Evaluation of Some Growth Parameters and Chemical Composition of *In Vitro* Grown Seedlings of *Rumex vesicarius* L. (Polygonaceae).

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Abstract: The aim of this research is to evaluate differences in growth and chemical composition of *in vitro* grown seedlings (10, 20 and 30 days old) of *Rumex vesicarius* L. (Polygonaceae) on either solidified MS medium or agar. Percentage of germination increased with time from 2 days till 16 days in case of seedlings grown on MS medium, and 10 days in case of seedlings grown on agar. Variations in seedlings length at 10, 20 and 30 days were non significant. Seedlings grown on agar were longer than seedlings grown on MS medium. Shoot: root ratio (%) decreased with time from 10 to 30 days, shoot: root ratio of seedlings grown on agar was less than these of seedlings grown on MS medium. Variation in shoot: root ratio of seedlings grown on either solidified MS medium or agar was highly significant. Fresh and dry weights of these seedlings increased with time in 10, 20 and 30 days old seedlings. Variations were highly significant in both fresh and dry weights. Fresh and dry weights of seedlings grown on MS medium were higher than seedlings grown on agar. Phytochemical screening of 10, 20 and 30 days old seedlings showed variations in the presence and / or amount of some biologically active constituents under investigation such as: flavonoids, saponins, alkaloids and tannins, chlorides and Sulphates, these variations indicated that, the formation of these active constituents is positively or negatively related to time. Regarding total phenolics, of seedlings grown on MS medium, 20 days old seedlings had the maximum concentration (3.833±0.334 mg GAE_s/g F.W.), followed by 10 days old seedlings (1.910±0.334 mg GAE_s/g F.W.), while 30 days old seedlings were found to contain the least amount of phenolics (1.167±0.334 mg GAE_s/g F.W.). Variations in the amount of total phenolics within different seedlings were non significant. Seedlings grown on agar contained low amount of phenolics till 30 days old, compared with seedlings grown on MS medium. Total flavonoids were determined also, highly significant variations were found between 10, 20 and 30 days old seedlings grown on either MS medium or agar. The maximum amount of total flavonoids was found to be in 10 days old seedlings grown on agar (106.350±3.849 µg/g F.W.); flavonoidal contents were negatively related to time. In wild young plantlets of *Rumex vesicarius* L. at vegetative stage, total phenolics were found to be lower than *in vitro* grown seedlings. Plantlets roots were found to be the richest organ (1.695± 0.178 mg GAE_s/g F.W.), however roots contains about less than half amounts found in *in vitro* grown seedlings on MS medium at 20 days old (3.833±0.334 mg GAE_s/g F.W.). Wild young plantlets were rich in flavonoids. There were highly significant variations between plantlets parts. Leaves were found to contain the highest amount of flavonoids (2835.000 ± 305.757 µg/g F.W.).

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Keywords: *Rumex vesicarius* L. - total phenolics - total flavonoids - phytochemical screening - *in vitro* grown seedlings.

Abbreviations:
GAE : Gallic Acid Equivalent.
HPLC: High Performance Liquid Chromatography.
µg/g, mg/g and mg : microgram/ gram, milligram/gram and milligram respectively.
D.W. : Dry Weight.
F.W. : Fresh Weight.

1. Introduction

Rumex vesicarius L. is a wild edible plant used as a sorrel and collected in spring time and eaten fresh (Batanouny, K.H., 1999), or cooked (Al-Quran, 2009). It was considered as a dietary complementary plant, since this plant is a rich source of ß carotenes (Bélanger *et al.*, 2010).

Rumex vesicarius L. has many important medicinal uses, the plant is stimulant, tonic, and acts as aphrodisiac agent (Gopal *et al.*, 2008).

The medicinal importance of this plant is a reflection to its chemical composition since the plant contains many bioactive substances such as flavonoids (vitexin, isovitexin, orientin and isorientin). The plant also is

rich in anthraquinones particularly in roots (emodin and chrysophanol). The plant also contains carotenoids, vitamins (especially vitamin C), proteins, lipids and organic acids. This plant is a good source of minerals such as; K, Na, Ca, Mg, Fe, Mn and Cu (Saleh, 1993; Al–Rumaih *et al.*, 2002; Alfawaz, 2006; Filho *et al*., 2008).

