Biochemical screening and pharmacological evaluations for the *Rumex veicaruies* extracts

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Abstract: *Rumex vesicarius* L. (Hummayd), of the family Polygonaceae, are edible herbs growing wild in Egypt. The proteins and soluble carbohydrates contents of different parts of hummayd were determined. The amino acids compositions consisted mainly of valine; arginine; cystine and glutamic acid. Phenolic compounds were examined by both liquid chromatography/mass spectrometry (LC/MS) and by gas chromatography/mass spectrometry (GC/MS). Pyrogallol was rich and pyrocatechin was moderate amount, while p-hydroxybenzoic acid; ferulic acid and syringic acid were low amount in plant extract. The crude extract of hummayd showed strong antibacterial activity against both Gram negative and Gram positive bacteria. Antibacterial activities were mostly associated with the presence of high level of phenols compound. In addition, gel electrophoreses of pollen grains showed obvious dense bands which used in preparing the allergen for skin prick test. High Immunoglobulin (IgE) in the patient group compared to the control group showed the ability of skin prick test to predict prescience of *Rumex vesicarius* allergy.

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Key words: *Rumex veicaruies*; Gram positive bacteria; Gram negative bacteria; antibacterial

1. Introduction

Rumex vesicarius, locally called hummayed and hammad (sour-wort), is wild leafy plant that belongs to the family of Polygonaceae (Mandaville, 1990). Hummayed is a shrub that grows in the central and northern regions of Egypt and Saudi Arabia. This wild leafy plant grows annually during the fall and spring rainy seasons. Both the leaves and tender stems are consumed by some people of Saudi -Arabia and other parts of the world. The raw leave may be used as a salad ingredient added during the preparation of (dried milk shared) to increase the acidity. (Tukan *et al*., 1998). Green leafy vegetable are, in general, good sources of vitamins, minerals, and fibers (FAO, 1988). This leafy wild plant is common in different parts of the world (Tukan *et al*., 1998), e.g. in India (Rao *et al*, 2011). The whole plant is medicinally important and cures several diseases, the plant is stimulant, tonic, and acts as aphrodisiac agent (El-Bakry *et al.*, 2011).

An allergy is sensitivity to a normally harmless substance, one that does not bother most people. The allergen (the foreign substance that provokes a reaction) can be a food, dust particles, a drug, insect venom, or mold spores, as well as pollen. Certain plants produce prodigious amounts of pollen. A single plant can produce 1 million to several million pollen grains in a day. The pollinating season of the various plants depends on the individual species and on the geographical location. Pollen sampling and morphological identification can be routinely performed to determine the plant source and allergen load (Esch *et al.*, 2001). So, the present work was aimed to screen the biochemical constituents of *Rumex vesicarius* L. (Hummayd) and to evaluate the antibacterial activity, in addition to investigate the anti IgE components antigenenic and allerginic activities of *Rumex vesicarius* and reactivity in an atopic population using *vivo & vitro* methods.

2. Materials and Methods

2.1. Collection of plant sample

Rumex vesicarius L plants (Fig. 1) were collected randomly from different fields around Cairo at May and August (2007). The plant was well preserved and identified by Botany Department, Faculty of Science, Al-Azhar University.

Determination of total soluble proteins was made a according to method of (Lowery *et al.*, 1951) using Cassein (ug /ml) as standard protein. Contents of total soluble carbohydrates were determined by another technique according to (Umbriet *et al.*, 1969). Phenols determination was carried out according to (Danial and George, 1972). Phenolic compounds were measured at 280 nm using Shimadzu HPLC (SCL-10 AVP, Shimadzu Co.,) separation was achieved with a TSK gel column ODS-80Tm (4.6mm X15mm) a gradient elution was performed with solvent A (water: acetic acid, 99:1) and solvent B (methanol: acetonitrile: acetic acid, 95:4:1), (Emmons *et al.*, 1999). Amino acid standard mixture was evaluated by Epp. Biotronik, LC.3000, amino acid Analyzer (Beckman Instrument).

