GC-MS and HPLC as the Analytical Tools in solving the Taxonomic Controversies of Plants

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***Abstract*—The science of chemical taxonomy is used for the classification of plants on the basis of their chemical constituents which are deeply concerned with the molecular characteristics. Five locally available plant taxa of *Solanum nigrum* Complex viz.: *S. americanum* Mill., *S. chenopodioides* Lam., *S. nigrum* L., *S. retroflexum* Dunal and *S. villosum* Mill. were investigated. GC-MS and HPLC were used as the analytical tools to resolve the international taxonomic controversy about these plants. Comparative qualitative and quantitative analyses of these plant samples were undertaken keeping Alkaloids, Flavonoids and Epicuticular wax as potential characters. The glycosides of alkaloids and flavonoids were determined by HPLC whereas their aglycones and epicuticular waxes were analysed using GC-MS. HPLC and GC-MS analyses of these constituents from *S. nigrum* Complex had not been reported previously. Statistical cluster analyses of results grouped taxa into different clusters on the basis of similarity index and Euclidean distance.**

I. INTRODUCTION

Family Solanaceae comprises of about 84 genera and 3000 species[1] and *Solanum (S.)* is one of its most important and largest genera. *Solanum nigrum* is the largest and most variable species of the genus *Solanum* and is now named as "*Solanum nigrum* Complex" because it is composed of a large number (about 30) of morphologically distinct taxa[2]. The species have been re-classified many times on the basis of morphological characters but no satisfactory revision of the whole section has yet been devised. The boundaries between many of the species are still ill-defined, with many of the 'new' taxa proving to be no more than slight morphological variants of those already described. The situation has been further complicated by a number of authors, who have persistently treated different members of the section as belonging to one species, *S. nigrum.*

The plants of *S. nigrum* Complex has been traditionally used as an analgesic, antispasmodic, for disorders of neuro-vegetative system, antiseptic, antidysentric, antinarcotic, emollient, diuretic, tonic, soporific, laxative, anticancer, antiulcer, etc. (Saijo et al, 1982; Akhtar and Muhammad, 1989; Schilling et al., 1992; Manoko et al., 2007). But each taxa of this complex had been found to be having its own specific medicinal and nutritional value (Edmonds and Chweya, 1997).

This medicinal value is mainly attributed to the alkaloidal contents of the plants. *S. nigrum* is especially known for its toxicity because it contains solanine, a neurotoxic glycoalkaloid (Abbas, 1998). Flavonoids are polyphenolic compounds that occur widely in foods and medicines of plant origin. They are extremely important because of their health effects. Quercetin and its derivative glycosides make up most of the flavonoid content in *S. nigrum*[11]. But the reports mostly did not take into account the morphological taxonomic complication associated with *S. nigrum* Complex and hence caused uncertainty. Waxes had consistently been found to contain hydrocarbons, alcohols, fatty acids, esters, aldehydes and ketones (Tulloch and Hoffman, 1971; Bianchi *et al.*, 1979; Reynhardt and Riederer, 1994; Koch *et al.*, 2005). Alkaloids, flavonoids and epicuticular waxes are said to be excellent taxonomic markers by a number of researchers (e.g. Izaddoost, 1975; Tetenyi, 1987; Greinwald *et al.*, 1995; Jamil *et al.*, 2007; Dinchev *et al.*, 2008; Loaiza *et al.*, 2008). [12-14]. (Evans et al., 1975; Griffiths et al., 1999; Tsydendambaev et al., 2004).

High performance liquid chromatography (HPLC) is famous for its precise qualitative and quantitative analyses of steroidal glycoalkaloids (SGA) Sotelo and Serrano (2000) and flavonoids glycosides (Wang and Li, 2007). Gas chromatography-Mass spectrometry (GC-MS) technique is cited (Suau *et al.*, 2002) for the easy determination and identification of alkaloids aglycones, flavonids aglycone and epicuticular waxes in addition to the application of the technique to chemotaxonomic studies. In one study[18], it was used to characterize the flavonol aglycons in tomatoes (*Solanum lycopersicum* L.). Also GC-MS was claimed to be useful in chemosystematics helping, for example, to characterize species on the basis of their cuticular wax[15,20].

