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Systematics Implications of GC-MS Analysis of Secondary Metabolites in the Ethanol Extract of *Solanum* Species from South West Saudi Arabia

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THIS INVESTIGATION deals with assessment of the diversity of 14 populations representing eleven species of *Solanum* from southwest Saudi Arabia based on differences in the secondary metabolites by using GC-MS analysis. The analysis was carried out using ethanol extract of the examined *Solanum* species/populations and 87 different phyto-constituents were detected at six different retention times. The highest M.wt. for the identified compounds was 641 and was recorded in *S. villosum* at a retention time of 15 min; its formula is $C_{38}H_{35}N_5O_5$ and its decided name is N-benzoyl-9-(2,3,5-tri-O-benzyl pentofuranosyl)-9H-purin-6-amine. On the other hand, the lowest M.wt. for the identified compounds was 84; its formula is C_6H_{12} and its decided name is 1Hexane and it was recorded in all *Solanum* species/populations except the two populations of *S. incanum*, *S. coagulans* and *S. schimperianum* at the retention time of 5 min. Based on differences in the phyto-constituents, genetic similarity coefficients were calculated and two distance trees were constructed to illustrate the relatedness of the examined species. The results support a hypothesis that *S. villosum* and *S. nigrum* can be regarded as one complex species. The results also revealed that *S. coagulans* is related to *S. macracanthum* and *S. glabratum* and also *S. schimperianum* is related to *S. incanum*. The results also revealed that *S. torvum*, *S. sisymbriifolium* and *S. dulcamara* are closely related species. This is generally congruent with the relatedness of the examined specie based on morphological variation and to some extend agree with their systematic treatments.

Keywords: GC-MS analysis, *Solanum*, Saudi Arabia, Systematics Implications.

Introduction

Solanum L. is a complex and large genus of the family Solanaceae. It contains between 1,500 and 2,000 species (Bohs, 2001). The species exhibit a wide diversity of habit with trees, shrubs, creepers, herbaceous, perennials and annual. Morphological characters including general habit vegetative characters, leaf architecture, epidermal orientation, inflorescence types and fruit types are used for diagnosis the different species belonging to genus *Solanum*. Many species bear some edible parts such as fruits, leaves, tubers such as tomato, potato and egg plants. In Saudi Arabia, the genus is represented by about 16 species, mainly in the west and southwest side of the country (Chaudhary, 2001 and Collenette, 1999). Mountainous southwestern Saudi Arabia are recognized remarkable for their comparably dense vegetation and species diversity. Floristic explorations have resulted in reporting of many new taxa and records (Alfarhan, 2000; Alfarhan et al., 1997, 2001 and Al-Turki et al., 2001). Recently, El-Shaboury et al. (2016) reported three new records of *Solanum* species in southwest of Saudi Arabia, which have been defined as *Solanum dulcamara* L., *Solanum sisymbriifolium* Lam., and *Solanum torvum* Swartz.

Limited work has been done on the nature of genetic diversity and characterization of wild and cultivated *Solanum* is Saudi Arabia. Haroun & Al-Wadi (1999), Al-Wadi (2002) and Al-Wadi & Lashin (2007) have studied some cytological characters of few species of *Solanum* from the Aseer region, southwest Saudi Arabia and their taxonomic significance. Their results have

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indicated that *S. forsskalii* and *S. glabratum* are cytologically stable, while, *S. macracantum* showed irregular meiosis indicating its hybrid situation. The studies of the above authors however, have not resolved the problems of synonymy and taxa misidentification common to the genus in Saudi Arabia. Classification of *S. nigrum* and *S. villosum* as varieties or distinct species is a long taxonomic controversy on the taxonomic identity of these two species (Stebbins & Paddock, 1949; Symon, 1970; Schilling & Andersen, 1990 and Edmonds & Chweya, 1997). Ahmad & Fadl (2015) addressed the genetic diversity of some *Solanum* species from Taif highlands using RAPD and SDS-PAGE.

Chemotaxonomic approaches to the classification of the Solanaceae was based on the excellent taxonomic markers provided by the analysis of alkaloids (Tetenyi, 1987). Cardoso et al. (2008) indicated that secondary metabolites profile can contribute to the taxonomic position of species or tribes which suffer morphological controversies. Mohy-UD-Din et al. (2010) tried to resolve the international taxonomic controversy based on morphological characters by using HPLC and GC-MS for the analysis of alkaloids in *Solanum nigrum* complex, where qualitative and quantitative comparison by cluster analysis demonstrated significant distances among *Solanum chenopodioides* and *Solanum villosum* as well as in *Solanum americanum* and *Solanum nigrum,* in their respective clusters, indicated them as distinct species. But *Solanum retroflexum* did not show such a marked difference and hence might be regarded as a variety or subspecies of *Solanum nigrum*.

Gheewala et al. (2013) analyzed the presence of the phyto-constituents with the use of analytical methods like HPLC and GC-MS with crude extract of dried fruit of *Solanum nigrum*. They were also indicated the presence of glycoalkaloid Solasonine which was in higher concentration than other glycoalkaloid α-Solamargine, β-Solamargine, α-Solanine and for aglycone solasodine was significantly present with higher percentage. As outcome, the study identified different phytoconstituents which can be applied for pharmacological screening. Akilan et al. (2014) studied the presence of various phytochemicals in *Solanum esculentum*, *Solanum trilobatum*, *Solanum nigrum* and *Solanum tuberosum* and recorede the presence of compounds such as tannins, flavonoids, alkaloids, phenols etc. The methanol extract of *Solanum esculentum* showed more antibacterial activity in all bacterial cultures. They used HPLC and GC-MS techniques to find active compound responsible for the antimicrobial activity and identified compounds such as ferrulic acid, caffeic acid etc.

Recently, Deepak & Gopal (2014) determined the essential chemical constituents in the bark of *Solanum verbascifolium* Linn; a total of 21 phytocompounds were identified in three different extracts from the bark using Gas Chromatography-Mass Spectroscopy (GC-MS) analysis. In solanum, the plants of *Solanum nigrum* complex has been traditionally used as an analgesic, antispasmodic, antiseptic, antidysentric, antinarcotic, emollient, diuretic, tonic, soporific, laxative, anticancer, antiulcer and for disorders of neuro-vegetative system etc. (Saijo et al., 1982; Akhtar & Muhammad, 1989; Schilling et al., 1992; Edmonds & Chweya, 1997 and Manoko et al., 2007). Though *Solanum americanum* Mill., *Solanum chenopodioides* Lam. and *Solanum retroflexum* Dunal have morphological resemblance with *Solanum nigrum*, yet no chemotaxonomic relationship has so far been established due to lack of a comprehensive study of their chemical composition. In the current investigation deals with assessment of the diversity of 14 populations representing eleven species of *Solanum* from southwest Saudi Arabia based on the differences in the secondary metabolites as revealed using GC-MS analysis.

