

Effect of Antioxidants of Sage tea and Marjoram tea on Advanced Renal Disease

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Abstract

Chronic kidney disease (CKD) is a worldwide public health problem. Oxidative stress is the causative factor for a wide variety of diseases, including CKD. Medicinal plants used in the management of CKD are effective in renal detoxification and help the effects of dialysis treatment. This study was conducted to investigate the effect of aqueous extract of sage (*Salvia Officinalis*) and marjoram (*Origanum Majoranum*) on advanced chronic kidney patients under dialysis. The experiment was carried out on sixty patients (40-50 years old), diagnosis based on detailed clinical history, clinical examination and other relevant biochemical investigations. The patients were divided into 6 groups (each group contain 10 CKD patients under treatment with (hemodialysis and regular medical treatment for 3 months) as followed: control patients were treated with regular medical treatment, other patients consumed aqueous extracts of (5g sage, 5g marjoram, 10g sage, 10g marjoram, mixture of 5g sage +5g marjoram) twice today respectively. Results illustrated that the aqueous extract of marjoram or sage are rich in antioxidants components (Phenolic acids, Flavonoids, Oxygenated monoterpenes, Diterpenoids and Triterpenes), antioxidant capacity and phenolic content. Results clarify that glomerular filtration rate (GFR) increased, while erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) significantly decreased ($P < 0.05$) in all treated patients compared to the control group. Malondialdehyde (MDA) decreased and superoxide dismutase (SOD) increased. The mixture of aqueous extracts of marjoram (5g) and sage (5g) recorded the best drink. Therefore, this study recommends the use of the aqueous extracts of marjoram (5g) and sage (5g) in decreasing the oxidative stress and improve kidney health in hemodialysis patients.

Key words: hemodialysis, oxidative stress, antioxidants, sage, marjoram superoxide dismutase, malondialdehyde, patients.

Introduction

The kidneys filter plasma in the glomerulus to form a protein-free ultrafiltrate. This ultrafiltrate passes through the various tubular segments where reabsorption of essential constituents and secretion of unwanted products occur (*Byham-Gray et al, 2014*). CKD is a worldwide public health problem characterized by either reduced glomerular filtration rate or the presence of kidney damage that leads to abnormal kidney function and higher proteinuria, blood urea nitrogen, and serum creatinine levels (*Romagnaniet al., 2017*).

Oxidative stress is the causative factor for a wide variety of diseases, including CKD. Antioxidants are the molecules that combat the oxidative stress developed from an imbalance between the rate of production and removal of produced oxidants (*Amarasiria et al., 2020*).

Medicinal plants are rich sources of bioactive compounds that have been reported to exert nephron protective mechanisms, such as antioxidant (mainly in the form of phenolic compound), anti-inflammation, diuretic, and immunomodulation. Hence, (*EI-Wakfet et al., 2020*) predicted that these plants are of utmost importance to protect renal function and slow the occurrence and the progression of CKD.

Among such plants, sage (*Salvia officinalis L.*) and marjoram (*Origanum majorana L.*) have been described to contain high percentage of biologically active polyphenolic compounds which are useful to human health and have effective therapeutic benefits (*EI-Wakfet et al., 2020*). Sage is considered as one of the most popular herbs consumed widely and traditionally as an herbaceous infusion. The incorporation of sage infusion in the daily diet can provide considerable benefits, being anti-mycotic, anti-carcinogenic, antidiabetic, antimicrobial, anti-inflammatory, antioxidant, and anti-proliferative. In addition to these effects, sage infusion exhibits antiradical activity which correlates strongly with their high level of total phenolic content. Rosmarinic acid, salvianolic acid K, and luteolin-7-O-glucuronide were detected as the main phenolic compounds of sage aqueous extract (*Sotiropoulou et al., 2020*). Marjoram is widely utilized as a spice and for better food flavor. It is a strong remedy for coughs, respiratory infections, cardiovascular disorders, skin lesions, and digestive problems. Marjoram could also assist in managing liver and kidney diseases (*EI-Wakfet et al., 2020*).

Aim of the study

The aim of this study was to investigate the effect of aqueous extract of sage (*Salvia Officinal*) and marjoram (*Origanum Majoranum*) on advanced chronic kidney patients under dialysis.

