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MORPHOLOGICAL AND IR FINGER PRINTING OF *SOLANUM* SPP. FROM KERALA

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Solanum, is the largest genus within the Solanaceae with 1000-1200 species. It is rich in steroidal alkaloids such as tomatidine and solasodine and therefore pharmaceutically important. In Kerala *Solanum* is represented by 30 species, of which 15 species were characterized in the present study. Taxonomically *Solanum* is a complex genus due to the presence of a number of hybrids with controversial taxonomic status. In the present study morphological and infrared spectral techniques were utilized to evaluate the taxonomic status of the genus. All the fifteen species are differentiated by using morphological characters like stem, leaf nature, prickles, flower colour and fruit nature. The infra red finger printing analysis provides peaks between 3200-3300 and another unique peak at 1635 suggests *Solanum* alkaloids. In addition there are many other peaks with different size and shape can be employed to discriminate the species of *Solanum.*

Keywords : Analysis; Infra red spectra; *Solanum;* Taxonomic significance.

Introduction

In orthodox taxonomy morphological characters provide fundamental basis for the classification of plants[1]. Solanaceae is an economically and medicinally important family. Specialized features of stem, leaf, flower, fruit and seed mostly characterise 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 for the identification of species. Jennifer and James[2] and Edward[3] used these characters for the differentiation of species. Qualitative characters play a key role compared to quantitative characters for the identification of the species. Comparison of the species on the basis of quantitative characters suggests that these characters are important markers for inter generic levels. However, they become least important at intra generic level studies. Within genera,variation among the quantitative characters are minimum, therefore it is difficult to distinguish species of same genus on the basis of these characters.

Qualitative characters help to differentiate species as they showed high variability among the taxa of *Solanum.* Taxonomically important quantitative characters are sepal length/width, petal length/width, anther length and leaf length/width. These characters have highly significant relationship with each other. The morphological markers such as plant habit, petiole status, fruit type, and pubescence were proved to be an important genus predicting characters. Whereas, leaf shape, floral characters, fruit colour, seed shape, seed colour are also considered important diagnostic characters for the identification of species. However Symon[4] utilized floral characters for the subdivision of Solanaceae into two different groups. Whereas Edward[3] differentiated genera of the family on the basis of floral and fruit characters.

Solanum is one of the largest and hyper diverse genus of Solanaceae[5]. In Kerala *Solanum* is represented by 30 species, of which 15 species have medicinal properties[6]. Most of the taxonomic information accumulated so far is based solely upon morphometry and it created many issues unresolved.

Material and Methods

Plant materials -Fresh specimens of 15 species of *Solanum* were collected (Table 1) in different places from Kerala, India, and were identified by comparison with the voucher specimen from Kerala Forest Research Institute (KFRI, Kerala).One voucher specimen of each species was deposited at the herbarium of the Department of Botany, University College, Thiruvananthapuram, Kerala.

Morphological analysis - Fertile aerial parts of plants were prepared according to standard techniques for herbarium preparation[7]. The morphological parts such as leaves, inflorescence, flower, calyx, corolla, androecium and

Table 1. Distribution of *Solanum* species from Kerala.

Plant name	Places
S.aculeatissimum	Wayanad, Idukki, Kollam
S.capsicoides	Wayanad, Malappuram, Idukki, Kottayam, Alappuzha, Pathanamthitta
S.melongena var. insanum	All districts
S.macrocarpon	Wayanad, Thrissur
S.giganteum	Kasargode, Kannur, Wayanad, Malappuram, Palakkad, Idukki , Pathanamthitta, Kollam,Thiruvananthapuram
S.mauritianum	Idukki
S.wendlandii	Wayanad, Idukki
S.trilobatum	Wayanad, Palakkad, Idukki
S.mammosum	Wayanad, Malappuram, Idukki, Thiruvananthapuram
S.violaceum ssp violaceum	All districts
S.pseudocapsicum	Idukki
S.torvum	All districts
S.nigrum	All districts
S.jasminoides	Idukki
S.virginianum	Kasargode, Kannur, Wayanad, Kozhikkode, Malappuram, Palakkad, Idukki, Kollam, Thiruvananthapuram

gynoecium were analyzed using stereoscopic Olympus microscope.

IR spectral analysis -The dried powdered leaf samples were analyzed by IR spectroscopy to know the functional groups using FTIR Shimadzu Prestige 21. Potassium bromide was used as the standard.