Materials and Methods

The examined *Solanum* species/populations were collected from their natural habitats in different sites in the southwest of Saudi Arabia (Table 1). The plant samples were washed with running water twice and air dried under shade for 5-15 days. After drying, the plant specimens were crushed to a dry powder using mortar and pestle. An amount of 50 g of the dried plant powder were soaked in 200 ml of 85% ethanol as organic solvent. To extract the active compounds, the samples were kept in shaking incubator at 35-40^{o}C for 24h at 100-150 rpm. After 24h, the plant samples were filtered through Whatman filter paper No.1 using micro filtration unit, and then centrifuged

and the supernatant was collected and stored at 4°C until use. Ten ml of the filtrate were evaporated in rotary vacuum evaporator and the crude filtrate was dissolved in petroleum ether three times for defatting using 2 ml each time.

TABLE 1. Scientific names, codes and sites of collection of the examined *Solanum* species/populations collected from south west of Saudi Arabia.

Ser.	*Solanum* species	Species code	Site of collection
1	*Solanum nigrum* L.	S1A & S1B	Abha (El-Soda) and Najran
2	*Solanum villosum* Mill.	S2	Abha (El-Soda)
3	*Solanum incanum* L.	S3A & S3B	Abha (El-Soda) and Najran
4	*Solanum glabratum var. sepicula* Dun.	S4A & S4B	Jazan and Wadi El-Dawaser
5	*Solanum villosum* (L.) Lam. ssp. *puniceum* (Kirsch.) Edmonds.	S5	Wadi El-Dawaser
6	*Solanum coagulans* Forssk.	S6	Wadi El-Dawaser
7	*Solanum schimperianum* Hochst. ex A. Rich.	S7	Abha- El-Arin District
8	*Solanum macracanthum* A. Rich.	S8	Abha- Al-Andalus District
9	*Solanum torvum* Swartz.	S9	Jazan
10	*Solanum sisymbriifolium* Lam.	S10	Jazan
11	*Solanum dulcamara* L.	S11	Bisha

The GC separation and MS analyses were performed by using GC Shimadzu QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column. The GC-MS Conditions were as follows: Column: (Varian Chrompack CP-Sil 8, 30m length x 0.25mm ID). Carrier gas: Helium with constant flow, 1.0 ml/min. Injector Temp. = 250°C, Split Ratio = 2. Oven Temp: Program: Start at 40°C withhold time of 1 min, then, 40 to 150°C at a rate of 10°C/min, with no hold, then, 150 to 280°C at a rate of 5°C/min with a hold for 5 min. Total Runtime = 30 min. Injected Volume of the extract = 1 µL. Interface Temperature = 280°C.

The interpretation of the mass spectrum GC-MS was carried out using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of each sample were ascertained using NIST Ver. 2.1 MS data library. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library version (NIST Chemistry Web Book) (Joulain & Koenig, 1998).

Data analysis

The separated compounds by GC-MS analysis were scored in binary matrices, where 0 stands for the absence and 1 stands for the presence of a compound for all examined *Solanum* species/populations; these codes were detailed in Table 2. Statistical analysis of the data of the compounds identified was carried out by using two software programs; the software package NTSYS-pc version 2.02 (Rohlf, 2002) and the online program Dendro UPGMA (A dendrogram Construction Utility) using SM coefficient and RMSD coefficient respectively (http://genomes.urv.cat/UPGMA/index.php?-entrada=).

Results

The GC-MS analysis of the examined materials separated 87 compounds from the 14 *Solanum* species/populations at six different retention times of 5, 10, 15, 20, 25 and 30 min (Table 2). The highest number of separated compounds recorded 31 different molecular weights, scored at the retention time of five minutes. On the other hand, the lowest number of separated compounds was 6 different molecular weights, scored at a retention time of 15 min. The highest M.wt. was 641 scored in *S. villosum* (S2) at retention time of 15 min., on the other hand the lowest M.wt. was 84 scored for all *Solanum* species/ populations except *S. incanum* (S3A & S3B), *S. coagulans* (S6) and *S. schimperianum* (S7) at retention time of 5 min.

Retention time 5 min

The molecular weights of the 31 compounds separated at the retention time of 5 min ranged from 84 to 442 including two molecular weights of 114 and 128 scored in the all *Solanum* species/populations. These have molecular formula of $C_7H_{14}O$ and $C_7H_{12}O_2$ and decided names of 2-(pentan-3-yl) oxirane and 2-ethoxy-3,4-dihydro-2H-pyran, respectively, and one M.wt. of 120 scored only in *S. schimperianum* (S7) with molecular formula C_8H_8O and decided name phenyl acetaldehyde (Table 2). The other molecular weights at this retention time were polymorphic. The lowest M.wt. was (84) has a formula C_6H_{12} and decided name 1hexene was scored in all *Solanum* species/ populations except *S. incanum* (S3A & S3B), *S. coagulans* (S6) and *S. schimperianum* (S7). On the other hand, the highest M.wt. (442) has a formula of $C_{22}H_{45}CL_3Si$ and a decided name trichloro (docosyl) silane, scored in all *Solanum* species/populations except for the two populations of *S. incanum* (S3A & S3B) and *S. schimperianum* (S7).

The most prominent polymorphism in molecular weights of the 31 compounds separated at the retention time of 5 min include the absence of a compound that has a M.wt. of 103, formula $C_5H_{13}NO$ and a decided name O-(3-methylbutyl) hydroxylamine from *S. villosum* (S2) and *S. schimperianum* (S7). A compound with a M.wt. 110 has a formula of $C_7H_{10}O$ and a decided name 4.4-Dimethyl-2-pentynal was present in only the two populations of *S. incanum* (S3A & S3B) and *S. schimperianum* (S7). A compound with M.wt. of 178, a formula $C_{11}H_{14}O_2$ and a name methyl eugenol was scored only in *S. villosum* (S2) and *S. torvum* (S9). Also, a compound with M.wt. of 194 a formula of $C_{13}H_{22}O$ and a decided name (E)-geranyl acetone was scored only in *S. glabratum* (S4A & S4B). For more examples of the compounds molecular weights, formulas, names and distribution in the examined *Solanum* species/populations at this retention time see Table 2.