Materials, Patients and Methods

Materials: Sage (*Salvia officinalis*) and marjoram (*Origanum majoranum L.*) were obtained from Family Pharmacia Company for Herbs, 38 Sharkia ind. Zone, Belbis – 10th of Ramadan road.

Patients: Sixty (40-50 years old) patients suffering from chronic kidney disease selected from Al-Azhar University Hospital, New Damietta, Egypt. The patients were diagnosed after detailed clinical history, clinical examination and other relevant biochemical investigations. Patients were divided into 6 groups (each group contain 10 CKD patients under treatment with hemodialysis) and regular medical treatment as followed: control patients treated with regular medical treatment, other patients consumed aqueous extracts of (5g sage, 5g marjoram, 10g sage, 10g marjoram and mixture of 5g sage +5g marjoram) respectively twice daily for 3 months.

Methods

A. Aqueous extracts preparation:

The air parts of plants were dried (5 grams) and treated with double distilled water at 100 degrees C^o, for a period of 15 minutes. The samples were filtered. The resultant filtrates were made off up to 50 ml with double-distilled water. Three samples for each analyzed condiment plant were done according to (*Al-Turkiet et al., 2007*).

B. Determination of antioxidant activity by DPPH method:

Antioxidant activity was determined in the extracts using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. This method was taken from (*Buřičova and Reblova, 2008 and Lo Scalzo, 2008*).

C. Determination of total phenolic compounds:

The content of total phenolics was determined spectrophotometrically at 760 nm by using Folin-Ciocalteu reagent. The results were expressed as the content of gallic acid per unit mass of the sample (*Dorman et al., 2003 and Stratilet al., 2008*).

D. Biochemical Assay

Blood samples were collected at zero time and at the end of the study. The serum was separated within 2 hours after blood withdrawal, by centrifugation at 3000 rpm for 10 minutes. The serum samples were kept frozen at -20°C until determination parameters in the laboratory of Al –Azhar University Hospital in New Damietta. Hemoglobin (HGB), red blood cell (RBC), white blood cells (WBC) and platelets (Plt) count were measured using automated hematology analyzer (Sysmex, Kobe, Japan). ESR was measured using (*Westergren, 1926*) method. Serum creatinine was determined according to (*Kaplan, 1984 and Murry, 1984*). Malondialdehyde (MDA) determined according to (*Satoh, 1978*). Superoxide dismutase was determined according to the Indirect Method (*Flohe and Ötting, 1984*). Serum C-reactive protein (CRP) was analyzed by an automated analyzer (Olympus AU400) with a latex turbidimetric immunoassay kit (CRP-UL assay, Wako Chemicals, Neuss, Germany). The glomerular filtration rate (GFR) was estimated according to criteria of the **National Kidney Foundation/ Kidney Disease Outcomes Quality Initiative**, using the equation proposed by (*Levey et al., 1999*).

$$\text{GFR} = 175 \times \text{standardized Scr}^{-1.154} \times \text{age}^{-0.203} \times 1.212 \text{ (if black)} \times 0.742 \text{ (if female)}.$$

E. Statistical analysis:

Data was statistically analyzed using (SPSS) software version (18). Analysis of variance (ANOVA) was used to show the significance $P < 0.05$ among groups (*SPSS, 1986*).

Results and Discussion

Chemical composition of aqueous extract of marjoram and sage:

The results illustrated that the aqueous extract of marjoram and sage are rich in antioxidants components as (Phenolic acids, Flavonoids, Oxygenated monoterpenes, Diterpenoids and triterpenes). Marjoram aqueous extract are rich in Terpinen-4-ol (20.23%), followed by vanillic acid (14.44%), ursolic acid (13.09 %), α -terpineol (7.64%) and transpinene hydrate (6.63%) and other components as shown in Fig (1). The main compounds in sage aqueous extract were, rosmarinic acid (26.03%), luteolin-7-O-glucuronide (16.5%), caffeic acid (12.69%), ursolic acid (11.43%) and apigenin (6.6%) as shown in Fig (2). These results agree with that reported by (*Beddows et al. 2000, 2001; Exarchou et al. 2002; Fasseas et al. 2007; Celikel and Kavas 2008; Calikoglu et al. 2009; Chrpova et al., 2010; Abdel-Massih et al., 2010; Miron et al., 2011 and Bina and Rahimi, 2017*) who approved the high phenolic content and high antioxidants components of sage and marjoram. They detected that rosmarinic acid is the main phenolic compounds of sage aqueous extract and terpinen-4-ol is the main component in marjoram aqueous extract with varying proportions of the other mentioned ingredients.

