

PHENETIC ANALYSIS OF MEDICINALLY IMPORTANT SPECIES OF THE GENUS *SOLANUM* FROM PAKISTAN

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Abstract

Solanum is one of the largest and hyper diverse genera of the family Solanaceae. In Pakistan *Solanum* is represented by 15 species, of which 11 species have the medicinal properties. Taxonomically this is a complex genus because of the presence of number of hybrid and controversial taxonomic status of *S. nigrum* complex. In the present study numerical techniques were utilized to evaluate the taxonomic status of the genus *Solanum*. Cluster analysis was employed to work out the relationship among the taxa of the genus *Solanum*. The Euclidean distance measured similarity matrix and a dendrogram was constructed by using the complete linkage method. This analysis showed that all the species of genus *Solanum* can easily be divided into two groups at hundred percentage linkage distance. Co-relation of quantitative characters showed that floral characters had highly significant relationship with the stem characters, these characters plays a significant role in the identification of the species of the genus *Solanum*.

Introduction

Solanum is one of the major genera of a cosmopolitan family Solanaceae. Though the species of this genus are distributed throughout the world, they occur in their greater concentrations in tropical and warm temperate regions with centers of diversity occurring in the Southern Hemisphere, particularly in South America. Other center of speciation occurs in Australia and Africa, with relative few and less diverse species being found in Europe and Asia (Symon 1981; D'Arcy 1991; Hawkes *et al.*, 1992).

Most of the taxonomic information of the genus relies on morphological markers. All taxonomists are agreed that the differences between plants and the similarities that plants may possess in common are measurable to large degree by the morphological characters of those plants (Lawrence, 1971). The value of morphological characters is measured by its consistency. The more constant the character greater is the reliability that can be placed on it (Lawrence, 1971). When the plants are highly variable and contain large number of hybrid, identification based on morphological characters is quite difficult. There are many taxonomic problems with the medicinally important species of the genus *Solanum*. Hence morphological markers as used in past are insufficient for their correct and proper identification. *Solanum nigrum*, *Solanum americanum* and *Solanum villosum* are three medicinally important species of *Solanum* genus. In the past their taxonomic status remained highly controversial. Clarke (1885) did not mention *S. nigrum*, *S. villosum* and *S. americanum* separately; however he considered morphological characters of three species only for *S. nigrum*. Hawkes & Edmond (1972) gave the rank of subspecies to *S. villosum*, Nasir (1985) considered *Solanum nigrum* as species and *villosum* as the variety while Jennifer & James (1997) gave rank of species to all of these. Morphologically these species are closely related. There are only a few morphological characters to distinguish them.

Table 1. Medicinally important species of genus *Solanum* from Pakistan.

S. No.	Species names	Distribution in Pakistan	Phyto geographical zone
1.	<i>S. nigrum</i>	Rawalpindi, Attock, Mianwali, Gujranwala, Mansehra, Hazara, Peshawar, Chitral, Gilgit, Bannu, Quetta, Dera Nawab Khan, Sibbi, Mirpur, Muzaffarabad	Saharo-Sindian Sino-Japanese
2.	<i>S. villosum</i>	Kotli, Muzaffarabad, Poonch, D. I. Khan, Multan, Quetta, Rawalpindi, Murree, Attock, Jhang, Rahim Yar Khan, Lahore, Bannu, Hazara, Gilgit, Sibi.	Saharo-Sindian Sino-Japanese
3.	<i>S. americanum</i>	Muzaffarabad, Poonch, Rawalpindi, Attock, Sahiwal, Sargodha, Lahore, Chitral, Sibbi, Hyderabad, Larkana	Saharo-Sindian Sino-Japanese
4.	<i>S. erianthum</i>	Rawalpindi, Hazara, Mirpur, Muzaffarabad, Poonch	Saharo-Sindian Sino-Japanese
5.	<i>S. torvum</i>	Bannu	Saharo-Sindian
6.	<i>S. surattense(p)</i>	Lahore, Mianwali, Rahim Yar Khan, Rawalpindi, D.I.Khan, Attock, Gujranwala, Jhang, Jhelum, Hazara, Kohat, Poonch, Khuzdar, Makran, Quetta, Loralai, Karachi,	Saharo-Sindian Sino-Japanese
7.	<i>S. surattense (W)</i>	Rawalpindi, Hazara	Saharo-Sindian Sino-Japanese
8.	<i>S. incanum</i>	Rawalpindi, Sargodha, Attock, Jhelum, Kohat, Hazara, Swat, Mirpur, Kotli, Muzaffarabad	Saharo-Sindian Sino-Japanese
9.	<i>S. melongena</i>	Kotli, Mianwali, Khyber, Hyderabad, Quetta, Skardu cultivated through out the Pakistan	Saharo-Sindian Sino-Japanese
10.	<i>S. pseudo-capsicum</i>	Rawalpindi, Hazara, Dir, Abbottabad, Poonch	Saharo-Sindian Sino-Japanese
11.	<i>S. anguivii</i>	Rawalpindi and Islamabad	Saharo-Sindian
12.	<i>S. cordatum</i>	Attock, Jhelum, Hyderabad, Kohat, Karachi	Saharo-Sindian

