



# Investigation of the Effect of *Origanum Majorana* on Polycystic Ovary Syndrome Patients

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**Abstract:** *The polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age. Many studies indicated the health-supporting effects of phenolic compounds in reducing the symptoms of PCOS. The richness of Origanum Majorana in phenolic compounds may enable it to have a role in the treatment of PCOS when it is consumed as a functional drink. This study aimed to investigate the effect of Origanum Majorana on the number and size of cysts in patients with PCOS. Origanum Majorana tea (2 g of Origanum Majorana in 200 ml water) was consumed daily by ten PCOS women for two months, with monitoring of the size and number of cysts. The consumption of Origanum Majorana tea decreased the number of cysts, since there were no more patients with high cyst numbers (more than 15 cysts). Additionally, the percentage of patients with 11-15 cysts decreased to 20%, while an increase in the number of patients with fewer than 11 cysts was recorded (p-value < 0.05). On the other hand, giving Origanum majorana tea to women with PCOS reduced cyst size; the percentage of patients with very small cysts increased to 50%, with no patients having cysts between 8 and 15 mm. Consumption of Origanum majorana tea reduced the number and size of the PCOS lesions, indicating a therapeutic role for O. majorana in PCOS when consumed as a herbal tea. It could be said that the phenolic compounds present in Origanum Majorana tea improved the condition regardless of age. Diet and BMI can affect patients' outcomes after consuming Origanum majorana tea.*

**Keywords:** *Origanum Majorana, Phenolic compounds, Polycystic Ovary Syndrome.*

## Nomenclature:

PCOS: Polycystic ovary syndrome

FDA: Food and Drug Administration of America

BMI: Body Mass Index

FDA: Food and Drug Administration of America

GAE: Gallic Acid Equivalents

## I. INTRODUCTION

The polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders among women of reproductive age, where it is estimated to affect about 3-15% of women around the world. The primary cause of this disorder is an ovarian defect [1], along with other factors reflecting interactions among genetic, metabolic, embryonic, and environmental factors. However, the relative importance

of these factors varies among women with PCOS. One of these factors is a disorder of reproductive gland secretion resulting in hormonal disorders, Hyperandrogenism, Insulin resistance, and dysfunction in the ovary [2].

Rotterdam standards are widely used to diagnose PCOS [3], where the syndrome is diagnosed by the presence of two of the following three criteria: Hyperandrogenism - that is confirmed by clinical and/or biochemical tests -, defect in ovulation with the presence of 12 cysts or more on one ovary with dimensions of 2-9 mm, or the presence of one cyst larger than 10 mm, all determined by ultrasound [2].

According to the Rotterdam criteria, PCOS is classified into four types based on the pattern of hyperandrogenism. First pattern, the ideal pattern that combines the three criteria: Hyperandrogenism, polycystic ovary, and scarcity or absence of ovulation, the prevalence rate of this pattern is 66%. The second pattern has an 11% prevalence and combines hyperandrogenism with a natural appearance of the ovary and a scarcity or absence of ovulation. The third pattern (13% prevalence rate) combines hyperandrogenism and polycystic ovaries with a regular menstrual period. The fourth pattern shows interruption or absence of ovulation, polycystic ovaries, and normal androgen levels; the rate of prevalence of this pattern is 9% [4]. PCOS is a syndrome, not a specific disease, that manifests in various clinical forms. Women can be fully asymptomatic or may experience any of the following symptoms. The first symptoms are typically a result of hyperandrogenism and are clinically reflected by Hirsutism, Acne, and baldness. Laboratory findings are also indicative of high circulating androgens. In some rare cases, women may suffer from skin roughness or an increase in the size of muscles or any manifestations of other potency. Second, ovulation and menstrual disorders are clinical symptoms of PCOS and are reflected by scant menstrual periods or menopause, thus the scarcity of ovulation. Usually, these disorders begin in adulthood and are associated with an increased risk of endometrial cancer, cancer of the uterus, as well as infertility [1]. In addition, mood changes can be clinical symptoms for PCOS, as studies show that PCOS women are more likely to have fluctuations of mood such as depression, nervousness, bipolar disorder, bulimia, anxiety, and others [5].

