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STUDY OF POLYPHENOLS AND FLAVONOIDS CONTENTS OF SOME HALOPHYTES SPECIES COLLECTED FROM DOBROGEA REGION

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Abstract: *The aim of this study was to investigate some biochemical attributes with an adaptive role for the soil salinity for nine halophyte species known as medicinal plants. Halophytes developed different mechanisms for adapting to abiotic stress action, by increased antioxidant activity. Thus, the content of total polyphenols and flavonoids was investigated in several halophyte species collected in vegetative and flowering phenophases in summer of 2012 from saline habitats located in Sulina, Murighiol and Histria (Tulcea county).*

Key words: *flavonoids, halophyte, medicinal species, total polyphenols.*

1. Introduction

Halophyte plants growing near seashores have been collected since ancient times as food, for their medicinal qualities, and for their high salt contents [12]. Halophytes show immense diversity in habitat and behavior, tolerate the abiotic stress conditions with uneven distribution across the taxa of flowering plants [8].

The *Salicornia sp.* and *Plantago sp.* crops, with their extreme salt tolerance and long history of human consumption have been appreciated for the salty taste and high nutritional values.

Several species of *Suaeda* have been selected and developed as an alternative for traditional oil seed crop for regions under saline irrigation [15].

Halophytes are known for their ability to withstand and quench these toxic ROS, as they are equipped with a powerful antioxidant system that includes enzymatic and non-enzymatic components.

Halophytes have developed various mechanisms of adaptation to stress tolerance including an increase of antioxidant enzyme activities [9].

Enhanced synthesis of secondary metabolites under stressful conditions is believed to protect the cellular structures from oxidative effects [10].

In plants, the synthesis of total polyphenols and flavonoids is generally stimulated in response to biotic or abiotic stresses, such as salinity [14].

In this context the secondary metabolites may play a role in the adaptation of halophytic species to salinity stress [11].

Natural antioxidants occur in all plant parts, and the typical compounds that exhibit antioxidant activities include

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phenolics, flavonoid, carotenoids and vitamins [4].

Phenolic compounds, ubiquitous in plants are an essential part of the human diet, and are important due to their antioxidant properties. In recent decades, particular attention has been paid to the antioxidant effect of flavonoides, their ability to bind free radicals and remove them from the body [3], [17].

In addition to their role as antioxidant, these compounds exhibit a wide spectrum of medicinal properties, such as antiallergic, hepatoprotective, antiviral, anticancer, activities antiulcer, anti-arthero-genic, anti-inflammatory, antimicrobial, antithrombotic, cardio-protective and vasodilator effects [2], [15].

Many synthetic drugs protect us against oxidative damage by scavenging free radicals but they cause adverse side effects. In this way the only solution to this problem is to consume natural antioxidants from food supplements and to use traditional medicines [5].

On the saline soils a large flora of halophytic species was identified, which can be exploited for multiple interests: food, fodder, oilseed, wood, medicines, chemicals, landscaping, ornamentals and fibers production [16], [12], [14], [20].

In order to valorize local halophytes as a new source of bioactive substances, the aim of our study was to evaluate the total polyphenols and flavonoids contents of the plants collected from different saline habitats of Dobrogea region.

2. Material and Methods

Halophytic species were collected in different phenophases during the summer of 2012 from the saline habitats located in Sulina, Murighiol and Histria situated in the South-East of Romania (Dobrogea region).

Thus, species collected from Sulina, *Plantago coronopus, Spergularia media, Limonium gmelinii,* and *Bassia sedoides* come from two habitats: a littoral area (Z I) and a habitat located 1000 m away from the littoral area (Z II).

The first mentioned species were collected in various stages of development (vegetative and flowering phases). The species collected from Histria are: *Plantago maritima*, *Bassia sedoides, Spergularia media, Suaeda maritima* and *Salicornia europaea.* The species *Plantago lanceolata*, *Spergularia media, Atriplex litoralis, Suaeda maritima, Salicornia europaea, Bassia sedoides* was collected around the Lake ”Saraturile” (Murighiol).

The plants from these species were dried and powdered using a blender and sieved to obtain uniform particles. This powder was used for the extraction of active constituents.