The previously mentioned bioactive phytochemicals (such as polyphenols, flavonoids, carotenoids, tocopherols and ascorbic acid) have a role as antioxidant and detoxifying agents. The intake of dietary antioxidant phytochemicals like carotenodis, phenolic compounds and flavonoids leads to protection against non-communicable diseases i.e. cancer, cardiovascular diseases and cataract. Phenolics and flavonoids were very important biologically active constituents, since they considered to be anticancer, antioxidant and antimicrobial agents etc., (Rao, 2003; Alberto *et al.*, 2006; Matkowski, 2008; Abd Ghafar *et al*., 2010 ; Imran *et al.*, 2011).

The main aim of this study is to evaluate differences in seedlings growth parameters as manifested by seedling length, shoot : root ratio, fresh and dry weights and chemical composition (particularly, total phenolics and total anthraquinones) of *in vitro* grown seedlings of *Rumex vesicarius* L. (Polygonaceae) at different stages of growth (10, 20 and 30 days) on either solidified MS medium or agar. Wild young plantlets were also studied for their chemical composition.

2. Materials and Methods

Plant material:

Seeds of *Rumex vesicarius* L. were obtained from plant samples collected during August 2010 at the ripening fruiting stage. Wild young plantlets at the vegetative stage of growth (February 2011) were collected from 60 km, Quatamia- Ain Sokhna desert road, Egypt. Plant specimens were botanically identified and authenticated by comparing with herbarium specimens, and the identified plant specimen was deposited in the herbarium of Botany and Microbiology Department, Faculty of Science, Helwan University, Helwan, Egypt (Number:1057). All experimental studies were carried out in Prof. Dr. / Hisham Afifi Lab., Plant Physiology Unit, Botany Department and Central Services Lab., National Research Centre, Giza, Egypt.

***In vitro* germination of seeds:**

Seeds were surface sterilized by immersion in 70 % ethanol for 30-60 seconds, then soaked in 20% of commercial Clorox for 15-20 minutes, then washed 3 times with sterile double distilled water. Sterilized seeds were aseptically transferred to either 1% (w/v) agar in double distilled water or hormone free MS medium (Murashige and Skoog, 1962) supplemented with 1% agar, then incubated at 25± 2°C in light (16 hours). Percentage of germination was determined every 2 days till 30 days. Seedlings of 10, 20 and 30 days old were collected and growth parameters estimated (seedling lengths "cm", fresh and dry weights "mg". Replicates = 300 seeds for determination of germination percentage and 30 seedlings for determination of growth parameters except shoot: root ratio (3 seedlings).

Chemical examination of *in vitro* grown seedlings and early vegetative wild plantlets:

1- Preliminary phytochemical screening:

Preliminary phytochemical screening of *in vitro* grown seedlings at different stages of growth (10, 20 and 30 days old) on either solidified MS medium or agar and early vegetative wild plantlets was carried out as follows: Flavonodis (Mabry *et al.*, 1970); Anthraquinones (Farnsworth *et al.*, 1969); Tannins (Trease and Evans, 1978); Alkaloids (Shellard, 1957); Saponins (Hungund and Pathak, 1971); Carbohydrates and / or Glycosides (Stank *et al.*, 1963); Irodoids (Weiffering, 1966); Coumarins (Feigl, 1960); Chlorides and Sulphates (Islam *et al.*, 1993); Sterols and / or Triterpenes (Claus, 1967 and Schmidt, 1964) and Cardiac glycosides (Balbaa *et al.*, 1981).

2- Determination of total phenolics and total flavonoids:

a- Assay for total phenolics:

Total phenolics were estimated according to Gursoy *et al.*, (2009), involving Folin–Ciocalteu reagent and Gallic acid as standard. 1 ml of each extract of either *in vitro* grown seedlings at different stages of growth (10, 20 and 30 days) on either solidified MS medium or agar and early vegetative wild plantlets contained 150 mg F.W.. Each sample was done in triplicate. Concentrations of phenolic compounds were calculated according to the following equation that was obtained form the standard Gallic acid graph.