PCOS can lead to many complications, such as infertility and abortion, difficulty in pregnancy, as well as difficulty in maintaining pregnancy, with a 30-50% rate of abortion in the first trimester of pregnancy [6]. The risk of cardiovascular diseases and arterial calcification increases in PCOS women [7]. Insulin resistance and diabetes type 2 diabetes are also complications of PCOS, especially in women who have a higher prevalence of diabetes. Some studies indicate the

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ratio of diabetes in women with PCOS is 15% versus 2-3% in healthy women [6].

The Food and Drug Administration of America (FDA) defined the term "natural ingredients" as the components that are derived directly from plant or animal products, where herbs are good sources for natural components [8]. According to the Codex Alimentarius Commission, the herbs come from leaves and flowering parts of plants, while spices come from the rest of the plant, such as seeds and roots. Spices and herbs are used in traditional and popular medicine because of their therapeutic properties; a World Health Organisation questionnaire indicated that 70-80% of the world's population relies on herbal sources for health care [9].

Herbs and spices are rich sources of phenolic compounds, which have many effects on food, plants, and health [10]. Many studies indicated the health-supporting effects of phenolic compounds [11] as natural antioxidants [12], anti-inflammatory effects [13] by several mechanisms [14], anti-bacterial effects [15] which enable them to be used even to preserve food [16], anti-diabetic effects [17], and anticoagulants [18] and even from many sources such as Rosa Damascene [19] and Ocimum Basilicum [20]. Phenolic compounds are considered one of the functional ingredients that support health [21]. Functional ingredients can be found naturally in a certain food [22] or added to it [23]. Functional foods and drinks are similar to conventional foods and drinks [24]; however, they demonstrate beneficial effects beyond their nutritional value [25]. The richness of Origanum Majorana in phenolic compounds may enable it to be an internal functional drink, especially since it has long been used in popular and traditional medicine. Origanum Majorana is related to the Lamiaceae and the genus Origanum and known as sweet Origanum. The origin of Origanum majorana is the Mediterranean region; it is also cultivated in many countries across Asia, North Africa, and Europe. The length of Origanum Majorana ranged between 30-60 cm; it has oval, dark green leaves and a cluster of white or red flowers in [26].

Phenolic compounds, including those from Origanum majorana, are a valuable source for the discovery of new drugs [27]. In recent years, it has been found that phenolic compounds can reduce the symptoms of PCOS, such as oxidative stress, insulin resistance, Hyperandrogenism, ovarian malformations, obesity, interruption of ovulation or scarcity of menstruation, abortion, infertility, and others by different mechanisms [28]. Therefore, this study aimed to investigate the effect of Origanum majorana on women diagnosed with PCOS by monitoring cyst counts and dimensions after drinking Origanum majorana tea once daily for two months.

## II. METHODS AND MATERIALS

### A. Materials

Folin-Denis (Sigma- Aldrich, Switzerland), Sodium carbonate (BDH, England), Gallic acid (Biotech Ltd), distilled water. Spectrophotometer (Jasco V-530 UV).

Dried Origanum majorana was purchased from the local market in Latakia, ground in a Mill, divided into portions, and given to patients with PCOS.

### B. Study Design

The study was carried out on ten PCOS patients who live in Baniyas city who visit the gynaecology clinic; their data were collected [age, family history of PCOS, body mass index (BMI), style diet, psychological status (stress), see table 1]. The age of the patient women ranged between 15 and 30 years old, 50% of them were 15-20 years old. 60% of the patients had normal BMI, while 40% were overweight with no case of obesity or underweight. 50% of the patient women had a family history of PCOS. 70% of patients followed an unhealthy, high-fat, high-sugar diet. Four patients (40%) answered that they have stress and mood disorders.