2.1. Extraction and Estimation of Total Polyphenols and Flavonoids Contents

The total polyphenols content was determined by using a modified Folin-Ciocalteau method [18]. The appropriately diluted sample was added a Folin-Ciocalteau reagent and mixed thoroughly. After four minutes, 15% Na_2CO_3 was added. The absorbance of the resulting bleu-coloured solution was read at 760 nm after two hours, against the blank (distilled water). The amount of the total phenolic content was expressed as mg galic acid equivalents (GAE) per g of dry weight (mg GAE/g DW) (R^2=0.99). Three readings were taken for each sample and the result averaged.

2.2. Total Flavonoids Content

The flavonoids content was measured following a spectrophotometric method [6].

Briefly, methanol extract were appropriately diluted with distilled water.

Initially, 5% $NaNO_2$ solution was added to each test tube; after five minutes, 10% $AlCl_3$ solution was added and then after six minutes 1.0 M NaOH was added. Finally water was then added to the test tube and mixed well.

Absorbance of the resulting pink-coloured solution was read at 510 nm against the blank (distilled water). The flavonoid content was expressed as mg catechin equivalents (CE) per g of dry weight (mg CE/g DW) (R^2 =0.98). Three readings were taken for each sample and the result averaged. To calculate and to graphically represent the statistical indices, the Microsoft Office Excel 2003 software of the Windows XP operating system was used.

3. Results and Discussions

Polyphenol compound are important plant constituents because of their free radical scavenging ability, facilitated by their hydroxyl groups [2].

In our study, Figures 1 and 2 show the heterogeneous responses of non-enzymatic biologically active compounds represented by total polyphenols and flavonoids contents in nine halophytes species belonging to different families collected from three distinct saline areas, located in the South-East of Romania (Dobrogea).

Previous studies have shown that the plants' phenolic content and antioxidant activities depend on biological factors (genotype, organ, and physiological development stage) and the type of solvent used for extracting as well as on the extraction methods [13].

The total polyphenols content of all halophytes species subjected to analysis from these areas, have registered a high variability. Thus, the total polyphenols content of the halophytes collected from the Dobrogea region recorded values between: 6.22 mg GAE/g DW for *Spergularia media* (collected from Murighiol) and 54.42 mg GAE/g DW for *Limonium gmelinii* (collected from Sulina).

Considering the physiological development stage at *Plantago maritima* collected from the Histria area, the total polyphenols content has been almost identical in vegetative and flowering phenophase. Our results are not in accordance with those obtained by [13] in an another halophyte species *Limonium densiflorum* collected from one of the semi-arid regions of Tunisia where the polyphenol content was lower in vegetative stages as compared to those during flowering. Another study released by [19] indicated that the peak concentration of total phenol was during the flowering stage in *Boerhavia diffusa* and *Sida cordifolia*.

On the other hand, [1] showed that the total phenol content in an extract of shahsparam (*Tanacetum balsamita L.*) was not significant during growing stages (vegetative, flowering and after-flowering stages) but they were influenced by the different drying (microwave, oven, sun and shade) methods used.

Both *Plantago coronopus* and *Limonium gmelini* from the Sulina litoral area (ZI) recorded high values for the total polyphenols content than those from the habitat located 1000 m away from the littoral (Z II). The evapotranspiration is higher than precipitations and influences the concentration of soil salinity as well as shallow water with a high degree of mineralization. Therefore the species from this area perhaps have a higher concentration of phenolic compounds, very important for the protection of cells against oxidative stress.

