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FORMULATION AND EVALUATION OF DARK CHOCOLATE ENRICHED WITH *VITEX AGNUS CASTUS AND MENTHA SPICATA* FOR PCOS

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder characterized by hormonal imbalance, insulin resistance, oxidative stress, and metabolic abnormalities. Conventional therapies often cause side effects and poor patient compliance, highlighting the need for safer alternatives. The present study aimed to formulate and evaluate phyto-enriched dark chocolate containing *Vitex agnus-castus* and *Mentha spicata* as a novel nutraceutical approach for PCOS management. These herbal extracts were selected for their hormonal regulatory and anti-androgenic properties, while dark chocolate served as an antioxidant-rich and palatable carrier. The formulation was prepared using the melting–tempering–molding method, and three batches (F1, F2, and F3) were developed with varying extract concentrations. Evaluation studies included organoleptic characteristics, weight variation, hardness, melting point, pH, moisture content, and phytochemical screening. Results demonstrated acceptable physicochemical properties and formulation stability. Among all batches, F2 was identified as the optimized formulation due to its superior palatability, stability, and therapeutic potential. The developed formulation represents a safe, innovative, and patient-friendly nutraceutical strategy for PCOS management and may serve as a promising alternative for future clinical application.

KEYWORDS: Polycystic Ovary Syndrome (PCOS), Nutraceuticals, Dark Chocolate, Vitex agnus-castus, Mentha spicata, Herbal Formulation, Antioxidant Activity, Phytochemicals, Functional Food, Hormonal Regulation, Chocolate-based Drug Delivery.

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is one of the most frequently occurring endocrine and metabolic disorders affecting women during their reproductive years. It is considered a heterogeneous disorder characterized by hormonal imbalance, ovarian dysfunction, insulin resistance, and metabolic disturbances. The prevalence of PCOS has been reported to range from approximately 6% to 20% worldwide, depending on the diagnostic criteria and population studied. Women affected by PCOS commonly present with symptoms such as menstrual irregularities, infertility, obesity, acne, hirsutism, and ovarian cyst formation. In addition to reproductive complications, PCOS is also associated with metabolic abnormalities including type 2 diabetes mellitus, dyslipidemia, cardiovascular disorders, and psychological stress.

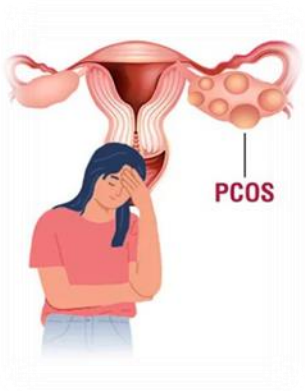

Fig 1: PCOS.

The pathophysiology of PCOS is multifactorial and involves complex interactions among genetic, endocrine, and environmental factors. Insulin resistance is considered one of the major contributors to disease progression, resulting in compensatory hyperinsulinemia that stimulates excess androgen production. Furthermore, oxidative stress and chronic low-grade inflammation have been recognized as significant contributors to hormonal imbalance and metabolic dysfunction. These interconnected mechanisms create a cycle that worsens both reproductive and metabolic symptoms of the disorder.

Conventional therapeutic strategies for PCOS include lifestyle modification, hormonal contraceptives, insulin-sensitizing agents such as metformin, and ovulation induction therapy. Although these treatments may provide symptomatic relief, prolonged use can lead to adverse effects such as gastrointestinal disturbances, weight gain, and poor patient adherence. As a result, there is increasing interest in identifying safer and more patient-friendly therapeutic approaches with improved long-term outcomes.

Fig 2: ***Vitex agnus-castus.***

Fig 3: ***Mentha Spicata.***

Herbal medicines and nutraceutical systems have emerged as promising alternatives because of their ability to target multiple pathological pathways simultaneously while demonstrating minimal side effects. Among the medicinal plants investigated for PCOS management, *Vitex agnus-castus* and *Mentha spicata* have gained considerable attention due to their therapeutic potential. *Vitex agnus-castus* exhibits endocrine-modulating activity by influencing dopamine receptors and reducing prolactin secretion, thereby helping regulate menstrual cycles and hormonal balance.

Conversely, *Mentha spicata* possesses anti-androgenic activity and has been reported to reduce testosterone levels while improving symptoms such as acne and hirsutism. Additionally, both plants contain antioxidant and anti-inflammatory constituents that may contribute to metabolic regulation and reduction of oxidative stress.

Dark chocolate has recently gained importance as a functional food and nutraceutical carrier because of its high content of cocoa polyphenols, flavonoids, and antioxidant compounds. These bioactive components possess antioxidant, anti-inflammatory, and insulin-sensitizing properties that may be beneficial in managing metabolic disturbances associated with PCOS. Furthermore, the lipid-rich matrix of dark chocolate can enhance the stability and bioavailability of herbal constituents while improving taste and patient compliance.

