Comparative Pharmacognostic evaluation of some species of the genera *Suaeda* and *Salsola* leaf (Chenopodiaceae)

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Abstract: The genera *Suaeda* and *Salsola* are halophytic plants belong to the family Chenopodiaceae. Species of these genera have been extensively used in traditional medicines against many diseases due to their various bioactive compounds such as carotenoids, vitamins, sterol, phenolic compounds etc. The present research was carried out to establish detailed pharmacognosy of *Suaeda fruticosa, Suaeda monoica, Salsola imbricata and Salsola tragus,* which included macroscopy, microscopy, physico-chemical parameters and qualitative phytochemical screening of leaf samples extracted with methanol and chloroform. It was observed that macroscopic and microscopic characteristics were diagnostic features and can be used for distinction and identification of these closely related plant species. Phytochemically, these plant species are rich in constituents like anthraquinones, alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, phenolic compounds and terpenoids. Physico-chemical parameters revealed that in all investigated plant species; methanol extractive values were higher than that of chloroform. Moreover, total ash values were found to be higher than other acid insoluble and water-soluble ash values, while a considerable amount of moisture was present in the species of both genera. On the basis of pharmacognosy, species of *Suaeda* were found to be more promising than *Salsola*. Present investigation will contribute towards establishment of pharmacognostic profile of these medicinally effective plants species.

Keywords: Halophyte, Pharmacognosy, Phytochemical, *Suaeda*, *Salsola*.

INTRODUCTION

Since long time, plants have been used as a remedy for many diseases because they have therapeutically active compounds (Cohen, 2002). Plant drugs have been significantly prescribed by physicians (Cowan, 1999). Increasing demand of medicinal plants is due to the presence of remedial natural products having no side effects (WHO, 2001), these natural products as a crude drug can be included in herbal pharmacopoeia after establishment their pharmacological standards (Mahendra *et al*., 2009).

Halophytic plants have been used for medicinal purposes because of the presence of health promoting bioactive compounds (Ksouri*et al*., 2012). *Saueda* and S*alsola* are two important halophytic genera of the family Chenopodiaceae. The family Chenopodiaceae comprises of 103 genera and c. 1300 species worldwide (Mabberley, 1997). In Pakistan, it is represented by 35 genera and 106 species. These genera are widely distributed in semi-desert, desert and along sea-shores (Freitag *et al.,* 2001). The extract of *Suaeda monoica* has been used against hepatitis because of the occurrence of triterpenoids and sterolsand these biomolecules have also been reported as an ointment for wounds (Ravikumar *et al.,* 2010). The leaf extract of *Suaeda fruticosa* is used in the treatment of opthalmia (Rashid *et al.,* 2000) and also been reported to possess antibacterial, antioxidant and anticancer activities (Sami-Ullah *et al*., 2012). *Salsola tragus* (synonym: *Salsola kali*) specifically has salsolin and salsolidin as active compounds and has been effectively used in the treatment of hypertonia by stimulating sleep activity (Borkowski and Wrocinski, 1959). *Salsolaimbricata* (synonym: *Salsola baryosma*) is used against inflammation and also as a diuretic (Al-Saleh *et al*., 1993).

Few reports are available on pharmacognosy of *Suaeda* species such as pharmacochemical characterization of *Suaeda monoica* leaf (Lincy *et al.,* 2013) and of *Suaeda maritima* by using stem and root part of the plant (Singh *et al* 2012, 2013; Patra *et al*., 2011).

Likewise, no detailed pharmacognostic work on *Salsola* species has been reported, except the phytochemical screening of triterpene saponins from *Salsola imbricata* (Hamed *et al*., 2011). Therefore, in present research, standardization of these plants was carried out by their systematic pharmacognostical studies, this would be helpful in developing standards for sample identification, quality and purity.