Recently taxonomist starts to use number of techniques to solve the taxonomic confusion which could not be resolved by morphological markers. One of these could be the use of various computer software for the better understating of phylogeny of Angiosperms (Gilani *et al.*, 2003). The main objective of the present study was to evaluate the taxonomic problems of the genus *Solanum* by using numerical technique based on the concept of “affinity” which can be measured by considering all characters.

Materials and Methods

Morphology: The taxonomic characters of the medicinally important species of the genus *Solanum*, were studied from the specimens of the major herbaria of Pakistan including Quaid-i-Azam University, Herbarium (ISL), National Herbarium, NARC, Islamabad (RAW) and Pakistan Museum of Natural History (PMNH), Karachi University Herbarium, Karachi (KCH). About 500 specimens of 11 different medicinally important species of the genus *Solanum* (Table 1) were studied.

Taxonomically important morphological characters were examined under the dissecting microscope. These characters are of two main types i.e., 1. Vegetative (Characters related to root, shoot and leaf) and 2. Reproductive (Characters related to inflorescence, flower, fruit and seeds).

Numerical analysis: For the numerical analysis 12 operational taxonomical units (OTU) were selected. This selection was based on the morphological variation. Forty-three morphological characters were identified that appear to show variation between OTU's. Characters were coded according to the method of the Boratynski & Davis (1971). For the most of the quantitative characters there was considerable range of variation. Therefore minimum and maximum values were taken as separate characters. The data matrix or the spreadsheet was prepared in Microsoft Excel, so that each row represents one taxon and each column represents one character (Yousaf *et al.*, 2004). The final matrix was subjected to the analysis. Cluster analysis is employed to work out the relationship among these taxa. The Euclidean distance measured similarity matrix and a dendrogram was constructed by using the complete linkage method. Using the Statistical (version 5.0), Excel and SPSS 10.0 (Statistical Procedure for Social Science) computer package did this analysis. By using the same software co-relation between the quantitative characters to know the extent to which they are contributing in the identification of species was calculated.

