Research Article

Qualitative and Quantitative Steroidal Alkaloids of *Solanum* Species Distributed Widely in Syria by TLC and HPLC

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ABSTRACT

Solanum Genus plantsare belong to the Solanaceae Family, and there are three species belong to this Genus, and they are widely distributed in Syria: *Solanum nigrum* L., *Solanum villosum* Mill., *Solanum elaeagnifolium* Cav. This plants are an important species and widely spread. However, the plants had not studied in Pharmacognostic and Medicine. In addition, they are a major source for steroidal alkaloids compounds. The aims of this study are the extraction of steroidal alkaloids from the fruits of *Solanum* plant by ultrasonic method and butanol. Then, they had separated and determent by using Thin layer chromatography TLC, HPLC and chemical reagents. Samples of *Solanum* plant have been collected from various sites in Syria. The study showed that the extraction give (4.5, 2.5 and 2.5% w/w) for butanol extract of fruits (BEF) of (*S. nigrum*), (*S. villosum*) and (*S. elaeagnifolium*), respectively.

Keywords: *Solanum*, steroidal alkaloids, Solanine, Solanidine, Extraction, HPLC, TLC.

INTRODUCTION

The Solanaceae family is widely distributed in different regions of the world. It is composed of approximately 84 genera and 3000 species[1].

Within Solanaceae family, *Solanum* constitutes the largest, variable and most complex genus. It is consists of annual and perennial plants, forbs, vines, sub-shrubs, shrubs, and small trees. They often have attractive fruit and flowers. Together with many other plants of both poisonous and medicinal value *Solanum* constitutes the largest and most complex genus of the family. It is composed of more than 1500species, many of which are also economically important throughout their cosmopolitan distribution[2,3]. *Solanum* is one of the most commercially and economically important genus of Solanaceae which had been extensively studied.

The steroidal alkaloids are considered among the most important compounds, which are found in the butanol's extracts of fruits (BEF) of the wild *Solanum* species in Syria. The (*S. nigrum*), (*S. villosum*) and (*S. elaeagnifolium*) belonging to the Solanaceae family according to Syria's Poisonous Plants Information System (SPPIS)[2].

The *Solanum* contains steroidal alkaloids that showed biological effects like antifungal[3,4] and antiviral ones[5], but the most important feature that it also shows considerable anticancer effects. For example, The Solamargine causes the human hepatoma cells death (Hep3B) by apoptosis[6]; α-solasonine from *S. crinitum* and *S. jabrense* has a cytotoxic effect on the leukemia cells[7]; chaconine, solanine, tomatine, and their derivatives inhibit the human colon (HT29) and liver (HepG2) cancer cells to grow[8]; β-2-solamargine from (*S nigrum* L.) has a toxic effect on the cell lines: HT-29 (colon), HCT-15 (colon), LNCaP (prostate), PC-3 (prostate), T47D (breast), and MDA-MB-231 (breast)[9]. The aims of this research are the investigating in qualitative and quantitative steroidal alkaloids contents in the three Species belonging to *Solanum* genus which distributed wildly in Syria.

MATERIALS AND METHODS

Material

The alkaloid standards (α-Solanine and Solanidine) were purchased from Sigma Co. The steroidal alkaloids were analyzed using HPLC apparatus consisting of Shimadzu LC-10A system equipped with a model LC-10AT pump, an SPD-10A variable wavelength detector, a CBM-10A interface module with class LC-10 HPLC software using a EMR C-18 column (250×4.6, i.d., 5 µm particle size). The steroidal alkaloids were determined using P-TLC scanner (027.6200, Camag, Münster, Germany).