Figure 1. Showing *Rumex vesicarius* L., of the family Polygonaceae, is edible herbs growing wild in Egypt.

2.2. Experimental condition

LC 3000 standard H1, Readymade buffers H1 (4-buffer system), column type H 125 X4 mm, Pre-column type H 60 x 4 mm. were used. This analysis was performed in the Al-Azhar University–(The Regional Centre for Mycology and Biotechnology) for identification of the prepared allergen extract.

HPLC apparatus Beckman system Gold, dual pump, Module 125 was used. This test was carried out by using HPLC- Beckman system gold, dual pump, Module 125, Kanauer Injector, with a 20 µl loop. Module 166 Variable UV detector. HPLC column–Phenomenex (Lichrosorb RP-18; 5 µl M, 250 x 4.6 mm ID USA). The purified allergen samples (the prepared allergen extract were passed through the apparatus in the Medical Research Center, Ain Shams University to detect fractionation of the crude allergens, according to (Islam *et al.*, 2011).

The antibacterial activity was carried out following disc diffusion method (Arya *et al.*, 2010).

Preparation of the Rumex vesicariuse extract for skin prick test was done in according to (Kwaasi *et al.*, 1992).

2.3. Detection of Specific I immunoglobulin E in Serum

This done using the Enzyme Linked Immuno sorbent assay (ELISA) technique according to (Bennich, *et al.*, 1978).

2.4. Allergen skin testing

Although there is a variety of in vitro and *in vivo* methods of assessing the presence of specific IgE antibodies, skin testing is performed because it is simple, sensitive, and less expensive and the results are immediately available (Nelson, 1983).

3. Results and Discussion

3.1. Biochemical Studies

The proteins and soluble carbohydrates contents of different parts of hummayd (*Rumex vesicarius*) are presented in figure (2). The proteins concentrations were within the range from 4.7 in roots to 7.9 and 9.8 mg/g in stems and leaves, respectively. In hummayd leaves, the concentration of soluble carbohydrates was higher as compared with stems or roots contents. These results are in conformity with those of Alfawaz (2006) who found that the protein value of hummayd was 17.1–20.1 g/100 and lipids were 3.1–3.8 g/100 g. Also, Londonkar and Tukappa (2013) studied the principal constituents of *Rumex vesicarius* and reported that reducing sugars, proteins, lipids and carbohydrates were included in various parts like leaf, stem, and root of the plants.

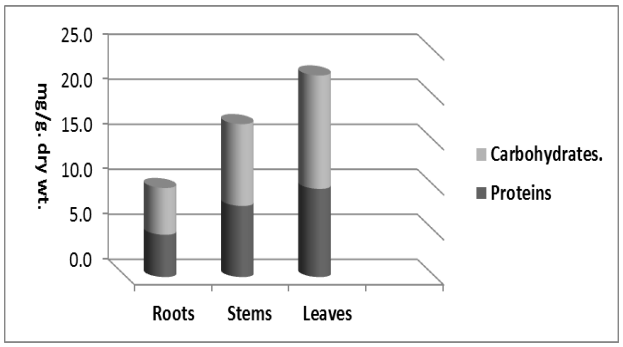

Figure 2. Carbohydrates and proteins contents in different parts of *Rumex vesicarius*.

The odtained results showed that amino acid analyzer (eppndorf–Biotromik/column (P) 125X4mm, percolumn 60 x 4 mm) of *Rumex vesicarius* exhibited eighteen different amino acids and related compounds. The data also included that hummayd contains high level of valine (20%), beta-aminoisobutryic (16%), arginine (12%), cystine (11%) and glutamic acid (7%) as seen in table (1). *Rumex vesicarius* extract was rich in some amino acids such as valine; arginine and cystine and it was minor in other amino acids such as glycine and phenyl alanine. Hummayd extract also showed the presence of moderate concentration of amino acids such as arginine; cystine; glutamic acid and aspartic acid. In this respect, Hariprasad and Ramakrishnan (2011) carried out the gas chromatography-Mass spectrometry

(GCMS) analysis of *Rumex vesicarius* L. in four solvents like ethyl acetate extract (EE), ethyl alcohol extract (EAE), chloroform extract (CE), and hexane extract (HE)) to identified the phytochemical constituents. In hexane 61 compounds, chloroform 56 compounds, ethyl alcohol 49 compounds and ethanol extract 45 compounds were identified.

