

Anatomical and chemo-taxonomical investigations within some *Salsola* L. species grown in the western costal region of Egypt

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Abstract

Fourteen stem anatomical characters, beside twelve minerals; Na, Mg, Al, Si, P, S, Cl, K, Ca, Fe, Cu, Zn; and organic matters ;flavonoids, Kaempferol, quercetin, quercetin-3-glucoside, quercetin-3-rhamnoside and quercetin-3-rutinoside; were identified in six *Salsola* species; *S. inermis*, *S. kali*, *S. longifolia*, *S. tetragona*, *S. tetrandra* and *S. volkensis* grown in the Mediterranean coastal strip from Alexandria to El-Dabaa district in Egypt, in order to better understanding of the relations between the studied species and elucidate the arguments about their taxonomic position. The work based on light microscope for anatomical investigations, X-ray analyses in the leaves for minerals compositions and paper chromatography in all plant parts for chemical organic constituents. The anatomical results obtained showed that *S. kali* and *S. inermis* are closely related and separated from the rest of the studied species, while *S. tetrandra* and *S. volkensis* are related as well and meanwhile in relation with the other two species; *S. tetragona* and *S. longifolia*. These characters revealed that the genus exhibit abnormal secondary growth with variations within the studied species.. The mineral contents in the leaves of these species differs between the taxa. The maximum records of Mg, Al, Si, P, Ca, and Fe were found in *S. kali* While the maximum records in Na and K were found in *S. inermis*. Flavonoids, Kaempferol, quercetin, quercetin-3-glucoside, quercetin-3-rhamnoside and quercetin-3-rutinoside identified. No flavone aglycons and glycosides or C-glycosides were detected. Hypogallic acid, gallic acid, phloroglucinol, gentisic acid, protocatechuic acid and (-)-epigallocatechin have been also detected. The biochemical data indicate that *Salsola kali* is highly different and support its anatomical aspects. Mineral and organic compounds analyses revealed that the studied species are under stress and their variations cannot be used in the taxonomy of the group. All the obtained results indicate that *S. kali* has its own characters which support its separation in a separate section as indicated by previous authors.

Keywords: Anatomy- Chemotaxonomy- Minerals-Organic compounds- *Salsola*-Taxonomy

1. Introduction

The Chenopodiaceae. of about 122 genera and about 1500 species (Cronquist 1981; Shmida, 1985; Prato 1986; Thorne 1992; Kühn *et al.*, 1993) is one of the core centrosperous families. It has great number of halophytes and xerohalophytes plants (Breckle 1986; Aronson, 1989; Akhani and Ghorbanli, 1993; Le Houerou, 1993). Genus *Salsola* L. is one of the halophyte plants belong to tribe *Salsoleae*. The genus *Salsola* L. *s.l.* includes the segregation genera *Caroxylon* Thunberg, *Clinacoptera* Botschantsev, *Hypocyclix* Woloszewak = *Darniella* Maire, *Neocaspia* Tzvelev, *Nitrosalsola* Tzvelev, and *Xylosalsola* Tzvelev, comprises 130-150 species, which are especially numerous in the arid and coastal zones of Eurasia, including Central Asia, the Middle East and the Mediterranean region (Mosyakin, 1996). The genus is a cosmopolitan group of plants which distributed and naturalized all over the world. Species belong to this genus found in sea beaches, in grassland, wastelands, roadsides and desert communities (Krzaczek *et al.*, 2009). The detailed revision of most species groups of the genus *Salsola* *s.* carried out by (Botschantsev, 1989). Based on the earlier works of (Fenzl, 1851, Ulbrich, 1934 and Iljin, 1936), as well as on morphological features of vegetative organs (Botschantsev and Akhani, 1989) recognized six systematic sections of *Salsola*; Section *Caroxylon* (Thunb.) (Fenzl), Section *Malpigila* Botsch., Section *Cardiandra* Aellen, Section *Belanthera* Iljin, Section *Coccosalsola*, sub. *Arbuscula* (Fenzl) and Section *Salsola*, sub. *Kali* (Ulbrich). The classification of genus *Salsola* *s.* worked by (Botschantsev and Akhani, 1989) is still widely accepted and in the present study .

Anatomical and chemical works on the genus are rare; from them are those of **Bisalputra (1962)** on the primary vascular system and nodal anatomy of 32 species of the family Chenopodiaceae. His results showed that the family has a unilacunar node, and the structure of the primary vascular system is classified into three types by reference to the number of leaf traces at a node and their relationships with each other or with cauline bundles within the axis. The *Kochia-Bassia* type, which is characteristic of *Kochia*, *Bassia*, *Malacocera*, *Threlceldia*, *Babbagia*, *Enchylaena*, *Suaeda*, and *Salsola*, is the most primitive and these genera are considered to be closely related. While **P'yankov et al. (1997)** made a comparative anatomical and biochemical analysis in *Salsola* species with and without a Kranz type leaf anatomy they studied the leaf anatomy by light and electron microscopy. **Bercu and Bavaru (2004)** studied the anatomical aspects of *Salsola kali* subsp. *Ruthenica* (Chenopodiaceae) and they comprised an investigation of the root, stem and leaf anatomy of *Salsola kali* subsp. *Ruthenica* (Chenopodiaceae) from the Dobroudzha region (Romania). Afterward **Idzikowska (2005)** examined the morphology and anatomy of generative organs of *S.kali* Ssp. *Ruthenica* using both light and Scanning Electron microscopy. The whole flower, fruit and their parts were observed in different developmental stages. From chemo- taxonomic investigations are those done by **Turki (1999)**, **Oeuslati et al. (2006)**, **Krzaczek et al. (2009)**, **Jafari et al. (2011)** and **El sharabasy and Hosny (2013)**. All these works dealt with few analyses of certain components only. For those both anatomical and chemical investigations within some of the wildy grown *Salsola* species grown in the western coastal strip of Egypt.