Plant materials

The botanical materials that are studied in this research have been collected in September 2010 where the (*Snigrum*) and the (*S.villosum*) fruits were collected from Ebla University's Garden (GPRS-data: 35° 54' 34" N and 36° 51' 17" E) while the (*S. elaeagnifolium*) fruits were collected from Al-Raqqa city (GPRS-data: 35° 57' 1" N and 39° 9' 40" E) and all documented by Dr. Amin Salkiny (ICARDA, Aleppo, Syria). The fruits are placed on stainless clean trays and stored at the room temperature for 7 days. The dry fruits are weighed, milled into fine powder and kept in a container and freeze-dried.

Methods

Extraction of steroidal alkaloids

The steroidal alkaloids were extracted from the *Solanum* species fruits by the ultrasound technique[12] using the butanol as a solvent[13]. A design of such extracting is found

in (Figure 1). The obtained butanol's extract is filtered through filtering papers and concentrated using the rotary evaporator (B465, Buchi, Switzerland). The (BEF) of *Solanum* species were stored in -20 °C until their use. The concentration used in the experiment based on the extract's dry weight (µg/ml).

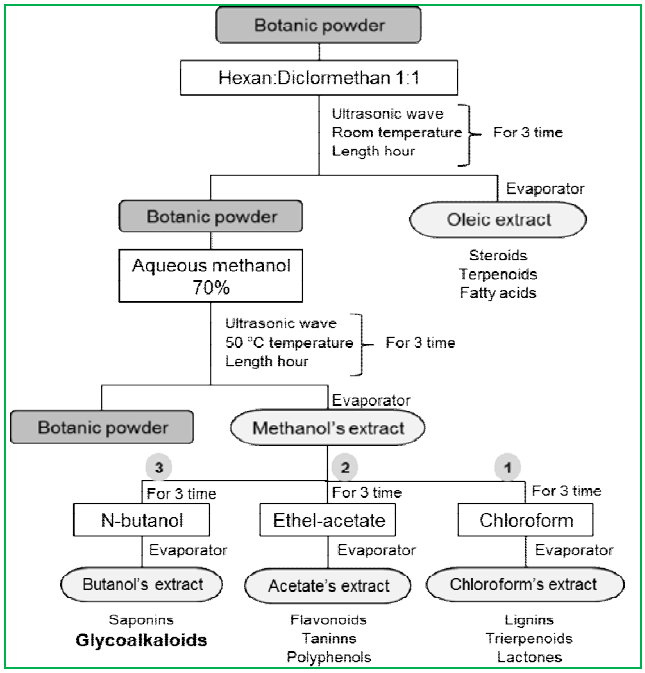

Figure 1: A design of extraction of the steroidal alkaloids

TLC analysis of steroidal alkaloids

The preliminary examination of the previously extracted steroidal alkaloids using TLC (Silica Gel 60 GF254, Merck, Darmstadt, Germany). The solvent system used was chloroform: methanol: water (14:6:1), the chromatogram is sprayed in Dragendorff's reagent[14], phosphomolybdic acid reagent[15], ninhydrin reagent 2% (then ultra-violet UV 365 nm)[16] (N.R. Farnsworth, 1962), antimony trichloride reagent 2% [17] and blood hemolysis reagent[18,19] (Figure 2).

HPLC analysis of α-solanine and solanidine

Butanol's extracts (20 mg/20 ml) prepared as described above were directly injected into an HPLC system. Stock solutions of reference compounds were prepared by dissolving 1.0 mg of each reference compound in 1 ml of ethanol. For the simultaneous determination of steroidal alkaloids, 20 ml of α-solanine and solanidine stock solutions were combined. All liquid chromatographic (LC) samples were filtered through a Millex-HV 0.45-mmfilter (Watford, Ireland) before injection. The detection wavelength was set at 205 nm. A reverse-phase LC separation method previously published[13] was performed with the following modifications. Separation was obtained with a reverse-phase column (EMR Cosmosil 120-5-C18, 250 mm - 4.6 mm) eluting at a flow rate of 1 ml/min; we used acetonitrile–triethylammonium phosphate (TEAP) buffer with a linear solvent gradient of from 20 to 70% acetonitrile in 20 min. The α-solanine (13.5 min) and solanidine (18.2 min) were used as the external standards to identify the active components in these extracts[20].

