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Medicinal food rich in phytochemical compounds and antioxidant power enhances the health of rural women who are exposed to pesticides

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Abstract

Antioxidants, antibacterials, anti-inflammatory, and perhaps protective compounds against cancer, heart disease, neurodegeneration, and type 2 diabetes are just a few of the health benefits linked to herbs and spices. Surprisingly few studies have attempted to investigate their consumed amounts through diet, and even fewer have searched for potential biomarkers of food intake, despite the ongoing interest in them as medicinal agents and their increasing application in various cuisines. The current study's objectives were to: A- thoroughly survey the used herbs and spices by urban women in Abo Hommes, El Beheira Governorate in Egypt, B- investigate their antioxidant scavenging activities and phytochemical components and, C- relate their consumption to the health protective effects against pesticide pollution. Antioxidant activity of spices was estimated using spectrophotometry and the phytochemical compounds were measured using GC-MS strategies. Results showed that black pepper, cinnamon, clove, cumin, nutmeg, cardamom, bay leaves, coriander, peppermint, and menthe were the ten herbs and spices used by women. Even though most people consume very little amounts of herbs and spices daily, these food supplements have protected women's health from pesticide exposure either directly or indirectly. Results showed the presence of specific pesticide residues in the blood samples of surveyed women. Along with that, they enhance women's health as indicated by the results of blood parameters and serum enzymes. Spices and herbs are rich in phytochemical components that coin them as highly important food additives and should not be undervalued, particularly considering the potential health benefits they may have.

Keywords: Survey, Herbs, Spices, Pesticide residues, Phytochemical components, GC-MS analysis, Human health.

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1. INTRODUCTION

For millennia, culinary herbs and spices have been used as preservatives and flavorings in food. Furthermore, herbs and spices have been used in medicine since ancient times due to their presumed and known health benefits. Spices are dried, smelling parts of plants. More precisely, "aromatic vegetable substances, in the whole, broken, or ground form, whose significant function in food is seasoning rather than nutrition" is a better definition for spices. [1] by the Food & Drug Administration (FDA). While herbs usually come from the leaves of

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the plant, spices can come from any part of the plant [1]. This is the primary distinction between a spice and an herb. Many known herbs and spices have been used since the time of ancient Egyptians and Romans, and the majority are native to Mediterranean, Middle Eastern, or Asian nations [2]. These substances have strong antioxidant qualities that aid in preventing DNA mutation and lipid peroxidation, as well as in turning healthy cells into malignant ones. Free radicals may be produced by pesticides [3]. Herbs and spices are rich in bioactive molecules, including tannins, alkaloids, sulfur-containing chemicals, phenolic diterpenes, vitamins, and particularly flavonoids and polyphenols [4, 5].

Table 1: different types of spices and Herbs used by Women at some of Abo Homes Villages

Name	**Classification**	**Scientific Name**	**name of plant part**	**Spices/Herbs shape**
Black pepper	Spices	*Piper nigrum*	Berry	
Cinnamon	Spices	*Cinnamomum verum*	Bark	
Clove	Spices	*Syzygium aromaticum*	Bud	
Cumin	Spices	*Cuminum cyminum*	Seed	
Nutmeg	Spices	*Myristica fragrans L.*	Seed	
Peppermint	Herbs	*Mentha piperita*	Leaf	

Mentha	Herbs	*Mentha pulegium L.*	Leaf	
Cardamomum	Spices	*Elettaria cardamomum*	Seed	
Coriander	Herbs	*(Coriandrum sativum L.)*	Seed	
Bay leaves	Herbs	*Laurusnoilis*	Leaf	

2. MATERIAL &METHODS

2.1. Study Area

El-Beheira has the largest agricultural activities in Egyptian governorates The research was conducted at Abu Hummus city which is located in the north-central of El-Beheira governorate in Egypt and located at 31.0834°N 30.3097°E (Fig 1).

Figure 1: Location of study in Abu Hummus City, Elbeheira Governorate, Egypt

2.2. Chemicals and Standards

Chemicals, Standards, and kits were provided by the Pesticides Residue Analysis &Toxicity Laboratory from the Faculty of Agriculture, Damanhur University.

2.3. Collection and Preparation of Spices

Based on the findings of a pre-survey, 10 herbs and spices—including black pepper, cinnamon, clove, cumin, nutmeg, bay leaves, coriander, cardamom, peppermint, and mentha—were first selected. The study's herbs and spices were all bought at the neighborhood market, air-dried, and then ground into fine powders for further analysis.

2.4. Spectrophotometric Examination of Chemical Components in Herbs

2.4.1. Methodology for Making Spice Crude Extracts

Weighing 100 mg of species powder with 2 ml of 90% methanol (MeOH) finished preparation of crude extracts for phytonutrient assays. The samples underwent a 60-second vortex at maximum speed, a 30-minute sonication (using a Branson 2200), and a 15-minute centrifugation at 3,500 rpm at 4°C. In 2 ml Eppendorf tubes, supernatants were gathered. The leftover pellet was extracted again using 1 m1 of 90% MeOH, and the supernatants were mixed.

2.4.2. Antioxidant Capacity Using DPPH Radical Scavenging Method

The procedure for performing radical scavenging capability assay was followed by Nassar et al. [5]. spice extract was diluted by around 100 µl in methanol, mixed with 1.5 ml of DPPH (2.5 µM in methanol), and thoroughly shaken. Using 1 cm disposable cells, the absorbance of the residual DPPH molecules was measured at 517 nm in a T80 UV-Vis spectrophotometer (pg Instruments, UK) during a 30-minute incubation period at room temperature. Three separate determinations were used to get the mean values. The measure of antioxidant activity was mg GAE/100 g DW [6].

2.4.3. Determination of Total Phenolic Content (TPC)

Using gallic acid (GA) as a reference, Chirinos' Folin-Ciocalteu (FC) reagent was used to assess the total extractable phenolic contents of spice extracts.

2.5. GC-MS Analysis of Extracts

Agilent gas chromatography combined with mass spectrometry (GC-MS) was used to identify the extracts [7].