Results

Key to medicinally important species of the genus *Solanum*

- 1a: Herbaceous, 40-100cm in height 2
- 1b: Under shrub-shrub, 100-300cm in height 6
- 2a: Prostrate herbs, stellate hairs, stem prickly and much branched 3
- 2b: Erect herb, prickles absent, hairs simple 4
- 3a: Flowers blue, stamens five, filament shorter than style *S. surattense*
- 3b: Flowers white, stamens 4-5, filaments longer than style *surattens acc.* 11612
- 4a: Stem purplish green, anthers winged *S. nigrum*
- 4b: Stem greenish brown, anthers simple 5
- 5a: Procumbent herb, 18-50cm long, Peduncle and petiole are equal in length flower yellow white. Berries orange *S. villosum*
- 5b: Erect herb, 37-80cm long, Peduncle is longer than petiole, flower white. Berries black *S. americanum*
- 6a: Undre shrub, stem cylindrical, flowers oblong 7
- 6b: Herbaceous- shrub, stem solid, flowers oval-lanceolate 8
- 7a: Stem green, flower violet, petals glabrous *S. melongena*
- 7b: Stem whitish brown, flower blue, petals hairy, hairs dense on margin
..... *S. incanum*
- 8a: Leaf colour same on the both side, petiole pubescence, hairs simple, seed sub reniform 9
- 8b: Leaf bifacial, petiole pubesent, stellate hairy, seed discoid 11
- 9a: Stem prickly, stellate hairs on leaves 10
- 9b: Stem smooth, leaves glabrous *S. pseudo-capsicum*
- 10a: Leaves orbicular -oblong, Inflorescence terminal/lateral, flower colour purplish
..... *S. cordatum*
- 10b: Leaves ovate-oblong, inflorescence axillary, flowers bluish purple *S. anguivii*
- 11a: Erect herb, leaf margin smooth-undulate, inflorescence terminal cymose, flowers white *S. erianthum*
- 11b: Shrub, leaf margin lobbed, inflorescence paniculate cymose, flowers pale white in colour *S. torvum*

Discussion

Solanum is one of the largest and hyper diverse genera of the family Solanaceae (Jennifer & James, 1997). In Pakistan *Solanum* is represented by 15 species, of which 12 species have the medicinal properties (Nasir, 1985). Geographical distributions of the *Solanum* species indicate that it is a bioregional genus, and found in two pytogeographical zones Saharo-Sindian and Sino-Japanese (Table 1). Most of the taxonomic information accumulated so far is based solely upon morphometry, and it has left many issues unresolved.

Morphological characters provided fundamental basis for the classification of *Solanum* are stem, leaf, flower, fruit and seed mostly characterize this family. Plant habits, stem structure and colour, petiole status, petiole pubescence, leaf shape, leaf margin, leaf apex, leaf base, flower shape, flower colour, fruit shape, fruit type and seed colour are greatly helpful in the identification of the species (Symon, 1985; Nasir, 1985; Pojarkova, 1997). In the identification of *Solanum* species qualitative characters play key role as compared to quantitative characters. Co-relation of the quantitative character was calculated and it was found that floral characters have highly significant relation (Table 2). Therefore they have significant contribution in the taxonomy of the genus *Solanum*. It is clear from the comparison of different medicinally important species of family Solanaceae based on quantitative characters that these characters are important morphological markers for inter generic studies. However they become least important for intra generic studies. Within the genera variation among the quantitative characters was least therefore it is difficult to differentiate species of same genus on the basis of quantitative characters (Symon, 1985; Hawkes & Edmond, 1972).

Table 2. Co-relation of quantitative characters of medicinally important species of the genus *Solanum*.

Characters	PH	PL	LL	LW	PeL	SL	SW	PetL	PetW	FL	AnL	FrL	SdL
PH	1.00												
PL	-0.07												
LL	0.45*	0.42*											
LW	0.21	0.43*	0.72**										
PeL	0.37	0.01	0.47*	0.19									
SL	-0.05	0.43*	0.23	0.44*	-0.06								
SW	-0.06	0.26	0.07	0.39	-0.05	0.86**							
PetL	-0.03	0.49*	0.21	0.48*	-0.04	0.98**	0.88**						
PetW	0.00	0.39	0.21	0.46*	-0.09	0.95**	0.86**	0.97**					
FL	0.01	0.37	0.35	0.37	-0.03	0.86**	0.50**	0.77**	0.76**				
AnL	-0.04	0.66**	0.25	0.39	-0.17	0.80**	0.53**	0.82**	0.79**	0.79**			
FrL	0.09	0.35	0.07	0.04	0.20	0.41*	0.22	0.41*	0.36	0.43*	0.60**		
SdL	-0.07	0.56**	0.20	-0.10	0.13	0.37	0.09	0.38	0.33	0.48*	0.58**	0.33	1.00