MATERIALS AND METHODS

Plant material

Fresh leaf samples of *Suaeda fruticosa*, *Suaeda monoica*, *Salsola imbricata* and *Salsola tragus* were collected and

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identified with the help of Flora of Pakistan (Freitag*et al.*, 2001). Herbarium sheets were prepared and deposited in the Karachi University Herbarium (Centre for Plant Conservation). The species names with their locality and voucher numbers are given in table 1.

Macroscopy

Macro morphological characters of leaf samples such as the apex,base, color, margin, phyllotaxy, size and shape were studied and recorded.

Microscopy

Qualitative microscopy

Free hand sectioning for all four investigated species was carried out, transverse sections of fresh leaf sample were mounted with glycerin and observed at 10X power of magnification under compound microscope (Thomas Scientific, USA).

Quantitative microscopy

Leaf constants such as type of stomata, number of stomata, stomata index number of upper and lower epidermis, vein islet number and vein termination number were estimated (Kokate, 1997; Khandelwal, 2005).

Physio-chemical parameters

Physico-chemical parameters were also determined such as total ash value, acid insoluble ash value, water-soluble ash value, moisture content (loss of weight on drying) and fluorescence analysis of powdered sample according to the standard methods described by Charles *et al*., (1958).

Determination of moisture content

About one gm of leaf powder was weighed in a silica crucible and placed in oven at 105˚C for a period until constant weight was obtained. Loss in weight was recorded as moisture content (w/w %).

$$\%\ \text{of moisture content} = \frac{\text{Loss in weight of powder sample}}{\text{Weight of powder sample}} \times 100$$

Determination of ash values

Total ash value

Leaf powder (3gm) was weighed in a silica crucible, to make sample free from carbon, ignited till red hot by gradually increasing flame and this step was repeated until constant value was obtained. Then total ash was calculated (w/w%) by using the following formula.

$$\%\ \text{of total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of powder sample}} \times 100$$

Acid insoluble and water soluble ash values

The total ash (which was obtained by above mentioned method) was mixed with 25mL of 2N HCl (for determination of acid insoluble ash value) and mixed with 25mL of water (for determination of water soluble ash value) and boiled for 5 min. The resultant material was collected on ash less filter paper, washed with water following the step of drying. The material was than subjected to ignition and weighed. Finally the acid insoluble and water-soluble ash values were calculated (w/w %) with the help of following formulas.

$$\%\ \text{of acid insoluble ash value} = \frac{\text{Weight of total ash - weight of acid insoluble ash}}{\text{Weight of powder sample}} \times 100$$

For fluorescence analysis, leaf powder after treatment with different chemicals was observed under ordinary light and ultra violet light at 366 nm following a previously reported method (Charles *et al*., 1958).

Qualitative phytochemical screening

Powdered leaf material of all four species (10g) was mixed separately in 100mL of solvents; methanol and chloroform and placed in a shaker for one week at 25°C. The mixture obtained was filtered, evaporated and used to perform preliminary qualitative screening of various phytochemical constituents such as alkaloids by Mayer's test (Evans, 1997) and Wagner's test (Wagner, 1993), proteins and amino acids by Biuret test (Gahan, 1984), carbohydrates by Benedict's test (Ramakrishnan *et al.*, 1994), fixed oils by spot test and saponins by foam test (Kokate, 1999), glycosides by Borntrager's test, phenolic compounds by alkaline reagent and lead acetate test and terpenoids by Salkowski's test. While tannins and anthraquinone derivatives were also determined according to the standard methods outlined by Evans (1997).

RESULTS

Macroscopy

Out of all macroscopic characteristics leaf base, leaf apex and leaf shape were found diagnostic, while leaf size was observed more variable all investigated characters are listed in table 2.