2. Materials and Methods

Field trips have been carried throughout three years (2012-2014) to the Mediterranean coastal strip, from Alexandria to Al-Dabaa, in Egypt by Shiha and Abdel-maged. Six species of genus *Salsola* were collected; *Salsola inermis* Forssk., *S.kali* L., *S.longifolia* Forssk., *S.tetragona* Delile, Descr., *S.tetrandra* Forssk. and *S.volkensii* Schweinf. & Asch. (Table 1). The collected specimens were identified by the aid of student's flora of Egypt (**Täckholm, 1974** and **Boulos ,1999**). The forth node from a well represented individuals has been chosen for anatomical investigations. Anatomy was done on the basis of customary method of **Johansen (1940)**. For mineral analyses, five leaves from each species were dried and grounded exposed to X-ray analyses in the scanning electron microscopy unit in Alexandria University, Egypt, each separately, to estimate the mineral contents in each leaf. The results are expressed as minimum, maximum and the mean of the five readings.

Chemical analyses has done by taken fixed weight of fresh materials; about 400 – 600 gm.; dried and powdered, defatted with ethyl acetate for 40 h, extracted with 100 ml methanol, and then concentrated by evaporation (**Markham,1982**). Hot distilled water (50 ml) was added to the extract and left over night to remove chlorophyll, lipids and waxes. The aqueous/methanolic filtered and the filtrate divided into two equal volumes (**Markham and Mabry, 1975**). One part of the aqueous/methanolic samples was concentrated and applied to a polyamide column with increasing polarity by using ascending sequencing concentrations; (70%, 80%, 85%, 90%, 95%, till absolute alcohol.). Further purification of the flavonoids was done by preparative thin layer chromatography. The other volume was evaporated to dryness then subjected to continuous ether extraction. It was then shacked several times with water. The ethereal layer contained the free aglycone. The phenolic acids and – (-) epicatechin were separated from the aqueous layer by preparative paper chromatography. All experiments were performed at least in triplicate.

Identification of phenolic compounds by both Column chromatography (CC) and Paper chromatography (PC), phenolic acids and flavonoids compounds according to **Harborne (1973 and 1984)**, Sugar moieties of flavonoids according to **Lewis and Smith (1967)**. UV- Spectroscopic analysis of flavonoids diagnosis according to **Mabry et al. (1970)** by using FT.IR Tensor 37.

Table 1 Collection data from the Mediterranean coastal strip in Egypt, North Africa

No	Species	Locations and date of collection	GPS data
1	<i>Salsola inermis</i> Forssk.	80 Km west of Alex; Alex-Matrouh road sides (Omayed) 16.10.2012	N 30 49.541 E 029 11.488
2	<i>S. kali</i> L.	Burg El-Arab road kilo 52 16.10.2012	N 30 56.519 E 092 30.145

3	<i>S. longifolia</i> Forssk.	80 Km west of Alex; Alex-Matrouh road sides (Omayed) 25.4.2013	N 30 49.541 E 029 11.488
4	<i>S. tetragona</i> Delile, Descr.	Burg El-Arab airport road 27.4.2014	N 30 59.251 E 029 39.331
5	<i>S. tetrandra</i> Forssk.	El-Hammam road, Mariout lake sides, Kilo 65 16.10.2012	N 30 52.866 E 029 22.170
6	<i>S. volkensis</i> Schweinf. & Asch.	El-Omayed road, El-Khashm hill 20.11.2013	N 30 48.293 E 029 11.485

3. Results

14 stem anatomical characters were investigated in the studied taxa as shown in table (2) and plate 1. The stem outline was irregular circle or circle except in *S. longifolia*, it was circular with four protrusions and two groves as shown in plate 1, Fig.5. Ribbed stem found in *S.kali* only, while undulate surface stem found in *S.inermis* and the rest of the taxa has straight stem surface. The stem enriched with either unicellular, multicellular hairs, or even both while in *S. longifolia* has glabrous stem. *S. tetragona* has unicellular and vesicular hairs (Plate 2, Fig. 9). The epidermal cells are radial except in *S. tetrandra* and *S.volkensis* they are tangential. The cortex tissue consists of one to six rows continuous or discontinuous, angular collenchymas, except *S.tetragona* (Table 2, Plate 1 & 2). Elongated palisade chlorenchyma layer followed by rounded chlorenchyma layer present in *S.kali* only, while lignified parenchyma found in *S.tetragona*. Paranchyma cells with druses ca-oxalate crystals in *S.kali*, *S.tetragona* and *S.tetrandra*, the rest of the studied taxa lack these crystals. The vascular bundles; from four to eighteen, exhibit anomalous secondary growth, in which the cambium divides giving secondary xylem introrse and fibers extrorse, then continued division giving secondary xylem entrorse and secondary phloem extrorse. The cambium divides giving 2^{ry} phloem to both the outside and inside, then starts to give 2^{ry} xylem to the inside and return again giving 2^{ry} phloem to the inside. The net result we found complete 2^{ry} bundles embedded in the 2^{ry} xylem or patches of secondary phloem in between the secondary xylem and the fibers (Plate 1 and 2).