Absorbance = 0.0167 Gallic acid (µg) + 0.017 (R^2: 0.99)

b- Assay for total flavonoids:

Total flavonoids were determined using the method of Gursoy *et al.*, (2009). 1 ml of each extract of either *in vitro* grown seedlings at different stages of growth (10, 20 and 30 days) on either solidified MS medium or agar and early vegetative wild plantlets contained 150 mg F.W.. Each sample was done in triplicate. Concentrations of flavonoid contents were calculated according to the following equation that was obtained from the standard Quercetin graph:

Absorbance = 0.0228 Quercetin (µg)–0.0045 (R^2: 0.9979)

Statistical analysis:

Statistical analysis was done using Fisher analysis of variance methodology. A least significant difference test was applied at 5% and 1% probability level to determine differences among treatment means (Steel and Torrie, 1984). The MSTAT computerized package

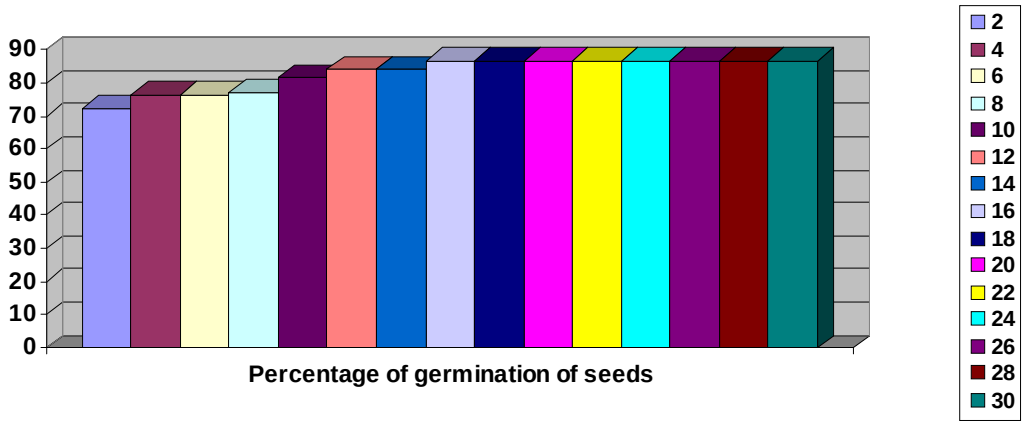
program was subjected to the regular statistical analysis of variance (Nissen *et al.*, 1985).

3. Results and Discussion:

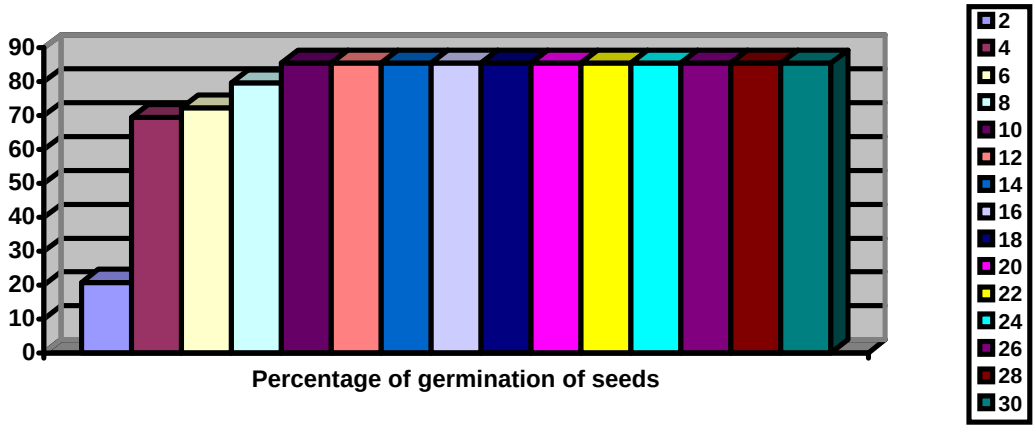
***In vitro* germination of seeds of *Rumex vesicarius* L.:**

Percentage of *in vitro* germination of *Rumex vesicarius* L. seeds (Figure:1.a) increased gradually with time from 2 days (72.333%), till 16 days (86.833%), then no change occurred in % of germination till 30 days in case of seeds germinated on solidified MS medium.