Microscopy

Qualitative and quantitative microscopy

Transverse sections of the *Suaeda* species (figs. 1-2) showed the presence of multi-layered epidermis (smooth in *Suaeda fruticosa* and wavy in *Suaeda monoica*) having no multi-cellular hair. Epidermis was followed by ground tissues, which were differentiated into double layered palisade parenchyma and loosely arranged spongy parenchymatous cells. Many vascular bundles were vertically arranged forming a row and in the center comparatively large vascular bundle was present. Vascular bundles were gradually small in size towards periphery, while in transverse sections of the genus *Salsola* species (figs. 3-4) multi-layered epidermis with multi-cellular hair was present. Multi-cellular hairs were observed to be long in the case of *Salsola imbricata* and

small in *Salsola tragus*. Ground tissues were observed below epidermis, which was differentiated into palisade parenchyma and spongy parenchyma. Palisade cells were double layered and spongy parenchymatous cells were irregular and loosely arranged. Single vascular bundle was present in the leaf center.

Observed quantitative microscopical characters are given in table 3.

Table 1: Voucher information of the plant samples

Name of species	Localities	Voucher number
Suaeda fruticosa	Near Arts Lobby, University of Karachi campus.	G.H.No: 86473
Suaeda monoica	Near Gharo on the way to Thatta.	G.H.No: 86471
Salsola imbricata	From Gulshan -e- Maymar near Super Highway, Karachi.	G.H.No: 86529
Salsola tragus	From Hunza valley, Gilgit.	G.H.No: 86530

For pharmacognostic evaluation anatomical, macro morphological, micro morphological, physio-chemical and phytochemical characters were investigated.

Table 2: Leaf macro morphological characters

Name of species	Color of fresh leaf sample	Color of leaf sample after drying	Apex	Base	Margin	Phyllotaxy	Shape	Size (mm)
Suaeda fruticosa	Dark green	Brownish black	Obtuse	Obtuse	Ciliate	Alternate	Oblong	3-15 x1.5-3
Suaeda monoica	Light green	Dark brown	Obtuse	alternate	Entire-Undulate	Alternate	Linear	7-21 x1-2.5
Salsola imbricata	Light green	Dark green	Acute	Hastate	Ciliate	Alternate	Hastate	3-9 x1-2
Salsola tragus	Yellowish green	Yellowish	Spinulate	Hastate	Undulate	Alternate	Hastate	3-7x1-2

Table 3: Quantitative Microscopy Of leaf

Parameters	*Suaeda fruticosa*	*Suaeda monoica*	*Salsola imbricata*	*Salsola tragus*
Number of stomata on upper surface	07-08	07-09	08-09	08-09
Number of stomata on lower surface	09-10	09-10	09-10	08-09
Stomata index of upper surface	9.20	8.60	8.45	8.45
Stomata index of lower surface	11.11	9.714	9.09	8.54
Vein islet number	08-10	08-09	07-09	10-12
Vein termination number	03-04	04	03	05

Table 4: Physico-chemical parameters

Ash Values (%w/w)

Parameter	*Suaeda fruticosa*	*Suaeda monoica*	*Salsola imbricata*	*Salsola tragus*
Total ash	9.15	8.3	6.65	7.95
Acid insoluble ash	1.4	1.53	3.0	1.8
Water soluble ash	2.3	2.16	1.86	1.6

Moisture content (% w/w)

Parameter	*Suaeda fruticosa*	*Suaeda monoica*	*Salsola imbricata*	*Salsola tragus*
Moisture content	16.1	15.7	14.7	14.4

Extractive values (% w/w)

Solvents	*Suaeda fruticosa*	*Suaeda monoica*	*Salsola imbricata*	*Salsola tragus*
methanol	24	22.8	23.6	25.6
chloroform	17.2	16.8	15.2	8.0