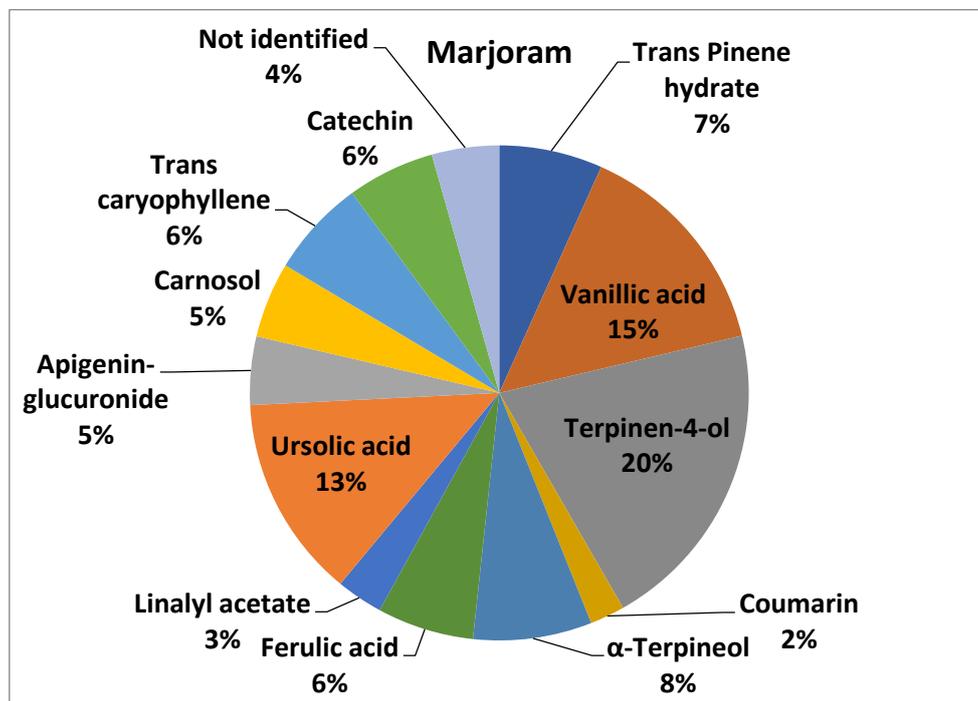


Fig (1):

The chemical constitutions of marjoram aqueous extract.

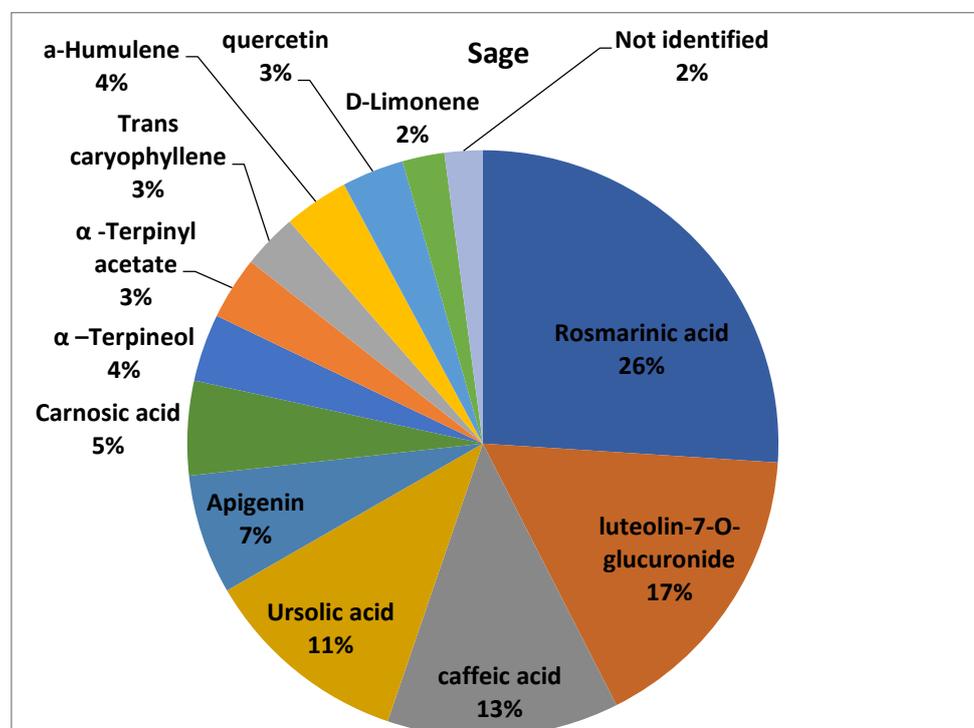


Fig (2):

