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Phytochemical Profiling and Antioxidant Activity of *Vigna mungo* Seeds: HPLC Quantification of Gallic Acid and Genistein with Potential Fertility-Enhancing Implications

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Abstract: *Vigna mungo* (L.) Hepper (black gram) is an important legume valued for its nutritional and medicinal properties. The present study evaluated the phytochemical profile and antioxidant potential of ethanolic seed extract of *Vigna mungo*. Seeds were extracted using the Soxhlet method with ethanol. Total phenolic content (TPC) was determined by the Folin–Ciocalteu method, antioxidant activity was assessed using the DPPH free radical scavenging assay, and selected phytochemical markers were quantified by High Performance Liquid Chromatography (HPLC).

The ethanolic extract exhibited a total phenolic content of 3.28 mg GAE/g extract and 21.43% DPPH radical scavenging activity, indicating moderate antioxidant potential. HPLC analysis confirmed the presence of gallic acid (0.04%) and genistein (0.61%). These compounds are known for their antioxidant and cytoprotective properties, while genistein has been reported to possess phytoestrogenic activity and potential relevance to reproductive health.

The findings provide baseline phytochemical data supporting the medicinal value of *Vigna mungo*. The presence of antioxidant phytochemicals and genistein suggests its potential relevance to fertility enhancement and reproductive health, warranting further in vivo and clinical investigations.

Keywords: *Vigna mungo, Antioxidant Activity, Total Phenolic Content, HPLC, Gallic Acid, Genistein, Fertility Enhancement, Reproductive Health.*

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I. INTRODUCTION

Medicinal plants have been used as primary sources of healthcare since ancient civilizations and continue to contribute significantly to modern pharmaceutical research. Plant-derived secondary metabolites including phenolics, flavonoids, alkaloids, tannins, terpenoids and isoflavones exhibit a wide spectrum of biological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer and immunomodulatory effects [1,2]. Scientific exploration of these bioactive compounds has gained considerable importance in the search for safer therapeutic agents with fewer adverse effects than synthetic drugs [3].

Among edible legumes, *Vigna mungo* (L.) Hepper (Family: Fabaceae) occupies an important position because of its nutritional richness and medicinal value. Commonly known as black gram or urad bean, it is extensively cultivated throughout India and several Asian countries [4]. The seeds are rich in proteins (20–25%), carbohydrates, essential amino acids, dietary fibre, vitamins and minerals, making them valuable in human nutrition [5]. Traditional medicinal systems such as Ayurveda describe *Vigna mungo* as a strengthening food possessing rejuvenating properties and recommend its use for improving vitality, tissue nourishment and reproductive health [6,7].

Recent phytochemical investigations have demonstrated that *Vigna mungo* contains numerous bioactive constituents including phenolic acids, flavonoids, isoflavones, tannins, saponins and phytosterols [8,9]. Among these, gallic acid and genistein are particularly

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important because of their documented antioxidant and pharmacological properties [10,11]. Gallic acid is a naturally occurring phenolic acid capable of scavenging reactive oxygen species and protecting cellular components from oxidative damage [12]. Genistein is an isoflavone recognized for its antioxidant activity and phytoestrogenic properties [13]. Due to its structural similarity to endogenous estrogens, genistein has been investigated for potential effects on reproductive physiology, endocrine regulation and fertility in experimental studies [14,15]. However, these effects are dose-dependent and context-specific, and the present study does not evaluate reproductive outcomes directly.

Oxidative stress has emerged as an important factor contributing to numerous chronic disorders, including reproductive dysfunction [16]. Excessive production of reactive oxygen species (ROS) may impair gamete quality, disrupt hormonal balance and damage reproductive tissues [17,18]. Natural antioxidants obtained from dietary plants are increasingly investigated because they can neutralize free radicals and reduce oxidative damage [19]. Phenolic compounds constitute one of the major classes of natural antioxidants responsible for the biological activities of many medicinal plants [20].

Assessment of antioxidant potential generally involves estimation of total phenolic content and free radical scavenging activity. The Folin–Ciocalteu assay is widely employed to estimate total phenolics [21], while the DPPH assay is a rapid and reliable method for evaluating antioxidant capacity [22]. These assays provide valuable information regarding the antioxidant potential of plant extracts and complement chromatographic identification of specific bioactive compounds.

