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TECHNICAL NOTE

Glycoalkaloid isolation from *Solanum linnaeanum* berries

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Abstract – Introduction. Glycoalkaloids are plant secondary metabolites that can be both harmful and beneficial to human health. They cause gastroenteric symptoms, coma and even death at high concentrations. It is thought that glycoalkaloids are toxic to human health as a result of their effects on the nervous system and destruction of cell membranes. On the other hand, glycoalkaloids can be effective drugs. For example, solasodine is used to treat skin cancer and tomatidine is used in cancer chemotherapy. Solasodine is also used as a precursor of steroidal drugs. The goal of the work was to isolate and separate efficiently these similar compounds. **Materials and methods.** Glycoalkaloids from *Solanum linnaeanum* berries were isolated using column chromatography and confirmed via NMR spectroscopy and MS/MS spectrometry. **Results and discussion.** The chemical structures of glycoalkaloids are nearly identical. For example, solamargine differs from solasonine in having a methyl group instead of a hydroxyl group bound to the sugar residue of the compound. Thus, their molecular weight and polarity are quite similar. In this work, the process yielded 37.1 mg solasonine, 92.3 mg solasonine and solamargine mix and 56.2 mg solamargine from 81.67 g dried *S. linnaeanum* berries. **Conclusion.** *Solanum linnaeanum* berries are a good source of these glycoalkaloids and the developed protocol proved efficient for purification of solasonine and solamargine.

Keywords: Turkey / nightshade / *Solanum linnaeanum* / glycoalkaloids / solamargine / solasonine

Résumé – Isolement de glycoalcaloïdes des baies de *Solanum linnaeanum* L.. Introduction. Les glycoalcaloïdes sont des métabolites secondaires végétaux qui peuvent être à la fois néfastes et bénéfiques pour la santé humaine. À des concentrations élevées, ils provoquent des symptômes de gastro-entérite, le coma et même la mort. On pense que les glycoalcaloïdes sont toxiques pour la santé humaine en raison de leurs effets sur le système nerveux et sur la destruction des membranes cellulaires. D'autre part, les glycoalcaloïdes peuvent être des médicaments efficaces. Par exemple, la solasodine est utilisée pour traiter le cancer de la peau et la tomatidine est utilisée en chimiothérapie du cancer. La solasodine est également utilisée comme précurseur de médicaments stéroïdiens. Le but de ce travail consistait à isoler et séparer efficacement ces composés analogues. **Matériels et méthodes.** Les glycoalcaloïdes extraits des baies de morelle de Linné (*Solanum linnaeanum*) ont été isolés par chromatographie sur colonne et leurs structures confirmées par spectroscopie à résonance magnétique nucléaire couplée à la spectrométrie de masse en tandem (RMNMS/MS). **Résultats et discussion.** Les structures chimiques des glycoalcaloïdes se sont révélées quasi identiques. La solasonine s'est distinguée de la solamargine par un groupe méthyle à la place d'un groupe hydroxyle lié au résidu carbohydrate du composé. Ainsi, le poids moléculaire et la polarité de ces deux composés sont assez semblables. Dans ce travail, le processus d'extraction et de purification a abouti à 37,1 mg de solasonine, à 92,3 mg d'un mélange de solasonine et de solamargine et à 56,2 mg de solamargine à partir de 81,67 g de baies séchées de morelle de Linné. **Conclusion.** Les baies de *S. linnaeanum* constituent une bonne source de ces glycoalcaloïdes et le protocole développé a prouvé son efficacité pour la purification de solasonine et de solamargine.

Mots clés : Turquie / morelle / *Solanum linnaeanum* / glycoalcaloïdes / solamargine / solasonine

1 Introduction

Most plants produce toxins against insects, pathogens and animals [1]. The largest class of such toxins is alkaloids. Alkaloids can be classified as benzylisoquinoline alkaloids, tropane alkaloids, terpenoid indole alkaloids, purine alkaloids, pyrrolizidine alkaloids, quinolizidine alkaloids and steroidal alkaloids [2]. Plants usually produce these alkaloids in glycosidic forms which are called glycoalkaloids. Glycoalkaloids are nitrogen-containing compounds and are found in the Apocynaceae, Buxaceae, Solanaceae and Liliaceae families [3, 4]. These compounds consist of three main portions: (i) a polar,