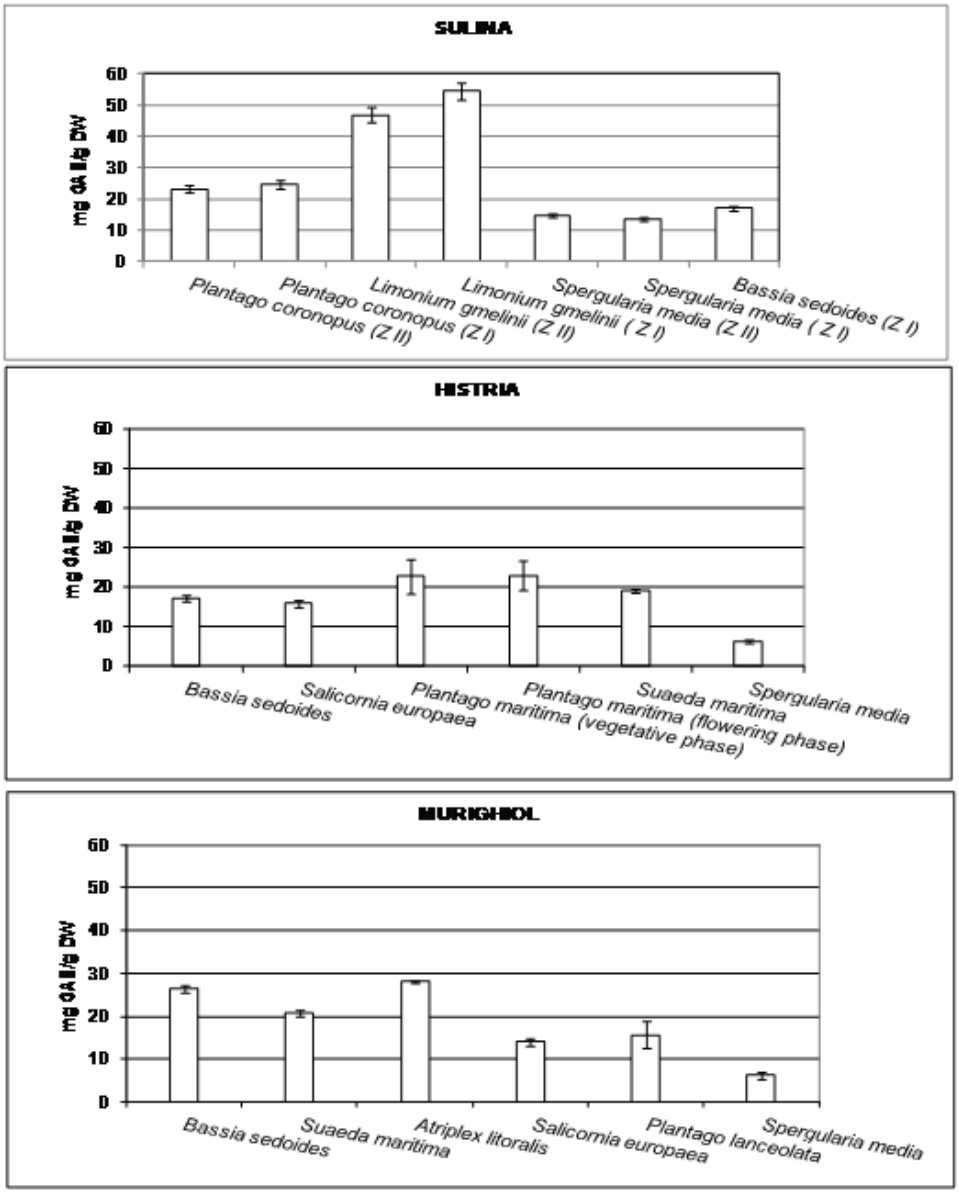

Fig. 1. *Total polyphenols content in halophyte species from different saline habitats of Dobrogea*

Flavonoids usually accumulate in the plant vacuole as glycosides, but they also occur as exudates on the surface of leaves and other aerial plant parts. One of the most actively studied properties of flavonoids is their protection against oxidative stress [15].

