of Mobile phase is used as Methanol, water and diethyl amine (55:45:0.2v/v) for HPLC.10mg of strychnine and 10 mg of brucine taken into a 10ml of volumetric flask and dissolved in methanol and filtered with 0.22 micron filter.

Instrument, column and method of gas chromatography- mass spectrometry

Identification of medicinally active compounds was done by using Gas Chromatography- Mass Spectrometry. Shimadzu GC-MS was used for analysis, model-QP2010 and operating in Electron Impact Ionization mode at 70electron volts. A Restek-5MS column (30 meters x 0.25 milli meter x 0.25µicro meter) was used, the helium flow rate was kept 1mL/min for analysis.

Instrument, column and method of HPLC

Quantification of Strychnine and Brucine was done by using HPLC. Agilent HPLC was used for analysis, model-1100 with Chemstation software. A Phenomenex-ODS column (250mm x 46mm x 5µicro meter) was used, the Column flow rate was kept at 1mL/min, Detector at 260nm, Run time 15 minutes for analysis.

Formula for HPLC calculation

The same formula used for quantification used for strychnine and brucine (Sheik *et al*., 2009).

$$\%c\ \text{Estimation} = \frac{At}{As} \times \frac{Ds}{Dt} \times \frac{Ws}{Wt} \times 100$$

At = Area count for sample solution.
As = Area count for standard solution.
Ds = Dilution factor for sample.
Dt = Dilution factor for standard.
Ws = Weight of standard (mg)
Wt = Weight of sample (mg).

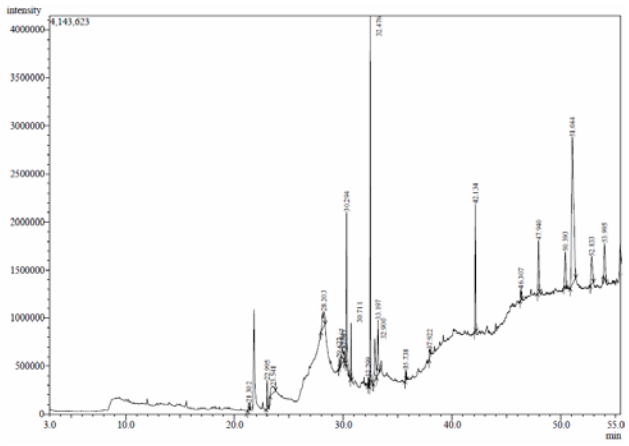

Fig. 1: GC-MS chromatograms shows the Retention Time of the compounds of *Strychnos Nux-vomica*

Instrument, column and method of gas chromatography- mass spectrometry

Identification of medicinally active compounds was done by using Gas Chromatography- Mass Spectrometry. Shimadzu GC-MS was used for analysis, model-QP2010 and operating in Electron Impact Ionization mode at 70electron volts. A Restek-5MS column (30meters x 0.25milli meter x 0.25µicro meter) was used, the helium flow rate was kept 1mL/min for analysis. The oven thermal program was initially kept at 50^{o}C and hold for 5 min, rise 10^{o}C per minute upto 150^{o}C and hold for 8 min, rise 8^{o}C per minute upto 250^{o}C and hold for 2 min, rise 10^{o}C per minute upto 280^{o}C and hold for 29.5min. The injector temperature was kept 280^{o}C and the injection volume was 2µL. The EI Source temperature kept was 250^{o}C, Quadrupole temperature kept was 150^{o}C, Interface Temperature kept was 250^{o}C, full scan mode, Scan range 40-600 m/z and solvent delay kept was 3 minutes.

Identification of components

Identification of mass spectra of GC-MS was done by using the National Institute of Standards and Technology (NIST) library. Unknown components of the mass spectrum were compared with to the NIST library components and identified. The name, mass and structure of the compounds of the test sample were determined.