Significant level* = 0.404, Highly significant level** = 0.515, PH= Plant height, PL= Petiole length, LL= Lamina length, LW= Lamina width PeL= Peduncle length, SL= Sepal length, SW= Sepal width, PetL= Petal length, PetW= Petal width, FL= Filament length, AnL= Anther length, FrL= Fruit length, SdL= Seed length

Solanum nigrum, *S. americanum* and *S. villosum* are three medicinally important species of genus *Solanum* (Jennifer & James, 1997). In the past their taxonomic status remained highly controversial. Clarke (1885) did not separate them and considered all of three species as *S. nigrum*. Hawkes & Edmond (1972) gave the rank of subspecies to *S. villosum* of *S. luteum*, however they considered *S. americanum* as the separate species. Baytop (1978) was in favor to take *S. villosum* as sub species of *S. nigrum*. Nasir (1985) considered *Solanum nigrum* as species with two varieties (*nigrum* and *villosum*). He totally neglected the *S. americanum*. While Jennifer & James (1997) gave rank of species to all of these. Morphologically these species are very much similar. However there are very few morphological markers for distinguishing them. *Solanum americanum* can be distinguished from *S. nigrum* by its smaller seeds, umbellate inflorescence rather than the raciform as in *S. nigrum*, smaller anthers and shiny fruit (Edward, 1990). *S. nigrum* can be separated from *S. villosum* by yellow, orange or red colored fruit, its rotate corolla, white or purplish flowers. Whereas *S. americanum* can be differentiated from *S. villosum* because of purplish colored stem, sub umbellate inflorescence, abaxially pubescent corolla, cup-shaped calyx, short filament and shiny black berry. Another important morphological marker is peduncle length in comparison of petiole length. *S. villosum* has peduncle and petiole of same length, while in *S. nigrum* and *S. americanum* peduncle is longer than petiole (Edward, 1990).

However Rechinger, (1958) findings were contradicted to it. According to him a plant sample with white flowers and black berry must be identified as *S. nigrum*. Whereas Edward (1990) mentioned *S. nigrum* with orange colour fruit. Edward misapplied the name of *S. alatum* Moench for *S. villosum*. As the characters he mentioned for *S. alatum* Moench are exactly the similar possessed by *S. villosum*. *S. alatum* has the same plant habit as that of *S. villosum* but it possess yellow colour flower with thin skin orange berries. Leaf size is also longer in *S. alatum* as compared to *S. villosum*. Hawkes & Edmond (1972) confused *S. villosum* with *S. luteum* and misapplied the name of *S. luteum* subsp *luteum*. Both of these species can be differentiated by Decumbent to erect stem, entire to sinuate-dentate margin, and longer deflexed pedicel. These morphological markers found in *S. luteum* and absent from *S. villosum*.

Solanum surattense is a medicinally important prostrate herb with scattered stellate hairs and prickly stem. It grows widely in Pakistan up to 1300m and also in other parts of world under the similar climatic and geographical conditions (Nasir, 1985). The nomenclature of this species has been remaining controversial in the past. Burmanii (1768) described it first of all and gave the name of *S. surattense*. Latter on taxonomist of different era gave it various names. The most commonly used synonym of *S. surattense* is *S. xanthocarpum*. Schard & Wendelbo gave this name in 1795. Clerk (1885) and Rechinger (1958) followed Schard & Wendelbo (1795).

Phenotypically this species is highly polymorphic. Morphological characters such as point of origin of spines, arrangement of spines, presence of hairs on petiole and stem, flower colour, length of filaments, length of style and presence of stellate hairs on ovary showed polymorphism. Because of this many taxonomist confuse this species with other species of the genus *Solanum*. As Bamber (1916) identified it as *S. indicum*, whereas the character description showed the specimen he examined was *S. surattense*. There was even not a single character which can be separated *S. surattense* from *S. indicum*. Moreover this species is not the part of Pakistani flora, no other taxonomist could record it from Pakistan.