2.6. Evaluation of Biochemical and Hematological Parameters

The blood samples were (N = 150) collected from surveyed women for the analysis of complete blood picture (CBC), (N = 90) oxidative enzymes, and pesticide residues [8].

2.6.1. Hematological Parameters

A Mindray BC-3000 hematology analyzer was used to measure the hematological markers, which included hematocrit (Hct %), total red blood cells (RBC) count, hemoglobin (Hb) content, total white blood cells (WBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (Plt) count.

2.6.2. Determination of Oxidative Stress Markers

Sera were used for the estimation of Catalase (CAT) [9], malondialdehyde (MDA) [10] , Glutathione (GSH) [11] & [12] And Carbonyl Protein (CP) [13].

2.7. Statistical Analysis

The questionnaire results were statistically analyzed using descriptive methods such as arithmetic mean, standard deviation, standard error, percentages, and frequencies. Quantitative statistical methods such as Pearson's simple correlation coefficient, correlation analysis model, and ascending progressive multiple regression were employed to explain and interpret the results. The Statistical Package for Social Sciences (IBM SPSS), version 25, was used.

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The Statistical Analysis System (SAS, Cary, USA, version 9.3) software evaluated the blood picture, oxidative stress, and pesticide residue data. The results were displayed as mean ± SD. Using Tukey's Studentized Range (HSD) post-hoc Test ($P \leq 0.05$), significant means were compared.

3. RESULTS:

3.1. Rural Women's Spices Consumption Behavior

Data in **Table 2** demonstrates the frequency of daily consumption of spices, and it was found that most rural women (97.4%) use spices in daily cooking and 2.2% of them sometimes add spices to the food while almost none (0.4 %) do not use any spices.

3.2. Total Antioxidant Capacity (DPPH Method) of Consumed Spices

Extracts of spices have many total phenolic contents (TPC) (**Table 3**) (**Fig 2**). The highest one of TPC is peppermint (5.60±0.01, mg/100 g GAE) followed by lauri (5.50±0.1 mg/100 g GAE) & pepper (5.45±0.1 mg/100 g GAE), the lowest one was coriander (3.18±0. mg/100 g GAE). A statistical study revealed substantial differences ($P < 0.05$) among the examined spices. The values of total phenolic were strongly impacted by both spice type & extraction solvent ($P<0.001$)

Concerning the antioxidant scavenging activity using the DPPH, nutmeg had the highest mean antioxidant activity (86.24±1.2 µg/ml) followed by mentha (85.60±1.2 µg/ml) and cloves (85.18±1.2 µg/ml). Coriander had the lowest mean antioxidant activity (6.32±1.2 µg/ml) (**Table 3 & Fig 2**).

Table 2: Frequency of consumption of spices by rural women

No of Times for adding spices to food	No of Participants	%
Always	264	97.4
Sometimes	6	2.2
Not	1	0.4
Mean=2.93, SD=0.458, SE=0.02	**271**	**100**

Table 3: total phenolic content (TPC) and antioxidant scavenging activity (DPPH values) of extracts of spices used by rural women expressed as gallic acid equivalent.

SPICES	TPC Mean±SD (mg/g)	DPPH Mean±SD (µg/ml)
Cardamom	3.31^{c}±0.2	40.80^{d}±1.2
Coriander	3.18^{c}±0.3	6.32^{e}±1.2
Cinnamon	4.52^{b}±0.07	$79.60^{b,c}$±1.2
Cloves	5.20^{a}±0.1	$85.18^{a,b}$±1.2
Cumin	4.60^{b}±0.03	77.61^{c}±1.2
Lauri	5.50^{a}±0.1	$84.44^{a,b}$±1.2
Peppermint	5.60^{a}±0.01	$83.40^{a,b,c}$±1.2
Mentha	4.44^{b}±0.3	$85.60^{a,b}$±1.2
Nutmeg	5.35^{a}±0.1	86.24^{a}±1.2
Pepper	5.45^{a}±0.1	6.81^{e}±1.2

.*Tukey's Studentized Range (HSD) post-hoc Test ($P < 0.05$) was used to compare means. The means that have the same superscript letter do not differ substantially.