Extraction of the aerial parts of *Salsola* species under investigation, according to their chemical properties were classified as flavonolaglycones and their O-glycosides. The distribution pattern of the identified flavonoids and phenolic compounds in the studied species is presented in table (3) and their chemical properties are shown in tables (4,5,6).

It could be seen that the flavonolaglycone (quercetin) and its O-glycosides; quercetin-3-glucoside, quercetin-3-rhamnoside and quercetin-3-rutinoside were the prevailing flavonoids. Quercetin-3-rutinoside was the most dominant flavonol glycoside in *Salsola* species under investigation. The flavonolaglycone kaempferol was identified while the kaempferol glycosides were not detected.

Flavone aglycons and their O-glycosides could not be detected in the studied species. Flavone, unlike flavonol occurs remarkably with sugar bound by a carbon-carbon to the aromating nucleus. Such glycosyl flavone was not found. It could be seen that the aerial parts of *Salsolas* species under investigation contain hypogallic acid (2,3-dihydroxybenzoic acid), gallic acid (3,4,5-Trihydroxybenzoic acid), phloroglucinol(1,3,5-trihydroxybenzene), gentisic acid (2,5-dihydroxybenzoic acid) and protocatechuic acid (3,4-Dihydroxybenzoic acid). Hypogallic acid was the most frequently detected and gallic acid was found only in *Salsola kali* as shown by the IR spectrum (Tab.5, Figs.1,2,3,4,5 & 6).

-(-)Epicatchin which is the condensed tannins precursor was found in the most of the *Salsola* species under investigation whereas gallic acid the precursor of hydrolysable tannins was only found in *Salsola kali*. (Tab.5, Fig.6). Hypogallic acid, gallic acid, phloroglucinol, gentisic acid, protocatechuic acid and -(-)epicatchin were detected for the first time in *Salsola* species.

The maximum records of Mg, Al, Si, P, Ca, and Fe were found in *S.kali* (1.7%, 3.45%, 9.25%, 5.25%, 34.1% and 2.95% respectively). While the maximum records in Na and K were found in *S.inermis* (7.7% and 39.6%). The minimum record in Na was in *S. longifolia* (2.3%) and it has the maximum records in Cu and Zn (3.75% and 3.35%). *S.tetragona* has minimum records of Mg, Si, Cl, Cu and Zn (0.6%, 0.3%, 20.55%, 1.65% and 0.8%),

while it has the maximum record of S (27.7%). *S.tetrandra* has the minimum record of S (2.6%) and maximum record of Cl (70.05%). *S.volkensii* has minimum amounts of Al (1%) and P (0.3%). The x-ray analyses indicate that *S. kali* is the only species that contain Fe in their leaves (Fig. 7).

These records express the percentage of that element according to the total amounts of minerals present in that species, i.e. the instrument suppose that the summation of all the mineral amounts represent 100 and each element represent how much from the 100.

4. Discussion

Anatomical characters have been employed for systematic purposes for over a hundred years **Radford et al. (1974)**. **Davis and Heywood (1973)** stated that anatomical characters play an increasingly important role in the formation of natural or phonetic groups. **Solereeder (1899)**, **Wilson (1924)** and all anatomical studies within both Amaranthaceae and Chenopodiaceae afterward concluded that there were no definite anatomical characters could be used to differentiate between the two families. The attention which has been focused on these two families is centered upon the peculiar secondary growth. The anatomy of the genus *Salsola* was studied by many authors, but all of these studies were in relation to C3-C4 Kranz anatomy in the leaves of the genus and the related genera. Stem anatomy of the genus was very rare and if found was in *Salsola kali* only. This is due to the difficulty in sectioning in the woody, hard stem and the abnormal secondary growth happened in many members of the Chenopodiaceae. The results showed great variations within the studied species, the anatomical features of *S.kali* are completely different than the other five species. *S.kali* has chlorenchyma cells in between the elevated striations which supported by layers of collenchymas cells. These characters coordinate with **Grigore et al. (2014)** who stated that the stem in cross section was ribbed containing collenchyma cells alternated with palisade tissue and short cell chlorenchyma. These characters are not common within the rest of the species, as *S.longifolia*, *S.tetragona*, *S.tetrandra*, *S. volkensii* and *S.inermis* have either irregular or flattened parenchyma in the cortex, no chlorenchyma cells found at all. The presence of chlorenchyma cells (palisade cells) in the cortex is an important character in the division of the genus as **Rilke (1999 a)** indicated and according to it the genus *Salsola* has divided into two sections; sect. *Kali* and sect. *Salsola*. The secondary growth is abnormal in cambium activity. In all the studied taxa anomalous secondary growth recorded in which the cambium divides giving 2^{ry} phloem to both the outside and inside, then starts to give 2^{ry} xylem to the inside and return again giving 2^{ry} phloem to the inside. The net result we found complete 2ry bundles embedded in the 2ry xylem fibers. These peculiar secondary growths were observed by **Solereeder (1899)** and **Wilson (1924)** and they described the primary vascular bundles of the Chenopod stem by being not circularly arranged and found in the medulla of the stem separated by several or many cell layers from the secondary growth. This is not found in this study as all the studied species are circularly arranged.