RESULTS

Extraction Results

The extraction gives (4.5, 2.5 and 2.5% w/w) for (BEF) of (*S. nigrum*), (*S. villosum*) and (*S. elaeagnifolium*), respectively. And shows the percentages of Oleic extracts, Chloroform's extracts, Acetate's extracts and Butanol's extracts in the dry weight of fruits three *Solanum* species which studied (Table 1).

Table 1: The percentages of extracts in the dry weight of fruits three *Solanum* species:

Parameter	The percentage (% w/w) in dry weight of Berries		
	S. nigrum	*S. villosum*	*S. elaeagnifolium*
Oleic extract	**11.7**	**13.8**	2.1
Chloroform's extract	0.95	1.3	1.2
Acetate's extract	1.3	1.2	0.2
Butanol's extract	**4.5**	2.5	2.5

Detection of steroidal alkaloids by TLC

It has been indicated through the TLC chromatogram the availability of several compounds in the butanol's extracts of the *Solanum* species fruits, some of these compounds are alkaloids with R_f values the following: (0.07, 0.13,0.17, 0.20, 0.27, 0.39, 0.52, 0.59, 0.63) and among such compounds there are ones with saponin properties called alkaloid steroid saponins having R_f values the following: (0.27, 0.52). It is noted here that the Dragendorff's reagent and the blood reagent are less sensitive to the little-amount concentrations than the rest of the used reagents (Table 2).

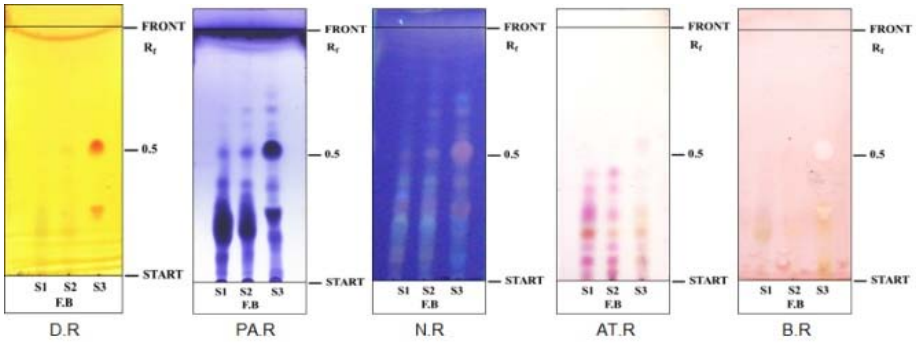

Figure 2: The TLC chromatogram of (BEF) of *Solanum* species

Where S1: *S. nigrum*, S2:*S. villosum*, S3 :*S. elaeagnifolium*; F.B: butanol's extract of *Solanum Species* fruits; Merck 60F Silica gel plate; mobile phase: chloroform: methanol: water (14:6:1); developed with PA.R: phosphomolybdic acid reagent, D.R: Dragendorff's reagent, N.R: ninhydrin reagent 2% (then UV365nm), AT.R: antimony trichloride reagent 2%, B.R: blood reagent.

Table 2: TLC of steroidal alkaloids of three of *Solanum* Species

R_f ±0.01	Fruits			Color				
	S1	S2	S3	PA.R	D.R	N.R	AT.R	B.R
0.07	+	+	+	Blue	-	Blue	purple	-
0.13	+	+	+	Blue	-	Blue	purple	-
0.17	+	+	+	Blue	-	Blue	purple	-
0.20	+	+	+	Blue	-	Blue	brown	-
0.27	+	+	+	Blue	Orange	brown	purple	White
0.39	+	+	+	Blue	-	brown	purple	-
0.52	+	+	+	Blue	Orange	brown	purple	White
0.59	+	+	+	Blue	-	brown	-	-
0.63	+	+	+	Blue	-	brown	-	-
0.69	+	+	+	Blue	-	brown	-	-
0.74	+	+	+	Blue	-	Blue	-	-