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water-soluble sugar residue composed of three or four monosaccharides, (ii) a nonpolar steroid portion called an aglycone and (iii) a basic portion which can be either indolizidine or oxa-azaspirodecane [1, 5]. Glycoalkaloids are formed when saccharides bind to the 3-hydroxy position of aglycones. These saccharides can be D-glucose, D-galactose, D-xylose and D-rhamnose and they can bind in different combinations as tri- or tetra-saccharides. Eggplant and its wild relatives contain six glycoalkaloids. These six glycoalkaloids are: the aglycone solanidine and its glycoalkaloids chaconine and solanine; the aglycone solasodine and its glycoalkaloids solamargine and solasonine. Chaconine is formed when two L-rhamnose and one D-glucose moieties bind to solanidine. Solanine is formed when L-rhamnose, D-galactose and D-glucose moieties bind to solanidine. Solamargine is formed when two L-rhamnose and one D-glucose moieties bind to solasodine. Solasonine is formed when L-rhamnose, D-galactose and D-glucose moieties bind to solasodine [6]. Glycoalkaloid synthesis in plants is affected by many environmental factors such as exposure to daylight, high or low temperature [7–9], physical wounding [7, 9], poor growth conditions, climate and storage conditions [10].

Glycoalkaloids are interesting metabolites because they can have both harmful and beneficial effects on human health. High doses of glycoalkaloids can cause gastroenteric symptoms, coma and even death. It is thought that they are toxic to human health as a result of their effects on the nervous system and destruction of cell membranes [11]. Their toxic effects can also increase synergistically with other glycoalkaloids. Food processing like grilling, baking or boiling does not affect the concentration of glycoalkaloids [8]. The toxic dose of glycoalkaloids is 2–5 mg kg^{-1} body mass and the lethal dose is 3–6 mg kg^{-1} body mass [12–14]. On the other hand, many beneficial effects of glycoalkaloids are reported. They are reported to decrease cholesterol level, protect against *Salmonella typhimurium* infection, have anticancer activity and increase the effects of malaria vaccine and cholinesterase inhibitor anesthetics [6]. In humans, glycoalkaloids inactivate viruses including *Herpes simplex*, *H. zoster*, and *H. genitalis* [15]. In humans, glycoalkaloids dampen the multidrug resistance of cancer cells [6]. Solasodine and tomatidine are glycoalkaloids found in solanaceous plants and are used for skin cancer and cancer chemotherapy, respectively [16]. In many countries, solasodine is used as a precursor of steroidal drugs, for example, as an alternative to diosgenin [17].

Because several glycoalkaloids are usually found in a single plant species, efficient methods must be devised for their individual purification. Once purified, the compounds can then be used for research and pharmaceutical purposes. In this study, a method was developed for efficient isolation of solamargine and solasonine from the fruit tissues of *Solanum linnaeanum*, a nightshade species and wild eggplant.

2 Materials and methods

2.1 General

Glycoalkaloid standards were obtained commercially from Sigma-Aldrich except solamargine which was obtained from Dr. Adelia E. Almeida, Sao Paulo State University, Sao Paulo, Brazil. Solvents, silica and sephadex resins were purchased from Sigma-Aldrich and thin layer chromatography (TLC) precoated Kieselgel 60 F254 plates were purchased from Merck Chemical Company.

2.2 Plant materials

Berries of *Solanum linnaeanum* (also named devil's apples) were obtained from Dr. Marie-Christine Daunay, INRA, Montfavet, France. Fresh fruits were harvested from four accessions of *S. linnaeanum* (MM 726, MM 1530 MM 602 and MM 1462) which were grown in four distinct isolation fields at the research station during summer and autumn 2008.

2.3 NMR and MS/MS conditions

Proton nuclear magnetic resonance (NMR) spectroscopy experiments were performed on a Varian AS-400 spectrometer in deuterated pyridine at 400 MHz. Tandem mass spectrometry (MS/MS) analysis was performed on a Bruker Maldi TOF-TOF mass spectrometer. For MS/MS, the spectra were acquired in positive reflection mode using 2,5-dihydrobenzoic acid as matrix.

2.4 Glycoalkaloid extraction and isolation

Glycoalkaloid purification for this work was based on a method modified from the literature [3, 18–22]. A total of 1 kg fresh *S. linnaeanum* berries was lyophilized until dry and ground to obtain a fine powder. A 81.67 g sample of freeze-dried fruit powder was twice extracted overnight with 250 mL methanol by reflux following 250 mL n-hexane treatment to remove lipids and 250 mL dichloromethane treatment to remove pigments. The extract was processed to liquid-liquid partition with water:butanol (1:1 v/v) to remove water soluble impurities. The butanol phase was evaporated and resuspended with methanol and applied to a reverse phase (RP) column. Gradient elution was achieved with 50% methanol, 60% methanol, 70% methanol, 80% methanol, 90% methanol and 100% methanol, respectively. Fractions were collected according to the volumes of gradient elution solvent used.