Malih (2010) studied the stem anatomy of four *Salsola* species, his results indicate to the importance of epidermal cell thickness, cuticles, chlorenchyma, collenchyma, sclerenchyma and also xylem row length in the separation of the studied species. From this work we can conclude that the most important anatomical characters in species circumscription are; the presence of chlorenchyma cells in the cortex (palisade cells), number of collenchyma layers and the type of secondary growth. The study of the anatomical characters in the stem, support the division of the *Salsola* species into two sections as mentioned by **Mosyakin (1996)** and **Rilke (1999a, b)**. In this investigation the type of secondary growth considered from the important characters in the circumscription of the species, as *S.volkensii* is completely different than the rest of the studied species. This conclusion supports the previous results obtained by **Carolin et al. (1975)** who found two anatomical types in the leaves of *Salsola*. Salsoloid type leaves are characterized by two continuous layers of chlorenchymatous cells (a layer of palisade mesophyll cells and an inner layer of very distinctive Kranz type bundle cells) on the periphery and water-storage parenchyma in the center. The main vascular bundle occupies the central position in the leaf, and only the small, peripheral vascular bundles are in contact with the chlorenchyma. The second type is the Sympegmoid type leaves are characterized by having two or three layers of palisade cells and a discontinuous layer of indistinctive bundle sheath cells (typically non-Kranz) around water-storage tissue.

The x-ray analyses indicate that all the studied species has no Fe at all, except *S. kali*. This data can aid in the conclusion of the identity in all the studied tools to separate *S.kali* in a separate section as indicated by **Rilke (1999a)**. It is perhaps interesting to see to which degree major taxa are characterized by certain substances and to which degree chemical data have influenced the classification of major taxa of plants. Meanwhile, **Kubitzki (1984)** pointed to the importance of the accumulation of plant substances in taxonomic studies as secondary metabolites considered defense substances against herbivores. The results of the study showed differences in both mineral and organic contents can be attributed to the ecologically stress present in the growing habitats of these species.

Extraction of the aerial parts of *Salsola* species under investigation, according to their chemical properties were classified as flavonol aglycones and their O-glycosides.

It could be seen that the flavonol aglycone (quercetin) and its O-glycosides; quercetin-3-glucoside, quercetin-3-rhamnoside and quercetin-3-rutinoside were the prevailing flavonoids. Quercetin-3-rutinoside was the most dominant flavonol glycoside in *Salsola* species under investigation. The flavonol aglycone kaempferol was identified while the kaempferol glycosides were not detected.

The presence of isorhamnetin-3-glucoside, 3-rutinoside, rhamnetin identified in *Salsola kali* (**Thomas et al.,1985; Hegnauer,1989**) was not confirmed in this investigation while the presence of quercetin, kaempferol, has been established (Tab.3). Quercetin-3-glucoside, quercetin-3-rhamnoside and quercetin-3-rutinoside were recorded for the first time in *Salsola* species. As the genus *Salsola* exhibits a large diversity in habitat and morphology, it could be also seen that there is a variety in the phenolic constituents. The biochemical data indicate that *Salsola kali* is highly different and support its morphological and anatomical aspects.

The presence of isorhamnetin-3-glucoside, 3-rutinoside, rhamnetin identified in *Salsola kali* by **Thomas et al. (1985) and Hegnauer (1989)** not confirmed in this investigation while the presence of quercetin, kaempferol, has been established. Quercetin-3-glucoside, quercetin-3-rhamnoside and quercetin-3-rutinoside were recorded for the first time in *Salsola* species. Quercetin and its 3-rutinoside (rutin) were the most widely spread among the species studied.

It could be seen that the aerial parts of *Salsola* species under investigation contain hypogallic acid (2,3-dihydroxybenzoic acid), gallic acid (3,4,5-Trihydroxybenzoic acid), phloroglucinol (1,3,5-trihydroxybenzene), gentisic acid (2,5-dihydroxybenzoic acid) and protocatechuic acid (3,4-Dihydroxybenzoic acid). Hypogallic acid was the most frequently detected and gallic acid was found only in *Salsola kali*. Gallic acid the precursor of hydrolysable tannins was only found in *Salsola kali*. Hypogallic acid, gallic acid, phloroglucinol, gentisic acid, protocatechuic acid were also detected.

-(-)Epicatchin which is the condensed tannins precursor was found in the most of the *Salsola* species under investigation it is detected for the first time in *Salsola* species. It was characterized by its chromatographic data and IR-spectral analysis.

As the genus *Salsola* exhibits a large diversity in habitat and morphology, it could be also seen that there is a variety in the phenolic constituents. The biochemical data indicate that *Salsola kali* is highly different and support its morphological and anatomical aspects. Although the number of species involved in this study is rather limited, yet the results obtained give an indication to the possibility of deducing a correlation between the presence or absence of certain flavonoid compounds and the different groups of species in the genus. This in itself may have significance in the hierarchical arrangement of the species in the genus *Salsola*. The data obtained, so far, show that all the glycosidic patterns are flavonol-O-glycosides of quercetin. The different phenolic patterns are related to their occurrence to the family chenopodiaceae.