High Performance Liquid Chromatography (HPLC) is considered one of the most accurate analytical techniques for qualitative and quantitative estimation of phytochemicals [23]. Quantification of marker compounds enables standardization of herbal materials and supports quality control of medicinal plants [24]. Identification of gallic acid and genistein in *Vigna mungo* may further enhance understanding of its therapeutic potential.

In the present study, ethanolic seed extract of *Vigna mungo* was evaluated for total phenolic content, antioxidant activity and HPLC-based quantification of gallic acid and genistein. The study also discusses the potential significance of these phytochemicals in relation to antioxidant defense mechanisms and their possible relevance to reproductive health based on published literature, while recognizing that dedicated biological studies are necessary to establish fertility-enhancing effects.

II. MATERIALS AND METHODS

➢ *Collection of Plant Material*

Healthy and mature seeds of *Vigna mungo* (L.) Hepper were collected from the local agricultural fields of Ahilyanagar District, Maharashtra, India. The seeds were washed thoroughly with distilled water to remove dust and foreign particles and then shade-dried at room temperature. The dried seeds were ground into a fine powder using a laboratory grinder and stored in airtight containers until further analysis [25].

➢ *Preparation of Ethanolic Extract*

The powdered seed material was extracted using the Soxhlet extraction technique. Approximately 10 g of seed powder was placed in a Soxhlet apparatus and extracted with ethanol until complete extraction was achieved. The extract was concentrated under reduced pressure and stored in amber-colored bottles at 4°C for further phytochemical and antioxidant analyses [26, 27].

➢ *Estimation of Total Phenolic Content (TPC)*

The total phenolic content of the ethanolic extract was determined by the Folin–Ciocalteu colorimetric method [21, 28].

- *Reagents*

✓ Folin–Ciocalteu reagent (10%)
✓ Sodium carbonate solution (7.5%)
✓ Gallic acid standard
✓ Distilled water

- *Standard Preparation*

A gallic acid stock solution (1 mg/mL) was prepared and diluted to obtain concentrations of-

✓ 20 µg/mL
✓ 40 µg/mL
✓ 60 µg/mL
✓ 80 µg/mL
✓ 100 µg/mL

A calibration curve was prepared using the absorbance values of these standards [21].

- *Procedure*

✓ 0.5 mL of plant extract was mixed with 2.5 mL of diluted Folin–Ciocalteu reagent.
✓ The mixture was incubated for 5 minutes.
✓ Subsequently, 2 mL of 7.5% sodium carbonate solution was added.
✓ The reaction mixture was mixed thoroughly and incubated in the dark for 30 minutes at room temperature.
✓ Absorbance was recorded at 765 nm using a UV–Visible spectrophotometer [21,28].

The total phenolic content was calculated using the following equation:

$$TPC = (C \times V) / M$$

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Where,

C = Concentration obtained from calibration curve (mg/mL)
V = Volume of extract (mL)
M = Weight of extract (g)

TPC = (0.0655 × 0.5) / 0.01 = 3.28 mg GAE/g extract.

The total phenolic content was expressed as mg Gallic Acid Equivalents (GAE)/g extract. Based on the calibration equation (y = 0.0087x + 0.02), the mean absorbance value of 0.59 corresponded to a gallic acid equivalent concentration of 65.52 µg/mL, which yielded a total phenolic content of 3.28 mg GAE/g extract.

➢ *Determination of Antioxidant Activity by DPPH Assay*

The antioxidant activity of the ethanolic extract was evaluated using the DPPH free radical scavenging assay [22, 29].

- *Principle*

The purple-colored DPPH radical is reduced by antioxidant compounds present in the extract, resulting in decreased absorbance measured at 517 nm [22].