For all fractions, reversed-phase thin-layer chromatography (RP-TLC) was performed and the TLC solvent was the same as the elution solvent for each fraction with 30% sulfuric acid used as a visualizing agent. According to the TLC results, the 80%, 90% and 100% methanol fractions were combined.

A silica gel column was constructed to separate glycoalkaloids. Isocratic elution was performed with a chloroform:methanol:water (61:32:7) (v/v/v) solvent system. In total, 137 fractions were collected and the volume of each fraction was 5 mL. TLC was performed for all fractions using chloroform:methanol:water (61:32:7) (v/v/v) solvent and 30% sulfuric acid as a visualizing agent with heating to 100 °C. According to the TLC results, fractions 9 through 32 contained solamargine and solasonine and were combined. To separate

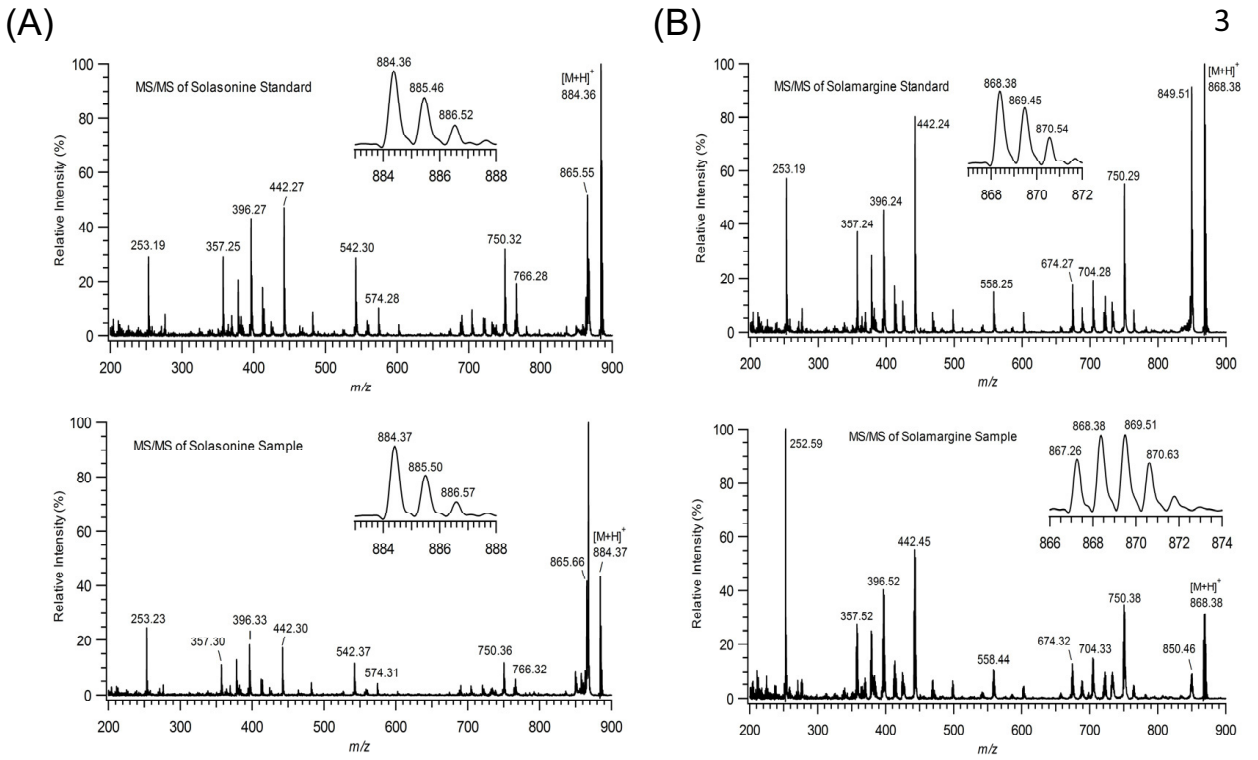

Figure 1. Tandem mass spectrometry (MS/MS) comparison of (A) solasonine and (B) solamargine.

fractions 9 to 32, another silica column was constructed. Elution was done as previously described and 147 2 mL fractions were collected. TLC was performed for all fractions as described above. Fractions 37 through 63 contained solamargine, fractions 64 through 92 contained a mixture of solamargine and solasonine and fractions 93 through 119 contained solasonine. Fractions for each glycoalkaloid and their mixture were separately combined and evaporated.