Results and Discussion

Morphological characters such as stem, leaf, flower, fruit and seed provided fundamental features for the identification and classification of *Solanum*. 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[8]. In the identification of *Solanum* species qualitative characters play key role as compared to quantitative characters. Co-relation of the quantitative character with floral characters was significant (Table 2 A, B, C). Therefore they have important contribution in the taxonomy of the genus *Solanum*. It is clear from the comparison of different species of *Solanum* based on quantitative characters suggest that these are less important morphological markers for inter specific studies. However they become important for intergeneric studies[9,10].

The entire *Solanum* species are grouped into two based on prickles.The section armed *Solanum* species was further categorized based on flower colour. *S. virginianum* and *S. aculeatissimum* are differentiated by their fruit colour. In the case of *S. wendlandii* and *S. trilobatum* flowers are purple, prickles present both on stem and leaves but can be differentiated by leaf nature *i.e.*, deeply lobed in *S.wendlandii*. In the case of *S. wendlandii,* spines are abundantly present on young stem rather than in mature stem. Similarly, *S. mammosum* the fruits are with nipple like projections.The unarmed section was further distinguished by the nature of erect or climbing stem.Both in *S. mauritianum* and *S. macrocarpon* flowers are purplish but fruits are either in cluster or solitary. Leaves are linear in *S. pseudocapsicum* whereas lobed in *S. capsicoides*. In the past the taxonomic status of *Solanum nigrum* remained highly controversial[2]. Clarke[7] did not separated *S. nigrum, S. americanum* and *S. villosum* from each other and considered all of them along *S. nigrum*. Rechinger's[11] 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[3] mentioned *S. nigrum* with orange colour fruit.

Key to species of the genus *Solanum*

1a: Plants armed 2
1b: Plants unarmed 10
2a: Flowers white 3
2b: Flowers purple 5
3a: Stem & leaves densely spiny 4
3b: Stem & leaves sparsely spiny ..... *torvum*
4a: Fruits yellow at maturity ..... *virginianum*
4b: Fruits red at maturity ..... *aculeatissimum*
5a: spines present both on stem & leaves..... 6
5b: spines present only on stem ..... *giganteum*

Table 2A. IR bonds of *S.nigum, S. macrocarpon, S. jasminoides, S. mammosum S. torvum, S. aculeatissimum* and *S. capsieoides.*

S.nigrum	*S.macrocarpon*	*S.jasminoides*	*S.mammosum*	*S.torvum*	*S.aculeatissimum*	*S.capsicoides*
342.37	358.77	349.12	345.26	356.83	345.26	362.62
356.84	369.37	376.12	405.05	376.12	389.62	378.05
480.28	480.28	480.28	480.28	480.28	480.28	480.28
435.8	391.56	424.34	513.07	420.48	514.99	426.27
475.53	423.38	451.34	588.29	441.7	584.43	453.27
517.9	576.73	460.99	653.51	551.34	1022.27	493.78
605.66	605.66	495.71	1016.49	462.92	1097.5	514.99
647.13	628.8	514.99	1064.71	518.85	1145.65	592.15
830.37	882.45	574.79	1317.38	545.85	1240.23	621.08
1024.22	1022.29	705.95	1400.32	574.79	1273.02	665.44
1062.8	1154.42	775.38	1521.84	597.93	1323.17	702.09
1237.36	1247	813.96	1535.34	617.22	1400.32	777.31
1328.98	1325.12	920.05	1618.28	663.51	1452.4	916.19
1394.56	1396.49	1070.49	1635.64	705.95	1531.48	1022.27
1630.84	1644.34	1111	1668.43	896.9	1627.92	1060.85
1731.14	1729.21	1159.22	1743.65	1037.7	1674.21	1101.35
2853.73	2455.42	1267.23	3120.82	1072.42	1739.79	1238.3
2925.1	2925.1	1313.53	3221.12	1114.86	2924.09	1321.24
3418.88	3345.59	1371.39	3251.98	1263.37	3115.04	1404.18
		1390.68	3282.84	1317.38	3290.56	1535.34
		1446.61	3304.06	1373.32	3321.42	1541.12
		1527.62	3334.92	1409.96	3354.21	1575.84
		1539.2	3361.93	1452.4	3379.29	1639.49
		1579.7	3392.79	1519.91	3402.43	1670.36
		1622.13	3419.79	1533.41	3431.36	1735.93
		1639.49	3441.01	1571.99	3491.16	2331.94
		1670.35	3473.8	1641.42	3514.3	2370.51
		1734.01	3500.8	1666.5	3558.67	2926.01
		2094.69	3523.95	1737.86	3597.28	3294.42
		2142.91	3549.02	2092.77	4688.95	3313.71
		2328.08	3593.38	2366.66		
		2362.8	4685.09	2920.23		
		2929.87		3010.88		
		3296.35		3284.77		
		3317.56		3325.28		
		3329.14		3360		
		3352.28		3387		
		3387		3419.79		
		4698.6		3441.01		
				3477.66		
				3498.87		
				3522.02		
				3579.88		
				3712.97		
				3865.35		