3.2. Antimicrobial Studies

The results of antibacterial activity studies on hummayd extract of shoot system (Table 2) revealed that the activities were high in cases of *Pseudomonas aeruginosa* and *Escherichia coli*, where the inhibition zones were 13.0 and 12.0mm, respectively. The effect were moderate in cases of *Staphylococcus faecalis* (inhibition zones =11.0mm) and *Sallmonella typhimurium* (inhibition zones =10.0 mm). However, the extract had poor effect against *Proteus mirabilis* and *Staphylococcus aureus*, where inhibition zones were 8.0 and 7.0 mm, respectively. The obtained results revealed that hummayd extract was potent antibacterial agents against a number of both Gram positive and Gram-negative bacteria. The present antibacterial activity results confirmed the findings of Elegami *et al*. (2001) who found that chloroform extract of R. vesicarius L. (whole plant parts) had positive effect against B. subtilis. Also, Mostafa *et al.*, (2011) and El-Bakry, *et al*. (2013) found that ethanol extract of *Rumex vesicarius* had antibacterial activity.

Rumex vesicarius in different solvents showed varied in the zone of inhibition from 5-17 mm against all the tested bacteria (Table 3). Hummayd ethyl acetate extract showed highest activity against *Pseudomas aeruginosa*, *Escherichia coli* and *Staphyloccus faecalis*. Ethanol and chloroform hummayd extracts showed almost similar antibacterial activity against *Pseudomas aeruginosa*, *Escherichia coli*, whereas lowest activity was observed against *Pseudomas aeruginosa.*

Such resulted suggested that phenol compound may be responsible for this activity. Conforming this, the results of phenol in *Rumex vesicarius* extract using different solvent (ethanol, chloroform, ethyl acetate and aqueous extracts) as seen in figure (3). Ethyl acetate extract showed the highest amount of phenol followed by ethanol extract. Aqueous and chloroform extracts exhibited the lowest amount of phenol. These results are in conformity with those of Hariprasad and Ramakrishnan (2011) who found that ethyl acetate extract of *Rumex vesicarius* showed maximum percentage of phenol (15.71%) while the chloroform extract showed minimum percent of phenol (1.65%).

The obtained results (Table 4) observed that the HPLC analysis of the extracts showed high amount of phenolic compounds; pyrogallol (291.40 mg/g) and moderate amount of pyrocatechin (43.20 mg/g) and low amount of p-hydroxybenzoic acid (4.04mg/g); ferulic acid (8.70 mg/g) and syringic acid (1.80 mg/g) . Our results run parallel with El-Hawary *et al.,* (2011) and Londonkar & Tukappa (2013) who reported that extract of *Rumex vesicarius* had high amount of phenolic compounds. Our results of antibacterial activity and total phenolics of *R. vesicarius* L. agreed with results of Tavares *et al.,* 2010, since they found that, polyphenolics compounds in *R. maderensis* were strongly associated with antibacterial capacity and total phenolics content reflect the antibacterial activity and antioxidant capacity of the plant.

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

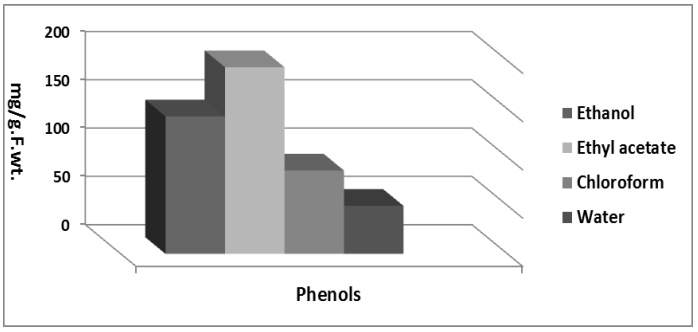

Figure 3. Total phenols compounds in different extracts of *Rumex vesicarius*.