The chemical constitutions of sage aqueous extract..

Antioxidant capacity and total phenolic contents of plants aqueous extract:

Table (1) shows the strongest antioxidant capacity of the aqueous extracts of marjoram and sage. The total antioxidant capacity (DPPH) of the aqueous extracts of marjoram and sage were (42.1 and 60.6), respectively and the phenolic content values were expressed as mg equivalent of gallic acid (GAE) were (27.7 and 24.3), respectively. This result exhibited the strongest antioxidant capacity of the aqueous extracts of marjoram and sage which agreed with (*Roby et al., 2013 and Dabija et al., 2018*).

**Table (1):
Antioxidant capacity and total phenolic contents of plants aqueous extract**

Herbs	Latin name	Family	DPPH (mg AAE/g)*	Phenols (mg GAE/g)**
Marjoram	Origanum majorana L.	Lamiaceae	42.1	27.7
Sage	Salvia officinalis L.	Lamiaceae	60.6	24.3

*AAE = ascorbic acid equivalent

** GAE = gallic acid equivalent

Effect of sage and marjoram extracts on some parameters of hemodialysis patients:

Table (2) compares the results before and after using the aqueous of herbs. The control group which includes the hemodialysis patients had increase in ESR and CRP which indicate inflammation occurrence, this result accepted with (*Fine, 2002*) with slightly improvement in GFR. But the treated groups by the aqueous extracts of sage and marjoram had a noticeable decrease in ESR, CRP with some variability among them and a noticeable increase in GFR for all other groups. Group (6) which was treated with mixture of the aqueous extract of marjoram (5 g) and sage (5 g) recorded the best result in GFR, which denotes the best glomerular function. Group (2) which was treated with the aqueous extract of sage (5 g) recorded the best decrease in ESR unlike CRP which had the best decrease with group (5) which was treated with the aqueous extract of marjoram (10 g) that denotes the best decrease in inflammation occurrence. However, this illustrates the good effect of using the aqueous of used herbs.

Table (3) showed the good effect of herbs that improve the kidney function as shown by the level of creatinine, whereas creatinine value increased in the control comparing to all treated groups which was had decreased in creatinine. The response in group (6) which consumed the mix of aqueous extract of herbs recorded the best decrease in creatinine.

Table (2):
Effect of the Aqueous Extract of Sage and Marjoram on Glomerular Filtration Rate (GFR), Erythrocyte Sedimentation Rate (ESR) and C - reactive protein (CRP) in hemodialysis patients:

Groups	Parameters					
	GFR (ml/min/1.73 m ²)		ESR (mm/hr)		CRP (mg/L)	
	Before	After	Before	After	Before	After
Group 1: (control)	7.57±0.36	8.48 ± 0.32	38.30±4.36	48.70±3.54	8.00±1.03	9.30±0.79
Group2: (5g sage)	10.62±1.03	12.86±0.70	22.30±1.91	12.40±2.02	9.00±1.04	7.20±0.80
Group3 : (10g sage)	9.93±0.74	12.85±1.08	44.60±7.62	13.60±2.74	9.80±0.86	6.85±0.58
Group4:(5gmarjoram)	9.13±0.41	11.26±0.66	33.10±3.03	19.00±2.38	7.80±0.91	6.82±0.53
Group5:(10gmarjoram)	10.04±0.37	11.11±0.59	55.10±4.95	20.40±3.02	8.60±1.07	6.78±0.58
Group6 : (5 g marjoram + 5 g sage)	10.08±0.56	15.22±0.28	34.30±3.01	15.4±1.72	9.50±0.87	6.89±0.24

Data expressed as Means ± SE.

Means with different superscript letters in the same column are significantly different at P< 0.05.