Three taxa belonging to *S. nigrum* Complex viz.: *S. americanum* Mill., *S. nigrum* L. and *S. villosum* Mill. had been reported in Pakistan [2]. *S. chenopodioides* Lam. and *S. retroflexum* Dunal are two other species that were found growing wild in and around the Botanical Garden, GC University, Lahore. On the basis of their morphological characters, classification of these taxa as being different varieties of *S. nigrum* or distinct species is controversial among the taxonomists. It is essential, for human health safety and quality control of the herbal medicine, to develop efficient methods for species identification/ delimitation. Their chemical analysis must be carried out to find out the relationship between them. We could not find any report on the analysis of alkaloids, flavonoids and epicuticular waxes of *S. nigrum* Complex by HPLC and GC-MS and their utilization in its chemotaxonomy. So we aimed to study their secondary metabolites profiles to search out the boundaries between the five taxa using the chemotaxonomy to help resolve the International morphological taxonomic controversy on *S. nigrum* Complex and to investigate the medicinal potential of each taxa.

II. EXPERIMENTAL

A. Plant material

Plant samples of morphologically different five plant taxa of *Solanum nigrum* Complex were grown under controlled conditions in Botanical Garden of GC University Lahore, Pakistan, each in specified area and third accession of each was taken (approx. 1 Kg each) at flowering-seeding stage for chemotaxonomic investigation. Voucher specimens were authenticated by Dr. Zaheer-ud-din Khan and deposited in Dr. Sultan Ahmad Herbarium of GC University Lahore, Pakistan.

B. HPLC analysis of steroidal glycoakaloids

The glycoalkaloids were analysed using HPLC apparatus consisting of Shimadzu LC-10A system as detailed previously. HPLC conditions were set as described by Sotelo and Serrano (2000) for *S. tuberosum* except that the buffer used was Ammonium dihydrogen Phosphate with pH 6.1 min and the UV absorbance detector was set at 205 nm. The mobile phase was acetonitrile-0.05 M and Ammonium dihydrogen Phosphate buffer (30:70 v/v). Solvent flow rate was 1.5 mL/. The injector loop was 20 µL. Mobile phase was prepared fresh, sonicated and filtered through a 0.45 µm polyamide filter. Solasonine, α-Solamargine, β-Solamargine and α-Solanine were used as internal standards. Results were presented in Table 5.

The validity was tested with glycoalkaloid standards and for a recovery study, different amounts (0.12-0.30 mg in triplicate) were added to 15 g of dried samples. The detector peak area response was linear over this range. The mean recoveries obtained from triplicate samples were 99.2 ± 0.47 to 99.6 ± 0.54%.

C. GC-MS analysis of alkaloid aglycones

Steroidal glycoalkaloid aglycones (SGAA) were obtained by the method reported by Laurila *et al.* (1999). The aglycon extracts were then evaporated to dryness. The SGAA derivatives were analyzed by the method recommended by Laurila *et al.* (1999), for *Solanum* species using Shimadzu GC-MS QP2010A system operating at an ionization voltage of 70 eV (EI mode) with ion source temperature of 180 °C. Samples were analyzed on an NB-54 fused-silica capillary column (15 m, 0.20 mm i.d., Nordion, Finland) using split sampling mode and an oven temperature of 180−285 °C heated at 7.5 °C/min. A 1 µL sample was taken for GC/MS analysis. Injector and detector temperatures were 285 °C. Helium was used as the carrier gas (flow rate = 0.5 mL/min). Identification of the aglycones in the plant materials was based on the GC-MS spectra of TMS derivatives of authentic standards. Concentrations of SGAA calculated were given in Table 6.

D. HPLC analysis of flavonoids glycosides

Two gradients were applied as described in an optimized method (Wang and Li, 2007) for the analysis of flavonoid glycosides. The standards used were quercetin-3-glucoside (isoquercitrin) and quercetin-3-galactoside. 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 and a Rheodyne injection valve with a 20 µL loop was used. Chromatographic separation was performed using a Merck C-18 column (250×4.6, i.d., 5 µm particle size). Injection volume was 20 µL. Samples were run for 30-50 min. Results were presented in Table 9.

Validity of the extraction procedure was assessed by measuring the peak area variation for flavonoid standards peaks in three replicate analyses. The RSD values for the peaks were less than 1%, which is quite acceptable for quantification.