Retention time 10 min

The molecular weight of 24 compounds separated at the retention time of 10 min ranged from 85 to 590 including four compounds scored in all *Solanum* species/ populations; these are a compound with M.wt. 118, formula $C_6H_{14}O_2$ and a decided name 2-butoxyethanol, M.wt. 130, formula $C_5H_7ClN_2$ and a decided name 1-aminopyridin-1-ium chloride, M.wt. 160 a formula $C_8H_{16}O_3$ and a decided name 3-ethoxy-4-methylpentanoic acid, and M.wt. 184 formula $C_{13}H_{28}$ and a decided name tridecane. The other separated compounds at this retention time were polymorphic. The lowest M.wt. was 85 for a compound that has the formula CH_3N_5 and a decided name 5-amino-2H-tetraazole and was scored in *S. incanum* (S3A & S3B), *S. glabratum* (S4A & S4B) and *S. macracanthum* (S8). The highest M.wt. was 590 has a formula $C_{42}H_{86}$ and a decided name dotetracontane was scored in all *Solanum* species/populations except *S. nigrum* (S1A & S1B), *S. villosum* (S2) and *S. villosum* ssp. puniceum (S5). On the other hand, a compound with a M.wt. of 111, a formula $C_7H_{13}N$ and a decided name 4-methylidenecyclohexan-1-amine was scored in *S. torvum* (S9) and *S. sisymbriifolium* (S10). Also, a 131 M.wt. compound with formula $C_6H_{13}NO_2$ and decided name 2-aminohexanoic acid was scored only in the two populations of *S. glabratum* (S4A & S4B). For more examples of the compounds in the examined *Solanum* species/populations at the retention time of 10 min (Table 2).

TABLE 2. The molecular weight, molecular formula and decided names for the compounds extracted by GC-Ms analysis at different retention times (5, 10, 15, 20, 25 and 30 min) for the *Solanum* species/populations (S1-S11); coded as given in Table 1.

Se.	M.wt	Mol. Formula	Decided Name	*Solanum* populations / RT 5 min													
				S1A	S2	S3A	S4A	S4B	S5	S3B	S1B	S6	S7	S8	S9	S10	S11
1	84	C_6H_{12}	1Hexene	1	1	0	1	1	1	0	1	0	0	1	1	1	1
2	89	$C_4H_{11}NO$	O-(2-methylpropyl) hydroxylamine	1	1	1	·	·	1	1	1	1	0	1	1	1	1
3	98	C_7H_{14}	5-Methyl-1-hexene	1	1	0	1	1	1	0	1	0	0	1	1	1	1
4	100	$C_6H_{12}O$	2-ethyl-2-methyloxetane	0	0	0	1	1	0	0	0	1	0	1	1	1	1
5	103	$C_5H_{13}NO$	O-(3-methylbutyl) hydroxylamine	1	0	1	1	1	1	1	1	1	0	1	1	1	1
6	110	$C_7H_{10}O$	4.4-Dimethyl-2-pentynal	0	0	1	0	0	0	1	0	0	1	0	0	0	0
7	112	C_8H_{16}	1-methylbutylcyclopropane	1	1	0	1	1	0	0	0	0	0	1	0	1	0
8	114	$C_7H_{14}O$	2-(pentan-3-yl)oxirane	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	116	$C_7H_{16}O$	3,3-dimethyl-1-pentanol	1	1	0	1	1	1	0	1	0	0	1	1	1	1
10	120	C_8H_8O	Phenylacetaldehyde (volatile oil)	0	0	0	0	0	0	0	0	0	1	0	0	0	0
11	128	$C_7H_{12}O_2$	2-ethoxy-3,4-dihydro-2H-pyran	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	140	$C_{10}H_{20}$	2,6-Dimethyl-2-octene	0	0	0	1	1	0	0	0	0	0	0	0	0	0
13	142	$C_{10}H_{22}$	Decane	0	0	0	0	0	0	0	0	0	0	1	1	1	1
14	143	$C_7H_{13}NO_2$	2-acetylpentanamide	0	0	0	1	1	0	0	0	0	0	1	0	0	0
15	154	$C_{10}H_{18}O$	4-Terpineol	1	1	0	1	1	1	0	0	0	0	1	1	1	0
16	156	$C_{10}H_{20}O$	1-(ethenyloxy)-6-methylheptane	1	1	0	0	0	0	0	1	0	1	0	0	0	1
17	168	$C_{12}H_{24}$	3-Undecene, 10-methyl	0	0	0	0	0	0	0	1	0	1	0	0	0	0
18	172	$C_{10}H_{20}O_2$	3-ethyl-5-methylheptanoic acid	1	0	1	0	0	0	1	1	0	1	0	0	0	0
19	178	$C_{11}H_{14}O_2$	Methyl eugenol	0	1	0	0	0	0	0	0	0	0	0	1	0	0
20	194	$C_{13}H_{22}O$	(E)-geranyl acetone (volatile oil)	0	0	0	1	1	0	0	0	0	0	0	0	0	0
21	198	$C_{14}H_{30}$	n-Tetradecane	0	0	1	1	1	0	1	0	1	0	0	0	0	0
23	210	$C_{15}H_{30}$	decylcyclopentane	1	1	0	1	1	1	0	1	0	1	1	1	1	1
24	214	$C_{14}H_{30}O$	Hexyl Octyl ether	1	1	0	1	1	1	0	0	0	0	1	1	1	1
25	224	$C_{13}H_{20}O_3$	methyl 2-[(1R,2S)-3-oxo-2-pent-2-enylcyclopentyl]acetate	1	0	0	0	0	0	0	1	0	1	0	0	0	0
26	238	$C_{15}H_{26}O_2$	[(2E)-3,7-dimethylocta-2,6-dienyl] 3-methylbutanoate	0	0	1	0	1	0	1	1	0	1	0	0	0	0
27	284	$C_{17}H_{32}O_3$	ethyl 13-methyl-10-oxotetra decanoate	0	0	1	0	0	0	1	0	0	0	0	0	0	0

28	334	$C_{21}H_{34}O_3$	4-methoxyphenyl tetra decanoate	0	0	1	0	0	0	1	0	0	0	0	0	0	0
29	354	$C_{14}H_{21}F_7O_2$	1-Heptafluorobutyryloxyd	1	1	0	0	0	0	0	0	0	0	0	0	0	0
30	388	$C_{22}H_{45}Br$	1-bromodocosane	1	1	0	0	0	0	0	0	0	0	0	0	0	0
31	442	$C_{22}H_{45}CL_3Si$	Trichloro(docosyl)silane	1	1	0	1	1	1	0	1	1	0	1	1	1	1