3.3. Pharmacological Studies

During flowering stage, pollen grains of hummayd were collected (Fig. 4). The provided pollen grains, totally weight 20 gm, were very minute as seen under scanning electron microscope (Fig. 5).

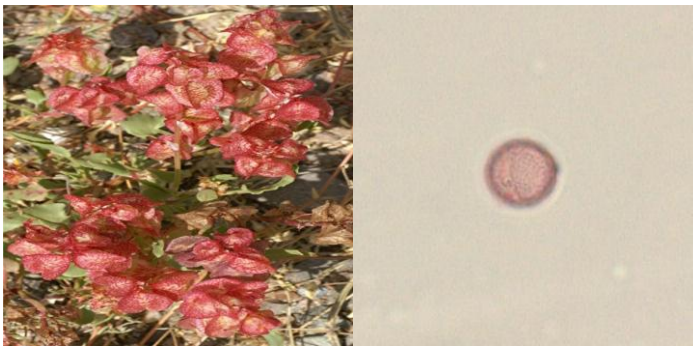

Figure 4. Showing image of *Rumex vesicarius* at flowering stage (on the right) and the pollen grains (on the left).

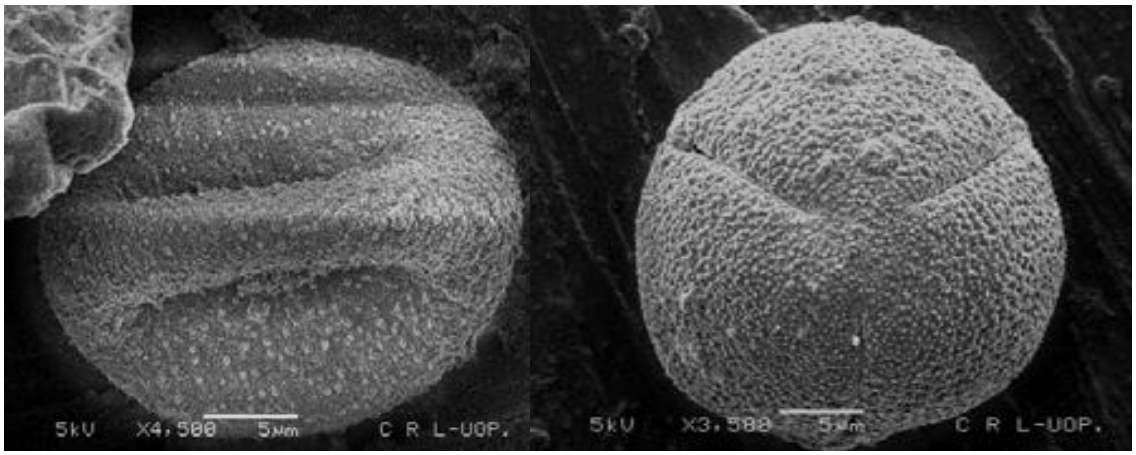

Figure 5. Pollen grains of *Rumex vesicarius* by Scanning Electron Microscope.

Table 1. Showing the different amino acid and related compounds in *Rumex vesicarius* extracts.

Peak numb.	Peak name	time	Conc. ug/ml	Conc.%
1	Phosphoserine	4.30	122.24	1.76
2	Phosphoethnolamine	6.95	96.46	1.38
3	Urea	9.03	115.32	1.66
4	aspartic acid	12.03	262.87	3.77
5	Threonine	16.93	221.84	3.19
6	Serine	18.42	152.13	2.18
7	glutamic acid	21.05	475.03	6.82
8	Glycine	32.88	81.29	1.17
9	Valine	35.83	1392.73	20.00
10	Cystine	39.58	765.00	10.85
11	Leucine	53.77	271.70	3.90
12	Tyrosine	57.60	199.85	2.87
13	phenyl alanine	64.68	57.84	0.83
14	Betaaminoisobutyric	83.50	1108.25	15.91
15	Carnosine	99.87	271.02	3.89
16	Lysine	105.62	123.02	1.77
17	ammonium sulfate	108.18	410.69	5.90
18	Arginine	115.68	846.59	12.16
TOTALS			6964.88	100.00

Table 2. Antibacterial activity of *Rumex vesicarius* extract against bacterial species tested by disc diffusion assay zone of inhibition (mm).