Table 2B. IR bonds of *S.violaceum, S.giganteum, S.pseudocapsicum, S.mauritianum, S.trilobatum, S.virginianum.*

S.violaceum	*S.giganteum*	*S.pseudocapsicum*	*S.mauritianum*	*S.trilobatum*	*S.virginianum*
354.9	356.83	383.83	366.48	383.83	376.12
428.2	399.26	480.28	395.41	420.48	395.41
480.28	480.28	462.92	480.28	480.28	480.28
516.92	441.7	514.99	464.84	495.71	422.41
582.5	551.34	578.64	495.71	514.99	588.29
624.94	462.92	704.02	514.99	545.85	626.87
704.02	476.42	837.11	547.78	580.57	779.24
773.46	501.49	906.54	580.57	588.29	1020.34
1323.17	518.85	1026.13	588.29	603.72	1055.06
1402.25	545.85	1068.56	624.94	636.51	1101.35
1458.18	574.79	1145.72	663.51	665.44	1317.38
1537.27	596	1269.16	702.09	702.09	1433.11
1739.79	698.23	1539.2	1068.56	914.26	1637.56
2926.01	779.24	1577.77	1099.43	1037.7	1668.43
3122.75	829.39	1635.64	1153.43	1060.85	1743.65
3280.92	914.26	1672.28	1238.3	1095.57	2852.72
3298.28	1020.34	1737.86	1319.31	1236.37	2922.16
3317.56	1066.64	2330.01	1388.75	1263.37	3012.81
3331.07	1099.43	2368.59	1458.18	1323.17	3035.96
3358.07	1236.37	2860.43	1533.41	1390.68	3059.1
3390.86	1263.37	2927.94	1637.56	1533.41	3074.53
3415.93	1290.38	3296.35	1670.35	1575.84	3122.75
3431.36	1329.31	3311.78	1735.93	1620.21	3284.77
3446.79	1392.61	3329.14	2856.58	1635.64	3313.71
3471.87	1450.47	3352.28	2922.16	1670.35	3331.07
3493.09	1535.34	3388.93	3076.46	1739.79	3360
3512.37	1571.99	3469.94	3122.75	2330.01	3390.86
3550.95	1624.06	3491.16	3284.77	2368.59	3419.79
3589.53	1639.49	3510.45	3302.13	2860.43	3441.01
	1670.35	3711.04	3313.71	2926.01	3477.66
	1743.65	3836.42	3331.07	3074.53	3500.8
	2326.15	3869.2	3358.07	3120.82	3523.95
	2370.51	4690.88	3390.86	3282.84	3547.09
	2854.65		3417.86	3298.28	3591.46
	2926.01		3441.01	3311.78	
	3010.88		3471.87	3331.07	
	3032.1		3498.87	3356.14	
	3062.96		3550.95	3388.93	
	3122.75		3585.67	3415.93	
	3223.05		3714.9	3439.08	
	3282.84		3836.42	3471.87	
	3305.99		3869.2	3496.94	
	3329.14			3520.09	
	3360			3550.95	
	3387			3583.74	
	3419.79			3741.9	
	3441.01			3836.42	
	3479.58			3869.2	
	3500.8			3967.57	
	3523.95				
	3554.81				
	3579.88				