Se.	M.wt	Mol. Formula	Decided Name	*Solanum* populations / RT 10 min													
				S1A	S2	S3A	S4A	S4B	S5	S3B	S1B	S6	S7	S8	S9	S10	S11
1	85	CH_3N_5	5-Amino-2H-tetraazole	0	0	1	1	1	0	1	0	0	0	1	0	0	0
2	88	$C_5H_{12}O$	2,2-dimethylpropan-1-ol	1	1	0	0	0	0	0	1	1	1	0	1	0	0
3	111	$C_7H_{13}N$	4-methylidenecyclohexan-1-amine	0	0	0	0	0	0	0	0	0	0	0	1	1	0
4	118	$C_6H_{14}O_2$	2- butoxyethanol	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	126	$C_7H_{10}O_2$	Methyl 2-methylidene cyclobutane-1-carboxylate	1	1	0	1	1	1	0	1	0	0	1	1	1	1
6	130	$C_5H_7CLN_2$	1-aminopyridin-1-ium chloride	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	131	$C_6H_{13}NO_2$	2-aminohexanoic acid	0	0	0	1	1	0	0	0	0	0	0	0	0	0
8	132	$C_7H_{16}O_2$	1-Tert-Butoxy-2-Propanol	0	0	0	0	0	0	0	0	0	0	0	0	1	1
9	138	$C_9H_{14}O$	2-Pentyl furan	1	1	0	0	0	1	0	1	0	1	0	0	0	1
10	148	$C_6H_{12}O_2S$	Butyl 2-sulfanylacetate	0	1	1	1	1	0	1	0	1	1	1	1	1	1
11	141	$C_7H_{15}N_3$	4-Azidoheptane	0	0	1	1	1	0	1	0	0	0	0	0	0	0
12	150	$C_6H_{11}CLO_2$	Chloromethyl 2,2-dimethylpropanoate	0	0	1	0	0	0	1	0	0	0	0	0	1	1
13	155	$C_8H_{13}NO_2$	2-{[1-(Furan-2-yl) ethyl] amino} ethan-1-ol	1	0	0	0	0	0	0	1	0	0	0	0	0	0
14	160	$C_8H_{16}O_3$	3-Ethoxy-4-methylpentanoic acid	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	162	$C_8H_{18}O_3$	2-(2-Butoxyethoxy)ethan-1-ol	0	0	0	1	1	0	0	0	1	0	1	0	0	0
16	184	$C_{13}H_{28}$	Tridecane	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	202	$C_{10}H_{18}O_4$	1,5-diethyl 2-methyl pentanedioate	0	1	0	0	0	0	0	0	1	0	1	1	1	1
18	204	$C_{15}H_{24}$	Germacrene D	1	1	0	1	1	1	0	1	1	1	1	1	1	1
19	226	$C_{15}H_{30}O$	Pentadecanal	1	1	0	1	1	1	0	1	1	1	1	1	1	1
20	240	$C_{17}H_{36}$	Hexadecane, 3-methyl	0	0	1	0	0	0	1	0	0	1	0	0	0	0
21	254	$C_{14}H_{22}O_4$	9-Oxabicyclo(3.3.1)nonan-2	1	1	1	0	0	0	1	1	0	1	0	0	0	0
22	268	$C_{19}H_{40}$	2,6,10,14-tetramethyl pentadecane	0	0	1	0	0	0	1	0	0	0	0	0	0	0
23	394	$C_{28}H_{58}$	Octacosane	1	1	1	0	0	1	1	1	1	1	1	0	1	1
24	590	$C_{42}H_{86}$	Dotetracontane	0	0	1	1	1	0	1	0	1	1	1	1	1	1

Se.	M.wt	Mol. Formula	Decided Name	*Solanum* populations / RT 15 min											
				S1A	S2	S3A	S4A	S4B	S5	S3B	S1B	S6	S7	S8	S9	S10	S11
1	170	$C_{12}H_{26}$	2,2,4,6,6-PentaMethylheptane	1	0	0	1	1	1	0	1	0	0	0	0	0	0
2	182	$C_{11}H_{18}O_2$	5-methyl-6-pentyl-3,4-dihydro-2H-pyran-2-one	1	1	0	0	0	0	0	1	1	1	1	1	1	0
3	218	$C_{12}H_{26}O_3$	1-(2-(2-Butoxyethoxy) ethoxy) butane	0	0	1	0	0	0	1	0	0	0	0	1	0	0
4	448	$C_{20}H_{20}N_2O_{10}$	1-methyl 3-[(3,4,5-trimethoxy phenyl) carbamoyl]methyl 5-nitrobenzene-1,3-dicarboxylate	0	0	1	0	0	0	1	0	0	0	0	0	0	0
5	470	$C_{31}H_{22}N_2O_3$	Ethyl 3-(2-naphthyl)-7-(naphthylcarbonyl) -8-hydro pyrrolo[1,2-e]pyrimidine-5-carboxylate	0	1	0	0	0	0	0	0	0	0	0	0	0	0
6	641	$C_{38}H_{35}N_5O_5$	N-Benzoyl-9-(2,3,5-tri-O-benzyl pentofuranosyl)-9H-purin-6-amine	0	1	0	0	0	0	0	0	0	0	0	0	0	0

Se.	M.wt	Mol. Formula	Decided Name	*Solanum* populations / RT 20 min													
				S1A	S2	S3A	S4A	S4B	S5	S3B	S1B	S6	S7	S8	S9	S10	S11
1	282	$C_{18}H_{34}O_2$	Furanon,dihydro-5-tetradecyl	0	0	0	0	0	0	0	0	0	0	1	0	0	0
2	422	$C_{30}H_{62}$	Triacontane	0	1	0	0	0	0	0	0	1	0	0	1	0	1
3	450	$C_{32}H_{66}$	Dotriacontane	1	1	0	0	0	0	0	1	1	1	0	1	1	0
4	558	$C_{28}H_{30}O_{12}$	[3,4,5-tris(acetyloxy)-6-[3-hydroxy-4-(2-phenylacetyl) phenoxy]oxan-2-yl]methyl acetate	0	0	0	0	0	0	0	0	0	1	0	0	0	0
5	570	$C_{35}H_{71}Br$	Pentatriacontane	0	0	0	0	0	0	0	0	0	0	0	0	1	0
6	604	$C_{43}H_{88}$	Tritetracontane	0	1	0	0	0	0	0	0	1	0	0	0	0	0
7	618	$C_{44}H_{90}$	Tetratetracontane	0	0	0	0	0	0	0	0	1	0	0	0	1	0