Regarding seeds germinated on agar, percentage of *in vitro* germination of seeds (Figure:1.b) increased gradually with time from 2 days (20.755%), till 10 days (85.535%), then no change occurred in % of germination till 30 days.



a) MS medium



b) Agar alone

Figure (1): *In vitro* germination of seeds of *Rumex vesicarius* L. on either solidified MS medium or agar during 30 days.

***In vitro* grown seedlings growth:**

Data in Table : 1 showed seedling length, shoot: root ratio and fresh and dry weights at 10, 20 and 30 days old seedling grown on either solidified MS medium or agar.

Length of seedlings grown on agar (7.080±0.587, 10.130±0.587 and 11.180±0.587 cm at 10, 20 and 30 days old respectively) was higher than that of seedlings grown on MS medium (3.960±0.587, 5.890±0.587 and 6.130±0.587 cm at 10, 20 and 30 days old respectively), this may be because seedlings tried to get more nutrients from this poor media (agar) by increasing roots length as absorbing organs, variation in length between seedlings grown on MS medium and

agar may be due to other factors such as gelling strengths of MS medium and agar and porosity of MS medium and agar particles etc.,. There were non significant differences in seedlings length grown either on solidified MS medium or agar.

Shoot: root ratio decreased with time from 10 to 30 days. Shoot: root ratio of seedlings grown on agar (50.000 ± 11.742, 36.500 ± 11.742 and 33.333 ± 11.742 at 10, 20 and 30 days old respectively) was less than that of seedlings grown on MS medium (66.667 ± 11.742, 61.111 ± 11.742 and 50.000 ± 11.742 at 10, 20 and 30 days old respectively). Variation in shoot: root ratio of seedlings grown on either solidified MS medium or agar was highly significant.

Fresh and dry weights increased with time in 10, 20 and 30 days old seedlings. Both fresh and dry weights of seedling were more in case of seedling grown on solidified MS medium (They reached 382.200±18.970 and 66.700±2.882 mg respectively) than those grown on agar (118.000±18.970 and 23.600±2.882 mg respectively). Variations were highly significant in case of fresh and dry weights of seedlings grown on either solidified MS medium or agar.

Table (1): Seedling length, shoot: root ratio (%), fresh and dry weights of *in vitro* grown seedlings of *Rumex vesicarius* L. (Polygonaceae) at different stages of growth (10, 20 and 30 days) on either solidified MS medium or agar.

***In vitro* grown seedlings**	**Seedling length (cm)**	**Shoot: root ratio (%)**	**Fresh weight (mg)**	**Dry weight (mg)**
a) MS medium				
10 days	3.960±0.587	**66.667 ± 11.742**	245.000±18.970	38.800±2.882
20 days	5.890±0.587	61.111 ± 11.742	331.200±18.970	49.500±2.882
30 days	**6.130±0.587**	50.000 ± 11.742	**382.200±18.970**	**66.700±2.882**
b) Agar				
10 days	7.080±0.587	**50.000 ± 11.742**	106.000±18.970	21.200±2.882
20 days	10.130±0.587	36.500 ± 11.742	115.5000±18.970	23.100±2.882
30 days	**11.180±0.587**	33.333 ± 11.742	**118.000±18.970**	**23.600±2.882**
L.S.D. (0.05)	**1.627**	**36.184**	**53.660**	**8.152**
L.S.D. (0.01)	**2.138**	**50.730**	**71.360**	**10.842**

Preliminary phytochemical screening of *in vitro* grown seedlings and early vegetative wild plantlets:

Phytochemical screening of 10, 20 and 30 days old seedlings (Table: 2 a) showed variations in the presence and / or amount of some biologically active constituents under investigation, in this regard, seedlings grown on MS medium were found to contain Sulphates in amounts increased with time, chlorides increased then decreased with age, flavonoids, alkaloids and tannins decreased with age then increased again. Saponins were formed between 10 and 20 days then increased with time, coumarins, sterols, carbohydrates and / or glycosides amounts did not change with time.