Table 2C. IR bonds of *S.melongena var insanum* and *S.wendlandii.*

S.melongena var *insanum*	*S.wendlandii*
354.9	345.26
395.41	383.83
480.28	480.28
478.35	516.92
514.99	582.5
574.79	619.15
698.23	700.16
779.24	775.38
1066.64	900.76
1263.37	1024.2
1317.38	1064.71
1390.68	1099.43
1535.34	1323.17
1571.99	1390.68
1637.56	1539.2
1668.43	1633.71
1741.72	1674.21
2924.09	1737.86
2956.87	2370.51
3037.89	2860.43
3072.6	2924.09
3120.82	3296.35
3282.84	3315.63
3302.13	3329.14
3313.71	3352.28
3329.14	3388.93
3358.07	3412.08
3390.86	3431.36
3419.79	3469.94
3441.01	3491.16
3477.66	3510.45
3500.8	
3522.02	
3589.53	

6a: Stem with creeping habit..... 7
6b: Stem with erect habit..... 8
7a: Leaves deeply lobed ..... *wendlandii*
7b: Leaves not deeply lobed..... *trilobatum*
8a: Fruits with nipple like projections .....*mammosum*
8b: Fruits without any projections 9
9a: Fruits erect at maturity ..... *violaceum* ssp *violaceum*
9b: Fruits drooping at maturity .....*melongena* var. *insanum*
10a: Plants not climbers..... 11
10b: Plants climbers ..... *jasminoides*
11a: Flowers purplish.....12
11b: Flowers whilte 13
12a: Fruits solitary..... *macrocarpon*
12b: Fruits in clusters ..... *mauritianum*
13a: Stem purplish green ..... *nigrum*
13 b: Stem not purplish green 14
14a: Leaves linear..... *pseudocapsicum*
14b: Leaves lobed..... *capsicoides*

IR spectral taxonomic key for *Solanum* spp. identification

1a. Species with peaks above 4000
2a. peak between 4690-4698
3a. peak at 4690 .....*S. pseudocapsicum*
3b. peak at 4698.....*S. jasminoides*
2b. peak between 4685-4688
4a.peak at 4685.....*S. mammosum*
4b. peak at 4688.....*S. aculeatissimum*
1b. Species with peaks below 4000
5a. . peak above 3866
6a. peak at 3867.....*S. trilobatum*
6b. peak at 3869..... *S. mauritianum*
5b. peak below 3866
7a. peak at 3865..... *S. torvum*
7b. peak below3865
8a. peak between 3579-3591
9a. peak at 3591.....*S. virginianum*
9b. peak below 3590
10a. a lower peak at 395.41.....
S. melongena var insanum
10b. a lower peak at 428.2.....
S. violaceum ssp violaceum
8b. peak between 3313-3510
11a. peak below 3346
12a. peak at 3313.....*S. capsicoides*
12b. peak at 3345.....*S. macrocarpon*
11b. peak above 3346
13a. peak at 3418..... *S. nigrum*
13b. peak above 3511
14a. peak at 3510.....*S. wendlandii*
14.b peak at 3579...*S. giganteum*

IR spectral analysis- Besides the morphological markers, we also propose infra red spectral markers that could be useful for identification of the samples. All the species of *Solanum* subjected to IR spectral analysis yielded peaks between 3200 - 3300 and also a peak range 1600-1700. This can be used as a marker character to identify the genus. The peak 3200- 3300 may represent NH group of *Solanum* alkaloids. Similarly the peak at 1635 forms C=N group contain alkaloids. The peaks above 3200 and below