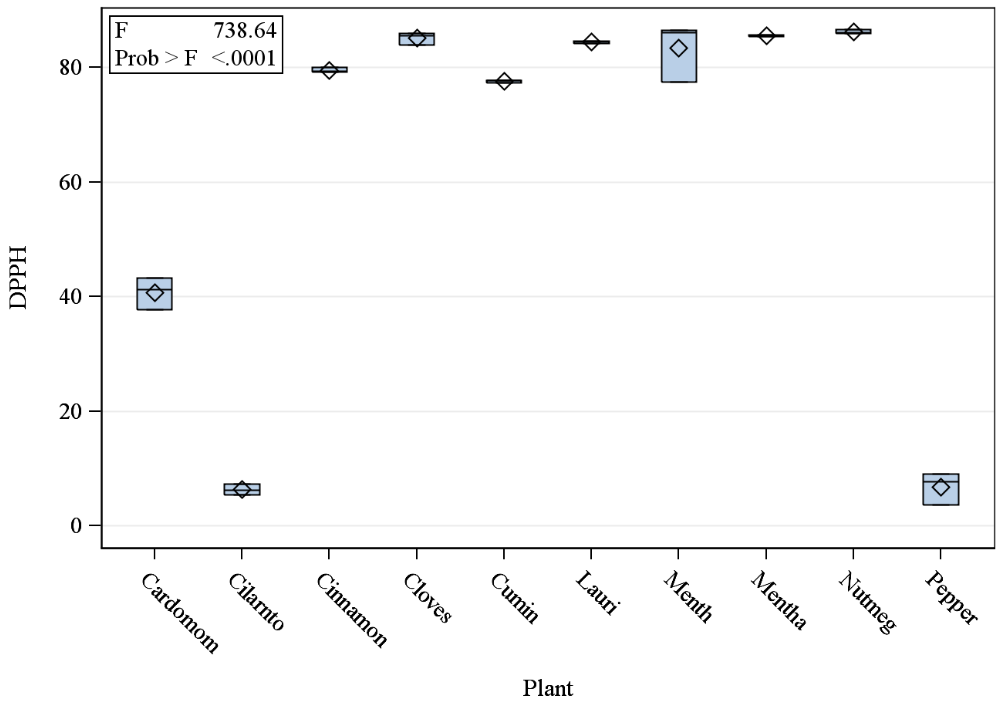

Figure 2: Mean antioxidant capacity using the DPPH technique of studied herbs

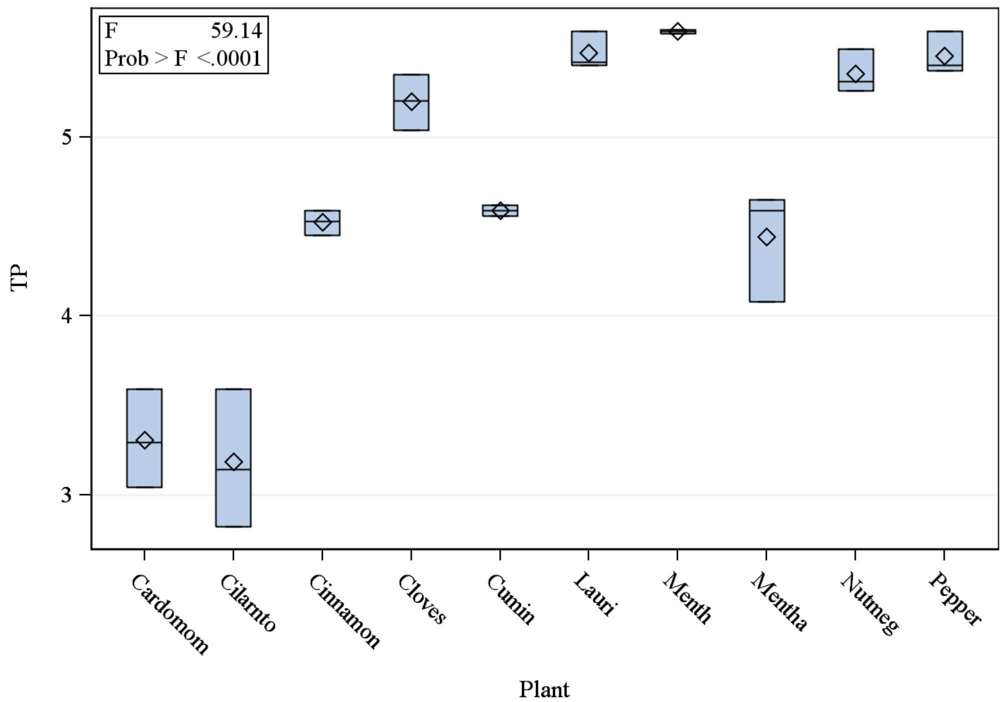

Figure 3: Mean of total phenolic content for studied herbs.

3.2.2. Chromatographic analysis of tested spices

Typically, flavonoids and phenolic acids were the main constituents in spices. **Table 4** demonstrates the chemical components of the methanolic extract of the ten studied spices. It was observed that the most abundant compounds were eugenol, cineole, β-caryophyllene, pinene, linalool, and limonene.

There was a variation between the components in spices where there were diterpene compounds such as phytol, and neophytadiene, monoterpenoids such as carvone, cineole, camphene, pinene, carene, eucalyptol, γ-Terpinene, myrcene, and sabinene, sesquiterpene such as germacrene, bisabolol, santamarine, humulene, caryophyllene and cubebene, and fat-soluble antioxidants such as vitamin E. Also, we found terpene compounds such as terpineol, ρ-mentha-1,3-diene-7-al, terpinyl acetate, linalool, cyclic monoterpene as limonene, humulene, phellandrene, phenylpropanoid compounds as safrole, eugenol, and estragole, long-chain fatty acids as myristicin, myristic acid, esters as cinnamyl acetate, cinnamaldehyde, and cumin aldehyde, and amine as piperidine and reynosin (**Table 4)**.

Table 4: Demonstrate Chromatographic analysis of spices Extracts Used by Rural Women

Spice	Rt	% of All	Components	Classification
1-Mentha	17.324	18.73	Pulegone	Monoterpene
	33.416	1.05	3-carene	Monoterpene
	30.253	1.61	Phytol	Diterpene
	17.435	5.48	Carvone	Terpenes
	20.264	0.76	α -terpinyl- acetate	Monoterpene
	20.486	4.00	Eugenol	Phenyl-propanoid
	27.755	2.83	**Neophytadiene**	Diterpene
2-Coriander	13.193	20.53	Linalool	Terpene alcohol
	11.158	5.70	Limonene	Cyclic monoterpenes
	15.542	0.28	myristic acid	Long-chain fatty acid
3-Cinnamon	15.914	1.37	cinnamyl acetate	Ester
	16.744	0.81	Cinnamaldehyde	Ester
	17.436	0.32	Carvone	Mono-terpenoid
	20.489	1.28	Eugenol	Phenyl-propanoid
	23.889	0.21	Germacrene	Sesquiterpene
	26.242	0.19	α –bisabolol	Sesquiterpene
	23.717	0.18	beta- bisabolene	Sesquiterpene
	30.518	0.36	vitamin e	Fat-soluble antioxidant
	22.143	0.87	caryophyllene,	bicyclic sesquiterpene
	30.681	0.93	γ –tocopherol	**(various methylated phenols) have vitamin E activity**
	36.694	0.17	thymol, tms derivative	Mono-terpenoid
	25.028	0.63	β-caryophyllene	Bicyclic sesquiterpene
4-Peppermint	17.435	5.48	Carvone	Mono-terpenoid
	11.238	2.25	Cineole	Monoterpene
	11.098	2.27	Limonene	Monoterpene
	11.242	0.16	1, 8-cineole	Mono-terpenoid
	17.324	18.73	Pulegone	Monoterpene
	18.618	1.05	cymen-7-ol	Alkylbenzene related to Monoterpene
	30.253	1.61	Phytol	Diterpene
	10.809	0.34	4-carene	Monoterpene
5-Cumin	25.027	0.10	Cuminaldehyde	Ester
	15.915	0.43	α –terpineol	Terpines
	15.543	1.39	terpinen-4-ol	Terpenes
	12.02	0.38	γ-terpinene	Terpenes
	37.120	6.21	licarin b	prenylated flavonol glycoside
	9.602	1.19	β-pinene	Monoterpene
	33.283	1.56	Camphene	Monoterpenes