Seedlings grown on agar were found to contain all substances under investigation in lower amounts than those grown on MS medium except flavonoids and carbohydrates and / or glycosides. Seedlings grown on both MS medium and agar were devoid of cardiac glycosides, irodoids, anthraquinones and sublimable substances, since they were not formed till 30 days.

These variations indicated that the formation of biologically active constituents is positively or negatively related to seedlings age and composition of growing nutrient media.

It was found also that, all plant parts of early vegetative wild plantlets of *Rumex vesicarius* L. (Table: 2 b) were rich in majority of active constituents under investigation, plantlets organs varied in the amount of these biologically active substances.

However, these plantlets were rich source of these active constituents; it is considered a limited source for two reasons:

1- Duration of vegetative stage of this plant is about one month then amounts of active constituents decreased when the plant turned to other developmental stages

2- Over collection of these young plantlets changed the ecological status of the plant gradually to being rare, taking in consideration that these plantlets are found normally in little numbers in the desert.

These results agreed with Mostafa *et al.,* 2011, since they found that, all plant parts of *Rumex vesicarius* L. at flowering and fruiting stages were rich in flavonoids, anthraquinones, alkaloids, tannins, sterols and / or triterpenodis, carbohydrates and/or glycosides, chlorides and sulphates and sublimable substances **.** There were variations in the presence and /

or amount of some biologically active constituents under investigation within different plant parts.

Table (2a): Preliminary phytochemical screening of *in vitro* grown seedlings of *Rumex vesicarius* L. (Polygonaceae) at different stages of growth (10, 20 and 30 days) on either solidified MS medium or agar.

	10 days old seedlings		20 days old seedlings		30 days old seedlings	
	MS medium	Agar	MS medium	Agar	MS medium	Agar
1-Carbohydrates and / or Glycosides	+	++	+	++	+	+
2- Saponins	-	-	+	+	++	+
3- Tannins	++	+	+	+	++	+
4- Sterols and / or Triterpenoids	+	+	+	+	+	+
5- Alkaloids	++	-	+	-	++	-
6- Cardiac glycosides	-	-	-	-	-	-
7- Flavonoids	++	++	+	++	+	++
8- Anthraquinones	-	-	-	-	-	-
9- Coumarins	+	+	+	+	+	+
10- Irodoids	-	-	-	-	-	-
11-a-Chlorides	+	+	++	+	+	++
11-b-Sulphates	+	-	++	+	++	++
12- Sublimation	-	-	-	-	-	-

- = Active ingredients under investigation were not found. + = Weak to moderate amounts of active ingredients under investigation. ++ = High amounts of active ingredients under investigation.

Table (2b): Preliminary phytochemical screening of different plant parts of early vegetative wild plantlets of *Rumex vesicarius* L.. (Polygonaceae) .

	Roots	Stems	Leaves	Whole plant parts
1-Carbohydrates and / or Glycosides	++	++	++	++
2- Saponins	++	+	++	++
3- Tannins	++	+	++	++
4- Sterols and / or Triterpenoids	+	+	++	++
5- Alkaloids	++	+	++	+
6- Cardiac glycosides	+	+	+	+
7- Flavonoids	+	++	++	++
8- Anthraquinones	++	++	+	++
9- Coumarins	+	+	+	+
10- Irodoids	+	++	++	++
11-a-Chlorides	++	++	++	++
11-b-Sulphates	++	++	++	++
12- Sublimation	++	++	+	++

Total phenolics and total flavonoids of *in vitro* grown seedlings and early vegetative wild plantlets:

Phenolics and flavonoids are important biologically active constituents, since they are considered to be anticancer, antioxidant and antimicrobial agents etc., (Alberto *et al.*, 2006; Abd Ghafar *et al.*, 2010 ; Imran *et al.*, 2011).

Results of total phenolics (Table: 3a) revealed that, in case of seedlings grown on MS medium, the maximum amount of total phenolics was found to be in 20 days old seedlings (3.833±0.334 mg GAEs/g F.W.), followed by 10 days old seedlings (1.910±0.334 mg GAEs/g F.W.), while 30 days old seedlings were found to contain the least amount of phenolics (1.167±0.334 mg GAEs/g F.W.).

This means that, there were nearly a duplication in the amount of total phenolics occurred in seedlings from 10 to 20 days, then a decline in the amount of total phenolics occurred again.