Se.	M.wt	Mol. Formula	Decided Name	*Solanum* populations / RT 25 min														
				S1A	S2	S3A	S4A	S4B	S5	S3B	S1B	S6	S7	S8	S9	S10	S11	
1	136	$C_{10}H_{16}$	α-Pinene	0	0	0	1	1	0	0	0	0	0	0	0	0	0	
2	165	$C_{10}H_{15}NO$	2-Mthoxyamphetamin	0	0	0	0	0	0	0	0	0	0	1	0	0	1	
3	176	$C_{12}H_{16}O$	3-(4-Methoxy-3-methylphenyl)-2-methyl-1-propene	0	0	0	1	1	0	0	0	0	0	1	1	0	0	
4	186	C_7H_7BrO	2-bromo-5-methylphenol	0	0	1	1	1	0	1	0	1	0	1	0	0	0	
5	196	$C_{13}H_{24}O$	2-(3-methylcyclohexyl) cyclohexan-1-ol	0	0	0	1	1	0	0	0	0	0	1	1	0	0	
6	250	$C_{12}H_{26}Se$	Dihexyl monoselenide	0	0	0	0	0	0	0	0	1	0	0	0	0	1	
7	372	$C_{16}H_{22}Br_2$	1-bromo-4-(1-bromo-2-cyclo hexylethyl)-2,5-dimethyl benzene	0	0	0	0	0	1	0	0	0	0	1	0	0	0	
8	498	$C_{33}H_{54}O_3$	24-Methyl-25,27-epoxy-9,19-cyclolanostan-3-yl acetate	0	0	0	0	0	0	0	0	1	0	0	0	0	0	
Se.	M.wt	Mol. Formula	Decided Name	*Solanum* populations / RT 30 min														
				S1A	S2	S3A	S4A	S4B	S5	S3B	S1B	S6	S7	S8	S9	S10	S11	
1	166	$C_{11}H_{18}O$	4-cyclopentylcyclohexan-1-one	0	0	0	1	1	0	0	0	0	0	1	0	0	0	
2	188	$C_{11}H_{24}O_2$	2-Methyl-2,5-decanediol	1	1	0	0	0	0	0	1	0	1	1	0	1	0	
3	220	$C_{15}H_{24}O$	3-methyl-5-(2,6,6-trimethyl cyclohex-1-en-1-yl)pent-1-yn-3-ol	0	0	1	1	1	0	1	0	0	0	1	0	0	0	
4	222	$C_{12}H_{14}O_4$	5-tert-butylbenzene-1,3-dicarboxylic acid	1	1	0	1	1	1	0	1	0	1	0	0	0	0	
5	265	$C_{11}H_{21}O_7$	L-Mannopyrnoside, methyl 6- deoxy-2,4-di-o-methyl -, acetate	0	0	0	0	0	0	0	0	0	1	0	0	0	0	
6	292	$C_{16}H_{20}O_5$	2-cyclohexen-1-one, 3-methoxy-2(2,4,5-trimethoxyphenyl)	1	1	0	1	1	0	0	1	0	0	1	0	0	0	
7	296	$C_{21}H_{44}$	Heptadecane, 2,6,10,14	1	1	1	0	0	0	1	1	1	0	1	0	0	1	
8	310	$C_{21}H_{26}O_2$	Estra-1,3,5(10)-trien-17-ol	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
9	379	$C_{23}H_{45}N_3O$	3-{[(1-cyclopentylpiperidin-3-yl) methyl][2-(piperidin-1-yl) ethyl] amino}-2,2-dimethyl propan-1-ol	0	0	0	0	0	0	0	0	0	1	0	0	0	1	
10	400	$C_{28}H_{48}O$	Cholestan, 3-ol-2-methylene	0	0	0	1	1	0	0	0	0	0	0	0	0	0	
11	578	$C_{30}H_{60}Br_2$	1,30-Dibromotriacontane	0	0	0	0	0	0	1	0	0	0	0	1	0	0	
Total number of identified chemicals (87 M.wt)				30	36	27	39	37	38	31	26	26	26	40	33	33	33	

Retention time 15 min

The retention time 15min, separated compounds with six different molecular weights ranging from 170 to 640 including three molecular weights unique to one species from the examined *Solanum* species. A compound with a M.wt. of 448 and formula $C_{20}H_{20}N_2O_{10}$ and decided name 1-methyl 3-[(3,4,5-trimethoxyphenyl) carbamoyl] methyl 5-nitrobenzene-1,3-dicarboxylate was scored only in the two populations of *S. incanum* (S3A & S3B). Also, two compounds with M.wt. of 470 and 641, formulas $C_{31}H_{22}N_2O_3$ and $C_{38}H_{35}N_5O_5$ and decided names ethyl 3-(2-naphthyl)-7-(naphthyl carbonyl)-8-hydropyrrolo[1,2-e] pyrimidine-5-ca ryboxylate and N-benzoyl-9-(2,3,5-tri-O-benzyl pentofuranosyl)-9H-purin-6-amine were scored only in *S. villosum* (S2). A 218 M.wt compound with formula $C_{12}H_{26}O_3$ and decided name 1-(2-(2-butoxyethoxy) ethoxy) butane was scored only in *S. incanum* (S3A & S3B) and *S. torvum*. A compound with lowest M.wt of 170 at this retention time has the formula $C_{12}H_{26}$ and decided name 2,2,4,6,6-penta methylheptane was scored in *S. nigrum* (S1A & S1B), *S. glabratum* (S4A & S4B) and *S. villosum* ssp. puniceum (S5) (Table 2).

Retention time 20 min

At a retention time of 20 min seven different compounds were separated. The molecular weights for these compounds ranged from 282 to 618 including three unique compounds for one species of the examined *Solanum* species/ populations. A compound with M.wt 282, formula $C_{18}H_{34}O_2$ and decided name furanon, dihydro-5-tetradecyl was scored only in *S. macracanthum* (S8). A 558 M.wt compound with formula $C_{28}H_{30}O_{12}$ and decided name [3,4,5-tris(acetyloxy)-6-[3-hydroxy-4-(2-phenylacetyl) phenoxy] oxan-2-yl]methyl acetate was scored only in *S. schimperianum* (S7). Also, a 570 M.wt compound with formula $C_{35}H_{71}Br$ and decided name penta triacontane was scored only in *S. sisymbriifolium* (S10). A compound with M.wt 604, formula $C_{43}H_{88}$ and decided name tritetracontane was scored only in *S. villosum* (S2) and *S. coagulans* (S6). The highest M.wt in this retention time 618 with formula $C_{44}H_{90}$ and decided name tetratetracontane was scored in *S. coagulans* (S6) and *S. sisymbriifolium* (S10)(Table 2).

