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THE REVIEW ON EFFECTIVENESS OF TAKING THE ANTHOCYNIN ACTIVE COMPOUND FROM HIBISCUS ROSA-SINENSIS AND ELLAGIC ACID FROM POMEGRANATE IN REDUCING THE SYMPTOMS OF POLYCYSTIC OVARIAN SYNDROME

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ABSTRACT

Pomegranate extract has demonstrated significant potential in supporting women's reproductive health, particularly in enhancing fertility, regulating the menstrual cycle, supporting pregnancy, and the managing polycystic ovary syndrome (PCOS). Its diverse bioactive compounds contribute to hormonal balance and promote regular menstrual cycles. The potent antioxidant properties of pomegranate extract help reduce oxidative stress, a key factor in impaired reproductive function and fertility outcomes. Evidence suggests that pomegranate extract may have a preventive effect against hormonal abnormalities associated with PCOS. This effect is primarily attributed to the presence of phytosterols and phenolic compounds, which help improve the metabolic and endocrine disturbances linked to the disorder. Hibiscus, a widely studied flowering plant, possesses significant therapeutic potential due to its rich phytochemical composition. The Anthocyanins and vitamin C found in hibiscus enhance its antioxidant capacity, helping to counteract the oxidative stress commonly observed in PCOS. Additionally, hibiscus exhibits alpha-glucosidase inhibitory activity, suggesting a potential role in regulating blood glucose levels and reducing oxidative damage. together,

pomegranate extract and hibiscus show promise as natural therapeutic agents that may help mitigate oxidative stress, hormonal imbalances, and metabolic dysfunction associated with PCOS, thereby supporting overall women's reproductive health. Together, pomegranate extract and hibiscus may serve as promising natural therapeutic agents for managing PCOS.

KEYWORDS: Medicinal Plant, polycystic ovarian syndrome, uterus, Female.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) and polycystic ovary disease (PCOD) are prevalent gynecological and endocrine disorders that affect millions of women globally. Among these, PCOS is recognized as one of the most common endocrine disorders in women of reproductive age, with an estimated prevalence of 8–13%. Also referred to as hyperandrogenic anovulation or Stein–Leventhal syndrome, PCOS is a chronic, heterogeneous, and multifactorial condition characterized by a broad spectrum of reproductive, metabolic, and endocrine abnormalities.

Clinically, PCOS manifests through menstrual irregularities, oligo- or anovulation, infertility, hyperandrogenic features such as hirsutism and acne, and metabolic disturbances including obesity and insulin resistance. Polycystic ovarian morphology, defined by increased ovarian volume (>10 mL) and the presence of multiple small follicles measuring 2–9 mm in diameter, is a hallmark diagnostic feature. In contrast, PCOD typically involves the accumulation of immature or partially mature ovarian follicles that form cysts and lead to symptoms such as irregular menstruation, abdominal weight gain, and fatigue, but it is not consistently associated with infertility or systemic metabolic dysfunction. PCOS, however, is increasingly regarded as a metabolic disorder driven by complex interactions between genetic susceptibility, hormonal imbalance, and environmental factors.

The pathophysiology of PCOS is primarily associated with hyperandrogenism, chronic low-grade inflammation, and insulin resistance, all of which impair normal folliculogenesis and ovulation. These mechanisms contribute not only to reproductive dysfunction but also to an increased risk of long-term comorbidities, including type II diabetes mellitus, cardiovascular disease, endometrial cancer, and psychological disorders. Notably, approximately 10% of women with PCOS develop diabetes before the age of 40. Although symptoms typically emerge between 18 and 39 years of age, delayed diagnosis remains common, often occurring only after significant impairment in quality of life.

Emerging evidence suggests that PCOS may be a lifelong condition with origins in early developmental stages, including fetal life. Current international guidelines recommend diagnosis based on the presence of hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology. In addition, environmental and lifestyle factors—such as diet, physical activity, socioeconomic status, and exposure to environmental pollutants—play a critical role in disease onset and progression. Lifestyle modification, particularly dietary management and regular physical activity, remains one of the most effective and cost-efficient approaches to managing PCOS and its associated complications. Early recognition and intervention are therefore essential to reduce the disease burden and improve long-term reproductive and metabolic outcomes.

AIM

To evaluate the effectiveness of anthocyanin, an active compound derived from *Hibiscus*, and ellagic acid obtained from *Pomegranate* in reducing the clinical and metabolic symptoms of polycystic ovarian syndrome (PCOS).