Regarding seedlings grown on agar it was found that, they contain low amounts of phenolic contents till 30 days old seedlings.

Variation in the amount of total phenolics within different seedlings grown on either MS medium or agar was non significant.

These results agreed with Mostafa *et al.,* (2011), since they found that, all plant parts were rich in phenolics, particularly fruits (15.633 mg GAE_s/g F.W.), while whole plant was found to contain 10.417±0.320 mg GAE_s/g F.W..

Results of total flavonoids in seedlings growing on MS medium (Table: 3a) showed that, the maximum amount of total flavonoids was found to be in 10 days old seedlings (42.930±3.849 µg/g F.W.), followed by 30 days old seedlings (37.868±3.849 µg/g F.W.), while 20 days old seedlings were found to contain the least amount of flavonoids (34.993±3.849 µg/g F.W.).

This may be explained physiologically on the basis that flavonoids is one of stress signs, so when seedlings at the first stage of formation and growth were under stress, so total flavonoids increased, and this followed by a decrease in total flavonoids of 20 days old seedlings, this may be due to that the stress may be reduced with further growth of seedlings, this decrease in total flavonoids is followed by increase in 30 days old seedlings, because plantlets may undergone further stress under growth and formation of leaves, other organs and more differentiation was occurred, so total flavonoids increased again as a sign of this stress, another reason for this increase may be also depletion of nutrient contents in the MS medium.

Regarding seedlings grown on agar, it was found that, flavonoidal content decreased with increasing seedling ages from 10 to 30 days, flavonoidal content of these seedlings was double of those found in seedlings grown on MS medium.

There were highly significant variations between 10, 20 and 30 days old seedlings grown on either MS medium or agar.

Total flavonoids content was higher than that in whole plant parts of wild samples, since Mostafa *et al.,* (2011) found that, whole plant parts collected from desert during flowering stage contain 11.223±1.850 µg/g F.W. only, at all cases seedling growing on either solidified MS medium or agar produced flavonoids (4-10 folds respectively) more than whole plant in the flowering stage.

Results of this research agreed with results of Astrid Kännaste, 2008, who found that age is an effective factor on the formation of phenolic volatile compounds (such as terpenoids; α-pinene, camphene, β-pinene, myrcene, α –phellandrene, terpinolene and limonene etc…,) in newly planted conifer seedlings in deforestation areas, thus small ("mini") seedlings, planted at the age of 7-10 weeks, are gnawed less by pine weevils than the larger, conventionally planted seedlings. So, it has been proposed that planting young conifer seedlings in clear-cut areas may reduce the damage caused by pine weevils.

Concerning total phenolics, early vegetative wild plantlets of *Rumex vesicarius* L.(Table : 3b) were found to contain lower amounts than *in vitro* grown seedlings. Plantlets roots were found to the richest organ in plantlets in this regard (1.695± 0.178 mg GAE_s/g F.W.), however roots contains about less than half amounts found in *in vitro* grown seedlings on MS medium at 20 days old (3.833±0.334 mg GAE_s/g F.W.). There were non significant variations between plantlets parts in this regard.

It was found that, early vegetative wild plantlets of *Rumex vesicarius* L.(Table : 3b) were rich in flavonoids. There were highly significant variations between plantlets parts. In this regard, leaves were found to contain the highest amount of flavonoids (2835.00 ± 305.757 µg/g F.W), followed by whole plant parts (1986.000 ± 305.757 µg/g F.W).

To conclude, chemical composition of seedlings varied according to seedlings age and composition of growing nutrient media. Seedlings grown on MS medium at 20 days old were a rich source of phenolics (3.833±0.334 mg GAE_s/g F.W.), these seedlings contained more than double the amount of phenolics found in roots (as the richest organ in the plantlet) of early vegetative wild plantlets of *Rumex vesicarius* L. (1.695± 0.178 mg GAE_s/g F.W.). Seedlings grown on agar at 10 days old and leaves (as the richest organ in the plantlet) of early vegetative wild plantlets of *Rumex vesicarius* L. were rich source of flavonoids (106.350±3.849 µg/g F.W. and 2835.000± 305.757 respectively).