RESULTS

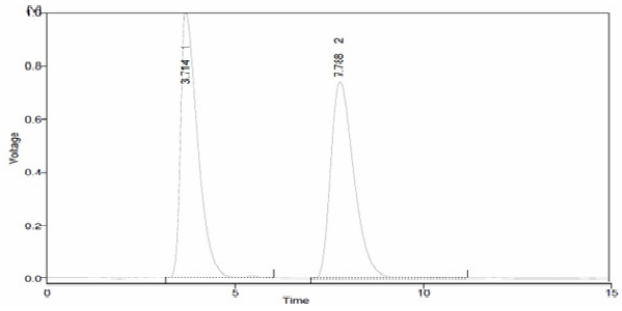

Fig. 2: HPLC analysis of Strychnine and Brucine standards

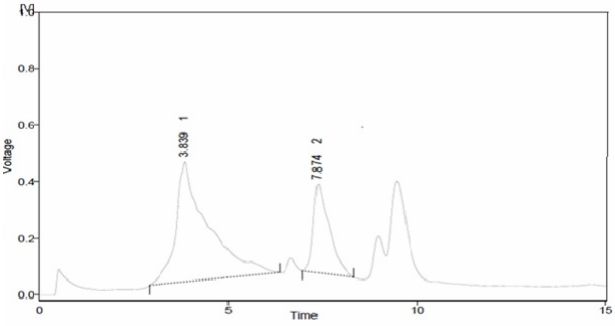

Fig. 3: HPLC analysis of Strychnine and brucine compounds in Strychnos Nux-vomica

DISCUSSION

In the present study, these biological compounds were found in GCMS analysis of Strychnos nux-Vomica. These compounds are Megastigmatrienone, 1,3,4,5-Tetrahydroxy- Cyclo hexane carboxylic acid, 3-O-Methyl-d-glucose, Hexadecanoic acid methyl ester, Cholest-5-EN-3-OL (3. Beta.), n-Hexadecanoic acid, 9,12,15-Octadecatrienoic acid methyl ester, 2-Hexadecen-1-OL, 1,4-Dioxaspiro[4.14] nonadecane, Hexadecanoic acid, 2,6,10,14,18,22-Tetracosahexaene, γ-Tocopherol, Vitamin E, Ergost-5-EN-3-OL, Strychnidin-10-ONE, Stigmast-5-EN-3-OL and Methyl Commate D (These are mentioned in fig. 1 for GCMS chromatogram, table 1 for list of the compounds). Strychnine and brucine content was quantified by HPLC, these are 0.6 and 1.6mg, respectively in the 500mg of the extract (These are mentioned in fig. 2 for HPLC Standard chromatogram and fig. 3 for HPLC Sample chromatogram).

Table 1: List of the Compounds present in *Strychnos nux-vomica* leaves extract of the GC MS analysis

Sl. No	Retention Time in minutes	Compound	Molecular Formula	Molecular Weight	Area Area %
1	21.302	Megastigmatrienone	$C_{13}H_{18}O$	190	415108 0.58
2	22.995	Megastigmatrienone	$C_{13}H_{18}O$	190	1403906 1.98
3	23.541	1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic acid	$C_7H_{12}O_6$	192	1680694 2.37
4	28.203	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	2220057 3.13
5	29.662	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	281869 0.40
6	29.867	Cholest-5-EN-3-OL (3.Beta.)	$C_{27}H_{46}O$	386	88151 1.24
7	30.087	Cholest-5-EN-3-OL (3.Beta.)	$C_{27}H_{46}O$	386	630306 0.89
8	30.294	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6887181 9.70
9	30.711	Hexadecanoic Acid, Ethyl ester	$C_{18}H_{36}O_2$	284	1496754 2.11
10	32.299	9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292	262921 0.37
11	32.476	2-Hexadecen-1-OL	$C_{20}H_{40}O$	296	10492917 14.78
12	32.900	Cyclopropaneoctanoic acid	$C_{22}H_{38}O_2$	334	3246047 4.57
13	33.197	9,12,15-Octadecatrienoic acid, ethyl ester	$C_{20}H_{34}O_2$	306	3298198 4.65
14	35.738	1,4-Dioxaspiro[4.14] nonadecane	$C_{17}H_{32}O_2$	268	418787 0.59
15	37.922	Hexadecanoic acid	$C_{19}H_{38}O_4$	330	477387 0.67
16	42.134	2,6,10,14,18,22-Tetracosahexaene	$C_{30}H_{50}$	410	4974663 7.01
17	46.307	γ-Tocopherol	$C_{28}H_{48}O_2$	416	557217 0.78
18	47.940	Vitamin E	$C_{29}H_{50}O_2$	430	3038192 4.28
19	50.393	Ergost-5-EN-3-OL	$C_{28}H_{48}O$	400	2683723 3.78
20	51.044	Strychnidin-10-ONE (Strychnine)	$C_{21}H_{22}N_2O_2$	334	20184057 28.43
21	52.833	Stigmast-5-EN-3-OL, (3.Beta.)-	$C_{29}H_{50}O$	414	2688057 3.79
22	53.995	Methyl Commate D	$C_{31}H_{50}O_4$	486	2769166 3.90