Table (3):
Effect of the Aqueous Extract of Sage and Marjoram on creatinine on patients:

Groups	Creatinine (mg/dl)	
	Before	After
Group 1:(-ve control)	5.74±0.64	8.47 ± 0.39
Group2: (5g sage)	8.14±0.61	6.38 ± .044
Group3 : (10g sage)	7.35±0.79	6.23±0.76
Group4 : (5g marjoram)	7.45±0.53	6.60±0.47
Group5 :(10g marjoram)	7.26±0.55	5.71±0.39
Group6 :(5 g marjoram + 5 g sage)	8.26±0.30	4.05±0.23

Data expressed as Means ± SE.

Means with different superscript letters in the same column are significantly different at P< 0.05.

Table (4) shows the blood picture results in groups before and after the experiment. It shows the decrease in Hb, RBCs, WBCs but not the platelets count in the control group. All other values increased after taking the extracts compared to the control group. This result agreed with (*Hakim et al., 2016*). The treated groups after the end of experiment compared with the control, showed an increase in Hb, RBCs, WBCs and Plts near the normal values. The best increase in Hb was in group (2) which consumed sage (5 g) aqueous extract, followed by group (3) which consumed sage (10 g)

aqueous extract. Group (6) which consumed the mix of aqueous extract of herbs recorded the best increase in WBCs and RBCs values. The last results indicate the improvement of hemodialysis patients' cases.

**Table (4):
Effect of the Aqueous Extract of Sage and Marjoram on Complete Blood Count in hemodialysis patients**

Groups	CBC							
	Hb (g/dL)		RBC ($\times 10^{12}/L$)		WBC ($\times 10^3/\mu L$)		PLT ($\times 10^3/\mu L$)	
	Before	After	Before	After	Before	After	Before	After
Group 1: (+ ve control)	10.65± 1.07	8.56 ± 0.44	3.92± 0.20	3.03± 0.15	8.37± 1.25	4.16± 0.37	108.10± 1.81	117.9± 2.47
Group2: (5g sage)	9.29± 0.24	11.11 ± 0.16	4.07± 0.15	4.45 ± 0.17	4.48± 0.25	5.56± 0.31	109.20± 5.03	140.1± 4.06
Group3 : (10g sage)	9.41± 0.18	11.02± 0.30	3.55± 0.12	3.77± 0.13	5.20± 0.31	5.87± 0.31	132.40± 3.27	160.6± 5.79
Group4 : (5g marjoram)	8.32± 0.57	10.09± 0.41	3.57± 0.31	3.90± 0.25	4.27± 0.54	5.92± 0.66	117.50± 3.20	131.9± 2.96
Group5 :(10g marjoram)	8.66± 0.30	10.16± 0.30	3.83± 0.25	4.33± 0.27	5.33± 0.34	6.24± 0.52	121.30± 6.09	134.6± 5.33
Group6 : (5 g marjoram + 5 g sage)	9.77± 0.65	10.59± 0.54	3.95± 0.17	4.95± 0.28	5.80± 0.41	6.66± 0.67	116± 5.11	151.8± 7.88

Data expressed as Means ± SE.

Means with different superscript letters in the same column are significantly different at $P < 0.05$.

Results in Table (5) showed a decrease in MDA and increase in SOD in all treated groups compared with the control group. The best result was recorded in group (6).

**Table (5):
Effect of the Aqueous Extract of Sage and Marjoram on MDA on hemodialysis patients:**

Groups	MDA (NMO/ml)		SOD (U/ml)	
	Before	After	Before	After
Group 1: (- ve control)	105.40±4.64	213.90±8.81	944.60±18.74	779.20±29.31
Group2: (5g sage)	405.20±10.82	194.50±3.29	607.50±7.47	911.80±44.48
Group3 : (10g sage)	266.10±5.66	175.9±9.10	682.80±10.35	940.40±16.72
Group4 : (5g marjoram)	287±13.83	161.6±7.82	554.7±19.70	959.6±35.35
Group5 : (10g marjoram)	264.6±10.08	142.7±3.17	724.7±14.94	949.4±12.11
Group6 : (5 g marjoram + 5	296.6±14.02	119.2±3.35	650±20.18	1050.2±22.55

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Data expressed as Means \pm SE.