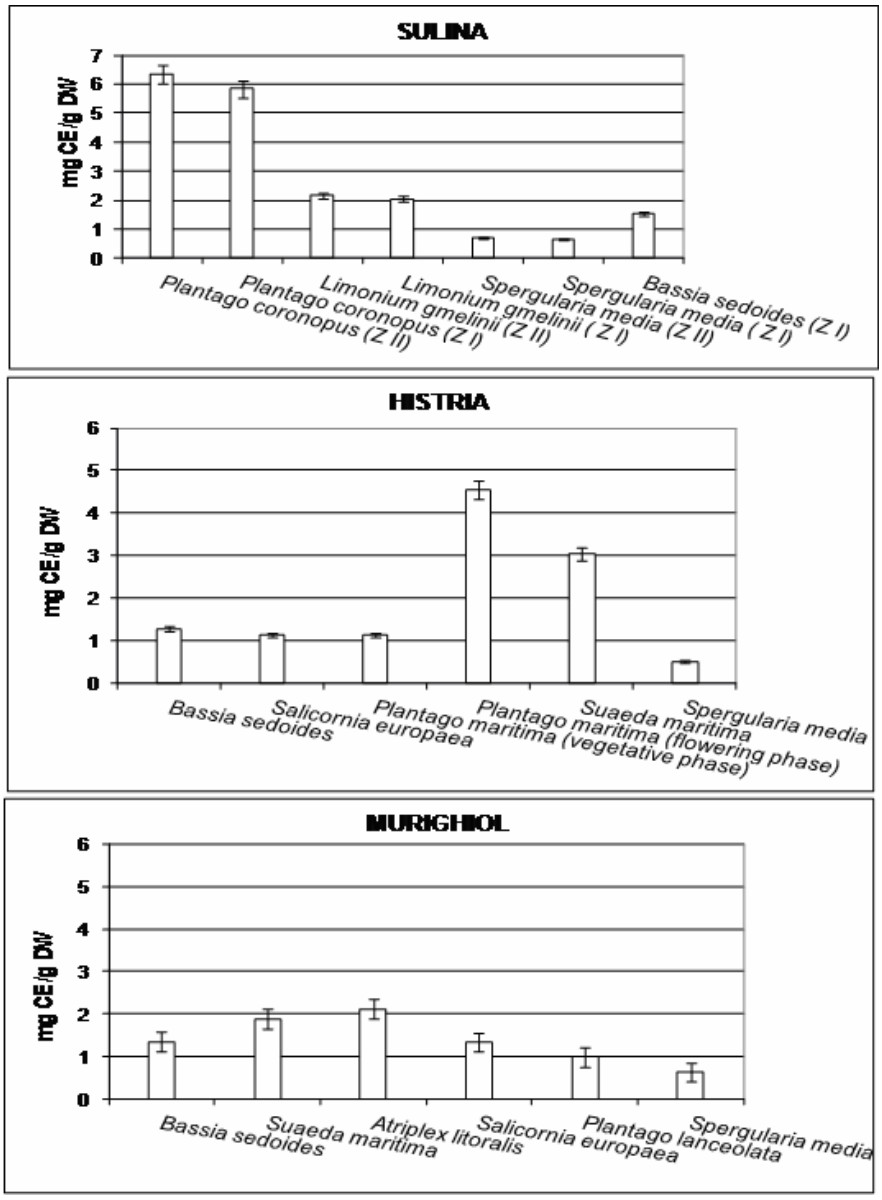

Fig. 2. *Flavonoids content in halophyte species from different saline habitats of Dobrogea*

The flavonoids content varied between 0.50 mg CE/g DW (*Spergularia media*) and 6.35 mg CE/g DW (*Plantago coronopus*). In comparison with the total value of polyphenols, the flavonoids content varied according to the phenophase thereby, *Plantago maritima* registered in the vegetative stage a content of 1.13 mg CE/g DW while during the flowering stage it recorded a content of 4.54 mg CE/g DW. The highest flavonoid amount in the flowering phase could maybe be associated

with an increase of the UV radiation content. It is known that stress conditions caused an accumulation of secondary metabolites like the flavonoids and thus both polyphenol and flavonoid contents have an important role in plant defense mechanisms [7].

The flavonoids content of *Bassia sedoides* did not vary significantly according to the three collection areas: 1.27 mg CE/g DW (Histria), 1.34 mg CE/g DW (Murighiol) and the highest level of flavonoids 1.55 mg CE/g DW (Sulina).

The halophytes *Spergularia media* and *Limonium gmelinii* collected from Sulina registered very close values of flavonoids content in both the littoral area (ZI) and in those located 1000 m away from littoral (Z II).

Only two species from the colected halophytes were found in all three areas (Sulina, Murighiol and Histria) namely *Spergularia media* and *Basia sedoides.* These species displayed variations of the total polyphenols and flavons contents. Thus, *Spergularia media* from Sulina registered a high content of total polyphenols (14.76 mg GAE/g DW) and 0.68 CE/g DW flavons. *Bassia sedoides* from Murighiol had 20.82 mg GA/g DW total polyphenols and the same plant but from another area,Sulina, had 1.55 CE/g DW flavons (Figure 3 and Figure 4).