Table 5: Fluorescence analysis of leaf powder

powder treatment	*Suaeda fruticosa*		*Suaeda monoica*		*Salsola imbricata*		*Salsola tragus*	
	normal light	U.V 366 nm	normal light	U.V 366 nm	normal light	U.V 366 nm	normal light	U.V 366 nm
Powder as such	Dark green	Brown	Light brown	Light brown	Light green	Brown	Light yellow	Brown
Powder + HCl	Greenish brown	Dark brown	Dark brown	Yellowish brown	Dark green	Dark brown	Light brown	Light brown
Powder + H_2SO_4	Brown	Dark brown	Light brown	Yellowish brown	Brownish green	Fluorescent green	Yellowish brown	Brown
Powder + 5% iodine solution	Grayish brown	Blackish brown	Yellowish brown	Brown	Light green	Brown	Light brown	Grayish brown
Powder + 1N NaOH	Brown	Dark brown	Dark brown	Dark brown	Light green	Fluorescent green	Light brown	Dark green
Powder + 5% $FeCl_3$	Light brown	Brown	Brown	Dark brown	Light green	Dark brown	Greenish brown	Black brown
Powder + glacial acetic acid	Dark brown	Dark Brown	Green brown	Brown	Dark green	Brown	Light brown	Dark brown

Table 6: Qualitative phytochemical screening

Test for	*Suaeda fruticosa*		*Suaeda monoica*		*Salsola imbricata*		*Salsola tragus*	
	methanol	chloroform	methanol	chloroform	methanol	chloroform	methanol	chloroform
Alkaloids								
Mayer's test	+	-	-	-	+	-	-	-
Wagner's test	-	-	-	-	-	-	-	-
Carbohydrate Benedict's test	+	-	+	-	+	-	+	-
Glycosides Borntrager's test	-	-	-	-	-	-	-	-
Flavonoids	+	-	+	-	+	-	+	-
Saponins Foam test	+	-	+	-	+	-	+	-
Proteins& amino acids Biuret test	-	-	-	-	-	-	-	-
Fixed oils	-	-	-	-	-	-	-	-
Phenolic Compounds								
Gelatin test	+	-	+	+	+	-	-	-
Lead acetate test	+	+	+	+	+	+	+	+
Anthraquinons	+	+	+	-	+	-	+	-
Terpenoids	+	+	+	+	+	+	+	+
Cardiac glycosides	+	-	+	-	+	-	-	-

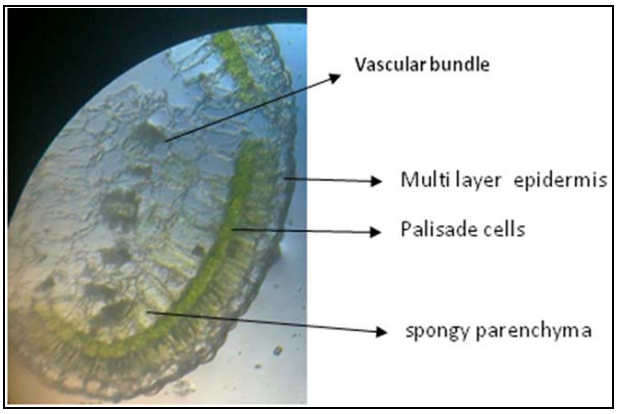

Fig. 1: Transverse section of *Suaeda fruticosa,*

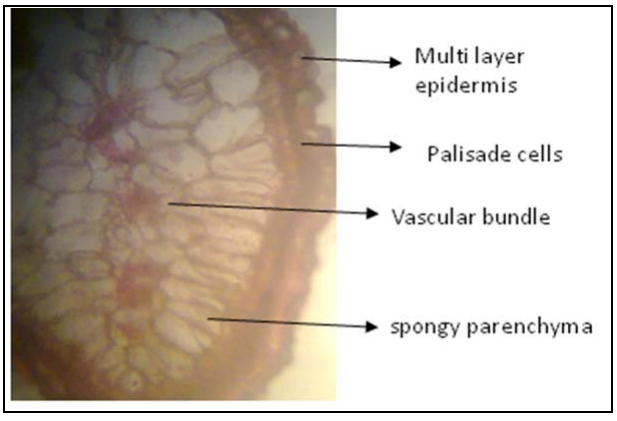

Fig. 2: Transverse section of *Suaeda monoica*

Physico-chemical parameters

In the current study total ash value was observed to be higher in *Suaeda fruticosa* (9.15% w/w) whereas water soluble ash values of *Suaeda* species were found to be higher than *Salsola* species. On the contrary, *Salsola* species have an increase amount of acid soluble ash values. Results obtained by Physico-chemical analysis are presented in table 4. The characteristic fluorescent colors emitted by the leaf powder of plant samples after treating with various reagents under normal and U.V light were recorded and are presented in table 5.