Retention time 25 min

The molecular weights of the eight compounds separated at the retention time of 25 min ranged from 136 to 498 including two molecular weights unique for one taxon from the examined *Solanum* species. The lowest molecular weight was 136 with formula $C_{10}H_{16}$ and decided name α-pinene was scored only in *S. glabratum* (S4A & S4B). The highest M.wt at this retention time was 498 and has a formula $C_{33}H_{54}O_3$ and decided name 24-Methyl-25,27-epoxy-9,19-cyclolanostan-3-yl acetate was scored only in *S. coagulans* (S6). Two compounds with M.wt 176 and 196 formulas $C_{12}H_{16}O$ and $C_{13}H_{24}O$ and decided names 3-(4-Methoxy-3-methyl phenyl)-2-methyl-1-propene and 2-(3-methyl cyclohexyl) cyclohexan-1-ol respectively were scored in *S. glabratum* (S4A & S4B), *S. macracanthum* (S8) and *S. torvum* (S9).

Retention time 30 min

At the retention time 30 min, eleven different compounds were separated. The molecular weights for these compounds ranged from 166 to 578 including three compounds unique to one species of the examined *Solanum* species/populations. A compound with M.wt 265, formula $C_{11}H_{21}O_7$ and decided name L-mannopyrnoside, methyl 6- deoxy-2,4-di-o-methyl -, acetate was scored only in *S. schimperianum* (S7). A 310 M.wt compound with formula $C_{21}H_{26}O_2$ and decided name estra-1,3,5(10)-trien-17-ol was scored only in *S. dulcamara* (S11). Also, a compound with M.wt 400, formula $C_{28}H_{48}O$ and decided name cholestan, 3-ol-2-methylene was scored only in *S. glabratum* (S4A & S4B). The compound with the lowest M.wt (166) and a formula $C_{11}H_{18}O$ and decided name 4-cyclopentylcyclohexan-1-one was scored in *S. glabratum* (S4A & S4B) and *S. macracanthum* (S8). While a compound with the highest M.wt (578) has the formula $C_{30}H_{60}Br_2$ and decided name 1, 30-dibromotriacontane was scored in *S. incanum* (S3B) and *S. torvum* (S9) (For more details see Table 2).

Systematic implications of GC-MS analysis on Solanum species relationship

In general, higher distance values were evident among the species/populations of *Solanum* while much higher similarity (lower distance values) characterized the populations

of the same species as indicated by the distance matrices among the examined species/ populations (Tables 3 , 4). A UPGMA tree based on the distance matrix between the 14 *Solanum* species/populations is presented in Fig. 1. In this tree, the two populations of *S. incanum* (S3A & S3B) were separated from all other species. The populations of *S. glabratum* (S4A & S4B) and *S. macracanthum* (S8) together as well as *S. schimperinum* (S7) were also separated from the remaining taxa. The remaining taxa were divided into two sub-cluster; one compressed the two populations of *S. nigrum* (S1A & S1B), the two populations of *S. villosum* and *S. villosum* ssp. puniceum (S2 & S5). The other sub-cluster included *S. coagulance* (S6), *S. torvum* (S9), *S. sisymibrifolium* (S10) and *S. dulcamara* (S11).

The similarity matrix for the 14 *Solanum* species/populations computed with SM coefficient based on the analysis of chemical constituents are illustrated in Table 3. The highest similarity level (98%), was scored for the two populations of *S. glabratum* (S4A & S4B), the same similarity level was scored also for the two populations of *S. incanum* (S3A & S3B). The two populations of *S. nigrum* (S1A & S1B) have a similarity level of 91%, whereas the similarity level for the two populations of *S. nigrum* and *S. villosum* ssp. puniceum (S1A & S5) was 83%. The two populations of *S. villosum* (S2 & S5) have similarity level of 76%. The two species *S. torvum* and *S. sisymbriifolium* (S9 & S10) have similarity level of 83%. Also *S. sisymbriifolium* (S10) was clustered with *S. dulcamara* (S11) at similarity level of 81%.

The relationship between the examined species based on the analysis of chemical constituents as indicated by the tree constructed based on the RMSD coefficient values is illustrated in Fig. 2. The two populations of *S. incanum* (S3A & S3B) and *S. schimperianum* (S7) were separated from the other *Solanum* species/populations. *Solanum coagulans* (S6) was also separated from the remaining taxa. The two populations of *S. incanum* (S3A & S3B) have the lowest genetic distance (0.108) the same distance was also scored for the two populations of *S. glabratum* (S4A & S4B). These two populations and *S. macracanthum* (S8) form a small cluster of the remaining taxa, where the two taxa of *S. glabratum* and *S. macracanthum* (S4A & S8) have genetic distance of 0.470. The two populations of *S. nigrum* (S1A & S1B), the two populations of *S. villosum* (S2 & S5) were efficiently separated from; *S. torvum*, *S. sisymbriifolium* and *S. dulcamara,* (S9, S10 and S11). The distance between the two populations of *S. nigrum* (S1A & S1B) was 0.28, while the distance between the two taxa of *S. villosum* and *S. villosum* ssp. puniceum was 0.48. The two populations of *S. torvum* and *S. sisymbriifolium* (S9 & S10) have a distance of 40 (Table 4).

Discussion

The GC-MS analysis separated all of the components in the examined samples and provided a representative spectral output. Each component ideally produced a specific spectral peak. The retention time can help differentiate between some compounds. The size of the peaks is proportional to the quantity of the corresponding substances in the specimens analyzed (Mohy-UD-Din, 2008). The chemical profile, as expressed by occurrence of the major categories of secondary metabolites (indole alkaloids, iridoids, triterpenes and anthraquinones) is remarkably distinctive (Young et al., 1996). As pointed out by Cardoso et al. (2008), secondary metabolites profile can contribute to the taxonomic position of some species or tribes which remain unclear due to morphological controversies.

The GC-MS analysis separated a total of 87 different compounds from the 14 *Solanum* populations belonging to different species at six different retention times (5, 10, 15, 20, 25 and 30 min). Similar to this study Mohy-UD-Din (2008) used GC-MS and HPLC analysis to solve some taxonomic problems in *Solanum nigrum* complex. Sundar & Justin (2014) also investigated the phytoconstituents present in petroleum ether and methanolic extract of *Solanum virginianum* L. leaves by GC-MS; identified five phytochemical components in the petroleum ether extract and seven phytochemical components in methanolic extract. Akintayo et al. (2013) reported that the essential oil obtained from the hydrodistilled leaves of *S. nigrum* var. virginicum L. from Nigeria was characterized by 37 volatile constituents accounting for 97.6% of the total oil contents.