Means with different superscript letters in the same column are significantly different at $P < 0.05$.

These results indicate that chronic kidney patients treated by hemodialysis suffered from increase oxidative stress which agreed with (*small et al., 2012*) and antioxidants improved kidney health which agreed with (*El-Ashmawy et al., 2005; 2007*). The aqueous extracts of sage and marjoram had antioxidant effect which agreed with (*Nakatani, 2000; Novak et al., 2000; Heo et al., 2002; Kelly, 2004; Hazzit et al., 2006; Ayatollahi et al., 2009; Ahmed et al., 2009 and Hossain et al., 2010*) and improved kidney function that agreed with (*Halliwell and Gutteridge, 2007; Nasri and Rafieian-Kopaei, 2014*) This appeared in the improvement of GFR and the decline in CRP, ESR and creatinine with an increase in Hb, WBCs, RBCs and platelets. It is worth noting that the groups that registered the improvement was group (6) which consumed the mixture of aqueous extract of sage (5g) and marjoram (5g) with the difference in the positive effect of other doses used in the experiment on patients (*El-Ashmawy et al., 2005*).

Conclusion

The study confirmed the antioxidant effect of the aqueous extract of sage and marjoram on chronic kidney patients treated with hemodialysis since it include (flavonoids, phenolic acids and terpenes) The best drink was the mix of aqueous extract of both sage (5g) + marjoram (5g), It is recommended to repeat the study on higher number of patients.

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تأثير مضادات الاكسدة لشاي البردقوش وشاي المريمية على مرضى الكلى المزمنة في المرحلة المتقدمة

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كلية الطب بدمياط - جامعة الأزهر²

الملخص العربي

مرض الكلى المزمن هو مشكلة صحية عالمية. الإجهاد التأكسدي هو العامل المسبب لمجموعة متنوعة من الأمراض، بما في ذلك مرض الكلى المزمن. النباتات الطبية المستخدمة في علاج مرض الكلى المزمن فعالة في إزالة السموم من الكلى وتقليل آثار علاج الغسيل الكلى. تم إجراء هذا العمل لمعرفة تأثير المستخلص المائي للمريمية والبردقوش على المرحلة المتقدمة لمرضى الكلى المزمن المعالجين بالغسيل الكلوي. تم إجراء التجربة على ستين مريضاً (40-50 سنة) مقسمة إلى 6 مجموعات (كل مجموعة تحتوي على 10 مرضى الكلى المزمن يتم علاجهم بالغسيل الدموي) وعلاج طبي منتظم لمدة 3 أشهر على النحو التالي: مجموعة ضابطة تحتوي على مرضى تم علاجهم بعلاج طبي منتظم وتناول المرضى الآخرون مستخلصات مائية كالأتي: (5 جم مريمية ، 5 جم بردقوش ، 10 جم مريمية ، 10 جم بردقوش ومزيج من 5 جم مريمية + 5 جم بردقوش) مستخلصات مائية مرتين في اليوم. تم تشخيص المرضى على أساس التاريخ الطبي المفصل والفحص الاكلينيكي والتحاليل البيوكيميائية الأخرى ذات الصلة. أوضحت النتائج أن مستخلص البردقوش المائي وخالصة الميرمية غنية بمكونات مضادات الأكسدة مثل (الأحماض الفينولية، الفلافونويد، أحادي التربينات المؤكسدة، الديتيربينويدات والترايتيربين). أوضحت النتائج أن معدل الترشيح الكبيرقد زاد بشكل ملحوظ ، وانخفضت سرعة ترسيب كرات الدم الحمراء والبروتين المتفاعلي سفي جميع المجموعات المعالجة مقارنة بالمجموعة الضابطة. انخفض المالنوندي الدهيدوزاد السوبر اكسيددزموتيز كما سجل مزيج المستخلصات المائية من البردقوش (5 جم) والمريمية (5 جم) أفضل نتائج. لذلك أوصت هذه الدراسة بأن استخدام المستخلصات المائية من البردقوش (5 جم) والمريمية (5 جم) مفيدة جداً في تقليل الإجهاد التأكسدي وتحسين صحة الكلى لدى مرضى الغسيل الكلوي. هناك حاجة إلى مزيد من الدراسات لتحديد تأثير مضادات الأكسدة للأعشاب والأطعمة على أمراض الكلى المزمنة.