	11.162	0.93	Limonene	**cyclic monoterpene**
	18.666	2.43	Safrole	Phenylpropanoid
	22.837	0.33	Humulene	**cyclic monoterpene**
	20.265	1.70	α -terpinyl- acetate	Monoterpene
	20.501	5.08	Eugenol	Pheny-lpropanoid
	16.080	0.25	β –phellandrene	**Cyclic monoterpene**
	22.314	1.08	p-mentha-1,3-diene-7-al	Terpenes
6-Lauri	17.433	0.21	d-carvone	Terpenoids
	12.911	0.18	Careen	Mono-terpene
	20.486	1.48	Eugenol	Phenyl-propanoid
	8.359	1.15	α-pinene,	Monoterpene
	31.33	2.21	**Santamarine**	Sesquiterpene
	11.227	18.89	Eucalyptol	Mono-terpenoid
	31.691	5.26	**Reynosin**	Amino acids
	13.218	0.50	Camphene	Monoterpene
	22.314	1.08	trans-p- mentha-2,8-dienol.	Terpenes
	11.144	0.14	Limonene	Monoterpene
	22.648	0.19	trans- isoeugenol	Phenyl-propanoid
	20.248	4.31	alpha-terpinyl acetate	Terpenes
	15.545	0.60	terpinene-4-ol	Terpenes
7-Cardomom	15.919	1.13	alpha,-terpinol	Terpenes
	15.454	0.19	terpinene-4-ol	isomer of terpineol
	20.271	0.57	α-terpinyl acetate	Terpenes
	13.819	0.05	gamma-terpinene	Monoterpene
	11.238	2.25	Cineole	Monoterpene
	11.206	0.31	Linalool	Mono-terpenoid
	15.535	0.06	l-alpha- terpinol	Terpenes
	20.715	0.81	Eugenol	Phenyl-propanoid
	17.446	0.24	Carvone	Monoterpene
	9.749	0.47	Myrcene	**Monoterpene**
	21.785	0.04	Isoeugenol	Phenyl-propanoid
	17.630	0.22	linalyl acetate	Mono-terpenoid
	34.733	0.21	Estragole	**Phenyl-propenoid**
8-Pepper	32.821	1.17	Piperine	Alkaloid
	13.181	0.81	Linalool	**terpene alcohol**
	22.847	1.43	Humulene	Sesquiterpenes
	22.178	11.23	β-caryophyllene	bicyclic sesquiterpene
	18.665	0.46	Safrole	Phenyl-propanoids
	15.540	0.27	terpinen-4-ol	isomer of terpineol
	11.100	3.79	Limonene	**cyclic** Mono-terpenoid
	21.055	1.06	Cubebene	Sesquiterpenes
	9.605	2.32	α-pinene	Monoterpene
	10.554	5.76	δ-3-carene	Monoterpene
	22.659	0.33	trans –eugenol	Phenylpropanoids

	10.388	0.54	β-pinene	Monoterpene
	9.947	0.43	α-phellandrene	cyclic Monoterpene
	30.497	0.43	Piperidine	Heterocyclic amine
	9.749	0.47	Myrcene	**Monoterpene**
	10.388	0.54	γ –terpinene	**Monoterpene**
	17.433	0.42	Carvone	Mono-terpenoid
	20.264	0.35	α-terpinyl acetate,	Terpenes
9-Nutmeg	20.486	4.00	Eugenol	Phenyl-propanoid
	9.948	0.26	Myrcene	Monoterpene
	18.985	0.27	**Sabinene**	**Monoterpene**
	15.535	0.08	terpinen-4-ol	Terpenes
	11.987	3.14	γ –terpinene	**Monoterpene**
	11.192	0.37	Linalool	Terpenes
	17.438	0.51	Carvone	Mono-terpenoid
	11.986	2.30	Myristicin	long-chain fatty acid
10-Cloves	20.501	18.24	Eugenol	Phenyl-propanoid
	17.426	0.26	Carvone	Mono-terpenoid
	17.630	1.76	linalyl acetate	Phytochemical
	24.548	1.11	Nerolidol	Sesquiterpene alcohol
			eugenyl acetate	Phenols
	22.834	0.47	α-humulene	Monocyclic sesquiterpene
	22.136	3.17	β-caryophyllene	Bicyclic sesquiterpene
	18.666	2.43	Safrole	Phenyl-propanoid

Biochemical Assays of Blood Samples of Rural Women

3.3. Hematological Parameters

In **Table 5**, the mean values of RBCs, MCH, MCV, MCHC, MPV, and Plt were not significantly different between rural women and reference values. However, Hb and Hct were significantly different among women from different villages. The RBC distribution width (RDW) of women from V2 values was slightly higher than a reference value (11.6-14.5%).

3.4. Oxidative Stress Parameters in the Serum of Rural Women

The results illustrated the mean activity of serum GSH and catalase enzymes and mean amounts of MDA and CP in blood samples of rural women. There were no significant differences among stress-related parameters, whereas MDA, CAT, and CP activity were at normal range except for GSH activity which was different among rural women.

Table 5: Hematological parameters RBC, Hct, HG, MCV, MCH, MCHC, RBC RDW and, WBC.