- *Procedure*

✓ Freshly prepared 0.1 mM DPPH solution in methanol was used. Two reaction mixtures were prepared-

- Control - DPPH + Methanol
- Test - DPPH + Ethanolic extract of *Vigna mungo*

✓ The mixtures were incubated for 30 minutes in the dark**.**
✓ Absorbance was measured at 517 nm using a UV–Visible spectrophotometer.
✓ The percentage inhibition was calculated using the following equation:

% Inhibition = [(A control – A sample) / A control] × 100

Where-

- A control = Absorbance of the control
- A sample = Absorbance of the sample

% Inhibition = [(0.700 − 0.550) / 0.700] × 100

% Inhibition = 21.43%

Therefore, the ethanolic seed extract of *Vigna mungo* exhibited 21.43% DPPH radical scavenging activity. This assay is widely used for evaluating the free radical scavenging capacity of plant extracts and natural antioxidants [22, 29].

➢ *HPLC Analysis*

Quantitative estimation of selected phytochemical markers was performed by 3S Analytical Technologies Pvt. Ltd., Pune, Maharashtra, India**,** using High Performance Liquid Chromatography (HPLC). The analysis was carried out using a validated analytical protocol for the quantification of gallic acid and genistein in the ethanolic seed extract of *Vigna mungo*.

The chromatographic separation was achieved using a reverse-phase HPLC system. The mobile phase consisted of Solvent A (water containing 0.1% acetic acid) and Solvent B (methanol/acetonitrile). A gradient elution program was employed, starting with 40% Solvent B and gradually increasing to 70% Solvent B over 20 min. The flow rate was maintained at 1.0 mL min^{-1}**,** and the injection volume was 20 µL. Detection was performed at 260 nm for genistein and 270 nm for gallic acid. The analysis was carried out in a single determination (n = 1)**.** Chromatograms and quantitative data generated by the laboratory were used for identification and estimation of the marker compounds. HPLC is widely recognized as a reliable analytical technique for the identification, quantification and quality assessment of phytochemicals in medicinal plants and herbal formulations [23, 24, 30].

III. RESULTS

➢ *Total Phenolic Content*

The Folin–Ciocalteu assay revealed that the ethanolic seed extract of *Vigna mungo* possessed measurable quantities of phenolic compounds. The calibration curve prepared using gallic acid standards exhibited a linear response within the selected concentration range. Based on the calibration equation, the total phenolic content of the extract was calculated as 3.28 mg GAE/g extract**.**

Table 1 Gallic Acid Standard Curve

Concentration (µg/mL)	Mean Absorbance
20	0.19
40	0.36
60	0.54
80	0.71
100	0.88

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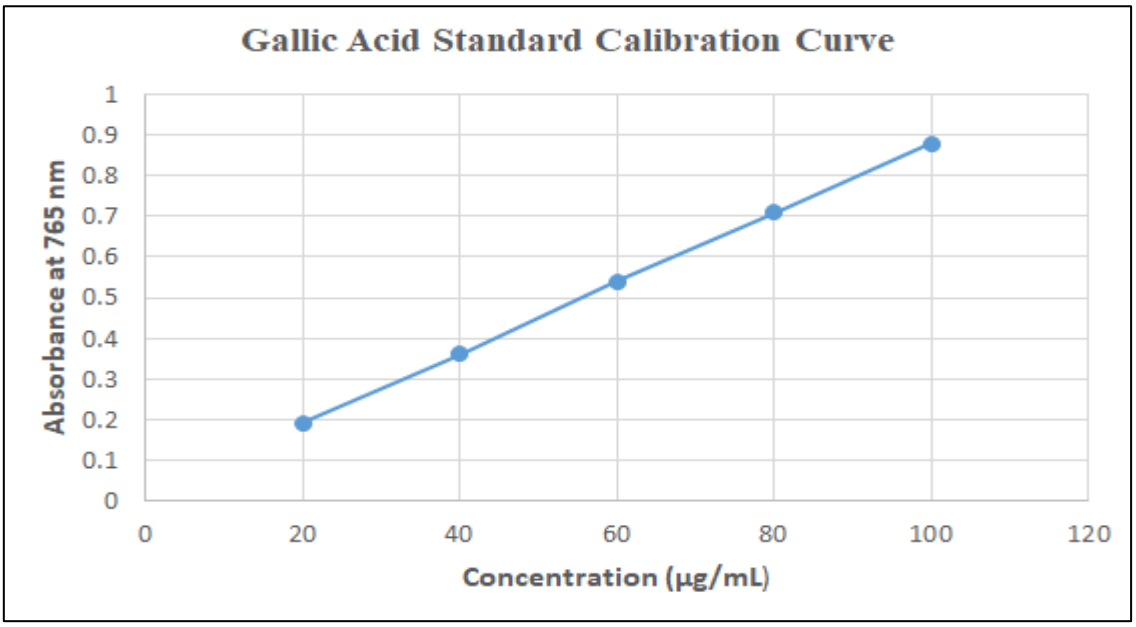