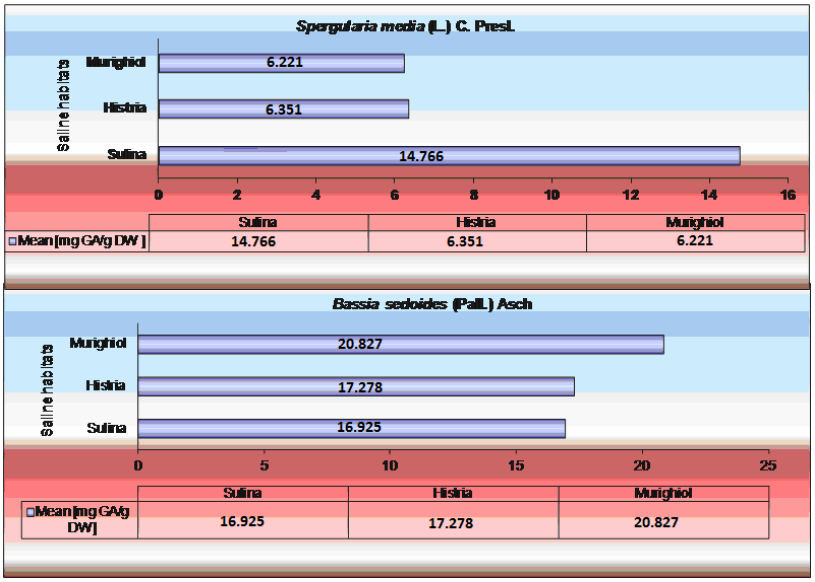

Fig. 3. *The variation of total polyphenols content of two halophyte species identified in three saline habitats of Dobrogea*

Spergularia media and *Basia sedoides* from the all three areas (Sulina, Murighiol and Histria) presented significant variations of the total polyphenols and flavons content. The plants from the first locations, Sulina and Murighiol, registered a high content in total polyphenols and flavons. *Spergularia media* from Sulina registered a high content of total polyphenols (14.76 mg GA/g DW) and 0.68 catechol/g DW flavons (Figure 3 and Figure 4). *Bassia sedoides* from Murighiol had 20.82 mg GA/g DW total polyphenols and the same plant but from another area, Sulina, had 1.55 catechol/g DW flavons.

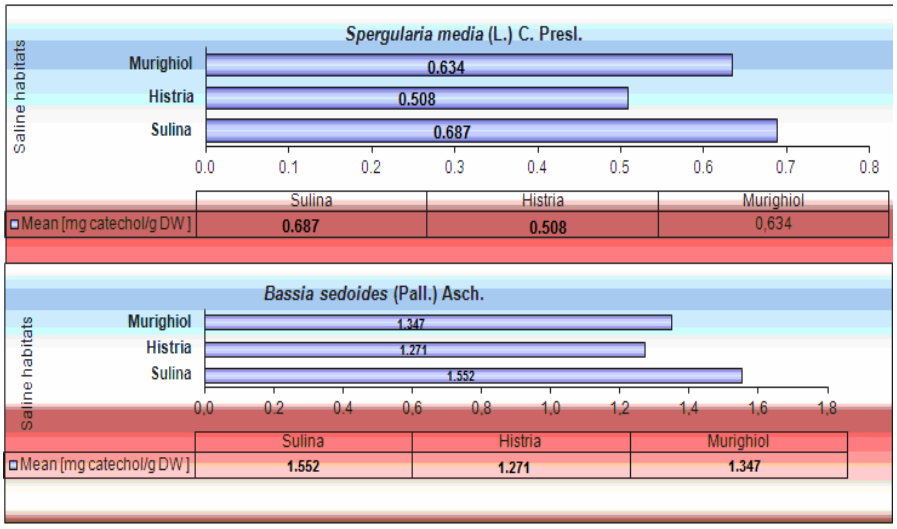

Fig 4. *The variation of flavonoids content of two halophyte species identified in three saline habitats of Dobrogea*

4. Conclusions

In conclusion, responses of non-enzymatic biologically active compounds represented by total polyphenols and flavonoids contents in those nine halophytes belonging to different families collected from three distinct saline Romanian areas (located in Sulina, Murighiol and Histria) varied according to the species, the vegetative phenophase and the area where the material was collected. Among the three areas, the halophytes that were collected from Sulina had the highest content in total polyphenols (*Limonium gmelinii*) and flavonoids (*Plantago coronopus*).