**Table I: Patients' Properties, Age, BMI, Family History, High-Fat Sugar Diet, Stress**

Age	Number	BMI	Number	Family History	Number
15-20	5	18.5 >	0	Yes	5
21-25	4	18.5-24.9	6	No	5
26-30	1	25-29.9	4		
30 <	0	30 <	0		
High-fat-sugar diet	Number	Stress	Number		
Yes	7	Yes	4		
No	3	No	6		

The number and dimensions of cysts were registered at the beginning of the study. The patient has been given Origanum majorana tea, prepared as a popular herbal tea. Briefly, 2 g of the dried plant was soaked in 200 ml of freshly boiled water, left at room temperature for 5 minutes, and then consumed. The women have taken Origanum Majorana tea daily for two months and were not given any drugs that treat PCOS during the study, with emphasis not to take it during the menstrual bleeding. The number and dimensions of cysts were reassessed at the end of the study, i.e., after two months of daily consumption of Origanum majorana tea. No negative control (patient women without the consumption of Origanum Majorana tea) or positive control (patient women with drugs that treat PCOS) were monitored, since the study was carried out on the same PCOS women before and after the consumption of Origanum Majorana tea.

### C. Determination of Phenolic Content

The phenolic content of Origanum Majorana extract was determined in the pharmacy faculty laboratories, Latakia University, using Folin-Ciocalteu reagent (Folin-Denis). After preparing the aqueous extract in a similar way to the consumption as herbal tea (as mentioned above, 2 g of dry Origanum Majorana was soaked in 200 ml of freshly boiling water and left for five minutes at room temperature), the extract was cooled in ice, filtered, and diluted twice before analysis. 0.1 ml of the previous extract was mixed with 2 ml of 2% sodium carbonate and incubated at room temperature for 5 min. After that, 0.1 ml of Folin-Denis' reagent (prepared by dilution of Folin-Denis' reagent with distilled water, v/v, 1:1) was added to the mixture and left at room temperature for 30 min before the absorbance reading. A blank was prepared in the same manner as the samples; however, 0.1 ml of distilled water was added instead of 0.1 ml of the extract, while





keeping the other steps. A serial dilution of Gallic acid was prepared and dissolved in distilled water at concentrations of 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 g/L [29]. The absorbance was measured at 750 nm for Gallic acid solutions, Origanum Majorana extract, and the blank. The linear equation was  $y=1.9246x+0.0249$  and  $R^2=0.9981$ . The total phenolic content was expressed as mg of Gallic acid equivalents (GAE) per 200 ml of herbal tea and was expressed as mg GAE in 1 g of dry Origanum Majorana [30]. The experiment was carried out in duplicate.

**D. Statistical Analysis**

All results were presented as means ± standard deviations. A Student's t-test was used to assess differences in women's states (cyst number and size) before and after consumption of Origanum majorana tea; a p-value < 0.05 was considered statistically significant. One-way Analysis of Variance (ANOVA) test was applied to investigate if women's properties (age, family history, BMI, and others) can affect the size and dimension of cysts if they consume Origanum Majorana tea or before that, where the presence of a statistically significant difference p-value will be < 0.05. Statistical analysis was carried out using IBM SPSS.

**III. RESULTS**

**A. Phenolic Content**

The content of phenolic compounds in 1 g of dry Origanum Majorana was  $8.85 \pm 0.12$  mg (GAE); this means the phenolic content in a cup of 200 ml was 17.7 mg (GAE).

**B. Effect of Origanum Majorana on the Number and Dimension of Cysts in PCOS Women**

The patients were classified into 4 groups based on the number of cysts (1-5, 6-10, 11-15, and >15). The number of cysts before giving Origanum Majorana tea was 11 to 15 cysts in 50% of the women, while 50% of them had more than 15 cysts. After administration of Origanum majorana tea, a decrease in cyst counts was observed, with no patient exceeding 15 cysts. At the same time, a decrease to 20% in the patients who have 11-15 cysts and an increase in the number of patients who have less than 11 cysts (p-value < 0.05, see table 2).