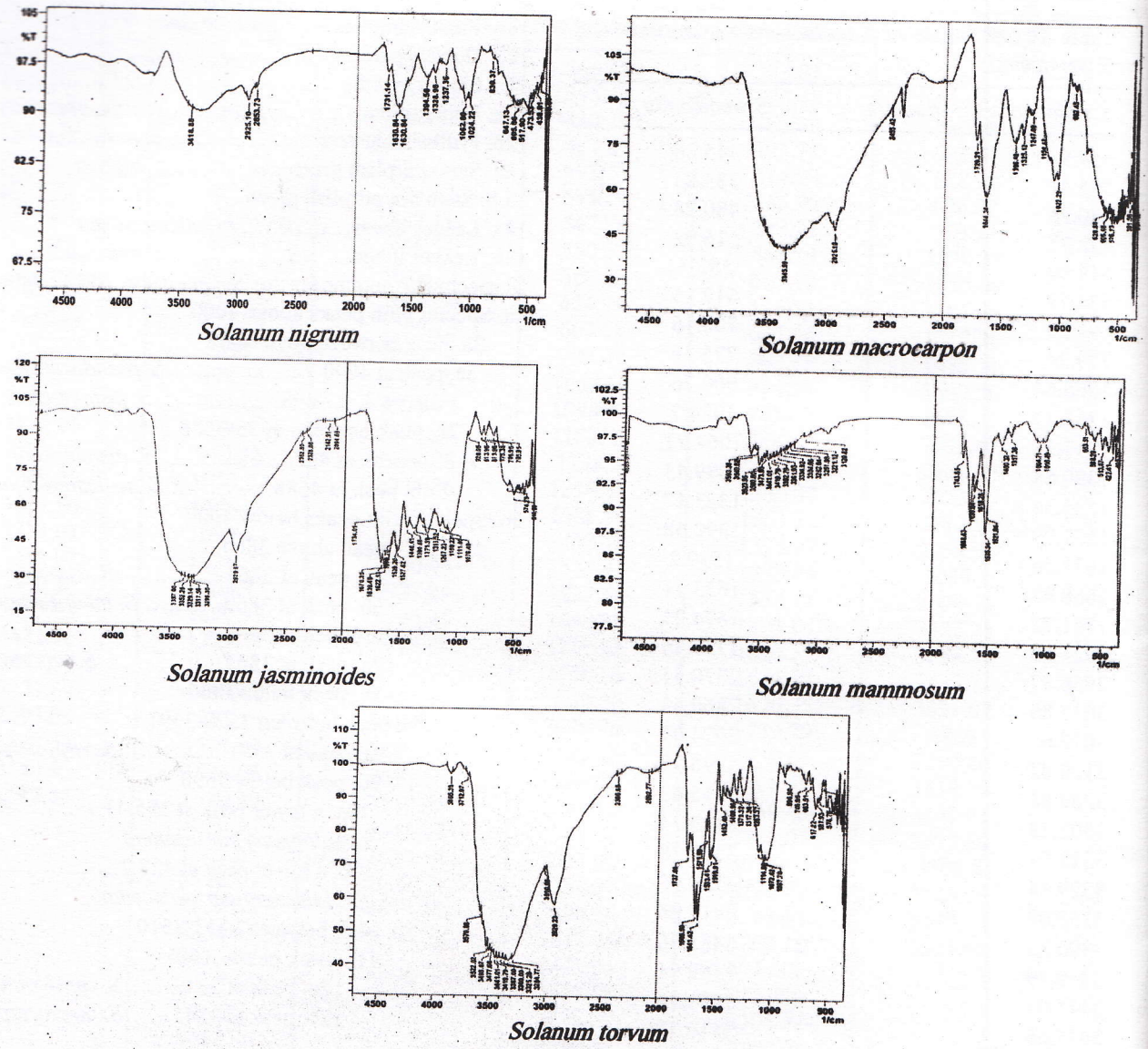


Fig.1a. IR spectra of *Solanum* species.

3300 may be alcohol, phenols or flavones like quercetin derivatives. The peak at 1700 represents C=O contains non conjugated compounds, while 1600 forms CH aromatic compounds. The peaks above 1700 and below 1800 represent unconjugated aliphatic ketones. The peaks at 1600, 1200 form the flavonoids and 1400 - 1500 forms C - O ether compounds or flavonoids (Table 2 A,B,C).

The correct botanical identification of plants is one of the pre-request for the success of herbal drug technology. Botanical identification is classically based on anatomical and morphological data. However, when the herbal products to be identified such as extracts, powder or the vegetative plant organs requires other tools for identification. Chromatographic techniques to analyse crude extracts, such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC) or gas chromatography (GC) hyphenated with detection techniques such as ultraviolet (UV), mass spectrometry (MS) and nuclear magnetic resonance (NMR) have been successfully employed[12-16]. Also, DNA-based techniques have been widely used for authentication of medicinal plants especially in the case when those plants are substituted or adulterated with other species morphologically or chemically indistinguishable[17]. In this context IR spectrum provides additional key for identifying the plants. In the present study the IR finger printing of