Variable	V#1 (n=50)	V2 (n=50)	V3 (n=50)	Total (n=150)	Reference value*
Hb(g/dL)	11.49±0.93	11.62±0.93	11.66±0.85	11.59±0.9	12-15
RBCs(10^6 / µL)	4.23±0.52	5.2±6.19	4.53±0.8	4.67±3.61	3.8-4.8
Hct(%)	34.53±3	34.87±3.37	35.12±2.84	34.84±3.07	36-46
MCV(fL)	79.39±10.06	79.06±8.43	79.94±6.01	79.46±8.29	76-96
MCH(pg)	27.97±8.23	27.33±2.51	26.44±2.32	27.24±5.15	27-32
MCHC(g/dL)	33.51±1.83	32.68±2.09	33.03±0.92	33.07±1.71	30-35
RDW(%)	13.59±0.93	14.78±9.64	13.69±0.89	14.02±5.6	11.6-14.5
WBCs(10^3/ µL)	9.79±3.28	9.94±3.16	9.13±3.33	9.62±3.25	/µL (4.000-10.000)
Lymphocytes(/µL)	23.84±8.13	23.34±7.19	27.02±9.95	24.73±8.6	/ µL(20-45%)
Monocytes (/µL)	3.02±1.31	3.4±1.65	3.5±1.95	3.31±1.66	/ µL (2-10%)
Plt(x10^6 /µl)	215.6±48.88	216±57.17	232.5±71.49	221.4±60.01	150-450/cm
MPV(fl)	9.91±1	10.55±1.13	12.28±13.3	10.91±7.74	8-15%

Data are expressed as numbers of individuals or means ±SD. *Reference value [14], #V: village.

3.5. Principal Component Analysis of Studied Hematological and Biochemical Parameters

Conducting a conclusive relationship analysis among studied hematological and biochemical was efficient in plotting a multidimensional preference analysis plot of the principal components analysis (PCA) procedure. This visual illustration tool showed the similarity classification of the dependent and independent variables with about 90% of the variance for studied variables of women from the three studied locations. Meaning the pattern of studied components were related negatively such as the MPV and WBC, neutrophils, MCH, and MCV. Moreover, the GSH enzyme and platelet number results would be used as some differentiated parameters among the responses of participants.

3.6. Pesticides Residue in the Serum of Rural Women

The presence of pesticide residues in blood samples of volunteering women was analyzed using the GC-MS technique (**Table 6**). This method was suitable for the determination of pesticides where the CV% as inter-assay and intra-assay values ranged from 4.33 to 7.89 and 5.37 to 11.12%, respectively. Also, the recovery percentages of detected compounds ranged from 88.28 to 96.55%, which indicated the specificity of the employed method.

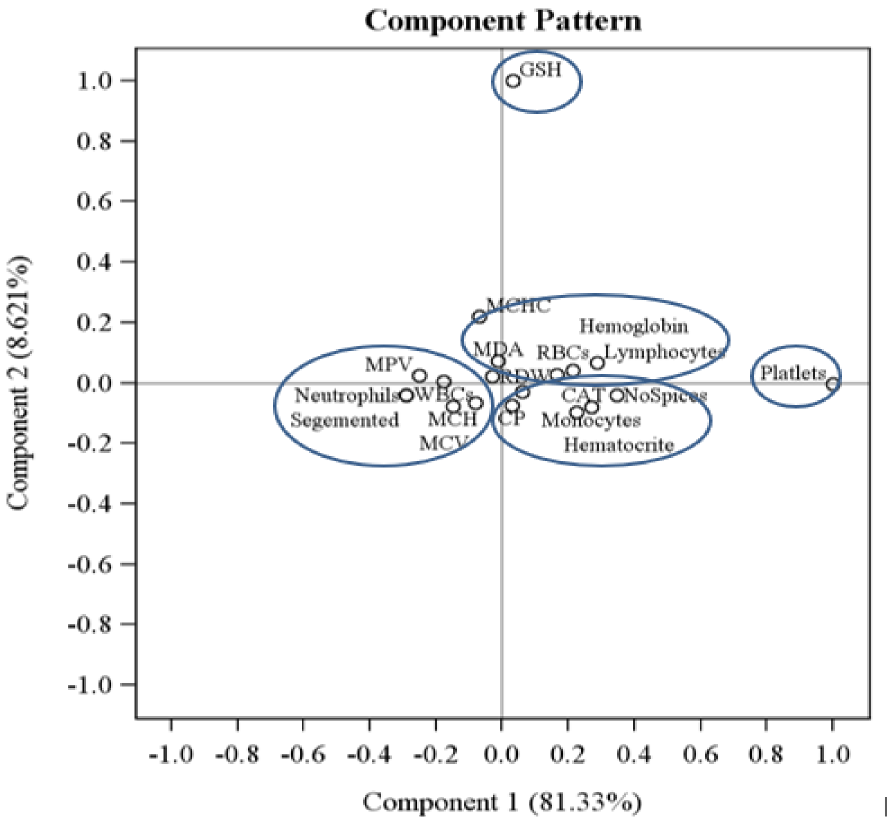

Figure 4: Principal component analysis (PCA) relationships among studied hematological and biochemical parameters of rural women in Abo Homes Villages

Table 6: Mean (ng/ml) ± standard deviation (SD) of detected pesticides in serum samples of studied women (150), experiment quality parameters including recovery percentages, LOQ (ng/g), and coefficients of variability (CV %)

Pesticide	Use[1]	Mean ± SD	Recovery[2] (%) ± SD	LOQ[3]	CV[4] (%)	
					Inter-Assay	Intra-Assay
Chlorfenapyr	A,I	0.057±0.0033	89.45±3.71	10	5.16	7.48
Ethofumesate	H	0.031±0.0012	94.27±4.35	50	7.89	9.64
Pyroquilon	F	0.002±0.0004	93.54±4.64	15	6.11	10.24
Azinphos – ethyl	I	0.037±0.0061	96.55±5.34	20	5.52	9.01
Azinphos – methyl	I	0.008±0.0016	96.28±5.16	20	5.08	8.43
Drazoxolon	F	0.002±0.0008	91.89±3.78	50	4.82	9.07
Malathion	I	0.078±0.0039	95.47±4.08	10	6.19	11.12
Ethoprophos	I, N	0.005±0.0002	92.33±4.27	10	5.82	8.04
Formothion	A, I	0.003±0.0015	88.28±4.97	10	5.67	9.13
Methidathion	I	0.016±0.0028	94.18±3.51	20	7.14	10.97
Bis (2-ethyl hexyl) phthalate	P	D	-	-	-	-
Desmedipham	H	0.027±0.0091	92.11±5.09	25	4.33	5.37

[1]A: acaricide, F: fungicide, H: herbicide, I: insecticide, N: nematicide, and P: plasticizer, [2]Recovery experiment was conducted with 50 ng/g concentration of each pesticide, LOQ: limit of quantification, Coefficients of Variability (CV) expressed as inter– and intra-assay precision.