**Table II: Number of Cysts Before and After Consumption of Origanum Majorana Tea**

Cysts Number	Before the Consumption of Origanum Majorana Tea		After Consumption of Origanum Majorana Tea		P-value
	Patient Number	(%)	Patient Number	(%)	
5-1	0	%0	3	%30	< 0.05
10-6	0	%0	5	%50	< 0.05
15-11	5	%50	2	%20	< 0.05
15 <	5	%50	0	%0.0	< 0.05
<b>Total Number</b>	10	100	10	100.0	

The cysts were divided into three categories according to the dimensions (very small cysts less than 1 mm, 1-7 mm, and 8-15 mm). At the beginning of the study, 20% of the women had very small cysts, 40% had cysts between 1 and 7 mm, and 40% had cysts between 8 and 15 mm. After the consumption of Origanum Majorana for over two months,

the dimensions of cysts decreased significantly, where no more patients had 8-15 mm cysts, and both the patients with very small cysts and 1-7 mm cysts increased to 50% (p-value < 0.05) (see Table 3).

**Table III: Dimension of Cysts Before and After Consumption of Origanum Majorana Tea**

Cysts Dimensions	Before Consumption of Origanum Majorana Tea		After Consumption of Origanum Majorana Tea		P-value (%)
	Patient Number	(%)	Patient Number	Patient Number	
Very small Cysts	2	%20	5	%50	< 0.05
1-7 mm	4	%40	5	%50	< 0.05
8-15 mm	4	%40	0	%0.0	< 0.05
Total number	10	100.0	10	100.0	

One-way Analysis of Variance ANOVA was applied to study the relation between cyst dimensions and the patient's properties (age, BMI, diet, stress, and family history of PCOS) before and after giving Origanum Majorana tea (see Table 4). No relation between each of family history, age, and stress and cyst dimensions, regardless of before or after the consumption of Origanum Majorana tea (p-value > 0.05).

**Table IV: Relation between Cyst Dimensions and Patient's Properties by One-Way ANOVA**

Patients Properties	p-value before Consumption of Origanum Majorana	p-value after Consumption of Origanum Majorana Tea
Age	0.311	0.419
BMI	0.23	0.004
Diet	0.13	0.04
Family History	0.422	0.512

There was a statistically significant relation between BMI and cyst dimensions after taking Origanum Majorana tea (p-value < 0.05). At the same time, there was no relation between them before starting the study (p-value > 0.05). There was also a relation between the diet (high fat and sugar) followed by the patients and cysts dimensions after giving Origanum Majorana tea (p-value < 0.05), with no relation between them before that.

The relation between the cyst number and women's properties (age, BMI, diet, stress, and family history of PCOS) before and after giving Origanum Majorana tea was also studied by the ANOVA test. As shown in Table 5, no effect of age, BMI, stress, and diet on the number of cysts before giving Origanum Majorana tea. Similarly, there was no effect of age, diet, stress, and family history of PCOS on the number of cysts after giving Origanum Majorana tea (p-value > 0.05). There was a relation between BMI and the number of cysts, after giving Origanum Majorana tea (p-value < 0.05). Additionally, there was a significant association between family history of PCOS and the number of cysts before consuming Origanum majorana tea (p-value < 0.05).



**Table V: Relation between Cyst Number and Patient's Properties by One-Way ANOVA**

Patients Properties	p-value before Consumption of <i>Origanum Majorana</i> Tea	p-value after Consumption of <i>Origanum Majorana</i> Tea
Age	0.519	0.324
BMI	0.141	0.024
Diet	0.54	0.115
Family History	0.001	0.124

## IV. DISCUSSION

The phenolic content in our study differs from that in other studies. Some research found that after aqueous and methanolic extraction, 1 g of *Origanum Majorana* contained between 2.85 and 10.27 mg or 4.93 and 11.48 mg (GAE), respectively [31]. Variations in phenolic content relative to other studies may be attributable to factors such as plant origin, extraction method, and harvest time. For example, differences in altitude above sea level, temperature, humidity, and rainfall all affect phenolic content [32]. Several phenolic compounds are present in the aqueous and alcoholic extracts of *Origanum Majorana*, including vanillic acid, gallic acid, rosmarinic acid, and others. Additionally, the most abundant flavonoids are hesperetin, catechin, quercetin, luteolin, and apigenin. *Origanum Majorana* also contains vitamins such as vitamin C and A [33, 34].