Where S1: *S. nigrum*, S2:*S. villosum*, S3 :*S. elaeagnifolium*; F.B: butanol's extract of *Solanum Species* fruits; Merck 60F Silica gel plate; mobile phase: chloroform: methanol: water (14:6:1); developed with PA.R: phosphomolybdic acid reagent, D.R: Dragendorff's reagent, N.R: ninhydrin reagent 2% (then UV365nm), AT.R: antimony trichloride reagent 2%, B.R: blood reagent. -: Absent, +: Present

HPLC analysis of α-solanine andsolanidine

When drawing chromatogram HPLC for each of the two compounds (α-Solanine and Solanidine) show that the retention time of the α-Solanine is min13.5, while the retention time Solanidine is 18.2 minutes.

Table 3: Percentages for each of the two compounds (α-Solanine Solanidine) in (BEF) of the three Species of *Solanum* and also in dry weight of fruits:

Parameter	Value (% w/w)					
	S. nigrum		*S. villosum*		*S. elaeagnifolium*	
	F.B	F.D	F.B	F.D	F.B	F.D
α-Solanine	9.7	0.4	26.6	1.4	**46.2**	1.2
Solanidine	0	0	3.9	0.2	**19.2**	0.5

FB = The percentage of (BEF), **FD**=The percentage of the dry weight of the fruits

DISCUSSION

This study showed that the highest percentage of oleic extracts were in the fruits of *S.villosum* (13.8%) in dry weight, followed the percentage of oleic extract in the fruits of *S. nigrum* (11.7%) in dry weight. It also shows that the highest percentage of butanol's extracts were in fruits of *S. villosum* (5.4%) in dry weight, as shown in the figure (3).

The separation of alkaloids and saponins showed in butanol's extracts that Dragendorff's reagent and blood reagent are sensitive for concentrations higher only, where gave good results with the compounds in (BEF) of the of *Solanum elaeagnifolium* Cav., In While not give tangible results with the rest of the extracts. In contrast it was ninhydrin reagent the more sensitive to the presence of alkaloids where it was able to show them in the rest of the extracts.

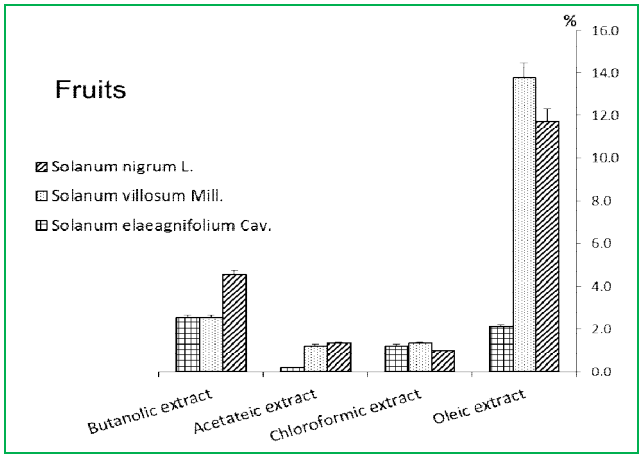

Figure 3: Scheme for percentage of extracts in the dry weight of fruits of the three species of *Solanum*

The compounds that have been separated and shown reagent alkaloids were with values of R_f (0.07, 0.13, 0.17, 0.20, 0.27, 0.39, 0.52, 0.59, 0.63, 0.69) in the fruits, which can speculate that be one of the following compounds [21]: α-Solanine &β-Solanine.

While the compounds with values of R_f (0.27, 0.52) in the fruits of the three species, considered steroid alkaloid saponins, so as to give it a white color with blood reagent. Compared with the reference studies could speculate that will be one of the following compounds[21]: Solamargine & Solasonine.