The solasonine-containing fractions were pure, however, the solamargine-containing fractions needed additional cleaning. Thus finally, a Sephadex column was constructed for the solamargine-containing fractions. Isocratic elution was done with methanol and the sample was redissolved in 1 mL methanol. A total of 139 1 mL fractions were collected. Solamargine-containing fractions 32 through 88 were combined (Supp. *figure 1*[1]). The solvents of the solamargine, solasonine, and mixed solamargine and solasonine samples were evaporated separately and lyophilized for two days. Samples were then dissolved in 1 mL of tertiary butanol.

3 Results and discussion

The identities of the isolated glycoalkaloids (solamargine and solasonine) were confirmed via the Rf values of TLC (Supp. *figure 2*[1]), proton NMR (Supp. *figure 3A-3B*[1] and *table I*) and liquide chromatography (LC)-MS/MS spectra (*figure 1A- 1B*). The results of these separate analyses were compared to literature values.

[1] Supplementary data are available online at www.fruits-journal.org.

Table I. Proton NMR results for isolated solamargine and solasonine measured in deuterated chloroform.

H	Solasonine	Solamargin	H	Solasonine	Solamargine
1	1.10, 1.72	1.17, 1.90	24	1.43, 1.56	1.37, 1.39
2	1.98, 1.94	1.89, 1.56	25	2.15	2.08
3	3.9	3.9	26	2.07, 1.95	2.06, 2.16
4	2.76, 2.77	2.41, 2.79	27	0.64	0.76
5	-	-	1’	4.71	4.71
6	4.98	4.98	2’	4.62	4.68
7	1.42, 1.88	1.42, 1.83	3’	4.6	4.67
8	1.47	1.43	4’	4.34	4.31
9	0.75	1.11	5’	3.9	3.9
10	-	-	6’	3.94	4.22
11	1.4	1.4	1”	6.68	6.56
12	1.10, 1.75	1.12, 1.75	2”	4.73	4.74
13	-	-	3”	4.81	4.76
14	1.05	1.09	4”	4.85	4.88
15	1.86- 1.11	1.39, 1.83	5”	4.97	4.91
16	4.51	4.5	6”	2.45	2.44
17	1.92	1.97	1”’	6.14	5.63
18	0.6	0.85	2”’	4.84	4.76
19	0.86	1.17	3”’	4.82	4.74
20	1.97	2.26	4”’	4.74	4.71
21	nd	2.37	5”’	4.73	4.68
22	1.42		6”’	4.6	4.6
23	1.94	1.98			

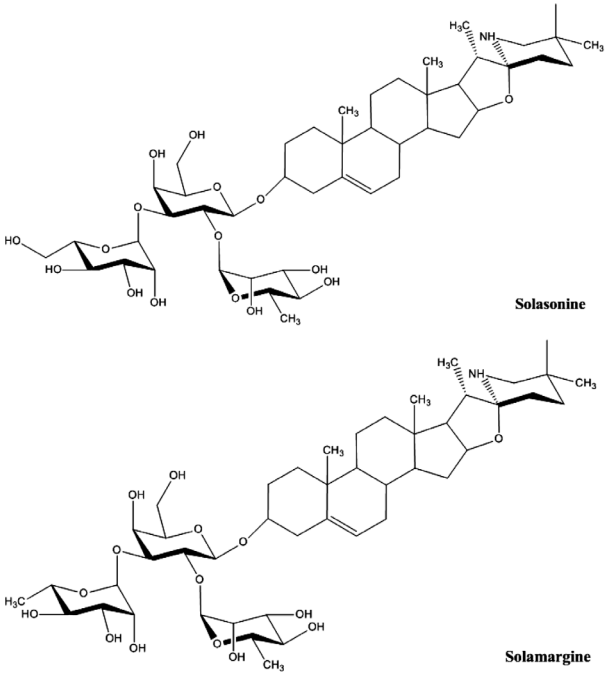

Figure 2. Chemical structures of solasonine and solamargine.