The collection from the coastal area was influenced only in the total polyphenols content of halophytes (*Plantago coronopus* and *Limonium gmelinii*) from Sulina area (Z I) which recorded higher values than those from the habitat located 1000 m away from the seaside (Z II).

Depending on the physiological development stage, the flavones content in *Plantago maritima* was higher in the flowering phase than in the vegetative phase while the total polyphenols content was relatively the same in both phenophases.

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References

1. Aghae M., Alizadeh M. et al., 2013. Effects of growing stages and drying methods on quality of shahsparam (*Tanacetum balsamita* L.). In: Intl J Agri Crop Sci., 5(2): 168-172.
2. Balasundram N., Sundram K. et al., 2006. Phenolic compounds in plants and agrindustrial by-products: Antioxidant Activity. Occurrence and Potential Uses. In: Food Chem, 99(1): 191-203.
3. Bors W., 1998. Oxidative Stress Discusses. In: Meth. Enzym., 1: 285-304.

4. Chanwitheesuk, A., Teerawutgulrag A. et al., 2005. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. In: Food Chem., 92: 491–497.
5. Daffodil D.E., Rajalakshmi K. et al., 2013. Antioxidant activity, total phenolics and flavonoids of *Salicornia brachiata Roxb*. Leaf extracts (*Chenopodiaceae*). In: World J. of Pharmacy and Pharmaceutical Sciences, 2(1): 352-366.
6. Dewanto V., Xianzhong W. et al., 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. In: J. Agric. Food Chem., 50(10): 3010-3014.
7. Dixon R.A., Paiva N., 1995. Stress-induced phenylpropanoid metabolism. In: Plant Cell, 7: 1085-1097.
8. Flowers T.J., Hanaa K. et al., 2010. Evolution of halophytes: multiple origins of salt tolerance. In: Funct Plant Biol, 37: 604–612.
9. Ivan M.A., Zamfirache M.M. et al., 2012. Determination of antioxidant enzymatic activity in several halophytes from Dobrogea area. In: Annals of „Alexandru Ioan Cuza" University, XIII (3): 47-52.
10. Jaleel C.A., Gopi R. et al., 2007. Antioxidative potentials as a protective mechanism in *Catharanthus roseus* (L.) G. Don. Plants under Salinity Stress Turk. In: J. Bot., 31: 245–251.
11. Ksouri R., Megdiche W. et al., 2007. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte Cakile maritima Plant Physiol. In: Biochem., 45: 244–249.
12. Lieth H., Mochtchenko M., 2003. Cash Crop Halophytes: Recent Studies. Kluwer Academic Publishers, London.
13. Medinim F., Ksourim R. et al., 2011. Effects of physiological stage and solvent on polyphenol composition, antioxidant and antimicrobial activities of *Limonium densiflorum*. In: Journal of Medicinal Plants Research, 5(31): 6719-6730.
14. Navarro J.M., Flores P. et al., 2006. Changes in the contents of antioxidant compounds in pepper fruits at ripening stages, as affected by salinity. In: Food Chemistry, 96: 66–73.
15. Parvaiz A. et al., 2009. Generation of ROS and non-enzymatic antioxidants during abiotic stress in plants. In: Botany Research International, 2(1): 11-20.
16. Pasternak D., Danon A. et al., 1985. Developing the sea water agriculture concept. In: Plant Soil, 89: 337-348.
17. Rice-Evans C.A., Miller N.J., 1996. Antioxidant activities of flavonoids as bioactive components of food. In: Biochem. Soc. Trans., 24(3): 790-795.
18. Singleton V.L., Orthofer R. et al., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: Methods Enzymol, 299: 152-178.
19. Verma V., Kasera K., 2007. Variations in secondary metabolites in some arid zone medicinal plants in relation to season and plant growth. In: Indian. J. Plant. Physio., 12: 203-206.
20. Vysochina G.I., Kukushkina T.A. et al., 2010. Flora of the Forest-Steppe zone of west Siberia as a source of biologically active compounds. In: Contemporary Problems of Ecology, 4(2): 202-211.