OBJECTIVES

1. To assess the impact of anthocyanins and ellagic acid on hormonal imbalances associated with PCOS.
2. To evaluate their antioxidant effects in reducing oxidative stress in PCOS.
3. To examine improvements in menstrual regularity and ovulatory function following supplementation.
4. To investigate the role of these compounds in regulating metabolic parameters, including insulin resistance and blood glucose levels.
5. To analyze their potential in alleviating common PCOS symptoms such as acne, hirsutism, and weight gain.

Literature Review

Hibiscus rosa-sinensis

Synonyms: China rose, Chinese hibiscus, shoe black plant, shoe flower, blacking plant

Biological Source: *Hibiscus rosa-sinensis*

Family: Malvaceae

Taxonomic Classification

- **Kingdom:** Plantae
- **Subkingdom:** Tracheobionta
- **Superdivision:** Spermatophyta
- **Division:** Magnoliophyta
- **Class:** Magnoliopsida
- **Subclass:** Dilleniidae
- **Order:** Malvales
- **Family:** Malvaceae
- **Genus:** *Hibiscus*
- **Species:** *Hibiscus rosa-sinensis*

Chemical Constituents of *Hibiscus rosa-sinensis*

Preliminary phytochemical analyses have revealed that *Hibiscus rosa-sinensis* contains a wide range of bioactive constituents, including tannins, anthraquinones, quinones, phenols, flavonoids, alkaloids, terpenoids, saponins, cardiac glycosides, proteins, free amino acids, carbohydrates, reducing sugars, mucilage, essential oils, and steroids. However, phytochemical screening of different extracts has shown variability in composition, with the presence of alkaloids, resins, glycosides, reducing sugars, lipophilic components,and sterols, and the absence of tannins and saponins in certain extracts.

Several studies have reported the isolation of four previously unidentified compounds from the leaves, including three sterols and one alkaloid, in addition to β-sitosterol and a taraxeryl acetic acid derivative. Investigations of the leaf composition have further identified hydrocarbons, unsaturated lipids, and oily constituents. Organic acids such as malvalic and streptacic cyclic acids have also been reported.

The flowers of *Hibiscus rosa-sinensis* are rich in vitamins and polyphenolic compounds, including cyanidin diglucoside, ascorbic acid, flavonoids, riboflavin, niacin, and thiamine. Several anthocyanin derivatives have been isolated from deeply colored yellow flowers, including cyanidin-3-sophoroside-5.glucoside, quercetin-3-diglucoside, cyanidin-3,5-diglucoside, and 3,7-diglucoside, which contribute to the plant's antioxidant activity.

Pharmacognostic Characteristics of Dried *Hibiscus* Flowers

Macroscopic characteristics

The dried flowers are purple in color with a slightly sweet and mucilaginous taste. The calyx is persistent and five-lobed with a five-part epicalyx. The corolla is polypetalous, while the stamens and pistil are monadelphous.

Microscopic characteristics

Microscopic examination reveals spherical, yellow, spiny pollen grains. Covering trichomes, including hooked and stellate glandular multicellular trichomes, are present. Numerous calcium oxalate crystals occur in cluster and rosette forms. The ovules are kidney-shaped and embedded with rosette crystals. Starch grains are abundant, occurring as both simple and compound (multicomponent) forms, and a few anomocytic stomata are observed.

punica granatum (pomegranate)

Synonyms

Punica granatum, pomegranate.

Taxonomic Classification

- **Kingdom:** Plantae
- **Division:** Magnoliophyta
- **Class:** Magnoliopsida
- **Order:** Myrtales
- **Family:** Lythraceae
- **Subfamily:** Punicoideae
- **Genus:** *Punica*
- **Species:** *Punica granatum*

Punica granatum was previously classified under the family Punicaceae but has been reclassified into the family Lythraceae based on phylogenetic evidence. The genus *Punica* comprises two species: *Punica granatum*, the most widely cultivated and economically important species, and *Punica protopunica*, a less common species native to the island of Socotra.

Chemical Constituents and Components

The chemical composition of pomegranate fruit and other plant parts varies depending on climatic conditions, geographical location, storage practices, and cultivar. Numerous bioactive compounds have been identified from different parts of the plant, contributing to its wide range of therapeutic properties.

Major phytochemicals include ellagitannins such as punicalagin and punicalin, ellagic acid and its derivatives (e.g., 3,3′-di-O-methylellagic acid, 3,3′-tri-O-methylellagic acid), and phenolic compounds including pedunculagin, punicacorteins A–D, granatins A and B, punicafolin, punigluconin, corilagin, and gallocatechins. Flavonoids such as luteolin, quercetin, kaempferol, and anthocyanins (delphinidin, cyanidin, and pelargonidin) are also abundant. Additional constituents include fatty acids, sterols, triterpenes, and other tannins.