E. GC-MS analysis of flavonoids aglycones

The standard procedure used for the hydrolysis of quercetin glycosides in *Solanum lycopersicum* L. has been described by Hertog *et al.* (1992). Each of the sample solution was filtered using 0.45µ polyamide filters (Sartorius, Germany) and was degassed by sonication for 3 min before injection.

GC-MS analysis was carried out using the conditions modified from the method of Tokusoglu *et al.* (2003) used for the characterization of quercetin aglycon from hydrolyzed extract of *Solanum lycopersicum* L. GC-MS spectra were recorded on Shimadzu GCMS-QP2010A system in EI mode (70eV) equipped with a split/splitless injector (280^{o}C), at a split ratio of 30/70 using DB-5MS column. Helium was used as a carrier gas at the rate of 1mL/min. 1 µL of sample was injected keeping ion source temperature 200°C and interface temperature at 250°C. The column temperature was kept at 100°C for 1 min after injection and then increased at the rate of 10°C min^{-1} to 275^{o}C which was held for 20 min. Standard stock solution 500 µg/mL of quercetin was prepared in methanol and the calibration curve was established using five dilutions of the standard solution in the concentration range of 0.1-2.0 µg/mL. R^2 value was 0.99. Concentration of quercetin aglycone was calculated as given in Table 10.

F. GC-MS of epicuticular waxes

The composition of the waxes was established using GC-MS technique with the method modified from that reported by Tulloch and Bergter (1981). GC-MS analyses were performed on a Shimadzu GCMS-QP2010A system given above in EI mode (70eV) equipped with injector at 250 ^{o}C, using DB-5MS column. Samples were injected at 250 ^{o}C with a split ratio of 50/50. Injection volume was 1 µl and electronic pressure programming was used to maintain a constant flow (0.67 ml/min) of the Helium carrier gas. The oven temperature was programmed from 150 ^{o}C (4 min) to 320 ^{o}C at a rate of 2 ^{o}C/min and held at this temperature for 2 min. The mass spectrometer was set to scan the mass range 40 amu to 600 amu with ion source temperature 200 ^{o}C and interface temperature 250 ^{o}C. Analyses were performed in triplicate with a blank run after every analysis. The resulting data was processed using Shimadzu Lab Solution GCMS Postrun Analysis software. The relative apparent percentage of each compound and of their classes was determined by area normalization method (Table 12 & 13). Compounds were identified by comparing the mass fragmentation pattern of the reported data and NIST 147 and NIST 27 libraries. Statistical analysis of the 34 compounds identified was carried out by Multivariate Cluster analysis using Minitab 3.2 Statistical software.

III. Results & Discussions

A. HPLC analysis of steroidal glycoakaloids

All the five taxa showed much similar SGA profile on HPLC. This is due to the fact that these taxa belong to the genus *Solanum* which is very well-known for the presence of SGA. So to make a chemotaxonomic comparison the quantitative analysis was required.

There had been many reports on the SGA of different species of genus *Solanum* but not a single one on *S. nigrum.* Therefore, different reported conditions that can affect selectivity of SGA of *Solanum* appreciably were applied. The best results were obtained by the method of Sotelo and Serrano (2000) so it is discussed here. Based on the relative areas obtained in the chromatograms, greater signal intensities were seen for standard analytes at 205 nm so it was selected for analysis. For this study, Solasonine, α-Solamargine, β-Solamargine and α-Solanine are the SGA of interest to our studies. The SGA were, therefore, further analysed qualitatively and quantitatively by the HPLC of the alkaloids extracted using the standard compounds. Four of the peaks were tentatively identified on the basis of retention time and peak response when standards were added (one by one) in each sample. The Solasonine, α-Solamargine, β-Solamargine and α-Solanine contents of each taxon is given in Table 1. According to our study β-Solamargine levels varied among different taxa of *S. nigrum* Complex (1.69-9.8 mg g^{-1}). Its concentration in *S. villosum* was distinctly higher than other taxa specially in contrast to *S. americanum* in which it was not detected. The α-Solamargine was detected at lower levels than the β-Solamargine. Infact it had not been detected in *S. chenopodioides* but its level in *S. nigrum* was slightly higher than other samples. Solasonine and α-Solanine were detected in all taxa with less concentration variations.