Bacterial species (test organism)	Activity (mm.)
Staphylococcus aureus ATCC 6538	7.0
Staphylococcus faecalis ATCC 8043	11.0
Sallmonella typhimurium ATCC 14028	10.0
Escherichia coli ATCC 8739	12.0
Pseudomonas aeruginosa ATCC 27853	13.0
Proteus mirabilis ATCC 29245	8.0

Table 3. Antibacterial activity of *Rumex vesicarius* in different solvent (ethanol, chloroform, ethyl acetate and dil. water) against bacterial species tested by disc diffusion assay zone of inhibition (mm).

Extracts Bact. disc	Ethanol	chloroform	Ethyl acetate	Dil water
Pseudomas aeruginosa	14	13	17	7
Escherichia coli	12	11	15	5
Staphyloccus faecalis	9	8	12	4

Table 4. Showing the phenolic compounds content (mg/g extract) of *Rumex vesicar* measured by HPLC.

Compound	Content (mg/g)	S.E
Pyrogallol	291.40	5.66
Pyrocatechin	43.20	0.23
p-Hydroxybenzoic acid	4.04	0.04
Ferulic acid	8.70	0.08
Syringic acid	1.80	0.34

3.4. Measurement of Immunoglobulin (IgE)

The results of Immunoglobulin (Ig E) showed that the level of IgE was very highly significantly increased in the patients group II compared to the control group I ($p<0.001$) using student as parallel group design as Total serum IgE is significantly elevated in group II as shown in table (5) (M 34.8 SD 1.116 M 559.45 SD 0.941) respectively (P-value = 0.0046). The enzymatic reaction of total IgE Ab was reading in ELISA reader using standard curve as shown in Fig. (6) converting the optical density (O.D) reading concentration of the sample.

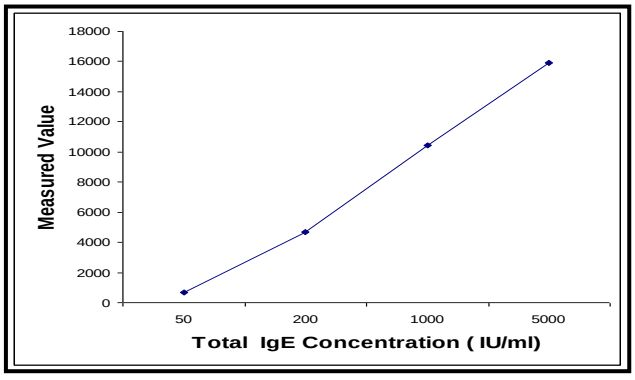

Figure 6. Standard curve of total IgE.

3.5. Measurement of specific Immunoglobulin E (IgE)

Ag-Ab–reaction test was carried out for confirmation antigenic property of the prepared allergen extracted using ELISA technique. The sera of patient have been used as Ab and the level of specific IgE was very highly significantly increased in the patients group II compared with control group I (Table 6).

Table 5. The total IgE in patients compared to the control.