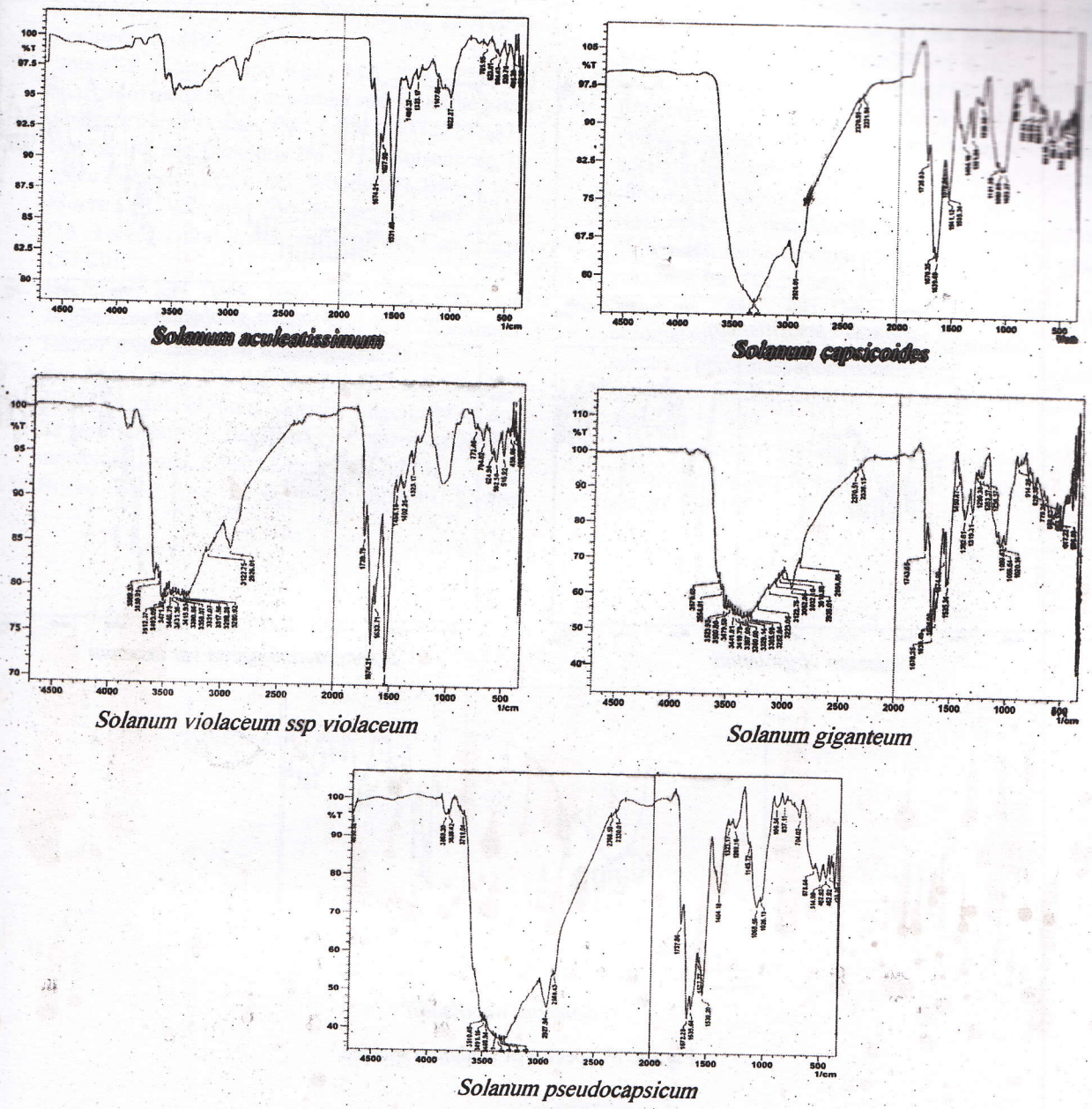
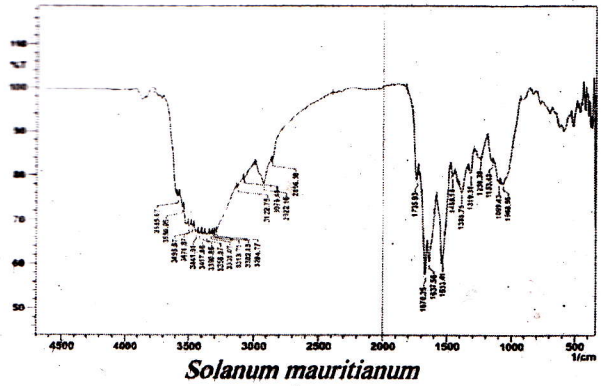