Statistical comparison (Fig. 1) of the taxa segregated *S. americanum* and *S. chenopodioides* more early than others but with a less similarity index. Then *S. nigrum* and *S. retroflexum* join this group at almost similar position. But *S. villosum* was unique and depicted very low similarity with rest of the taxa.

B. GC-MS analysis of alkaloid aglycones

The steroidal glycoalkaloid aglycones of interest to this work will be referred to as SGAAs. Only two SGAAs were important to our work, namely, solanidine and solasodine. Solanidine produced mono-TMS derivatives with molecular ion peaks at m/z 469. In its GC-MS spectrum, solasodine showed the di-TMS derivative after silylation with base peak at m/z 125 and at m/z 559 [M++2H+]. Quantification of aglycones was carried out using an external standard calibration method. The principal glycoalkaloid present in all taxa was solasodine with a percentage range of 66.94-85.67% (Table 2). Solanidine concentratration was much lower ranging from 8.85-20.31%. Solanidine is reported to be toxic so care must be taken in using *S .americanum* in herbal medicine and as food. Calibration was performed by injecting standard mixtures of solasodine and solanidine at levels ranging from 4 to 200 mg/L. Good linearity of response was found for solanidine and solasodine this concentration range belonging to cited interval, with correlation coefficients greater than 0.995.

Cluster analysis (Fig. 2) separated the taxa into three main groups. *S. nigrum* and *S. retroflexum* formed a much closely related group. *S. chenopodioides* and *S. villosum* constituted another group but with slight less similarity index. However *S .americanum* showed a characteristic behavior of its own with highest percentage of solasodine and lowest of solanidine, so it aligned distantly with the above two groups.

C. HPLC analysis of flavonoids glycosides

HPLC of the flavonoid glycosides was performed using two different gradient systems (Table 3).

TABLE I.
CONCENTRATION OF SGA IN *S. NIGRUM* COMPLEX

Compound	Concentration in species (Code)[a] (mg/g)				
	SA	SC	SN	SR	SV
β-Solamargine	Nd[b]	2.53	1.69	4.78	9.8
α-Solamargine	1.96	Nd	5.03	1.45	1.17
Solasonine	3.5	4.4	5.8	2.9	2.01
α-Solanine	3.29	3.08	4.7	5.7	1.5

[a] Abbreviation of species; SA: *S. americanum*, SC: *S. chenopodioides*, SN: *S. nigrum*, SR: *S. retroflexum*, SV: *S. villosum*
[b] Nd: not detected

TABLE II.
SGAA CONCENTRATION IN *S. NIGRUM* COMPLEX

Plant	Retention time[a] (min)		Percentage of aglycones[b] (%)	
	Solanidine	Solasodine	Solanidine	Solasodine
SA	21.613	26.645	8.85	85.67
SC	25.773	26.645	11.08	66.94
SN	25.779	29.843	16.03	75.90
SR	24.918	26.662	20.31	71.76
SV	25.337	28.672	10.15	74.70

[a] Results were presented as mean (n=3)
[b] Determined by area normalization method

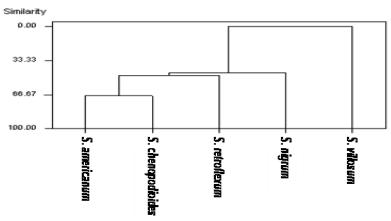

Figure 1. Affinity relationships among different taxa of *S. nigrum* Complex based on the distribution of SGA

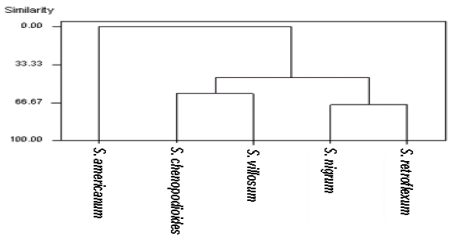

Figure 3. Affinity relationships among different taxa of *S. nigrum* Complex based on the distribution of SGAA

Gradient I: Detection of quercetin glycosides was carried out but the response of two standards was not linear. Therefore this gradient could not be used for quantification of these glycosides.