Finally, seedlings grown on MS medium at 20 days old (Photo: 1) were a rich source of phenolics, while seedlings grown on agar at 10 days old and leaves of early vegetative wild plantlets of *Rumex vesicarius* L. were a rich source of flavonoids (Photos: 2 and 3).

Table (3a): Total phenolics, total flavonoids of *in vitro* grown seedlings of *Rumex vesicarius* L. (Polygonaceae) at different stages of growth (10, 20 and 30 days) on either solidified MS medium or agar.

In vitro grown seedlings	Total phenolics (mg GAEs/g F.W.)		Total flavonoids (µg/g F.W.)	
	MS medium	Agar	MS medium	Agar
10 days old	1.910±0.334	0.074±0.334	42.930±3.849	**106.350±3.849**
20 days old	**3.833±0.334**	0.115±0.334	34.993±3.849	86.040±3.849
30 days old	1.167±0.334	0.130±0.334	37.868±3.849	76.460±3.849
L.S.D. (0.05)	**1.029**		**11.861**	
L.S.D. (0.01)	**1.443**		**16.629**	

Table (3b): Total phenolics and total flavonoids of different plant parts of early vegetative wild plantlets of *Rumex vesicarius* L.

Plant organs	Total phenolics (mg GAEs/g F.W.)	Total flavonoids (µg/g F.W.)
Leaves	0.405± 0.178	**2835.000 ± 305.757**
Stems	0.340± 0.178	545.000 ± 305.757
Roots	**1.695± 0.178**	458.000 ± 305.757
Whole plant parts	0.386± 0.178	1986.000 ± 305.757
L.S.D. (0.05)	0.580	997.127
L.S.D. (0.01)	0.845	1450.720

Photo (1): Seedlings grown on MS medium at 20 days old as a rich source of phenolics.

Photo (2): Seedlings grown on agar at 10 days old as a rich source of flavonoids.

Photo (3): Early vegetative wild plantlets of *Rumex vesicarius* L. as a rich source of flavonoids.

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References

1- Batanouny, K.H.: Wild medicinal plants in Egypt. Academy of Scientific Research and Technology, Egypt and International Union for Conservation (IUCN), Switzerland 1999:166- 167.

2- Al-Quran, S.: Ethnopharmacological survey of wild medicinal plants in Showbak, Jordan. Journal of Ethnopharmacology 2009; 123: 45- 50.

3- Bélanger, J.; Balakrishna, M.; Latha, P. and Katumalla, S.: Contribution of selected wild and cultivated leafy vegetables from South India to lutein and β-carotene intake. Asian Pacific Journal of Clinical Nutrition 2010; 19 (3): 417-424.

4- Gopal, *R.*; Vijayakumaran, M.; Venkatesan, R. and Kathiroli, S.: Marine organisms in Indian medicine and their future prospects. Natural Products Radiance 2008; 7(2): 139-145.

5- Saleh, N.A.M.; El- Hadidi, M.N. and Raafat, A.: Flavonoids and anthraquinones of some Egyptian *Rumex* species (Polygonaceae). Biochemical Systematics and Ecology 1993; 21 (2): 301- 303.

6- Al–Rumaih.; May, M.; Al-Saad, F.A. and Warsy, A.S.: Seasonal variation in mineral content of different organs development of *Rumex vesicarius* L.. Saudi Journal of Biological Sciences 2002; 9 (1): 69- 78.

7- Alfawaz, M.A.: Chemical composition of hummayd (*Rumex vesicarius*) grown in Saudi Arabia. Journal of Food Composition and Analysis 2006; 19: 552- 555.

8- Filho, J.M.B.; Alencar, A.A.; Nunes, X.P.; Tomaz, A.C.; Filho, S.J.G.; Petronio, F.A.; Silva, M.S.; Souza, M.F.V. and Cunha, E.V.L.: Source of alpha-, beta-, gamma-, delta-, and epsilon-carotenes: A twenties century review. Brazilian Journal of Pharmacognosy 2008; 18 (1): 135- 154.

9- Rao, B.N.: Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. Asian Pacific Journal of Clinical Nutrition 2003; 12 (1): 9- 22.