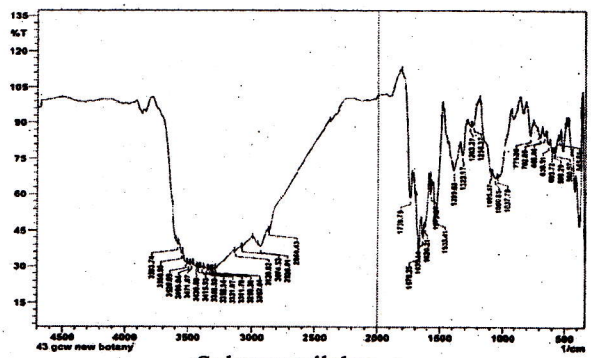
Fig.1b. IR spectra of *Solanum* species.

Solanum species show diversity in the size and shape of the peaks (Figs 1.a,b,c). *S macrocarpon, S. nigrum, S. aculeatissimum, S. capsicoides, S. jasminoides* and *S. mammosum* are differentiated by specific IR spectral peaks 3345.59, 3418.88, 4688.95, 3313.71, 4698.6, respectively. *S. torvum* can be distinguished from *S. giganteum* by the peaks 3865.35 and 3579.88. *S. wendlandii* and *S. pseudocapsicum* are differentiated by 3510.45 and 4690.88. Similarly, *S. mauritianum, S. trilobatum* and *S. violaceum* ssp. *violaceum* are differentiated by peaks 3869.2, 3867.57 and 428.2, respectively. *S. virginianum* and *S. melongena* var. *insanum* showed peaks at 3591.46 and 395.41.

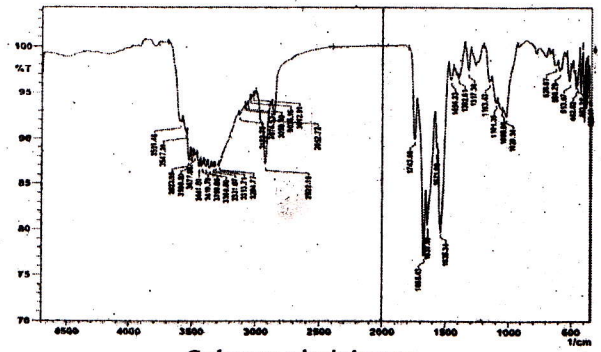
In conclusion, the *Solanum* species can be identified using morphological data. Prickles, flower colour, fruit characters can be used as morphological markers. However, when the plant material is powdered, it is necessary to investigate them by IR spectral profile



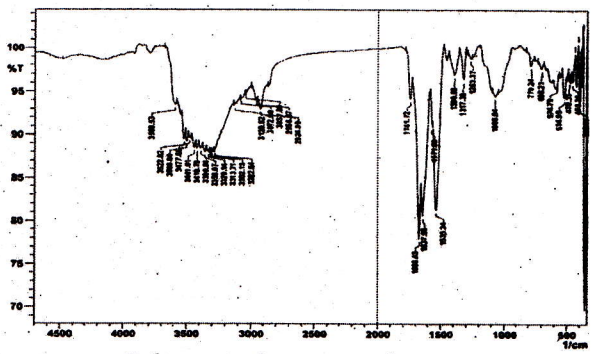
Solanum mauritianum



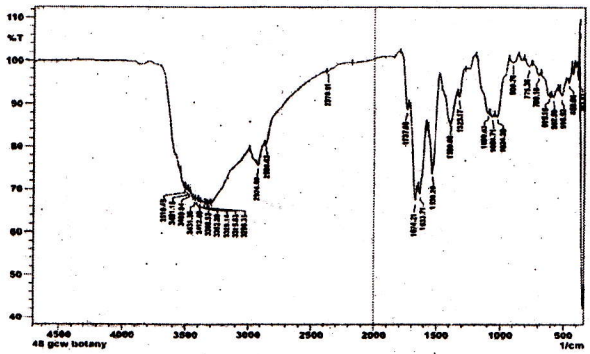
Solanum trilobatum



Solanum virginianum



Solanum melongena var insanum



Solanum wendlandii

Fig.1c. IR spectra of *Solanum* species.

of its crude powder.

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