Solasonine (compound 1) and solamargine (compound 2) are regarded as the common glycoalkaloidal constituents of Solanum species [24]. Both of these glycoalkaloids are similar to their aglycone, solasodine. Compounds 1 and 2 were observed as major components in methanol extraction of *S. linnaeanum*. Both compounds contain a trisaccharidic sugar moiety. Compound 1 has α-L-rahmnose, β-D-glucose, β-D-galactose and compound 2 has α-L-rahmnose, β-D- rahmnose, and β-D-glucose. The proton NMR spectra of compounds 1 and 2 showed four tertiary methyl groups. Additionally, three anomeric protons were observed for both solamargine (δ 4.71, 6.56 and 5.63) and solasonine (δ 4.71, 6.68, and 6.24). Thus, both compounds were considered to be 22αN-spirosal-5-ene monoglycosides. Because of glycosylation, H-3 signal was obtained (for solasonine 3.90 ppm, 2.76 ppm; for solamargine 3.90 ppm, 2.44 ppm). In both molecules, H-16 is a neighbor to oxygen, therefore its signal was obtained at the lower field. Also H-26 is a neighbor to nitrogen, (for solamargine 1.37 ppm, 1.39 ppm; and for solasonine 1.43 ppm, 1.56 ppm), therefore, its signal was obtained at the lower field. Consequently, the structure of compound 1 was established as (25R)-3β-{O-α-L-rahmnopyranosyl-(1-2)-[O-β-D-glucopyranosyl-(1-3)]-β-D-galactopyranosyloxy}-22αN-spirosal-5-ene. Compound 2 was established as (25 R)-3β-{O-α-L-rahmnopyranosyl-(1-2)-[O-α-L-rahmnopyranosyl-(1-4)]-β-D-glucopyranosyloxy}-22αN-spirosal-5-ene. Based on the proton NMR and mass spectra data, the chemical structures of the purified compounds were drawn (*figure 2*) with ChemBioDraw ultra 11 software program MS/MS data of solamargine and solasonine are compatible with their standards' MS/MS data. In *figure 1A* (M+H)+ ions at m/z 870 indicate compound 2 and in *figure 1B* (M+H)+ ions at m/z 884 indicate compound 1.

Table II. The content of solamargine and solasonine in different nightshade species.

Solanum species	Solamargine (mg kg^{-1})	Solasonine (mg kg^{-1})	Reference
S. linnaeanum[a]	688.0	454.0	This study
S. xanthocarpum[a]	66.9	23.0	[23]
S. lycocarpum[a]	59.0	87.0	[24]
S. lycocarpum[a]	115.0	63.2	[25]
S. aculeastrum	26.0	Not measured	[21]
S. khasianum	52.0	18.70	[22]
S. linnaeanum[b]	56.2	37.1	This study
S. sodomaeum	129.0	20.4	[26]
S. sycophanta[b]	7.8	7.8	[20]

[a] Calculated values based on fruit dry weight.
[b] Calculated values based on fruit fresh weight.

The development of efficient isolation methods for the nightshade glycoalkaloids solamargine and solasonine will help facilitate potential medicinal uses of these health-related compounds. Structure similarity of these molecules makes their separation and identification difficult. Moreover they are found in nightshade at low quantities. Thus, finding a new and/or good source of glycoalkaloids, and discovering efficient methods for isolation are strategic. Different extraction methods can be performed with ethanol [24] or methanol via either maturation [21, 26], reflux [25], alkalinisation after acidic hydrolysis [20, 22, 23] or ammonium hydroxide precipitation [24]. Methanol can be considered as the most efficient extraction solvent. On the other hand, although alkalinisation or precipitation provides a pre-purfication step for the glycoalkaloids, it is tedious and time-consuming. Chromatographic methods are applied for purfication of glycoalkaloids. Generally column chromatography with alumina [20, 22, 24] or silica [25, 26] is used for separation and purification. In addition, droplet counter current chromatography (DCCC) [21] or fast centrifugal partition chromatography (FCPC) [23] can be used. DCCC and FCPC are more automatized systems but require instrumentation. On the other hand, column chromatography is simple and as effective as other chromatographic systems. Another important factor in determining the efficiency of isolation is the source of the glycoalkaloids. Different nightshade species have been investigated to date including *S. sycophanta* [20], *S. aculeastrum* [21], *S. khasianum* [22], *S. xanthocarpum* [23], *S. lycocarpum* [24, 25] and *S. sodomaeum* [26]. In this work, we used *S. linneaenum* berries and obtained glycoalkaloids in higher quantities compared to all other nightshade species except *S. khasianum (table II)*. These results indicate that *S. linneaenum* is a good source of solamargine and solasonine.