Fig 1 Gallic Acid Standard Calibration Curve Showing the Linear Relationship Between Concentration (20–100 µg/mL) and Absorbance at 765 nm. The Regression Equation Obtained was y = 0.0087x + 0.02.

The calibration curve of gallic acid showed good linearity over the concentration range of 20–100 µg/mL and was represented by the regression equation y = 0.0087x + 0.02, where *y* represents absorbance and *x* represents gallic acid concentration. The equation was used to determine the phenolic concentration of the extract from its measured absorbance value.

Table 2 Total Phenolic Content of Ethanolic Seed Extract of *Vigna mungo*

Replicate	TPC (mg GAE/g extract)
1	3.24
2	3.31
3	3.29
Mean ± SD	3.28 ± 0.04

➢ *Antioxidant Activity*

The ethanolic extract exhibited free radical scavenging activity against DPPH radicals.

Control absorbance = 0.700
Sample absorbance = 0.550
Percentage inhibition = 21.43%

Table 3 DPPH Radical Scavenging Activity of Ethanolic Seed Extract of *Vigna mungo*

Replicate	% Inhibition
1	21.10
2	21.55
3	21.64
Mean ± SD	21.43 ± 0.29

The antioxidant activity of the ethanolic seed extract of *Vigna mungo* was evaluated using the DPPH free radical scavenging assay. The extract exhibited a mean radical scavenging activity of 21.43 ± 0.29%**,** indicating moderate antioxidant potential. The observed activity may be attributed to the presence of phenolic compounds and other antioxidant phytochemicals capable of donating hydrogen atoms or electrons to neutralize free radicals. The results suggest that the seed extract possesses measurable antioxidant capacity and may contribute to protection against oxidative stress.

➢ *HPLC Quantification of Bioactive Compounds*

HPLC analysis confirmed the presence of two important phytochemical markers in the ethanolic seed extract of *Vigna mungo*.

Table 4 HPLC Quantification of Bioactive Compounds in Ethanolic Seed Extract of *Vigna mungo*