10- Alberto, M.R.; Canavosio, M.A.R. and Nadra, M.C.M.: Antimicrobial effect of polyphenols from apple skins on human bacterial pathogens. Electronic Journal of Biotechnology 2006; 9 (3).

11-Matkowski, A.: Plant *in vitro* culture for the production of antioxidants – A review. Biotechnology Advances 2008; 26: 548- 560.

12- Abd Ghafar, M.F.; Prasad, K. N.; Weng, K.K. and Ismail, A.: Flavonoid, hesperidine, total phenolic contents and antioxidant activities from Citrus species. African Journal of Biotechnology 2010; 9 (3): 326-330.

13- Imran, M.; Raja, M. M. and Basith, J.A.: Determination of total phenol, flavonoid and antioxidant activity of edible mushrooms *Pleurotus florida* and *Pleurotus eous*. International Food Research Journal 2011; 18: 574-577.

14- Murashige, T. and Skoog, F.: A revised medium for rapid growth and bioassays with Tobacco tissue cultures. Physiologia Plantarum 1962; 15: 473-479.

15- Mabry, T.T.; Markhan, K.R. and Thomas, M.B.: The Systematic Identification of Flavonoids. Springer, Verlag, New York 1970: 46- 54.

16- Farnsworth, N.R.; Fong, H.H.; Blomster, R.N. and Draus, F.G.: Studies on *Vinca major* (Apocyaceae). Journal of Pharmaceutical Science 1969; 51 (3): 217- 224.

17- Trease, G.T. and Evans, W.C.: Text Book of Pharmacognsy, Baillier, France 1978.

18- Shellard, E.J.. Practical plant chemistry. Pitman. Medicinal Publishing Co., LTD, London 1957: 53- 54.

19- Hungund, B.I. and Pathak, C.H.: USDA forest. Services Research Paper, NE 1971: 201.

20- Stank, J.; Cerny, M.; Kocoursk, J. and Pacok, J.: The monosaccharides; Publishing House of the Czechosolovak. Academy of Sciences, Prague 1963: 22- 100.

21- Weiffering, J.H.: Aucubinartige glucoside und verwandte heteroside als systematische merkmale. Phytochemistry 1966; 5: 1053.

22- Feigl, F.: Spot tests in organic analysis. Elsevier Publishing Co, New York, 6th ed.; 1960:29- 59.

23- Islam, A.M.; Hassan, E.A. and Hannout, I.B.: Manual of Practical Chemistry, Dar Al- Maaref, Egypt, 2nd ed.; 1993: 19- 39.

24- Claus, E.P.: Pharmacognosy, Henery Krimpton, London, 5th ed.; 1967: 168.

25- Schmidt, J.: Text Book of Organic Chemistry. Olivar and Poyed ed., London; 1964: 673.

26-Balbaa, S.I.; Sayed, H.H. and Ashgan, Y.Z.: Medicinal plant constituents; General Organization for University and School Books, 3rd ed.; 1981:190- 255.

27- Gursoy, N.; Sarikurikcu, C.; Cengiz, M. and Solak, M.H.: Antioxidant activities, metal contents, total phenolics and flavonoids of seven *Morchella* species. Food and Chemical Toxicology 2009; 47: 2381- 2388.

28- Steel, R.G.D. and Torrie, J.H.: Principles and Procedures of Statistics, McGraw Hill Book Co. Inc, New York , USA, 2nd ed: 1984.

29- Nissen, O.; Eisensmith, S.P.; Freed, R.; Everson, E.H.; Smail, V.; Weber, M.; Tohme, J.; Anderson,

J.; Rorick, K.; Portice, G.; Rittersdorf, D.; Wolberg, P.; Bricker, B. and Heath, T.: A microcomputer program for the design, management and analysis research experiments. Version 4, Michigan State University and Agriculture University of Norway, USA: 1985.

30- Mostafa, H.A.M. , El-Bakry, A.A. and Eman, A. Alam. Evaluation of antibacterial and antioxidant activities of different plant parts of *Rumex vesicarius* L. (Polygonaceae). International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3(2): 109-118.

31- Astrid Kännaste. Volatiles of Conifer Seedlings Composition and Resistance Markers. Ph.D. Thesis, KTH Chemical Science and Engineering, Universities services US AB, Stockholm 2008: 33.

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