TABLE 3. Similarity matrix computed based on the analysis of chemical constituents by GC-MS for the *Solanum* species/populations (S1-S11); coded as given in Table 1.

Populations Code	S1A	S2	S3A	S4A	S4B	S5	S3B	S1B	S6	S7	S8	S9	S10	S11
S1A	1.00													
S2	0.86	1.00												
S3A	0.53	0.48	1.00											
S4A	0.61	0.56	0.54	1.00										
S4B	0.60	0.55	0.55	0.98	1.00									
S5	0.83	0.76	0.62	0.73	0.72	1.00								
S3B	0.52	0.47	0.98	0.53	0.54	0.61	1.00							
S1B	0.91	0.77	0.59	0.58	0.59	0.82	0.58	1.00						
S6	0.62	0.67	0.67	0.59	0.58	0.69	0.66	0.66	1.00					
S7	0.69	0.65	0.65	0.47	0.48	0.67	0.63	0.77	0.65	1.00				
S8	0.67	0.67	0.55	0.77	0.76	0.72	0.54	0.63	0.67	0.51	1.00			
S9	0.68	0.73	0.54	0.67	0.66	0.77	0.55	0.67	0.73	0.59	0.75	1.00		
S10	0.73	0.73	0.56	0.65	0.63	0.80	0.55	0.69	0.73	0.61	0.77	0.83	1.00	
S11	0.68	0.70	0.59	0.60	0.59	0.80	0.58	0.69	0.73	0.61	0.73	0.76	0.81	1.00

TABLE 4. Distance matrix computed using Dendro UPGMA based on the analysis of variation in chemical constituents by GC-MS analysis for the *Solanum* species/ population (S1-S11); coded as given in Table 1.

Pop. Code	S1A	S2	S3A	S4A	S4B	S5	S3B	S1B	S6	S7	S8	S9	S10	S11
S1A	0	0.374	0.682	0.619	0.629	0.403	0.690	0.285	0.610	0.550	0.571	0.560	0.517	0.560
S2		0	0.715	0.656	0.665	0.482	0.723	0.470	0.571	0.591	0.571	0.517	0.517	0.539
S3A			0	0.673	0.665	0.610	0.108	0.638	0.571	0.591	0.665	0.673	0.656	0.638
S4A				0	0.108	0.517	0.682	0.647	0.638	0.723	0.470	0.571	0.591	0.629
S4B					0	0.528	0.673	0.638	0.647	0.715	0.482	0.581	0.600	0.638
S5						0	0.619	0.418	0.550	0.571	0.528	0.470	0.445	0.445
S3B							0	0.647	0.581	0.600	0.673	0.665	0.665	0.647
S1B								0	0.581	0.470	0.600	0.571	0.550	0.550
S6									0	0.591	0.571	0.517	0.517	0.517
S7										0	0.699	0.638	0.619	0.619
S8											0	0.494	0.470	0.517
S9												0	0.403	0.482
S10													0	0.431
S11														0

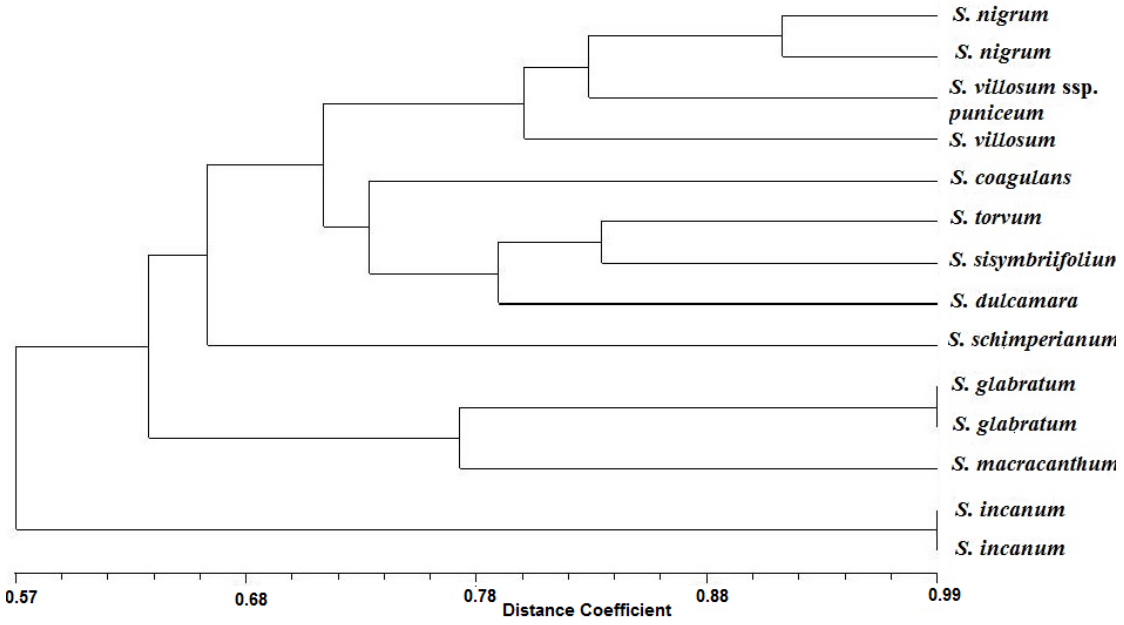

Fig. 1. UPGMA distance tree illustrating the relationships among the Solanum species/populations based on the analysis of chemical constituents revealed by GC-MS analysis.