The analysis revealed the detection of different types of pesticides: acaricides such as Chlorfenapyr and Formothion, fungicides (Pyroquilon and Drazoxolon), herbicides (Ethofumesate and Desmedipham), insecticides (Chlorfenapyr, Formothion, Azinphos–ethyl, Azinphos–methyl, Malathion, Methidathion, Ethoprophos, and Formothion), nematicides (Ethoprophos), and the plasticizer Bis-(2-ethyl hexyl)-phthalate (**Table 6**). Residue levels pesticides were detected in only 15 rural women with levels ranging from 0.002 to 0.078 ng/ml. The malathion insecticide was the greatest detected compound followed by chlorfenapyr (0.057 ng/ml), while the lowest concentration was for pyroquilon and drazoxolon pesticides with 0.002±0.0004 ng/ml.

4. Discussions

4.1. Rural women's spices consumption

Table 2 shows the frequency of daily spice consumption at home. It was discovered that 97.4% of rural women use spices in their cooking daily, which is a good practice because herbs and spices have been used for centuries as flavoring agents, food preservatives, and medicines [2].

Phenolic acids and flavonoids, primarily flavones and flavonols, make up the majority of the polyphenolic class present in herbs and spices [15].

Strong anti-inflammatory, anti-microbial, and antioxidant qualities have also been reported for herbs and spices [16]. Furthermore, many herbs and spices have flavoring qualities that tend to lessen the use of salt as a flavoring agent (i.e., reduced sodium consumption), which has further advantages for cardiovascular health [2]. Only one person did not use any spices in their cooking, and only a small percentage of them (2.2%) did not use them at all.

4.2. Antioxidant Activity Measured by the DPPH Method

The study found that the highest mean antioxidant activity was found in nutmeg, followed by cloves and mentha. The lowest mean antioxidant activity was found in coriander (Fig 2). (Table 3).

The properties of Mentha pulegium L. include antibacterial [17], antioxidant [18], treatment of infestations [18], metabolic [19], anti-steel corrosion [20], anti-hepatic [21], and insect repellent [18]. Black pepper and cloves have been used as cancer preventives [22].

4.3. Chromatographic measurement of chemical compounds in herbs

4.3.1. Total Polyphenols Content of chemical compounds in herbs

Plants such as polyphenols, which are secondary metabolites, protect from damaging elements. Plant extracts high in polyphenols, as per Jówko [23], have a greater antioxidant impact than vitamin C, tocopherol, or carotene. Biologically active compounds can be found in large quantities in herbs and spices [24]. They may be separated into flavonols, flavononols, flavones, flavanols, flavanones, anthocyanidins, and isoflavonoids based on the variations in their chemical structures [1, 16].

The majority of research examined the antioxidant potential of a small number of culinary spices or fresh [25, 26] or dried [1, 23, 27–29] herbs. This study examined the antioxidant activity of spices with their concentration of physiologically active ingredients (polyphenols). Furthermore, a varied concentration of total polyphenols was observed in spice extracts (Table 4). The biggest quantity was discovered in Menth, followed by Lauri and Pepper, although Cilantro had the lowest amount (Fig 3). Assefa et al. produced results that were entirely different [30].

A vast class of bioactive substances generated from plants that may have anti-disease properties are called phytochemicals. Flavonoids and other phenolic compounds, carotenoids, plant sterols, glucosinolates, and other sulfur-containing chemicals are the members of this category. Over 6000 flavonoids are now recognized [31].

All of the herbs and spices utilized in this research were bought at the neighborhood market, air-dried, and crushed into a fine powder before being used to make an extract. As in previous research, the extraction efficiency (type of solvent, temperature, and extraction time) may have an impact on the overall concentration of polyphenols in the products under analysis [32, 33].

We found a significant positive association between our test plants' total phenolic contents and the various antioxidant tests, indicating that variations in our test plants' antioxidant capabilities may be related to the phenolic contents of the spices. total phenolic content of plants & their antioxidant capabilities are strongly correlated by many studies in the past [34, 35], confirming the role of phenolic compounds in antioxidant activities.

4.3.2. Chromatographic analysis of spices Extracts Used by Rural Women

Flavonoids and phenolic acids are the most common phenolic compounds in spices with antioxidant activity [4]. The antioxidant activity of phenolic compounds is mostly owing to their redox characteristics, which include adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, and degrading peroxides. Flavonoids have stronger antioxidant activity against peroxyl radicals than phenolic acids due to their numerous hydroxyl groups [4].

Our research revealed that spices have distinct components from one another. Furthermore, diterpene compounds (phytol and neophytadiene), monoterpenoids (carvone and cineole), sesquiterpenes (germacrene and bisabolol), fat-soluble antioxidants (i.e., vitamin E), terpenes (terpin, terpinyl acetate, and linalool), cyclic monoterpenes (limonene and humulene), phenylpropanoids (safrole and eugenol), long-chain fatty acids (myristicin and myristic acid), estersOur findings coincide with Srinivasan[36], who noted that spices include natural substances such as vitamins, phytonutrients, mineral elements, alkaloids, flavonoids, terpenoids, and sesquiterpenes.

Cumin contains flavonoids and phenolics, which boost insulin production and have anti-obesity characteristics that lower glycemic levels and plasma glucose [37]. Peppermint leaves include fatty acids such as linoleic, linolenic, and palmitic acids. Several volatile chemicals have been found, including menthol, menthone, and isomenthone, as well as β-carotene, chlorophyll, α- and γ-tocopherols, and ascorbic acid [38].

Cardamom is effective in treating snake and scorpion poison. It also treats food sickness. It is also used in Chinese traditional medicine to treat stomach pain, constipation, dysentery in children, and other digestive issues. Cardamom pods are also beneficial when fried and combined with mastic and milk to treat bladder issues [39]. Coriander seeds are high in essential oils, triglycerides, carbohydrates, proteins, and vitamin C, and they are used as an enhancing agent in alcohols, teas, meat products, and pickles [40]. Coriander adds taste to foods while also delaying or preventing rotting [41]. Mentha pulegium L. is a mentha species also known as pennyroyal. The essential oil of pennyroyal is used in aromatherapy and contains pulegone, a very poisonous volatile organic molecule that affects liver and uterine function. Pennyroyal was a popular culinary herb among the Greeks and Romans [18].