Qualitative phytochemical analysis

Phytochemical analysis revealed the presence of anthraquinones, carbohydrates, flavonoids, saponins, phenolic compounds and terpenoids in all investigated plant species. The current analysis of phyto-constituents shows that both solvents (methanol and chloroform) failed to extract glycosides, fixed oils, proteins and amino acids from all plant samples. *Suaeda monoica* and *Salsola tragus* were tested negative for the presence of alkaloids and only *Salsola tragus* showed the absence of cardiac glycosides. Results of phytochemical screening are summarized in table 6.

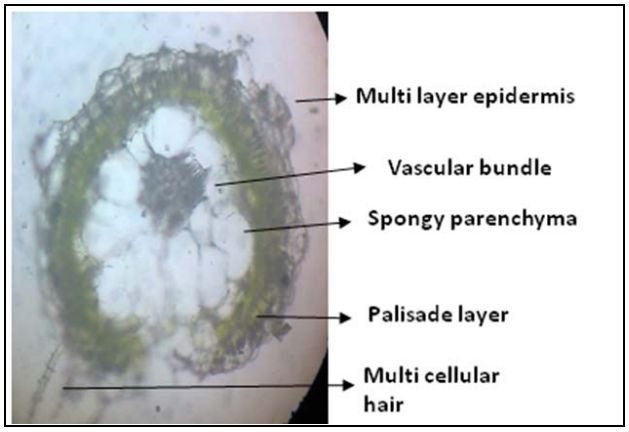

Fig. 3: Transverse section of *Salsola imbricata,*

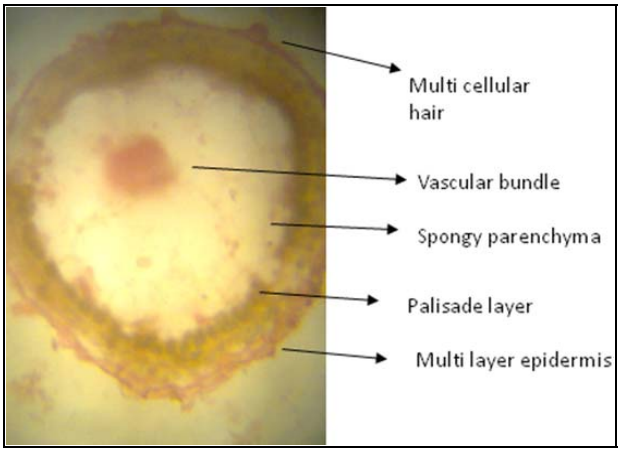

Fig. 4: Transverse section of *Salsola tragus.*

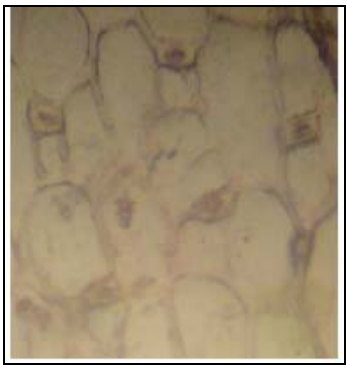

Fig. 5: anomocytic type of stomata in species of genus *Suaeda*

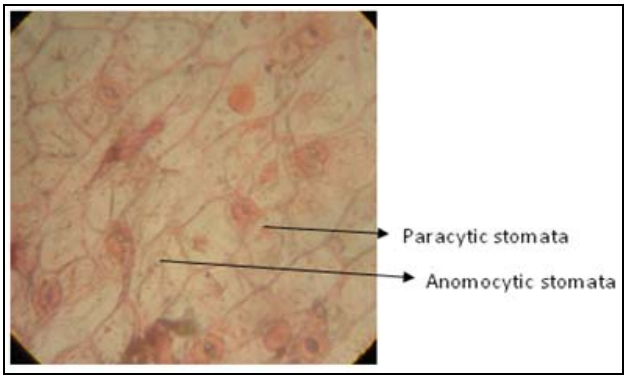

Fig. 6: Stomata type in the species of genus Salsola

DISCUSSION

For the identification of plant species, characters from other sources for instance, anatomy, cytology, palynology and genetics are also used but taxonomists still rely on morphological characters because these characters can easily differentiate closely related species (Naik, 2006). Likewise, in present research, macroscopic characters were distinguishable and found to be useful for the identification of all species. The finding of paracytic stomata in *Salsola* species is in accordance with the previous findings (Bercu and Bavaru, 2004; Saadoun and Decamps, 1990). However, Perveen *et al*. (2007) reported anomocytic stomata in the species of *Suaeda* and *Salsola*. The physico-chemical parameters are required to judge the purity and quality of a drug. High ash value is an indication of contamination and presence of impurities. Fluorescence analysis is also required in order to evaluate purity of plant crude drug (Kadam *et al*., 2012). During storage excess moisture may enhance the growth of yeast and fungi resulting breakdown of important bioactive compounds. For crude drug making, general requirement of moisture content should not be more than 14% w/w (Llanchezhian *et al.,* 2011). According to the data observed in this study, slightly increased value of moisture was observed in all species, therefore extra precautions would be required to use *Suaeda* and *Salsola*

as plant drugs. Extractive values of both solvents showed that methanol is more effective than chloroform for the extraction of phytochemical constituents such as anthraquinones, carbohydrates, flavonoids, saponins, phenolic compounds and terpenoids. Medicinally, these naturally occurring compounds have shown enormous potential in the prevention and treatment of cancer. Flavonoids and polyphenolics are found to be very effective against carcinogenesis (Ren *et al*., 2003). Anthraquinones, saponins and terpenoids were also reported to be effective in breast and liver cancer treatments (Beslija, 2003; Thoppil and Bishayee, 2011; Man *et al*; 2010). In medicine, saponin is also used as an antioxidant, anticancer and hypercholesterolemia agent (Price *et al*., 1987).

CONCLUSION

The present research established that macroscopical and qualitative microscopical characters are more useful for the identification and differentiation of closely related *Suaeda* and *Salsola* species. Preliminary phytochemical analysis revealed *Suaeda fruticosa* as a rich source of phyto chemical constituents among all species. The results obtained from comparative pharmacognosy of the genera *Suaeda* and *Salsola* species would serve as a reference for the identification and authentication of these species for drug manufacturing.

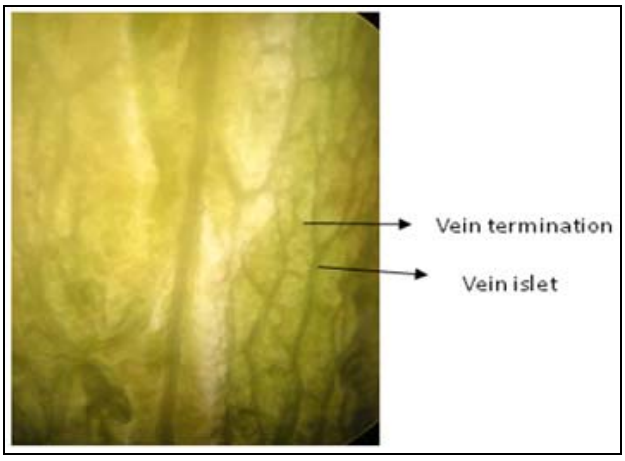

Fig. 7: Vein islet number and termination number

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