4 Conclusion

Solamargine and solasonine are known to be the most abundant glycoalkaloids found in the Solanaceae family. However, the quantity of these compounds shows variation among species in this family (*table II*). In our work, we obtained 37.1 mg solasonine, 92.3 mg solasonine and

solamargine mix and 56.2 mg solamargine from 81.67 g dried *S. linnaeanum* berries. This is more than was obtained for other Solanum species except for the dried berries of *S. khasianum*. These results indicate that *S. linnaeanum* berries are a good source of these glycoalkaloids and that the simple protocol we developed is efficient for purification of these compounds.

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Online Material

Supplementary Data

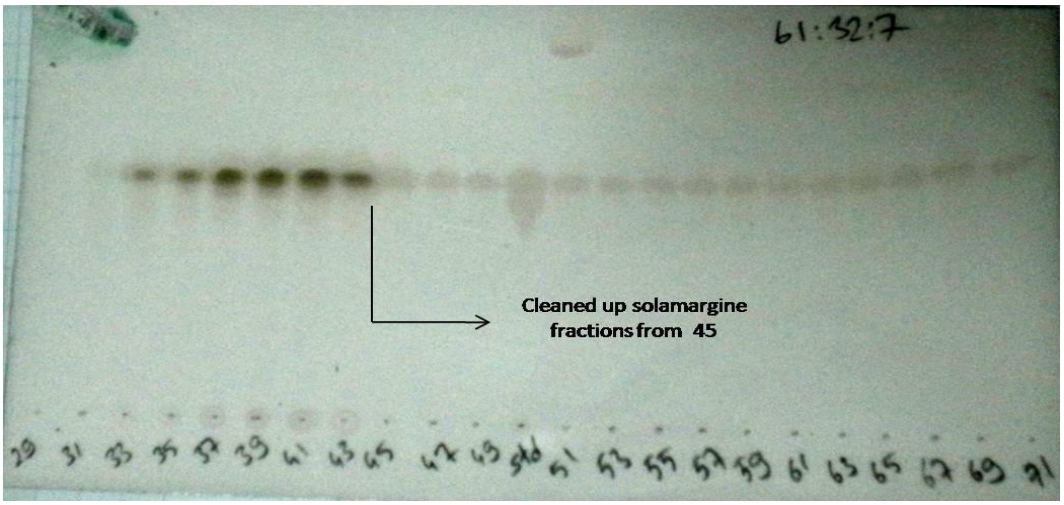


Figure 1. A. Thin layer chromatogram of solamargine from Sephadex column.