Compound	Method	Content (%)
Gallic Acid	HPLC	0.04
Genistein	HPLC	0.61

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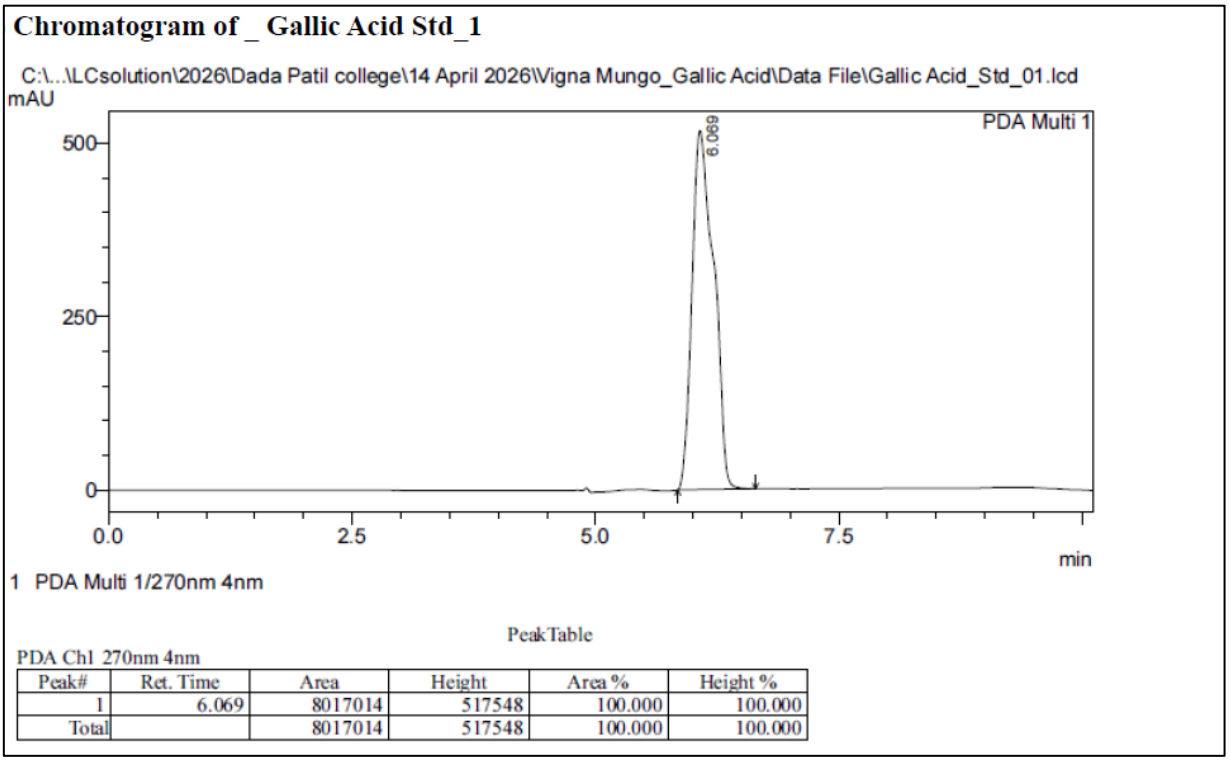
Representative HPLC chromatograms of gallic acid and genistein standards along with the corresponding *Vigna mungo* seed extract chromatograms are presented in Figures 2–5. Comparison of retention profiles confirmed the presence of gallic acid and genistein in the ethanolic seed extract. Quantitative analysis revealed gallic acid and genistein contents of 0.04% and 0.61%, respectively. The occurrence of these bioactive compounds, particularly genistein, a phytoestrogenic isoflavone, may provide a phytochemical basis for the traditional use of *Vigna mungo* in promoting vitality, reproductive wellness and potential fertility enhancement, although direct fertility-related effects were not evaluated in the present study.