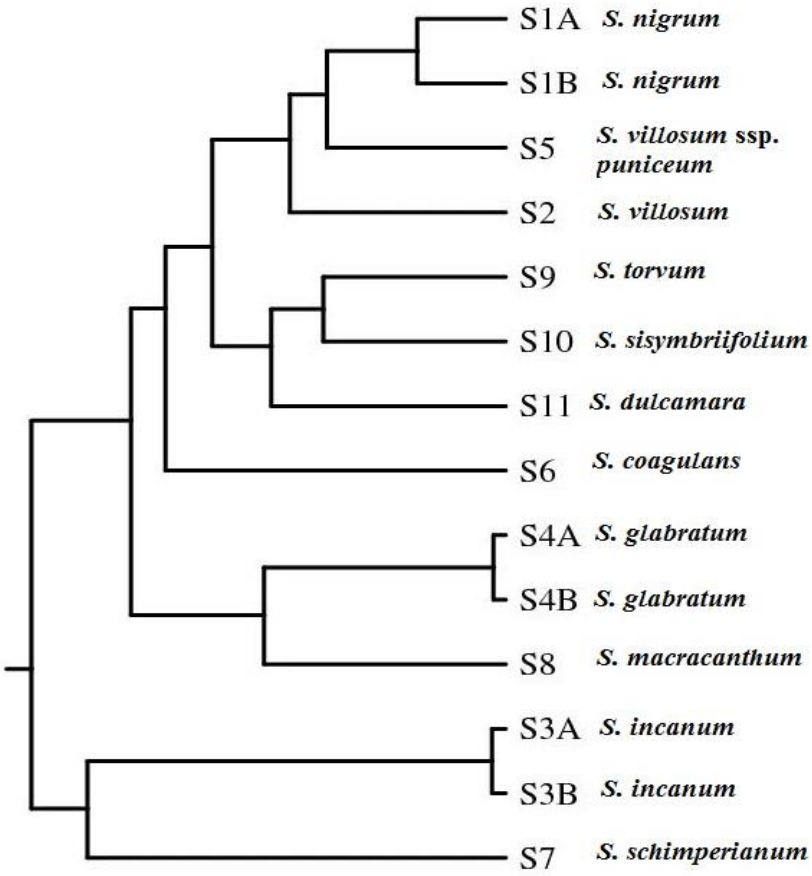

Fig. 2. UPGMA tree constructed with RMSD coefficient showing the relationships among the examined Solanum species/populations based on the analysis of chemical constituents by GC-MS analysis.

Similar to our results, Deepak & Gopal (2014) have determined the essential chemical constituents in the bark of *Solanum verbascifolium* Linn. They have identified a total of 21 phytocompounds in three different extracts from the bark using GC-MS analysis including eight different phytocompounds that were identified also in our study and have variable appearance in the investigated *Solanum* species/populations; they were, 116 and 172 compounds extracted at retention time of 5 min, 88 and 138 compounds at retention time of 10 min, 186 and 196 compounds extracted at retention time of 25 min and two compounds (222 & 296) extracted at the retention time of 30 min.

The cluster analysis of phytochemical data using the RMSD and SM coefficients showed that the two populations of *S. nigrum* (S1A & S1B) are clustered with the two populations of *S. villosum* (S2 & S5); this result confirmed that *S. villosum* is related to *S. nigrum*. Mohy-UD-Din et al. (2009), (2010a) and (2010b) used TLC, HPLC and GC-MS analysis to examine flavonoid glycosides content, alkaloids and epicuticular waxes and also morphological analysis in *S. nigrum* complex. The results suggested that *S. americanum*, *S. chenopodioides*, *S. nigrum* and *S. villosum* had significant differences and might be treated as separate species and not varieties/ subspecies of *S. nigrum*. The above works showed that *S. retroflexum* showed high similarities with *S. nigrum* and was regarded as a variety/subspecies of *S. nigrum*. However, the cluster analysis based on chemical composition cannot differentiate between the two populations of *S. nigrum* at similarity level of 91% using the two coefficients SM and RMSD.

The three species *S. torvum*, *S. sisymbriifolium* and *S. dulcamara* were efficiently separated with *S. coagulans* from *S. nigrum* and *S. villosum* in one cluster using SM coefficient but *S. coagulans* was separated individually when using RMSD coefficient. These results revealed that the three species are related to each other and also the presence of the three species in the same group with *S. nigrum* and *S. villosum* indicating that *S. torvum*, *S. sisumbriifolium* and *S. dulcamara* may be related to *S. nigrum* and *S. villosum*. The current results also revealed that the two populations of *S. glabratum* were clustered with *S. macracanthum* using the SM and RMSD coefficients indicating that the two species are related to each other. Also, the two population of *S. incanum* were separated in one cluster with *S. schimperianum* when using the RMSD coefficient but the two species were separated individually when SM coefficient was used (Fig. 1 and 2).

In conclusion, GC-MS analysis of ethanol extract of 14 *Solanum* species/populations revealed 87 phytochemical constituents detected at six retention times of 5, 10, 15, 20 25 and 30 min. All retention times revealed stable and reproducible polymorphism with the examined *Solanum* species/ populations. The highest molecular weight for the identified compounds was 641 scored in *S. villosum* at retention time 15 min. On the other hand, the lowest molecular weight was 84 and was scored in all *Solanum* species except the two populations of *S. incanum*, *S. coagulans* and *S. schimperianum.* The distance coefficients based on the analysis of chemical constituents separated the populations of *S. incanum* from all other species. The two taxa of *S. glabratum* and *S. macracanthum* together as well as *S. schimperianum* were also separated as two small clusters. Of the remaining taxa, the two populations of *S. nigrum* and the two populations of *S. villosum* were efficiently separated from *S. coagulans, S. torvum, S. sisymibrifolium* and *S. dulcamara.*

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الدلالات التصنيفية للمركبات الثانوية فى مستخلص الايثانول لأنواع السولانم فى جنوب غرب المملكة العربية السعودية

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أجريت هذه الدراسة لتقييم التنوع التصنيفي والوراثى لأربعة عشر من أنواع وجماعات جنس السولانم في جنوب غرب المملكة العربية السعودية بناءا علي التنوع بين المركبات الكميائية الثانوية باستخدام التحليل الكروماتوجرافي، وقد أظهر التحليل الكروماتوجرافي لمستخلص الايثانول ظهور 87 مادة طبيعية خلال 6 فترات مختلفة. تم تسجيل أعلي وزن جزيئي 641 في نوع سولانم فيلوسم *S. villosum* خلال 15 دقيقة، بينما كان أقل وزن جزيئي 84 وتم تسجيله في جميع أنواع السولانم محل الدراسة عدا سولانم انكانم، سولانم كوأجيولانس وسولانم شيمبريانم *S. incanum, S. coagulans and S. schimperianum* خلال 5 دقائق، واعتمادا علي الاختلافات في المكونات الطبيعية تم حساب معامل التشابه الوراثي وبناء شجرتين للبعد الوراثي لتوضيح العلاقة بين أنواع السولانم محل الدراسة حيث أكدت النتائج أن سولانم نجرم وسولانم فيلوسم (*S. villosum* and *S. nigrum*) قد ينتميان إلي نوع واحد. أظهرت شجرة المسافة أيضا أن سولانم كوأجيولانس (*S. coagulans*) يتبع كلا من سولانم ماكراكانثم وسولانم جلابراتم (*S. macracanthum* and *S. glabratum*)، وتشير نتائج الدراسة كذلك أن سولانم سيسيمبريفوليم وسولانم دلكمارا (*S. sisymbriifolium* and *S. dulcamara*) لهما منشأ واحد، ومن الجدير بالذكر أن هذه النتائج متوافقة إلى حد كبير مع علاقات الأنواع محل الدراسة بناءا علي الاختلافات المورفولوجية بينها.