Upon comparison between (BEF) of *Solanum* species in terms of the presence of the two compounds (α-Solanine and Solasonine) and their concentrations using HPLC, the following schemes have been drawn: Figures (4-5).

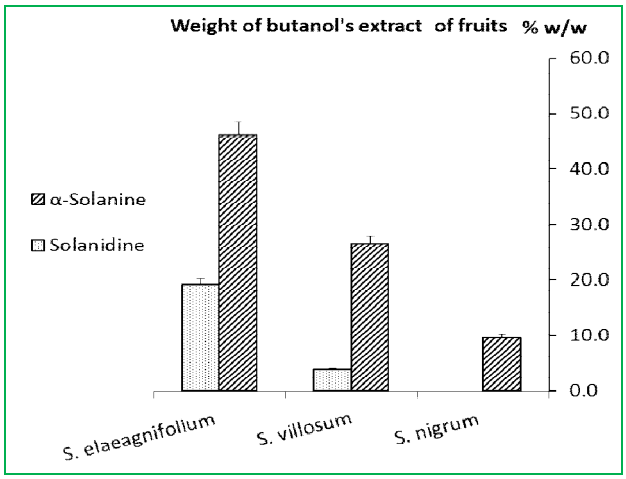

Figure 4: Scheme of the percentage of compounds (α-Solanine and Solanidine) in (BEF) of the three species of the genus *Solanum*

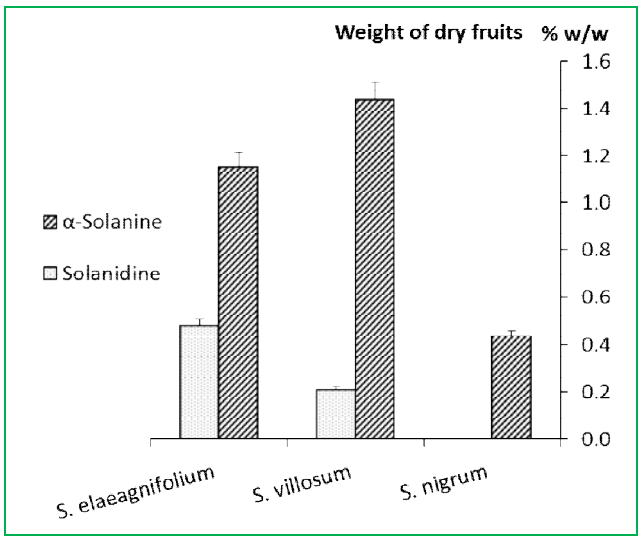

Figure 5: Scheme of the percentage of compounds (α-Solanine and of Solanidine) in the dry weight of the fruits of three species of the genus *Solanum*

It can be seen that the highest percentage of compound α-Solanine is in (BEF) of *S. elaeagnifolium* (46.2% w/w), followed in (BEF) of *S. villosum* (26.6% w/w) and Finally in (BEF) of *S. nigrum* (9.7%w/w), and this is identical to what came in the reference studies that the most toxic species is *S. elaeagnifolium*, followed *S. villosum* and followed *S. nigrum,* and according to the amount of the α-Solanine.

It also shows that the compound Solasonine found in fruits of *S. elaeagnifolium* and *S. villosum* and is not present in the fruits of *S. nigrum*.

CONCLUSION

Butanol's extracts of Species fruits of plants Genus *Solanum*: *Solanum nigrum* L., *Solanum villosum* Mill., *Solanum elaeagnifolium* Cav. Distributed Wildly in Syria contains steroid alkaloids and some saponins, which is possible to determine the identity and calibrated in if standard solutions has been made available, and the possibility of their use in the chemical and pharmaceutical experiments. In addition to that *Solanum* species toxicity studied closely linked with the concentration of α-Solanine in their extracts.

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