Most commonly found flower colour of *S. surattense* is Purplish however white colour flowers are occasionally present (Nasir, 1985). In the herbaria two different types of samples were studied. A specimen with accessions no 11612, 9447 collected from different part of Potohar flower color is white while the other specimen has purplish blue colour flower. Spines are alternate and one spine arises from one point while in *S. surattense* (P) spines are opposite and more than one spine arises from one point. Stamens are four in number (this is not the permanent characters mostly five stamens are present) while in *S. surattense* (P) stamens are five and carpel is usually longer than stamens but in this variety carpel is smaller than stamens. Anthers are of green colour, whereas in the usual parent this colour is yellow. The end of style and the top of ovary is covered with stellate hairs, however in *S. surattense* (P) style and ovary are smooth. And the last difference is in ovary in purplish flower variant is smooth while variety bears sand like dots due to which it is shinny. These differences indicated to separate these accessions as variety nova from the species *S. surattense*.

A dendrogram was constructed by using all the qualitative and quantitative characters. All the eleven species divided into two groups (Fig. 1). This lower order taxonomy of the *Solanum* genus is different from conventional classification (Table 3). Schenobeck-Temesy (1972) divided the genus into two subgenera and six sections. He placed *S. pseudo-capsicum* and *S. nigrum* together into subgenus *Solanum*, whereas *S. melongena*, *S. incanum*, *S. cordatum* and *S. surattense* into subgenus *Leptostemonum*.

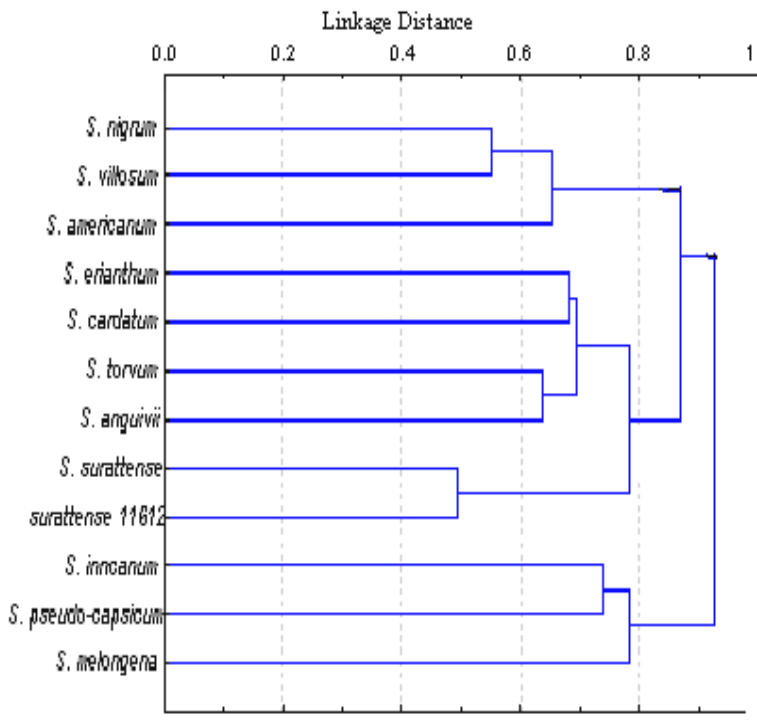


Fig 1. Cluster analysis of medicinally important species of the genus *Solanum* on the basis of morphological characters.

Table 3. Proposed classification of medicinally important species of the genus *Solanum* based on phonetic analysis.

Genus	Subgenus	Species
<i>Solanum</i>	A	<i>S. nigrum</i> , subspp <i>S. villosum</i> , <i>S. americanum</i> , <i>S. anguivi</i> , <i>S. torvum</i> , <i>S. erianthum</i> , <i>S. cordatum</i> , <i>S. surattense</i> , <i>S. surattense</i> var. <i>nova</i>
	B	<i>S. pseudo-capsicum</i> , , <i>S. melongena</i> , <i>S. incanum</i>

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