Number	Total IgE in Patients	Total Ig E in Control
1	450 ± 0.92	40 ± 1.2
2	600 ± 0.6	50 ± 0.8
3	900 ± 1.2	24 ± 1.1
4	570 ± 0.7	35 ± 0.98
5	325 ± 2.3	35 ± 0.68
6	230 ± 1.3	20 ± 1.3
7	375 ± 0.92	15 ± 1.1
8	756 ± 1.0	54 ± 1.8
9	516 ± 0.83	24 ± 1.0
10	450 ± 0.52	51 ± 1.2
11	280 ± 0.79	
12	310 ± 0.81	
13	900 ± 0.7	
14	690 ± 1.2	
15	538 ± 0.50	
16	299 ± 0.90	
17	657 ± 0.85	
18	1230 ± 1.1	
19	789 ± 0.70	
20	324 ± 0.98	
Mean	**559.45 ± 0.941**	**34.8 ± 1.116**

Groups	Mean	S.E	P-value
Controls	34.85	1.116	0.001
Patients	559.45	0.941	0.001

There is a high significant different between group I and group II by using independent t-test at $p< 0.001$.

Group I: ten apparently healthy controls with normal detectable serum total IgE (less than 150 Iu/ml).

Group II: twenty patients with highly detectable serum total IgE level (more than 150 Iu/ml).

Table 6. The specific IgE of patients compared to the control

Number of Patients	Specific IgE of Patients	Specific IgE of Control
1	180 ± 0.52	34 ± 0.41
2	214 ± 0.52	45 ± 0.61
3	210 ± 0.46	16 ± 0.34
4	201 ± 0.55	23 ± 0.21
5	145 ± 0.26	25 ± 0.43
6	162 ± 0.49	17 ± 0.31
7	90 ± 0.40	13 ± 0.40
8	102 ± 0.49	35 ± 0.42
9	234 ± 0.73	32 ± 0.49
10	89 ± 0.42	43 ± 0.46
11	135 ± 0.57	
12	210 ± 0.17	
13	300 ± 0.54	
14	134 ± 0.66	
15	89 ± 0.25	
16	112 ± 0.46	
17	290 ± 0.40	
18	265 ± 0.30	
19	100 ± 0.37	
20	67 ± 0.33	
Mean	**166.45 ± 0.44**	**28.3 ± 0.407**

Measuring Specific IgE Normal range up to 50 IU/ml

Groups	Mean	S.E	P-value
Controls	28.3	0.407	0.001
Patients	166.45	6.90.44	0.001

*= there is very high significant difference between groups by using independent t-test at P-value<0.001.

3.6. Measurement of Eosinophil Count

It is obvious that absolute eosinophil count was highly significantly increased in the patients group II compared to group I at probability ($p<0.05$) using student-T test parallel design as showing in table (7) and Fig. (7). The means were 241 in group I and 646.5 in group II.

N.B

From the graph we conclude that by increasing total IgE, specific IgE and absolute eosinophils also increase in allergic patients.

Our study indicated that the mean value of absolute eosenophilia count (241 SE 1.1) in group I and in group II the mean value of absolute eosenophilia count (646.5 SE 1.0 at P <0.005).

Table 7. Shows the count of absolute eosinophils in patients compared to the control.

	Eosinophils In Patients	Eosinophils in control
1	200 ± 0.9	100 ± 1.0
2	420 ± 0.9	160 ± 1.1
3	800 ± 1.1	230 ± 1.2
4	475 ± 1.0	200 ± 0.98
5	600 ± 0.96	260 ± 0.97
6	550 ± 1.1	390 ± 0.99
7	490 ± 1.2	300 ± 1.0
8	475 ± 0.9	270 ± 1.1
9	580 ± 0.97	320 ± 1.2
10	350 ± 0.95	180 ± 1.0
11	950 ± 1.1	
12	520 ± 1.0	
13	1000 ± 1.0	
14	820 ± 1.0	
15	1100 ± 1.2	
16	610 ± 0.97	
17	730 ± 0.97	
18	840 ± 0.95	
19	750 ± 1.1	
20	670 ± 0.97	
Mean	**646.5 ± 1.0**	**241 ± 1.1**

Normal range of absolute Eosinophile: 40 – 400

Groups	Mean	S.E	P-value
Controls	241	1.1	0.005
Patients	646.5	1.0	0.005

* =There is a high significant difference between group I and group II by using independent t-test at P-value < 0.005.