Pomegranate juice contains anthocyanins, glucose, ascorbic acid, ellagic acid, gallic acid, caffeic acid, catechin, epigallocatechin gallate, quercetin, rutin, iron, and amino acids. The seed oil is rich in punicic acid and sterols. The fruit rind contains punicalagin, flavones, flavanones, and flavanols, while the leaves are rich in tannins such as punicalin and punicafolin, along with flavone glycosides including luteolin and apigenin. The flowers contain ursolic acid, triterpenoids, and asiatic acid, whereas the roots and bark contain ellagitannins and piperidine alkaloids. The aril juice provides approximately 16% of the recommended daily intake of vitamin C, vitamin B5, potassium, and natural phenols such as ellagitannins and flavonoids.

Morphological features

Macroscopic evaluation is a primary step in the authentication of plant materials and includes assessment of size, shape, texture, color, taste, and odor. In the present investigation, dried peels of *P. granatum* were dark reddish-brown in color, varied in size, and brittle in nature. The peel fragments appeared concave-convex in shape with a smooth, woody external surface. The internal pith was yellowish-brown, septate, and membranous, bearing impressions of seeds. The powdered peel exhibited a faint aromatic odor and an astringent taste.

Microscopic features

Microscopic analysis provides detailed information on internal anatomical characteristics and is a rapid, cost-effective method for plant identification and differentiation of closely related species. In this study, both transverse section analysis and powder microscopy were performed to establish the identity and authenticity of *Punica granatum* peel.

MATERIALS AND METHODS

Pomegranate (*Punica granatum*)

MATERIALS

Pomegranate juice press waste was obtained from a local fruit juice processing unit. The peels were manually separated, thoroughly washed, and stored in a deep freezer at −20°C until further use. Amberlite XAD-4 is a non-polar adsorbent resin composed of a styrene–divinylbenzene matrix, with a particle size of 20–60 mesh, a surface area of approximately

725 m²/g, an average pore diameter of 40 Å, and a pore volume of 0.98 mL/g.

Extraction of Ellagic Acid from Pomegranate Peel Waste

To enhance extraction efficiency and ensure uniform particle size, industrial pomegranate peel waste was ground to approximately 2 mm. Extraction was performed using a hydro-ethanolic solvent system (ethanol:water, 1:1 v/v) at 85°C for 100 min. A solid-to-solvent ratio of 1:12.5 (w/v) was maintained, as determined from preliminary optimization studies.

The extraction process was conducted in a sealed glass container using a laboratory-scale shaking water bath (Jeio Tech BS-06/31, Seoul, Korea) at a stirring speed of 150 rpm. After extraction, the mixture was filtered through coarse filter paper, and the filtrate was stored at 20°C for further analysis. Crystallization was subsequently carried out, followed by filtration and drying to obtain ellagic acid as a pale yellow (flaxen) powder.

MATERIALS AND METHOD

Hibiscus (*Hibiscus Rosa-sinensis*)

MATERIALS

Laboratory apparatus used in this study included chromatographic columns, beakers, test tubes, conical flasks, a rotary evaporator,an analytical weighing balance, and filter paper. Thin-layer chromatography (TLC) was performed using Merck Millipore silica gel 60 F254 TLC plates. A 10% sulfuric acid (H_2SO_4) solution in methanol was used as a visualizing reagent. Additional equipment included a TLC chamber and a hot plate. All solvents used were of analytical grade and were distilled prior to use.

INSTRUMENTS

- Thin layer chromatography glass plates coated with silica gel 60 F254

- Silica gel of varying mesh sizes (60–120, 100–200, and 230–400) for column chromatography.

Method: Preparation and Extraction of Hibiscus Flowers

Fresh *Hibiscus rosa-sinensis* flowers were thoroughly washed with distilled water, cut into small pieces, and dried in a hot air dryer at 45°C for 48 h. The flowers are dried and were pulverized into a fine powder using a mechanical grinder.

The powdered hibiscus material was subjected to maceration using 70% ethanol as the extraction solvent. The mixture was stirred until homogeneous and allowed to stand for 24 h at room temperature. The extract was then filtered through a Buchner funnel lined with filter paper to remove insoluble impurities. The residual plant material was re-extracted twice with fresh ethanol following the same procedure to ensure maximum extraction of bioactive compounds.