Therefore, the present study was designed to formulate and evaluate phyto-enriched dark chocolate containing *Vitex agnus-castus* and *Mentha spicata* as a novel nutraceutical approach for the management of PCOS. The combination of herbal actives with a chocolate-based delivery system is expected to provide a synergistic therapeutic effect by targeting hormonal imbalance, oxidative stress, inflammation, and metabolic dysfunction while improving patient acceptability and compliance.

2. Plant Profile

Table no.1

Parameter	**Vitex agnus-castus**	**Mentha spicata**
Common Name	Chaste tree / Chasteberry	Spearmint
Scientific Name	*Vitex agnus-castus*	*Mentha spicata*
Image		
Family	Lamiaceae	Lamiaceae
Biological Source	Dried ripe fruits of Vitex agnus-castus	Dried leaves and aerial parts of Mentha spicata
Kingdom	Plantae	Plantae
Division	Angiosperms	Angiosperms
Class	Eudicots	Eudicots
Order	Lamiales	Lamiales
Genus	Vitex	Mentha
Species	Vitex agnus-castus	Mentha spicata
Major Chemical Constituents	Flavonoids (casticin, vitexin), iridoid glycosides, essential oils, diterpenoids	Menthol, carvone, flavonoids, phenolic acids, tannins
Pharmacological Activities	Hormonal regulation, antioxidant, anti-	Antioxidant, anti-androgenic activity,

	inflammatory, prolactin modulation	anti-inflammatory, digestive aid
Uses	Menstrual regulation, PMS treatment, PCOS support	Hormonal balance, reduction of androgen levels, digestive support
Category	Herbal endocrine modulator	Medicinal aromatic herb

3. MATERIALS AND METHODS

3.1. List of Ingredients

Table No.2

Sr. No.	Ingredient	Quantity	Role
1	Dark Chocolate (70–80%)	76.5 g	Base material; provides structure, taste, and antioxidant activity
2	Cocoa Butter	10 g	Improves texture, smoothness, and melting characteristics
3	*Vitex agnus-castus* Extract	2 g	Hormonal regulation and endocrine modulation (LH/FSH balance)
4	*Mentha spicata* Extract	2 g	Anti-androgenic activity and reduction of testosterone levels
5	Sweetener (Jaggery)	8 g	Improves taste and masks bitterness of herbal extracts
6	Flavoring Agent	1 g	Improves taste and aroma

3.2. List of Instruments

Table No.3

Sr. No.	Instrument	7	Digital pH Meter
1	Digital Weighing Balance	8	Hot Air Oven
3	Magnetic Stirrer	9	Texture Analyzer
4	Chocolate Molds	10	Incubator
5	Vernier Caliper / Monsanto Hardness Tester	11	Refrigerator

4. Formulation Table of Phyto-Enriched Dark Chocolate Batches

Table No.4

Ingredients	F1	F2	F3	Role
Dark chocolate	80 g	76.5 g	73 g	Base material and nutraceutical carrier
Cocoa butter	10 g	10 g	10 g	Improves smoothness and melting property
Vitex agnus-castus extract	1 g	2 g	3 g	Hormonal regulation and endocrine modulation
Mentha spicata extract	1 g	2 g	3 g	Anti-androgenic and antioxidant activity
Sweetener (Jaggery)	7.5 g	8 g	8.5 g	Improves taste and masks bitterness
Flavoring agent	q.s.	q.s.	q.s.	Improves aroma and palatability
Total	100 g	100 g	100 g	

Optimized batch: F2

F2 was selected as the optimized formulation because it showed better taste, acceptable hardness, smooth texture, and good antioxidant activity.

4.1. Procedure

- Accurately weigh the required quantities of dark chocolate, cocoa butter, *Vitex agnus-castus* extract, *Mentha spicata* extract, sweetener, Jaggery, and flavoring agent.
- Melt the dark chocolate and cocoa butter together using a water bath at a controlled temperature of approximately **45–50°C**.
- Stir the molten mixture continuously until a smooth and uniform chocolate base is obtained.
- Add the measured quantity of *Vitex agnus-castus* extract to the chocolate base and mix thoroughly.

- Add the *Mentha spicata* extract gradually into the prepared mixture while continuously stirring to ensure uniform distribution.
- Add sweetener (Stevia/Jaggery), Jaggery, and flavoring agent one by one with continuous mixing.
- Continue stirring the formulation for approximately **10–15 minutes** to obtain a homogeneous mixture.
- Subject the prepared mixture to a tempering process by controlled cooling and reheating to improve texture and crystal formation.
- Pour the prepared formulation into chocolate molds and allow it to cool under refrigeration conditions until complete solidification occurs.
- Finally, remove the prepared chocolate units from the molds, label them appropriately, and store in airtight containers at room temperature for further evaluation.