Gradient II: This gradient system was used for the quantification of flavonoids from the plant extracts. A calibration curve was plotted using peak areas against five different concentration levels (2.0-20 mg/20µL) of standards (quercetin-3-glucoside and quercetin-3-galactoside) and the concentration of flavonoid glycosides was determined (Table 9). The highest concentration of Quercetin-3-glucoside was observed in *S. americanum* and the lowest in *S. retroflexum*. Level of Quercetin-3-galactoside was highest in *S. americanum* and lowest in *S. chenopodioides.*

Concentrations of these two quercetin glycosides helped comparing the taxa statistically. *S. nigrum* and *S. retroflexum* resembled much closely and were first to segregate as a cluster. This is because of resemblance in their glycosides concentrations. But *S. villosum* and *S. chenopodioides* formed another cluster having slightly lower similarity index as of the previously discussed cluster. These two clusters were distantly related to *S. americanum* which showed relatively higher concentration of the quercetin glycosides under study (Fig. 13).

D. GC-MS analysis of flavonoids aglycones

GC-MS has been used in a number of occasions for the analysis of flavonoids in plant (Tokusoglu *et al.*, 2003). Quercetin in its different glycosidic forms has been the only flavonol reported from *S. nigrum* Complex (Nawwar *et al.*, 1984). The presence of quercetin aglycone in the hydrolyzed extracts of the five taxa under study was confirmed by GC-MS.

Retention time of quercetin standard was 13.012 ± 0.001 min. It showed molecular ion peak of *m/z* (relative intensity in %) at 304 (22.8), base peak at 153 (100) with other characteristics peaks at 286 (4.0), 275 (27.6), 195 (2.8), 165 (12.4), 152 (24.4), 150 (21.2) and 123 (42.4). Quercetin in the samples was detected by spiking the samples with 0.5 µg/mL of standard quercetin solution and identified on the basis of comparison with the retention time and mass fragmentation pattern of the standard, and of the mass spectrum with the data from NIST 147 and 27 Libraries linked to the mass detector. The extreme variation in its percentage composition in the extracts from the taxa grown, harvested and analyzed under similar conditions decides its part in their chemotaxonomy (Table 10). Comparing the area percentage, quercetin made up only 7.28% of the hydrolyzed flavonoid extract of *S. nigrum* and this continued to increase irregularly while moving to *S. retroflexum*, *S. villosum* and *S. chenopodioides*. But *S. americanum* stands alone in the group with such a high percentage (92.92%). The calibration curve of standard was linear with R^2 value of 0.99 in the concentration range of 0.1-2.0 µg/mL. Amount of quercetin varied from 3.06 ± 0.01 to 6.46 ± 0.01 mg/100g of plant (Table 10). Yang *et al.* (2008), reported the quercetin content of *S. nigrum* and *S. villosum*. Our results were in agreement with this study for *S. nigrum* (3.7 mg/100g) but there was contradiction for *S. villosum* (18.1 mg/100g).

The percentage variation of the flavonoids observed in the five taxa by GC-MS analysis was used to group them statistically. Here again *S. nigrum* and *S. retroflexum* formed a closely related cluster with a high similarity index showing much similarity in their flavonoid profiles. *S. chenopodioides* and *S. villosum*, although not so closely related to one another as compared to the previously discussed cluster, made another cluster. *S. americanum* aligned more distantly with above mentioned clusters (Fig. 14).

TABLE III.
FLAVONOIDS GLYCOSIDES CONCENTRATION IN *S. NIGRUM* COMPLEX

Plant	Retention time[a] (min)		Concentration[b] (mg/100mL)	
	Quercetin glucoside	Quercetin galactoside	Quercetin glucoside	Quercetin galactoside
SA	15.347	16.535	0.03520	0.00750
SC	15.089	16.992	0.00725	0.00051
SN	15.346	16.524	0.00550	0.00252
SR	15.265	16.817	0.00450	0.00256
SV	15.270	16.480	0.00957	0.00063

[a] Results were presented as mean (n=3)
[b] Determined from the calibration curves of standards

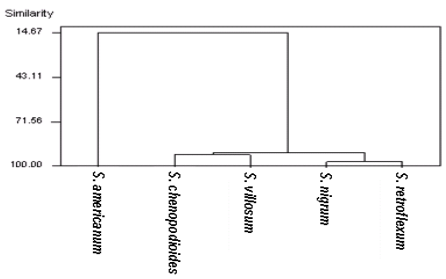

Figure 4. Affinity relationships among different taxa of *S. nigrum* Complex based on the distribution of flavonoid glycosides