From this study we conclude that the studied species can be described as a typical Chenopoid genus. Anatomical characters revealed that the genus exhibit abnormal secondary growth with great variations within the studied species. This study indicates that *S.kali* has its own characters which support its separation in a separate section as indicated by previous authors. Mineral and organic compounds analyses revealed that the studied species are under stress and their variations cannot be used in the taxonomy of the group.

Table 2 Stem anatomical characters investigated within the studied *Salsola* Sp.

Taxa→ Characters↓	<i>S.inermis</i>	<i>S.kali</i>	<i>S. longifolia</i>	<i>S. tetragona</i>	<i>S. tetrandra</i>	<i>S. volkensis</i>
S T E M outline	Irregular Circle	Irregular Circle	Circular with 4 protrusion	Circular	Circular	Circular
Surface	Undulated	Ribbed	Straight	Straight	Straight	Straight
Hair type	Unicellular & multicellular	Unicellular	-----	Unicellular & Vesicular	Unicellular	multicellular
Shape of epidermal cells	Radial	Radial	Radial	Radial	Tangential	Tangential
Collenchyma cells (angular)	Present	Present	Present	Absent	Present	Present
State of collenchyma cells	Continuous	Discont.	Continuous	-----	Continuous	Continuous
Number of collenchyma	1	6	6	-----	1	1
Palisade tissue	Abscent	Present	Abscent	Abscent	Abscent	Abscent
Lignified cortical parenchyma	Abscent	Abscent	Abscent	Present	Abscent	Abscent
Crystals in cortical parenchyma	Abscent	Present	Abscent	Present	Present	Abscent
Arrangement of vascular bundles	Symmetric	Asym.	Asym.	Symmetric	Asym.	Asym.
No. of vascular bundles	14-18	12	8	4-6	8	5
Lignified pith parenchym	Present	Absent	Present	Absent	Absent	Absent
Crystals in pith parenchyma	Absent	Absent	Absent	Absent	Absent	Present

Table 3 Distribution of flavonoids and Phenol compounds in *Salsola* species under investigation.

	Flavenoids					Phenol compounds					
	Q	K	Q-3-GLU	Q-3-RH.	R	H. Ga	Ga	Ph.	Ge.	Proto.	-(-)Epi.
<i>S.kali</i>	+	+	+	+	+	+	+	-	+	-	-
<i>S.longifolia</i>	+	+	-	+	-	-	-	-	+	+	+
<i>S.tetragona</i>	-	-	-	-	+	-	-	+	-	+	-
<i>S.tetrandra</i>	+	+	-	-	+	+	-	+	-	-	+
<i>S.volkenisii</i>	+	-	+	-	+	+	-	+	-	-	+
<i>S.inermis</i>	-	+	-	-	+	+	-	-	-	-	+

Key to flavenoids: Q=Quercetin; K=Kempferol; Q-3-GLU=Quercetin-3-glucoside; Q-3-RH=Quercetin-3-rhamnoside; R=Rutin.

Key to phenol compounds: H.Ga.=hypogallic acid; Ga.=gallic acid; Ph.=Phloglucin; -(-)Epi.=Epicatechin; Ge=Gentisic acid; proto.=protocatechuic acid.

Table 4 R_f values of flavonoids from *Salsola* species under investigation.

Flavonids	Solvent systems		
	PC	TLC	
		BAW	HOAcBPF
	R _f (X100)		
Q	71	04	16
K	64	05	20
Q-3-GLU	57	35	
Q-3-RH	31	58	
R	61	51	

Key: PC=Paper chromatography; TLC=Thin layer chromatography; Q=Quercetin; K=Kempferol; Q-3-GLU=Quercetin-3-glucoside; Q-3-RH=Quercetin-3-rhamnoside; R=Rutin. (BPF=Benzene-Pyridin-Formic acid). BAW=Butanol-Acetic acid-Water 4:1:5 ;HOAc=Acetic acid (15%).

Table 5 Absorption spectra and characterization of flavonoids isolated from *Salsola* species under investigation.

Flavonoids	Absorption spectra (nm) after addition of					
	MeOH	NaOMe	AlCl ₃	AlCl ₃ /HCl	NaOAc	NaOAc/H ₃ BO ₃
Quercetin(Q)	371.255	321.221	453.221.272	432.223.272	403.227.275	402.227.264
Kaempferol(K)	366.265	413.275	302.350.266	351.420.255.271	386.275	317.370.266
Quercetin-3-glucoside(Q-3-glu)	370.231	431.225.277	351.403.227	343.405.228	401.227.253	397.228.260
Quercetin-3-rhamnoside(Q-3-Rh)	350.253	391.267	330.428.275	401.270	372.272	365.257
Quercetin-3-rutinoside(Rrtin)	350.372 250.276	230.415 225.270	364.400 226.267	355.402 224.266	402.226.274	391.227.262

Key: MeOH= Methanol; NaOMe= Sodium methoxide; AlCl₃= Aluminium chloride; HCl= Hydrochloric acid; NaOAc= Sodium acetate; H₃BO₃=Boric acid.