Nutmeg essential oil is frequently utilized in the perfume and medicinal sectors. The volatile fraction comprises sabinene (21.38%), 4-terpineol (13.92%), and myristicin (13.57%), along with safrole, elimicin, terpineol, α-pinene d-camphene, limonene, linalool, and isoeugenol [42]. Clove contains significant amounts of eugenol, beta-caryophyllene, and sesquiterpenes such as β-caryophyllene [43]. Also, cloves and black pepper have been used to treat cancer [22]. It was observed that cloves contain more flavonoids than cumin [24], which is consistent with our findings. Pepper contains numerous health-related compounds, including monoterpene hydrocarbons (α-pinene, myrcene, limonene, αterpinene, p-cymene, β-pinene, and α-phellandrene), oxygenated monoterpenoids (1,8-cineole, linalool, and terpinen-4-ol), sesquiterpene hydrocarbons (β-sesquiphellandrene), and oxygenated sesquiterpenoids. The results for caryophyllene oxide and the phenylpropanoids safrole and eugenol were identical to those of Prased [44].

Flavonoids, tannins, eugenol, citric acid, vitamins, hormones, alkaloids, triterpenoids, and components of essential oils, such as linalool, terpinyl acetate, eugenol, pinene, phellandrene, and terpineol, are all included in laures [45]. The presence of cinnamaldehyde [45] and its derivatives, such as cinnamaldehyde, cinnamic acid, cinnamate, eugenol, and water-soluble polyphenols (catechin, epicatechin, procyanidin, quercetin, kaempferol, and polyphenolic polymers), is thought to be responsible for the spicy flavor and aroma of cinnamon. Cinnamtannin oligomers and proanthocyanidins are the two main types of flavonoids. It is believed that the bioactive element of glucose metabolism consists of doubly linked phenol type A polymers [46]. The results of this investigation are consistent with those reported in [45].

4.4. Alleviative role of spices in the rural environment

Black pepper, peppermint, cardamom, bay leaves, cinnamon, clove, nutmeg, coriander, mentha, and cumin are the most popular spices among rural women due to their distribution, significance, and active component content. Through the inhibition or quenching of free radicals and reactive oxygen species, piperine has been demonstrated to protect against oxidative damage in vitro. High levels of antioxidant activity were shown by both the oil and the oleoresins [47]. According to an animal model investigation, piperine decreased histamine release and eosinophil infiltration while also suppressing allergic airway inflammation and hyperresponsiveness [48]. In addition, piperine has been demonstrated to have strong inhibitory effects on airway inflammation in a mouse asthma model [48].

Peppermint oil and several of its components have antibacterial and antioxidant effects [49]. Mint's anti-pruritic or anti-itching characteristics, when combined with a little camphor, aid to relieve the irritation and rash produced by bug bites and stings. Mint's powerful diuretic properties aid in the elimination of toxins from the body. It inhibits the growth of dangerous bacteria and fungi in the body and can help treat asthma and other allergy disorders to some extent [50]. Peppermint contains substantial antibacterial and antiviral activity, as well as powerful antioxidant and anticancer properties [51].

Cardamom can help with digestion, retention, vomiting, digestive constipation, unusually high blood pressure, asthma, diarrhea, colic, dyspepsia, epilepsy, and carminative issues. It is also used to treat a variety of other conditions, including cardiovascular, stomach-related, aspiratory, kidney-related, lung-related, and liver-related disorders [52]. It is well-known for its carminative properties, diuretic effect, cough relief, cold treatment, and heart stimulation. It has traditionally been used to treat renal and bladder diseases, as well as gastrointestinal concerns [39]. Extracts of bay leaves demonstrated strong antioxidant activity [53]. Batool et al. [53] found that bay leaves contain antiviral and anticonvulsant properties. It is used to treat respiratory problems and infections, gastrointestinal discomfort and irregularity, diarrhea, and amenorrhea as an emetic, stimulant, and diuretic [54, 55].

Cinnamon intake has been linked to a considerable drop in fasting plasma glucose levels, which helps regulate glycemic illnesses [56-58] and treat Alzheimer's disease [59, 60]. It is used to treat spleen, breast, uterine, liver, and stomach indurations, as well as malignancies (particularly those of the abdomen, liver, and sinews) [2, 61]. Furthermore, cloves have been shown to have analgesic, antibacterial, antidotal, antiseptic, and stimulant characteristics, as well as anesthetic, anthelminthic, antiparasitic, antioxidant, antiperspirant, carminative, deodorant, digestive problems, and stomachic effects [39] [40]. Furthermore, its oil is used to treat a variety of ailments, including joints, muscles, sinewy tissue, and, in particular, rheumatoid arthritis [62]. Western studies have supported the use of cloves and clove oil for tooth pain. It exerts its effects through the presence of several phytochemicals [43].

Zheng et al. [63] discovered that in an in vivo animal investigation on mice, myristicin, the primary component of nutmeg essential oil, might boost the effectiveness of a possible cancer chemoprevention agent, and this discovery was reproduced in another study [64]. Coriander contains antioxidants that assist in extending the shelf life of goods, hence it is commonly utilized in the food business. Coriander adds taste to foods while also delaying or preventing rotting [41]. It has been reported to have a variety of potential therapeutic qualities, including antispasmodic, carminative, and stomachic characteristics [65]. Furthermore, it has been promoted as a diabetic treatment [66]. M. pulegium has also been described with antibacterial [17], antioxidant [18], metabolic [19], anti-hepatic [21], and repellent properties the effects of insects [18]

Cumin is frequently employed in the culinary and medicinal sectors because of its healing and sweet-scented qualities. Because of the presence of aromatic and antioxidant components, it has flavoring, purifying, and irritating resistance qualities. The essential amino acids found in cumin [67] have excellent protein qualities and exhibit antibacterial, antimicrobial, and antifungal characteristics [68].