TABLE IV.
QUERCETIN CONCENTRATION IN *S. NIGRUM* COMPLEX

Plant	Rt[a] (min)	Quercetin concentration	
		%age in extract[b]	mg/100g of plant[c]
SA	13.019	92.92 ± 0.45	6.46 ± 0.01
SC	13.008	38.31 ± 0.23	3.81 ± 0.02
SN	13.011	7.28 ± 0.33	3.06 ± 0.01
SR	13.013	13.03 ± 0.38	3.18 ± 0.01
SV	13.012	23.94 ± 0.28	4.91 ± 0.01

[a] Results were presented as mean (n=3)
[b] Determined by area normalization method
[c] Calculated from the calibration curve

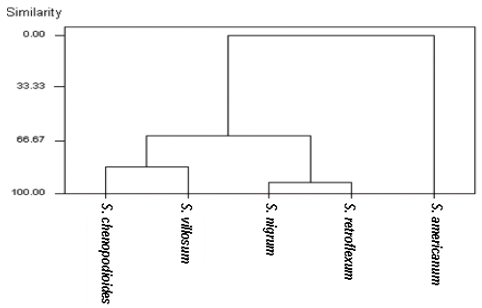

Figure 5. Affinity relationships among different taxa of *S. nigrum* Complex based on the distribution of flavonoid aglycones

E. GC-MS of epicuticular waxes

Epicuticular wax from each of the five taxa was extracted using *n*-hexane. The procedure used extracts only epicuticular wax without disturbing the internal chemical makeup (Medina *et al.*, 2006). Different classes of compounds were found by applying GC-MS analysis. In addition to the four compounds which had been reported previously (Hanna *et al.*, 1996), 30 more compounds were detected in quantifiable amounts in the five plant species analysed (Table 13).

1. Alkanes and Alkenes: In the present study, unlike the other plant species analysed, 1-chloroalkanes of C21, C25, C27 and aromatic hydrocarbons were also found in *S. americanum* (Table 13). The individual *n*-alkanes were composed of C14 to C28. The alkanes C15, C16, C18, C19, C20, C21, C23, C24 C25, C27 and C28 were found common in all the samples in varying amounts. But the alkanes C14 and C17 showed selective occurrence. The taxa can be distinguished from one another by the distribution of the *n*-alkanes, taking into account the two main alkanes (Skorupa, *et al.*, 1998), e.g. by quoting one main alkane outside and the second main alkane inside the parenthesis as: *S. chenopodioides* — C17 (C16); *S. nigrum* — C27 (C25); *S. retroflexum* — C20 (C18); *S. villosum* — C27(C18). The occurrence of the individual alkane was almost the same in *S. chenopodioides* and *S. villosum* qualitatively and matched closely with the alkanes of *S. nigrum* and *S. retroflexum*, but there were marked quantitative variations.

2. Alcohols, Aldehydes and ketones: Free alcohols are widespread components of plant waxes (Bianchi, 1995). In the present study, there was not much variety in long chain alcohol types. Phytol was detected in the four taxa and it accounted for 19.24% of the total wax in *S. chenopodioides*. Two more unsaturated alcohols, 3,13-Octadecadiene-1-ol and 9-Eicosen-1-ol, were found in *S. villosum* in minute quantities. In addition to 9-Eicosen-1-ol, *S. retroflexum* also showed the presence of *n*-Cetyl alcohol and an aldehyde that were not found in other taxa. Alcohols were not detected in *S. americanum*, instead 3-hydroxyspirost-8-en-11-one (14.07%) was detected (Table 13).

3. Free fatty acids and esters: Free aliphatic fatty acids are common components of leaf waxes, but are usually present in low concentrations (Bianchi, 1995). Stearic acid, detected in small quantity in the four taxa, matched in quantity in *S. chenopodioides*, *S. nigrum* and *S. retroflexum* but was more than double in *S. villosum*. α-Linolenic acid was also detected in reasonable amount (9.81%) in *S. villosum* and in a trace amount in *S. nigrum* but not found in detectable amount in other three taxa. Its methyl ester (0.63%) was also detected in *S. villosum*. γ-Linolenic acid (19.68%) was present in *S. villosum* only. Palmitic acid was previously reported in *S. nigrum* (Hanna *et al.*, 1996) and *S. tuberosum* (Szafranek and Synak, 2006) and seems to be a characteristic acid of genus *Solanum*. Concentration of Palmitic acid was relatively high in *S. villosum* (24.04%) and here its methyl and ethyl esters were also detected though in small amounts. *S. americanum* showed the unique presence of chlorinated ester of acetic acid in small quantity (Table 13).