Table 6: R_f values of phenolic acids isolated from *Salsola* species under investigation and sugar moieties.

phenolic acids	Solvent systems	
	PC	
	BAW	HOAc
	R _f (X100)	
Hypogallic	77	70
Gallic	85	62
Phloroglucinol	67	63
Protocatechuic	79	56
Gentesic	83	64
-(-)Epicatchin	68	51
Sugar moieties		
Glucose	18	
Rhamnose	30	
Aglycons Quercetin	71	04
Kaempferol	64	05

Key: PC=Paper chromatography; BAW=Butanol-Acetic acid-Water; HOAc=Acetic acid (15%).

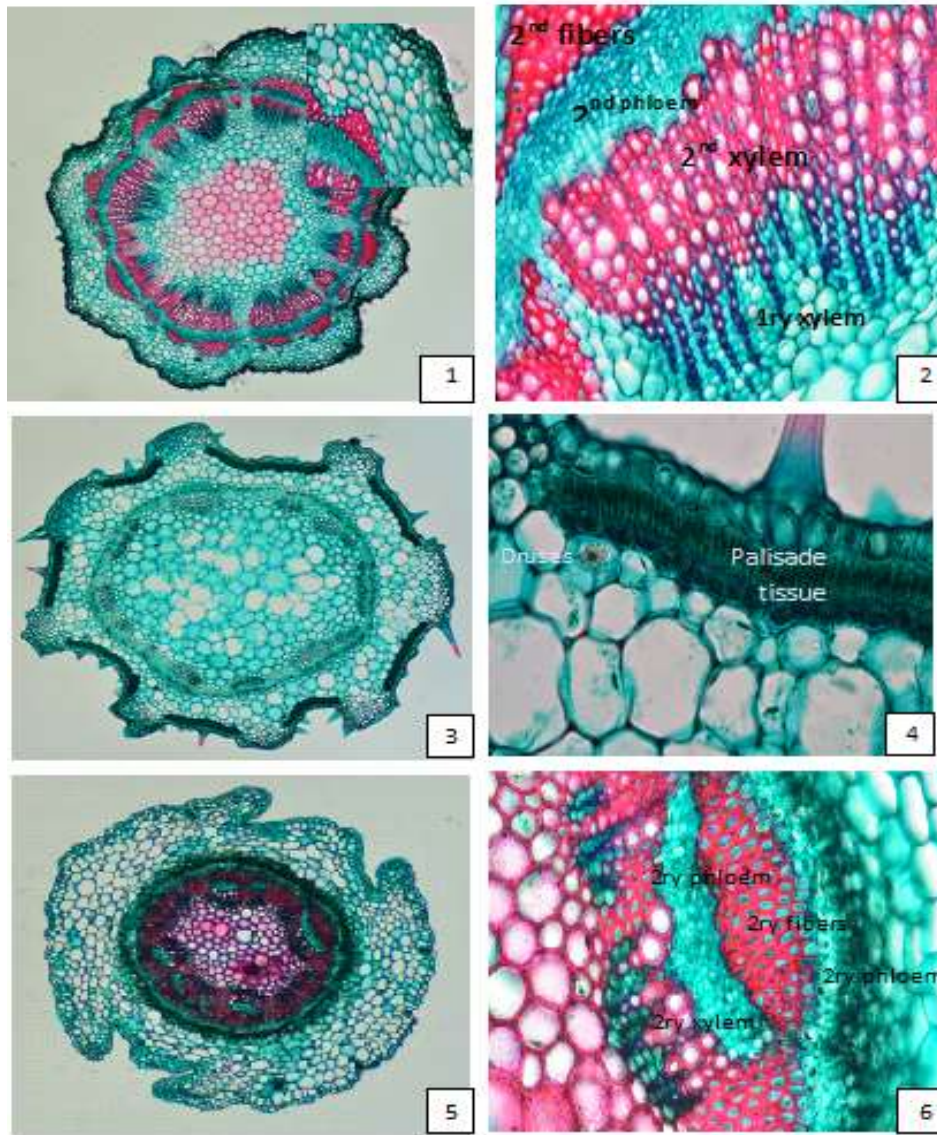


Plate 1 Light Microscope (L M) micrographs of T.S. in stem of *Salsola* studied species.

Figs 1 &2 *Salsola inermis* showing outline and vascular bundles (X.40,X400)

Figs 3 &4 *Salsola Kali* showing outline and palisade tissue (X 40,X 400)

Figs 5 &6 *Salsola longifolia* showing outline with 4 protrusion and V.B.(X 40,X 400)