4.5. Hematological parameters in rural women

RBCs, MCH, MCHC, WBCs, neutrophils, lymphocytes, monocytes, and Plt levels in our study did not differ statistically from reference values in rural women. Nonetheless, blood samples taken from rural women had MCV, Hb, and Hct levels that were marginally below the standard norm. Exposure to pesticides was linked to aberrant hemoglobin and MCV levels [69].

Our findings are consistent with those of other research [70, 71] that show lower hemoglobin levels in the sprayer population and Patil et al. [71] that show lower mean corpuscular volume (MCV) and hemoglobin levels. It has been suggested that binding of iron and organophosphate insecticides, as well as disruption of heme and hemoglobin production, might lead to reduced levels of hemoglobin, hematocrit, and MCV [72, 73]. After being exposed to pesticides, red blood cells were destroyed, which led to the development of anemia. The detrimental effects on the bone marrow also played a role [74]. Prior research has revealed aberrant blood indices and evident impairment of liver and kidney functions in agricultural workers and pesticide sprayers [14, 75].

Hematotoxicity is a biomarker that can show genotoxicity, oxidative stress, and occupational exposure [70]. Lower levels of RBCs and WBCs, especially lymphocytes and eosinophils, are linked to high pesticide usage seasons [76]. Additionally, agricultural laborers and pesticide sprayers frequently have abnormal blood indices as well as impairments to their liver and kidney functions [75–77].

Farmers had higher leukocyte counts than those who were not exposed, according to Egyptian research [78]. However, the observed inverse relationship with lymphocyte counts was consistent with Indian research [76] that found that compared to unexposed patients, a group of sprayers working on mango farms had lower lymphocyte counts. However, they also discovered that pesticide exposure was linked to changed numbers of erythrocytes, neutrophils, monocytes, hemoglobin, MCV, and MCHC. Furthermore, our findings about the present usage of pesticides partially corroborated Chinese research that revealed reductions in monocytes, hemoglobin, and platelets following pesticide exposure, indicating that acute exposures to pesticides may have hematotoxic effects [79]. Nonetheless, prolonged exposure was linked to a rise in the number of white blood cells in the Chinese study.

4.6. Biochemical parameters in rural women

Pesticides have the potential to cause oxidative stress by many methods, including increased production of highly reactive molecules (ROS) or modifications to the body's antioxidant system, which includes catalase (CAT) and glutathione reductase (GSH). The rise in protein carbonyl levels, a good indicator of oxidation of proteins and lipid peroxidation, reflects this [80]. The current study demonstrated that the levels of oxidative stress indicators were generally changed in rural women exposed to pesticides. Prior research has indicated that pesticide applicators experience oxidative stress as a result of exposure to many pesticide categories, including fungicides, carbamates, and organophosphates [81].

accumulation of oxygen free radicals in erythrocytes and other cells, which can cause lipid peroxidation of biological membranes and oxidative binding of important intracellular molecules containing thiol groups, such as GSH, which can ultimately cause cellular death and maybe most significant in the cytotoxicity of pesticides [82]. These results, in line with the majority of earlier research, suggest that pesticides alter the redox state and thus have negative health impacts on people. The idea that extended exposure to OPs generates reactive oxygen species, which simply consume and exhaust the body's supply of antioxidant agents, explains these results [83].

When oxidative stress parameters were measured in rural women's serum, stress-related metrics did not significantly vary from one another. Except for GSH activity, which varied across rural women, MDA, CAT, and CP activity were all within normal ranges. Our findings contradict those of prior research [84] that reported a considerable rise in malondialdehyde (MDA) levels. Rural women had differing GSH levels, which is consistent with the study's finding that reactive chemicals like ROS produced by pesticide exposure may be more frequently used for detoxification. Nevertheless, other research revealed that exposed patients' GSH concentrations remained unchanged [85].

4.7. Pesticides residue in serum and hematological parameters

The number of pesticides used in Egypt has increased overall, with fungicides and herbicides accounting for the majority of this rise [86]. By causing oxidative stress, pesticides can also disrupt the metabolism of proteins, fats, and carbohydrates [87]. Out of the 90 participants in our study, 15 (16.66%) were rural women between the ages of 18 and 55 who had pesticide residues in their blood. These results were consistent with those of Yawar et al. [88], who found that 20% of the 35 volunteers, all of whom were female aged between 22 to 51, had chlorpyrifos residues in their bodies. The blood samples from rural women in the current investigation included residues of chlorfenapyr and other pesticide categories, which might indicate exposure to the pesticide during spraying or ingestion of food that contained high concentrations of the pesticide [89].

Additionally, 48.3% of participants had inadequate knowledge of appropriate procedures for buying and transporting, preparing, applying, and storing pesticides, disposing of empty containers, maintaining appropriate hygiene, and avoiding the threat of pesticide residues. Rural women in Abo Homms Villages were in charge of storing newly purchased and partially used pesticides. Kumari and Reddy [90] reported similar outcomes. Furthermore, when it came to the storage of pesticides by package reading, our findings concurred with those of Lekei [91]. Recena et al. [92] also discovered that 54.4% of households kept empty containers in their houses. because they are not covered by protective gear such as long-sleeved overalls, safety goggles, rubber boots, masks, or gloves. Therefore, the blood of rural women contains pesticide residues, which is quite concerning and suggests that they have been exposed to the environment.

During their lives, women are usually exposed to a variety of pesticides, and several pesticides are applied concurrently or during the same growth season. Because of this, we are unable to completely rule out the potential that any of the relationships were caused by pesticide-related interactions. Confounding due to numerous pesticides being exposed to the group. Furthermore, data regarding dietary status and the history of immunological disorders, viral diseases, and allergic reactions that affect hematological parameters were not available for the study population. Even with its restrictions, Egypt is one of the world's biggest pesticide consumers, and many of the pesticides used there have already been outlawed elsewhere. This study is the first to examine the relationship between occupational exposure to pesticides and hematological changes in Egypt. The research population is a good representation of the target group, which is the rural Abo Hommus agricultural community. A comprehensive questionnaire and the measurement of many pesticides in serum were also used to evaluate recent and past exposure to current use pesticides.

Ethical approval

This study received ethical approval **(DUFA-2024-14), from Damanhour University, Faculty of Agriculture.**

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The authors have no conflict of interest

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