The combined filtrates were concentrated using a rotary evaporator at 70°C under reduced pressure. The concentrated extract was subsequently diluted with distilled water to obtain final concentrations of 2%, 4%, and 6% (w/v) for further experimental use.

Identification Tests

Identification Tests for Anthocyanins

1. Color Reaction (Acid–Base Test)

A small quantity of the sample was placed in a test tube and treated with 2 M hydrochloric acid (HCl), followed by heating for 5 minutes.

Positive result: Formation of a red color confirms the presence of anthocyanins. Subsequently, a few drops of 2 M sodium hydroxide (NaOH) were added.

Positive result: Development of a blue or green color that gradually fades further confirms the presence of anthocyanins.

2. Lead Acetate Test

To the anthocyanin solution, 1% lead acetate solution was added.

Positive result: Formation of a blue coloration, particularly for cyanidin-type anthocyanins, due to the interaction of lead ions with hydroxyl groups on the flavonoid nucleus.

3. Thin Layer Chromatography (TLC)

Anthocyanins were analyzed using cellulose TLC plates with acetonitrile:water:formic acid

(0.7:1.3:0.1, v/v/v) as the mobile phase.

OBSERVATION: Anthocyanins appeared as pink or red spots with characteristic Rf values under visible light.

Confirmation was performed by scraping the spot, eluting with methanol, and recording UV–visible absorption spectra, which showed a characteristic maximum between 520–550 nm, typical of anthocyanins.

Identification Tests for Ellagic Acid

1. Colorimetric Nitrosylation Reaction (Spectrophotometric Method)

The sample was treated with nitrous acid generated in situ by mixing sodium nitrite ($NaNO_2$) and hydrochloric acid. Ellagic acid reacts to form a pink to red nitrosylation product.

Confirmation: Absorbance measured at 538 nm indicated the presence of ellagic acid. This method is highly selective for ellagic acid compared to other phenolic compounds.

2. HPLC and UV–Visible Spectroscopy

Ellagic acid was identified and quantified using high-performance liquid chromatography (HPLC) equipped with a C18 column. Detection was carried out at 270 nm.

Confirmation: The retention time of the sample matched that of a standard ellagic acid reference.

3. Mass Spectrometry

Ellagic acid exhibited a pseudomolecular ion at m/z 301 ($[M–H^-]$) with characteristic fragment ions at m/z 257 and m/z 229 in ESI-MS analysis, providing definitive confirmation in complex mixtures.

CONCLUSION

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous disorder requiring an individualized and multidisciplinary treatment approach. Due to incomplete understanding of its pathogenesis, management primarily focuses on symptom control and prevention of long-term complications through lifestyle modification, conventional pharmacotherapy, and complementary therapies. Early diagnosis and sustained lifestyle interventions are essential to reduce the risk of metabolic syndrome, type II diabetes, and cardiovascular disease.

Emerging evidence suggests that ellagic acid, a polyphenol derived from pomegranate, may offer therapeutic benefits in PCOS management by improving insulin sensitivity and reducing oxidative stress and inflammation. Clinical studies have shown significant improvements in biomarkers such as MDA, TAC, and CRP at doses of 180–200 mg/day over an 8-week period. However, larger and long-term clinical trials are required to confirm these findings and establish optimal dosing strategies.

Additionally, hibiscus flower extract, rich in flavonoids and tannins, demonstrated notable bioactivity, including antibacterial effects, with a 6% concentration showing the highest inhibitory activity. Overall, natural bioactive compounds such as ellagic acid and hibiscus-derived anthocyanins show promise as complementary agents, warranting further clinical and translational research.

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REPORT

Recent studies indicate that natural bioactive compounds such as ellagic acid from pomegranate and anthocyanin-rich extracts from *Hibiscus rosa-sinensis* exhibit promising therapeutic potential in the management of polycystic ovary syndrome (PCOS). Ellagic acid has been reported to improve insulin sensitivity, reduce androgen levels, and attenuate oxidative stress and inflammatory responses—key pathological features of PCOS. Similarly, hibiscus contains high levels of anthocyanins and flavonoids that demonstrate antioxidant, anti-inflammatory, and glucose-regulating properties, which may contribute to the mitigation of metabolic and endocrine disturbances associated with PCOS. Although these findings support the potential role of these phytochemicals as complementary therapeutic agents, the current evidence is largely derived from limited clinical and experimental studies. Consequently, well-designed, large-scale, and long-term human trials are required to establish their clinical efficacy, safety, optimal dosage, and formulation before they can be recommended for routine use in PCOS management.

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