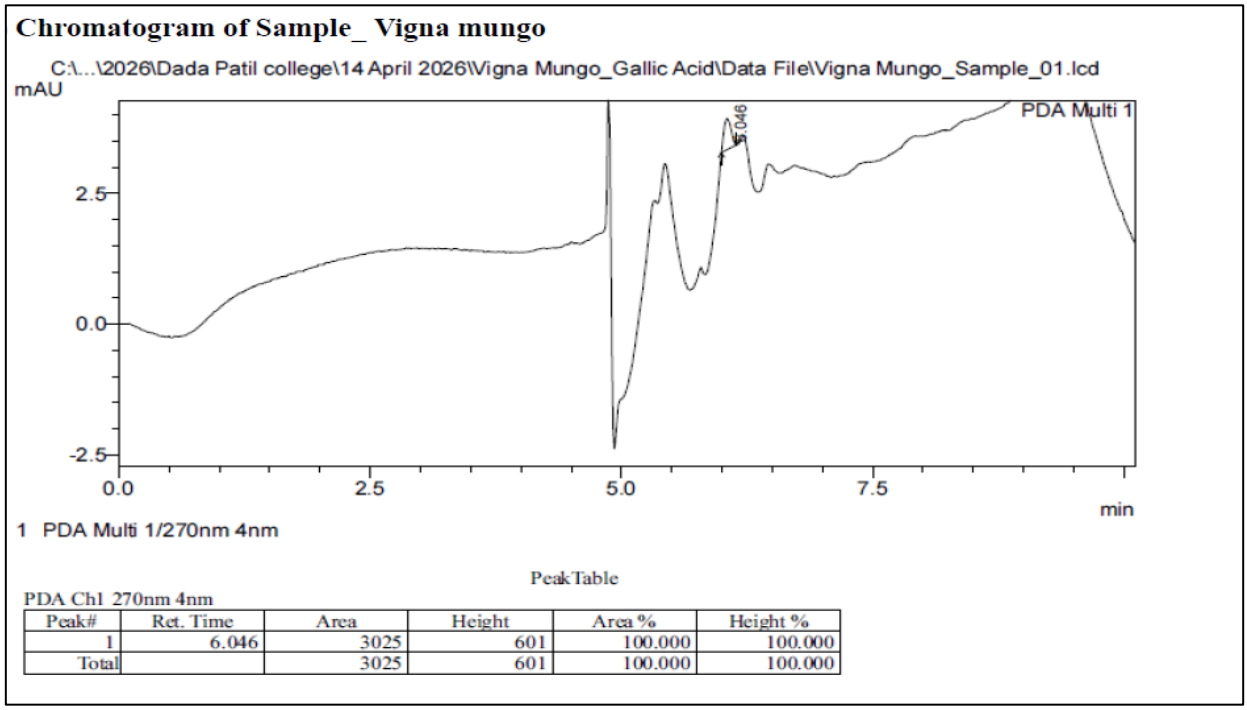


PeakTable

PDA Ch1 270nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.069	8017014	517548	100.000	100.000
Total		8017014	517548	100.000	100.000

Fig 2 HPLC Chromatogram of Gallic Acid Standard Used for Identification and Quantification of Gallic Acid.



PeakTable

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Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.046	3025	601	100.000	100.000
Total		3025	601	100.000	100.000

Fig 3 HPLC Chromatogram of Ethanolic Seed Extract of *Vigna mungo* Showing the Presence of Gallic Acid (0.04%) and Other Phytochemical Constituents.