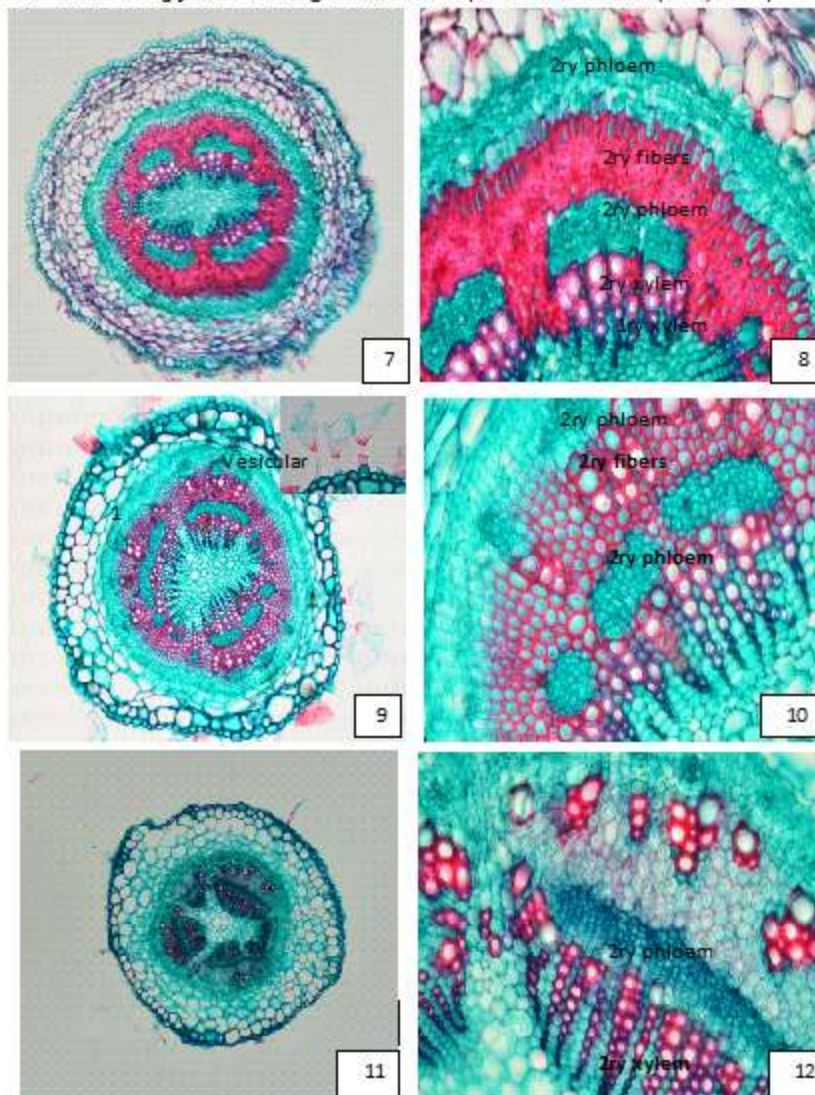


Plate 2: Light Microscope (LM) micrographs showing T.S. in stem of Salsola
Figs 7 &8 *Salsola tetragona* showing outline and secondary growth in V.B. (X 100,X400)
Figs 9 &10 *Salsola tetrandra* showing outline ,vesicular hairs and V.B.(X 100,X400)
Figs 11&12 *Salsola volkensisii* showing outline and V.B.(X40,X400)
Fig 1 IR spectrum of hypogallic acid (2,3-dihydroxybenzoic acid).

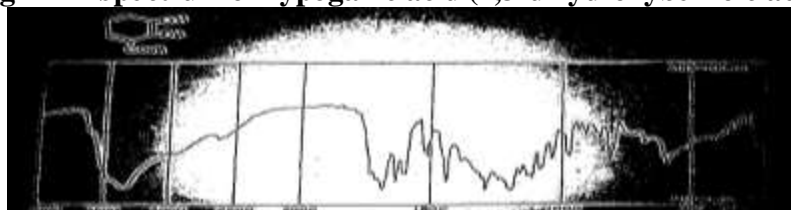


Fig 2 IR spectrum of gallic acid (3,4,5-Trihydroxybenzoic acid).

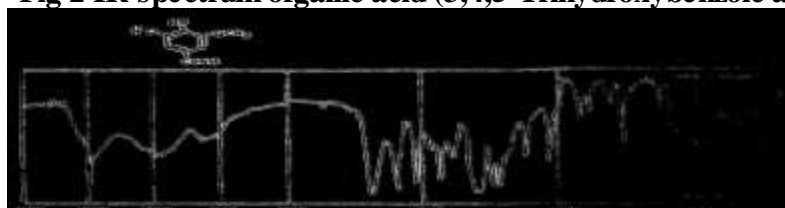


Fig 3 IR spectrum of phloroglucinol (1,3,5-trihydroxybenzene).

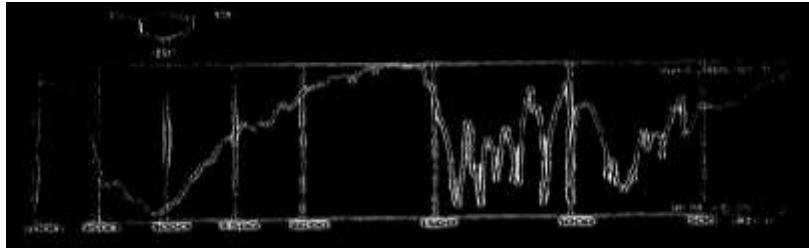


Fig 4 IR spectrum of gentisic acid (2,5-dihydroxybenzoic acid).

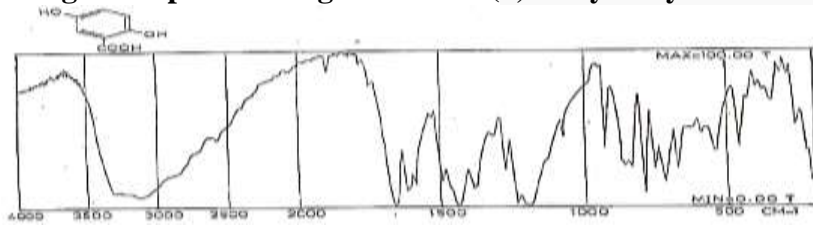


Fig 5 IR spectrum of protocatechuic acid (3,4-Dihydroxybenzoic acid).