Statistical analysis: Statistical analysis of the data presented in Table 13 was carried out using Minitab 3.2 Statistical Software. By cluster analysis (Fig. 15), the two samples *S. nigrum* and *S. retroflexum* were first to segregate. They showed many similarities in the distribution of epicuticular wax constituents except some minor differences like presence of 10-Methyl-1-octadecene, *n*-Cetyl alcohol and 9-Eicosen-1-ol in *S. retroflexum* that were not detected in *S. nigrum*. Also Tetradecane and Palmitic acid were present in trace amounts in *S. nigrum* but not detected in *S. retroflexum*. *S. americanum* deviated from the average distribution pattern of wax components due to unique occurrence of a ketone, aromatic hydrocarbon, chlorinated hydrocarbon and chlorinated ester and the absence of free hydrocarbons, some fatty acids and some esters occurring in other taxa. These features aligned *S. americanum* more distantly with above mentioned cluster as shown in Fig. 15. *S. chenopodioides* and *S. villosum*, although not much closely related to one another as compared to the previously discussed cluster, made another cluster. Their epicuticular wax showed all the hydrocarbons, Squalene, Phytol, Palmitic acid, Stearic acid and ester of Palmitic and oleic acid with different amounts. However, eight such compounds were present in *S. villosum* that were not detected in *S. chenopodioides*.

TABLE V.
DISTRIBUTION OF DIFFERENT COMPOUNDS IN WAX OF *S. NIGRUM* COMPLEX

Parameter	Percentage in species[a]				
	SA	SC	SN	SR	SV
Alkanes	-	42.19	15.48	23.58	15.06
Chloroalkanes	1.45	-	-	-	-
Aromatic hydrocarbons	2.07	-	-	-	-
Alkenes	24.86	1.76	13.52	12.1	0.83
Alcohols	-	19.24	7.04	12.85	2.72
Free fatty acids	1.18	6.1	4.24	5.15	67.37
Esters	53.91	30.7	59.72	46.34	11.25
Chloroesters	2.45	-	-	-	-
Aldehydes	-	-	-	-	2.78
Ketones	14.07	-	-	-	-

[a] Expressed as relative apparent percentages

IV. CONCLUSION

This research work attempted to identify the taxa related to the *S. nigrum/* black nightshade more accurately, by providing their chemical composition as identification key. The similarity index and the Euclidean distance among the clusters formed by Multivariate cluster analysis of the above discussed parameters helped drawing the conclusion that *S. americanum*, *S.*

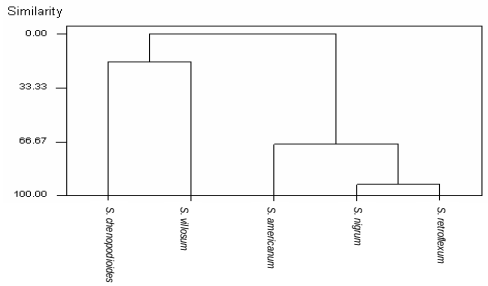

Figure 6. Affinity relationships among different taxa of *S. nigrum* Complex based on the distribution of flavonoid aglycones

chenopodioides, *S. nigrum* and *S. villosum* are distinct species of genus *Solanum* but *S. retroflexum* might be regarded as a variety/subspecies of *S. nigrum*. Because of the taxonomic misunderstanding surrounding the component species and the tendency to refer to all members as '*S. nigrum*', it is advisable that the information found in literature should be reinterpreted and any medicinal/commercial use of the taxa should be carried out in the light of above chemotaxonomic suggestion. While the taxonomic account compiled for this document covered a revision of the species concerned, it is hoped that it will stimulate continued biosystematic work on material of known origin so that these 'Solanums' can be understood, controlled and utilized appropriately throughout the world.

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