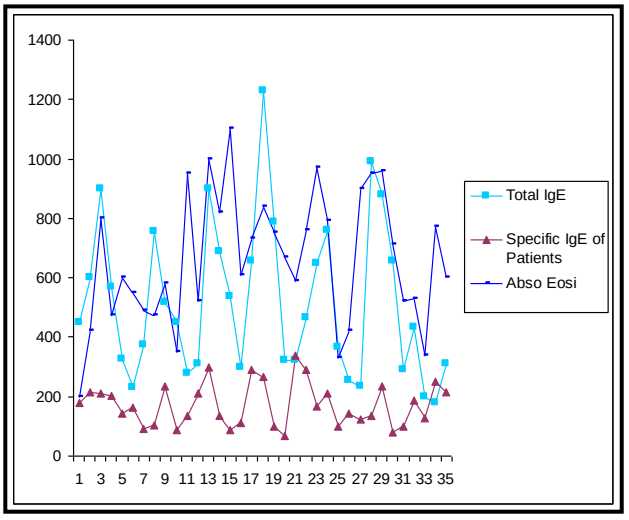

Figure 7. A correlation between T.IgE, Sp IgE, absolute eosinophils.

3.7. Skin prick test

Table 8. Showing the results of patients Skin prick test.

Case No.	Wheel mm	Flare mm	Positive control	Negative control
1	0(-ve)	0(-ve)	25	0
2	0(-ve)	0(-ve)	25	0
3	0(-ve)	0(-ve)	24	0
4	4	18	25	0
5	0(-ve)	0(-ve)	27	0
6	0(-ve)	0(-ve	25	0
7	0(-ve)	0(-ve)	22	0
8	5	18	30	0
9	3	0(-ve	28	0
10	4	0(-ve)	35	0
11	6	0(-ve)	32	0
12	5	0(-ve)	22	0
13	6	22	28	0
14	0(-ve)	0(-ve)	20	0
15	0(-ve)	0(-ve)	30	2
16	5	0(-ve)	20	0
17	0(-ve)	0(-ve)	18	0
18	6	15	28	0
19	5	2	30	0
20	0(-ve)	0(-ve)	22	0
21	5	2	28	0
22	5	0(-ve)	28	0
23	3	0(-ve)	30	0
24	3	0(-ve)	20	0
25	3	0(-ve)	28	4
26	0(-ve)	3	28	0
27	0(-ve)	0(-ve)	0	0
28	5	4	0	3
29	0(-ve	0(-ve)	0	2
30	2	0(-ve)	0	0
Mean	**2.5**	**2.85**	**22.6**	**0.36**

Mean T. IgE P	Mean Sp IgE P.	Mean Ab. Eo P	Mean T. IgE C	Mean Sp IgE C	Mean Ab Eo C
559.45	166.45	646.5	34.85	28.3	241

It is common knowledge amongst inhabitants in Egypt that *Rumex veicaruese* pollen is a causative agent of asthma and seasonal allergic rhinitis. The study of *Rumex veicaruese* it is not enough because the seasonal of pollination it will be appear with deferent type of plants and we recorded *Rumex veicaruese* caused the allergic diseases by the skin prick test. The importance of the local people is underscored by the fact that some clinicians in allergy clinic in Saudi Arabia, through patient request, found it necessary to obtain custom-made *Rumex veicaruese* from commercial sources abroad for inclusion in their Allergy skin pricks test (SPT) panel (Kwaasi *et al*., 1992). Although this study of IgE revealed presence of different components of type I hypersensitivity reaction in from of elevated total serum IgE.

In our result revealed positive linear correlation between total IgE measurement and Ag-Ab Serum IgE of *Rumex veicarues* pollens.

This study examined the ability of skin prick test to predict prescience of *Rumex veicaruese* allergy (Robert and Hamilton, 2003).The aim of this study was use in vivo and in vitro methods to assess the antigenic and allergenic activity of and identify their anti-IgE components and reactivates in an a topic population. The results of such a study will help draw the attention of the general public, general practitioners and allergists to the fact that pollen of *Rumex vecariuse* are allergenic and should be included in an allergy-testing panel.

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