Table 2: Results of HPLC analysis of Strychnine and Brucine standards

S. No	Retention Time (min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	W05
1	3.713	31712.159	994.058	49.6	57.3	0.48
2	7.787	32174.177	741.853	50.4	42.7	0.66
	Total	63886.336	1735.911	100.0	100.0	

Table 3: Results of HPLC analysis of Strychnine and Brucine compounds in *Strychnos nux-vomica*

S. No	Retention Time (min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	W05
1	3.838	25161.659	423.541	73.8	57.6	0.75
2	7.873	8946.179	311.664	26.2	42.4	0.44
	Total	34107.838	735.205	100.0	100.0	

The therapeutic properties of the significant component specify the veracity of the use of this medication. Megastigmatrienone is used as an Aroma compound and 3-O-Methyl-d-glucose is having Preservative properties. Hexadecanoic acid methyl ester is used in Antioxidant, Flavor, Hypo cholesterolemic Pesticide and 5-Alpha reductase inhibitor treatments. 9, 12, 15-Octadecatrienoic acid methyl ester are reported in Anti-inflammatory, Hypocholesterole, Cancer preventive and Hepatoprotective. 2-Hexadecen-1-OL showed the Antimicrobial, Anticancer and Anti-inflammatory activity. 2, 6, 10, 14, 18, 22-Tetracosahexaene is also having the Antibacterial and Antioxidant properties. γ-Tocopherol is a vitamin and used in the treatment for Anticancer, Antioxidant, Antitumor, Anti-inflammatory, Hypocholesterolemia and Cardioprotective sickness. Vitamin E is known compound used in Antiaging, Antialzheimeran, Antidermatitic, Antidiabetic, Antioxidant, Antitumor, Cancer-preventive, hypocholesterolemia and Immunostimulant. Ergost-5-EN-3-OL is an Antioxidant and also having hypocholesterolemia activity. Stigmast-5-EN-3-OL used in Antihepatotoxic, Antiviral, Antioxidant, Cancer, preventive and hypocholesterolemia illnesses. Methyl Commate D is a compound used in antimicrobial, anti-inflammatory diseases (Phytochemical and Ethnobotanical Databases, 2016). Cholest-5-EN-3-OL (3.Beta.) is essential for the biosynthesis of steroid hormones, bile acids, and vitamin D (Hanukoglu I, 1992). n- Hexadecanoic acid is having Antioxidant, hypocholesterolemia, Nematicide, Antiandrogenic, Hemolytic, 5-alpha reductase inhibitor and antipsychotic properties (Vijisaral ED and Subramanian A, 2014; Sermakkani M and Thangapandian, 2012). Strychnidin-10-ONE is a biologically active compound used in various treatments that is analgesic (Yin *et al*., 2003), apoptotic effect, antidepressant (Yarnell and Abascal, 2001), antidote for snake poisoning (M. Grieve, 2007), antitumor (Deng *et al*., 2006) and diabetic activity (Chitra *et al*., 2010). Strychnine can be a stimulant with laxative potential for stomach ailments at low dose levels (Patel *et al*., 2012). Antiinflammatory activity of Brucine was investigated and it was found in both central and peripheral mechanisms by inhibiting prostaglandin E_2 (PEG_2) and by increasing 5-hydroxytryindole-3-acetic acid (Wu *et al*., 2003). Both Strychnine and Brucine are proven for potential cytotoxic properties using $Hepg_2$ cell line (Deng *et al*., 2006). Strychnine is proven with antioxidant (Tripathi and Chaurasia, 2000) and antimicrobial potentials (Dutta and Roy, 1992).

CONCLUSION

Bio active compounds in Strychnos nux-vomica leaves were identified by using GCMS with NIST library and Strychnine and Brucine were quantified by HPLC. These naturally occurring compounds plays a crucial role in pharmaceutical and biological industries for new drug development for various diseases. Hence, a lot of attention and extensive research is going on this plant ensuring the chances of developing new drugs from this plant in near future.

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