The consumption of *Origanum Majorana* tea significantly decreased the number of cysts. Furthermore, a relationship was observed between intake of *Origanum majorana* and cyst size. According to statistical analysis, the phenolic compounds in *Origanum majorana* tea may improve patients' conditions, resulting in a clear reduction in both cyst size and number, regardless of age. Patients' diets influenced cyst dimensions after treatment, emphasising the importance of a healthy diet [35, 36] to boost the effectiveness of *Origanum Majorana*. There was also a significant correlation between BMI and condition improvement, specifically, a decrease in cyst number and size following tea intake, which may be due to obesity or excess weight, particularly in the abdominal area, contributing to peripheral tissue resistance to insulin [37, 38]. Moreover, the study found a link between family history of PCOS and the initial number of cysts, indicating that cyst count relates to prior medical history [39]. Our findings align with other studies showing that aqueous extracts of *Origanum Majorana* can improve insulin sensitivity and hormonal balance in 25 PCOS patients, with rosmarinic acid, luteolin glycosides, and hydroquinone playing key roles [28, 40].

Similar results were observed in a PCOS rat model treated with *Origanum Majorana* alone or combined with metformin, resulting in decreased ovarian weight, estradiol, IL-6 and improved levels of ovarian SOD and GPx [37, 41]. These positive effects on PCOS may stem from phenolic compounds, such as flavonoids (quercetin, apigenin, and luteolin), present in the extract. Quercetin, extensively studied, exhibits antioxidant, anti-inflammatory, antitumour, hypoglycaemic, and cardioprotective effects [42], and also plays roles in regulating ovarian function and protecting ovarian morphology [1, 43]. Research shows that quercetin reduces the expression of 17 $\alpha$ -hydroxylase and 17,20-lyase, key enzymes in the conversion of progesterone to

testosterone, thereby decreasing their activity. Quercetin also modulates androgen receptors, helping correct androgen excess and ovulation issues in women with PCOS [44, 45]. Clinical data indicate that administering quercetin (1 g for 12 weeks) improves insulin resistance and metabolic disorders by increasing total and high-molecular-weight adiponectin and reducing resistance levels associated with insulin resistance and hyperinsulinemia [46]. Insulin resistance is a critical pathogenic factor in PCOS. Oxidative stress is another hallmark of PCOS, with studies showing that quercetin enhances antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, glutathione transferase, and glutathione itself, which help mitigate oxidative damage in ovarian tissue, especially in postmenopausal mice. Quercetin also reduces inflammatory cytokines (TNF- $\alpha$ , IL-6, IL-1 $\beta$ ) by modulating pathways such as oxidised LDL, Toll-like Receptor 4, and NF- $\kappa$ B, thereby improving ovarian inflammation and reversing insulin resistance in PCOS mice [27, 47]. Apigenin, a flavonoid from *Origanum Majorana*, has been investigated for its antioxidant, anti-inflammatory, and anti-tumour effects. It also contributes to the treatment of PCOS by reducing oxidative stress and inflammation [26]. Studies show that apigenin can lower testosterone, estrogen, and FSH/LH levels in mice with induced PCOS, thereby promoting hormonal balance, reducing oxidative stress, and restoring ovarian tissue with increased normal follicles [48]. Luteolin, another flavonoid found in *Origanum Majorana*, has demonstrated significant effects in PCOS mice by restoring glucose regulation and improving insulin sensitivity through PI3K/Akt pathway modulation. Like quercetin and apigenin, luteolin exhibits antioxidant properties, primarily via Nrf2 activation—a key transcription factor in antioxidant defence [49]. These findings elucidate how the main phenolic compounds of *Origanum Majorana* (quercetin, apigenin, and luteolin) can beneficially influence PCOS.

## V. CONCLUSION

Consumption of *Origanum majorana* tea resulted in a decrease in the number and size of individuals. It could be said that the phenolic compounds present in *Origanum majorana* tea improved the condition. It should be noted that only a few studies have been conducted on women to evaluate the role of *Origanum Majorana* in treating PCOS, so there is a need to establish more studies to clarify its role in women. In addition, other studies should investigate the extent of its toxicity on long-term use by women.

## DECLARATION STATEMENT

After aggregating input from all authors, I must verify the accuracy of the following information as the article's author.

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