Fig 4: Mixture of all ingredients and Product.

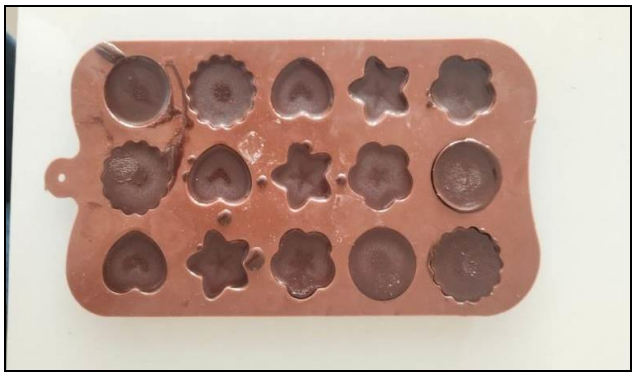

Fig 5: Molds/Products.

Fig 6: Products.

5. Evaluation Parameters

- Organoleptic properties
- Weight variation
- Hardness
- Moisture content
- pH
- Stability study

6. RESULT AND DISCUSSION

Table No. 5.

Evaluation Parameter	F1	F2	F3	Discussion
Color	Dark brown	Dark brown	Dark brown	All formulations showed uniform appearance without visible defects.
Taste	Good	Very good	Slight bitterness	F2 exhibited optimum sensory acceptability, while higher herbal concentration in F3 produced mild bitterness.
Texture	Smooth	Smooth	Slightly rough	Increased extract concentration slightly affected texture characteristics.
Weight variation (g)	9.89 ± 0.12	10.01 ± 0.08	10.15 ± 0.11	Minimal variation indicated good uniformity among prepared formulations.
Thickness (mm)	7.2 ± 0.2	7.4 ± 0.1	7.6 ± 0.2	Thickness slightly increased with increased herbal concentration but remained within acceptable range.

7. Comparative Phytochemical Screening of *Vitex agnus-castus* and *Mentha spicata* Extracts

Table No. 6

Sr. No.	Phytochemical Constituent	Qualitative Test	*Vitex agnus-castus*	*Mentha spicata*
1	Alkaloids	Mayer's test	+ Present	+ Present
2	Flavonoids	Shinoda test	+ Present	+ Present
3	Phenolic compounds	Ferric chloride test	+ Present	+ Present
4	Glycosides	Keller–Killiani test	+ Present	+ Present
5	Terpenoids	Salkowski test	+ Present	+ Present
6	Iridoid glycosides (Agnuside)	Legal’s Test	+ Present	− Absent
7	Tannins	Ferric chloride test	+ Present	+ Present

8. Stability Study

Table No.7

Evaluation Parameter	Initial	15 Days	20 Days	30 Days
Hardness (kg/cm²)	4.7	4.6	4.5	4.4
pH	6.5	6.5	6.4	6.4
Moisture content (%)	1.65	1.69	1.72	1.75
Melting point (°C)	34.8	34.7	34.5	34.4
Physical appearance	Smooth	No change	No change	No significant change

9. DISCUSSION

The prepared phyto-enriched dark chocolate formulations were successfully developed and evaluated for physicochemical properties and antioxidant activity. All formulations showed acceptable appearance, texture, and stability characteristics. An increase in herbal extract concentration resulted in increased antioxidant activity and total phenolic content due to the presence of bioactive phytoconstituents. However, higher concentrations slightly affected

sensory properties by producing a mild bitter taste. Among all formulations, F2 exhibited the best balance of taste, texture, hardness, stability, and therapeutic potential. Therefore, F2 was selected as the optimized formulation for further studies.

10. CONCLUSION

The present study successfully formulated and evaluated phyto-enriched dark chocolate containing *Vitex agnus-castus* and *Mentha spicata* for the management of Polycystic Ovary Syndrome. The developed formulations demonstrated satisfactory physicochemical properties, acceptable organoleptic characteristics, and significant antioxidant activity.

Among the three prepared formulations, F2 exhibited the most balanced profile in terms of sensory properties, hardness, stability, and therapeutic potential. The combination of *Vitex agnus-castus*, *Mentha spicata*, and dark chocolate provided a synergistic approach by targeting hormonal imbalance, oxidative stress, and metabolic disturbances associated with PCOS. The findings suggest that phyto-enriched dark chocolate may serve as a novel nutraceutical delivery system with improved patient compliance and therapeutic benefits. However, additional in vivo studies and clinical investigations are necessary to confirm long-term efficacy, safety, and clinical applicability of the formulation.

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