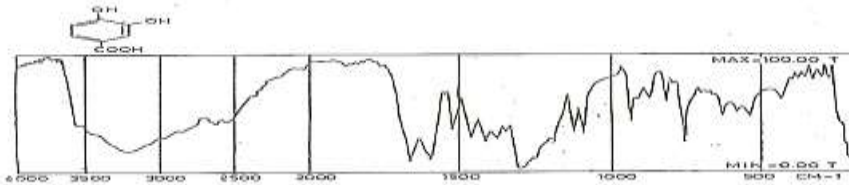


Fig 6 IR spectrum of (-)-epicatchin.

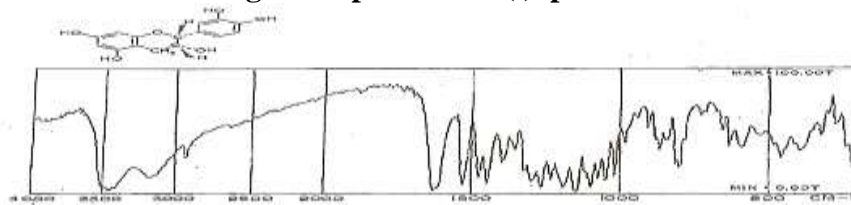
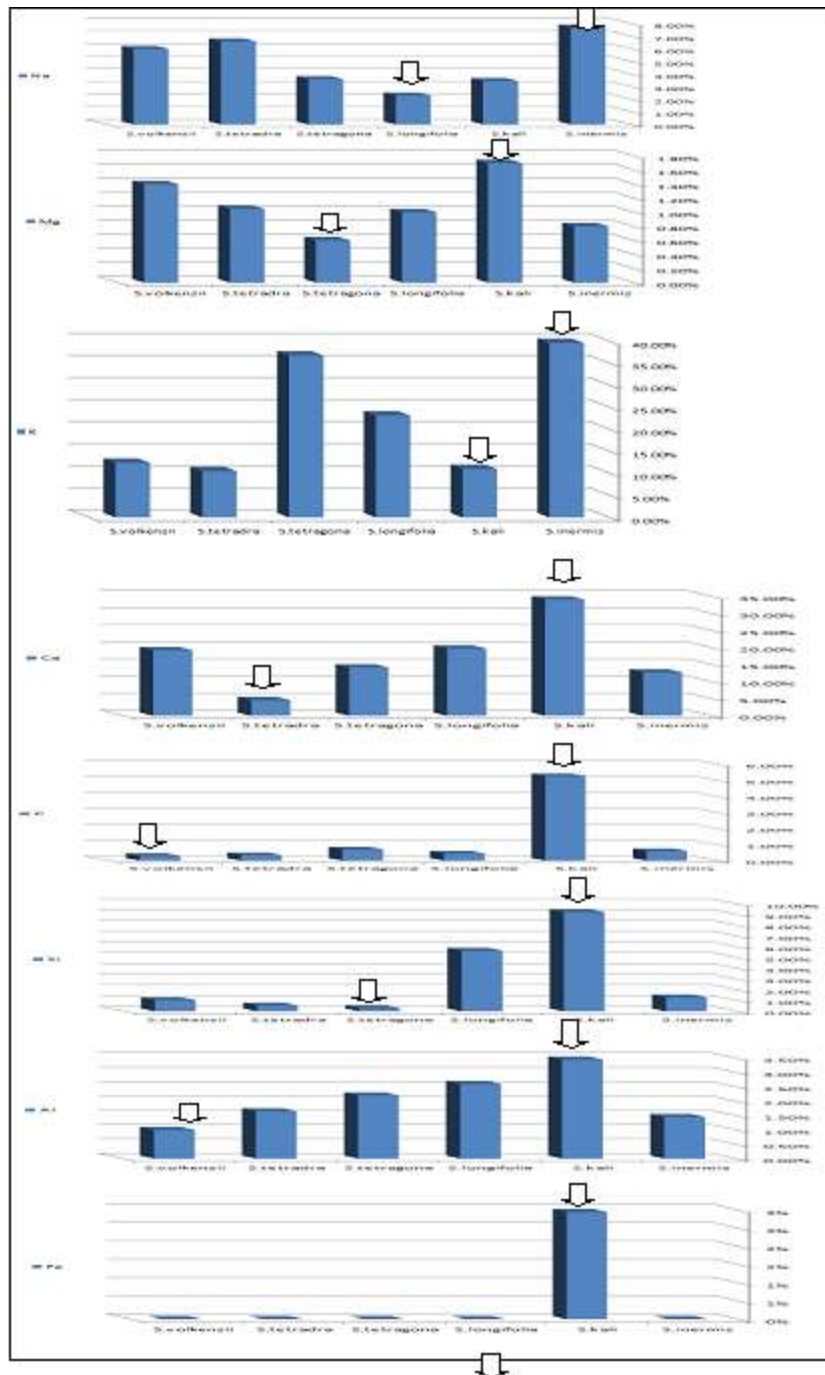


Fig. 7 Variation in mean records of the different minerals investigated within the studied *Salsola* species. The highest and lowest ratios indicated by arrows. The figures arranged as follows: Na, Mg, K, Ca, P, Si, Al, Fe, Cu, Zn, S, Cl.



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