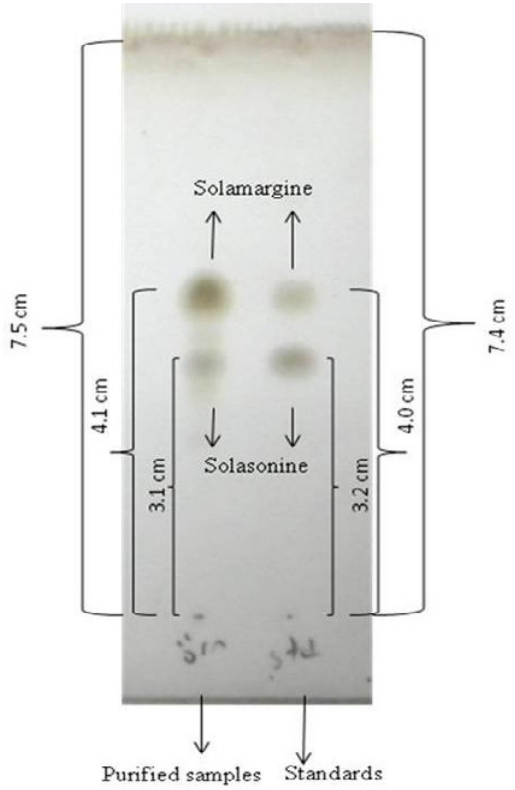
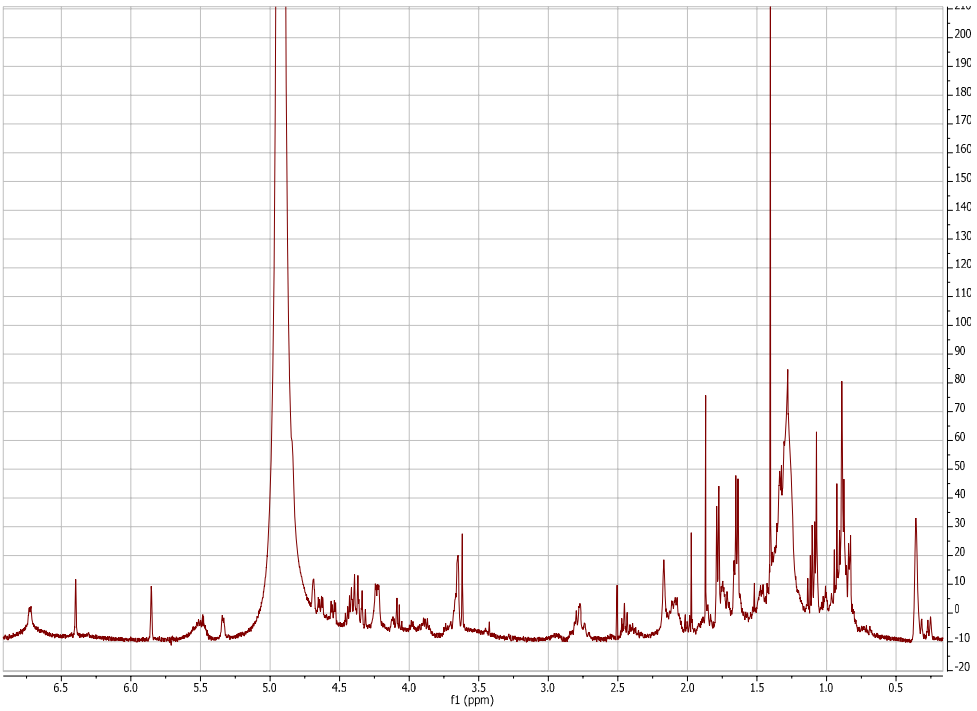
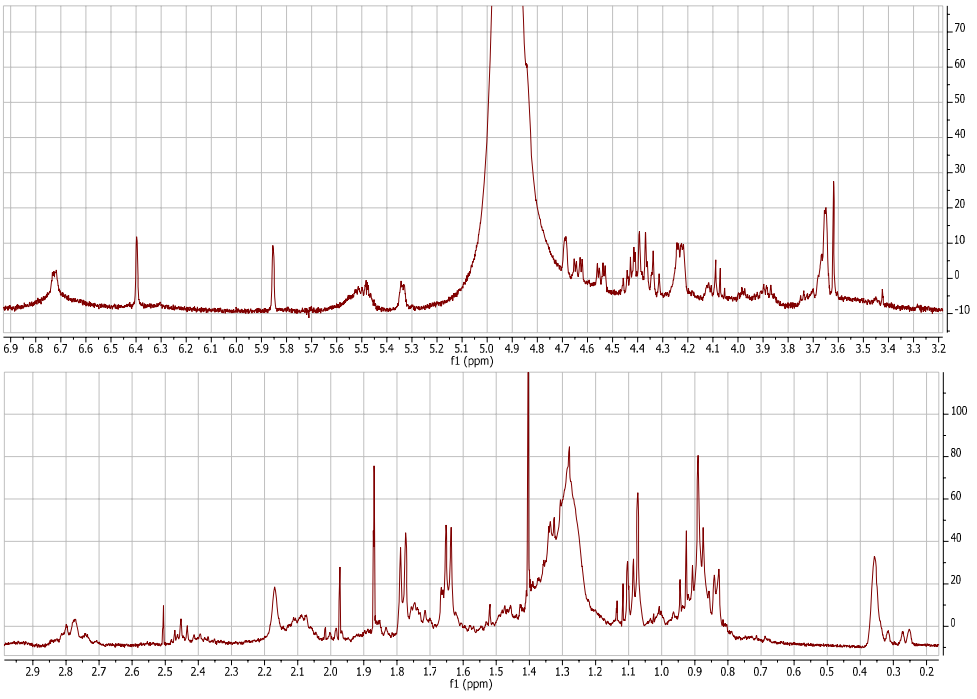


Figure 2. Thin layer chromatogram of solamargine from sephadex column
Rf calculation of standard and purified solamargine and solasonine:
Rf *values for solamargine:*
Solamargine standard: Rf: 4.0/7.4= 0540
Purified solamargine: Rf: 4.1/7.5 = 0.546
Rf *values for solasonine:*
Solasonine standard: Rf: 3.2 / 7.4= 0432
Purified solasonine: Rf: 3.1/7.3 = 0.426

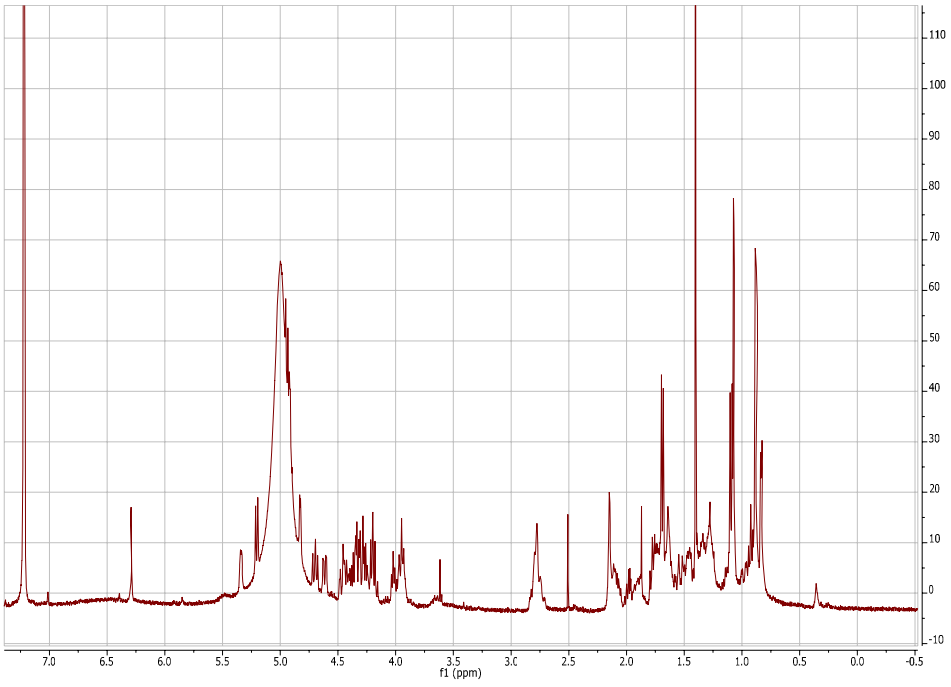


(1)

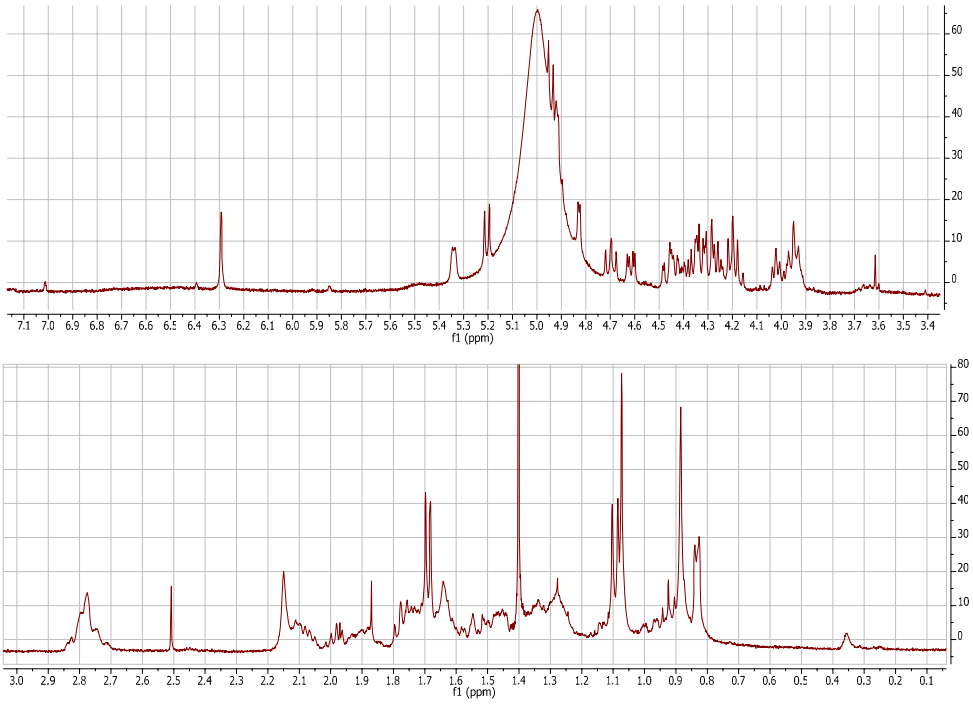


(2)

Figure 3. A. (1) Proton nuclear magnetic resonance (NMR) spectrum of solamargine, (2) expanded version of proton NMR spectrum.



(1)



(2)

Figure 3. B. Proton nuclear magnetic resonance (NMR) spectrum of solasonine, (2) expanded version of proton NMR spectrum.