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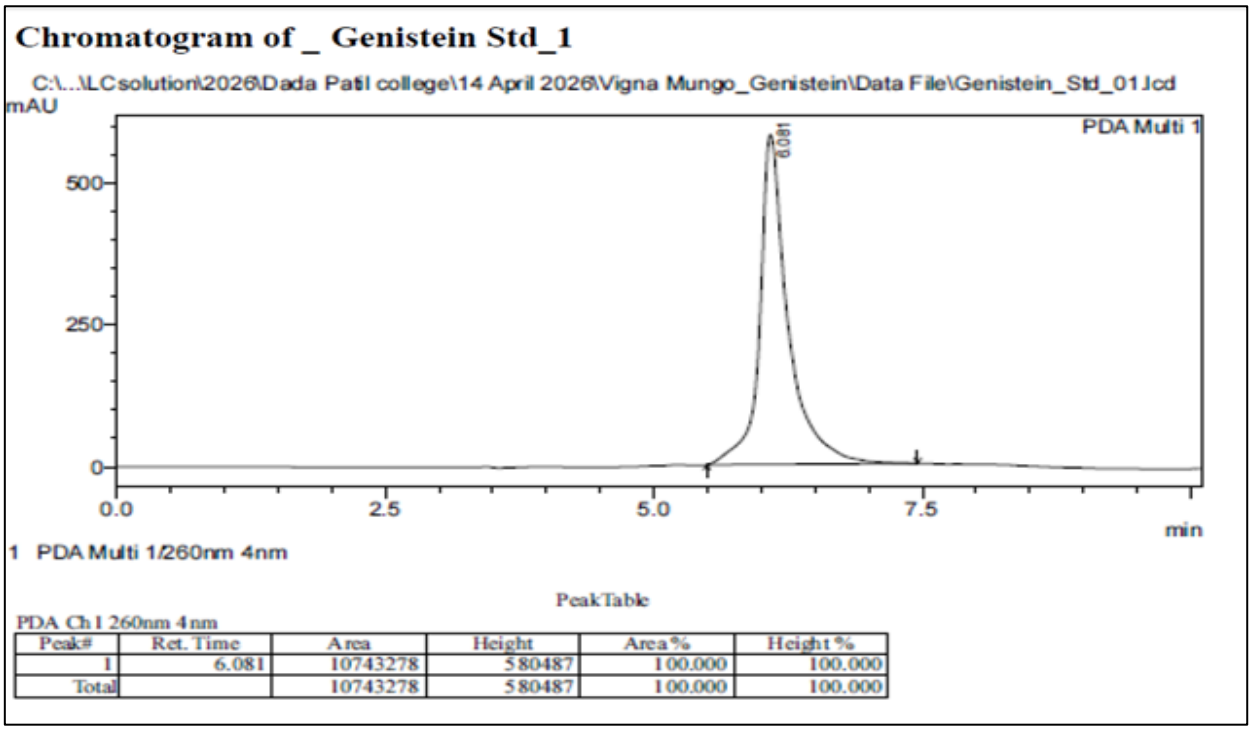
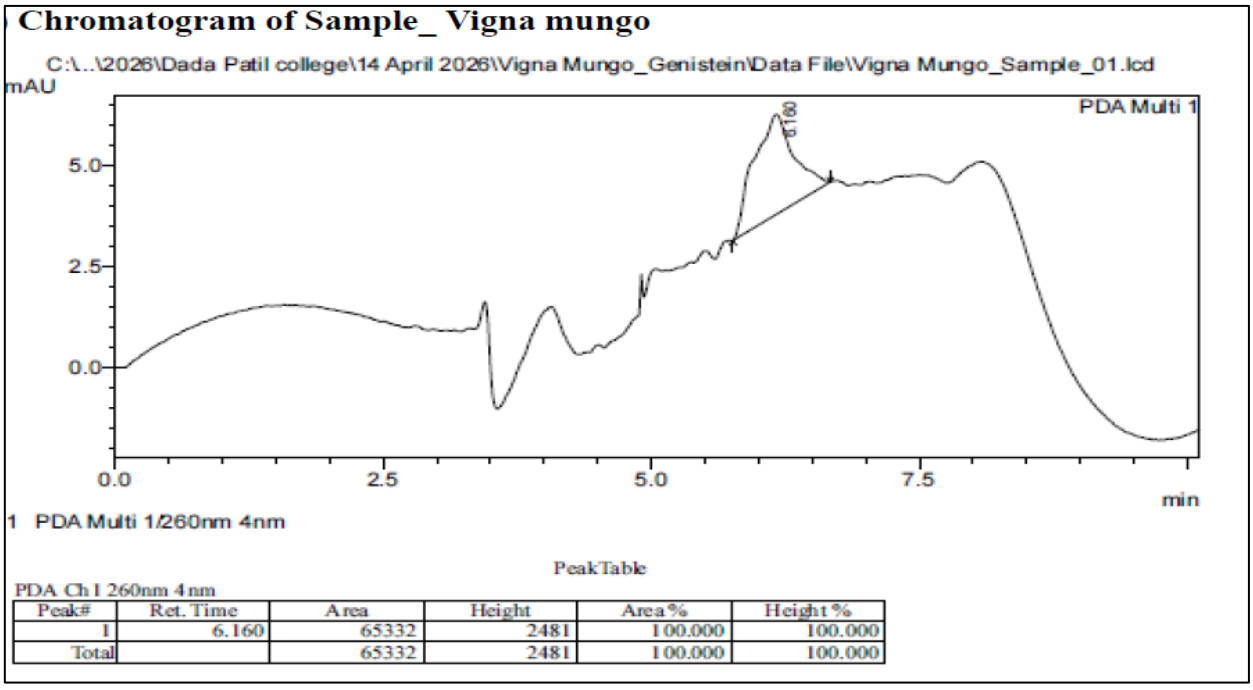


PeakTable

PDA Ch1 260nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.081	10743278	580487	100.000	100.000
Total		10743278	580487	100.000	100.000

Fig 4 HPLC Chromatogram of Genistein Standard Used for Identification and Quantification of Genistein.



PeakTable

PDA Ch1 260nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.160	65332	2481	100.000	100.000
Total		65332	2481	100.000	100.000

Fig 5 HPLC Chromatogram of Ethanolic Seed Extract of *Vigna mungo* Showing the Presence of Genistein (0.61%).

Gallic acid is a naturally occurring phenolic acid known for its antioxidant, anti-inflammatory and cytoprotective activities. Although detected in a relatively lower concentration, its presence contributes to the overall phenolic profile and antioxidant potential of the extract. Genistein, an isoflavone commonly found in legumes, has attracted significant scientific interest because of its antioxidant and phytoestrogenic properties. The relatively higher concentration of genistein observed in the present study suggests that *Vigna mungo* seeds may serve as a valuable dietary source of biologically active isoflavones.

The HPLC findings support the results obtained from the total phenolic content and DPPH antioxidant assays, indicating that the antioxidant activity of the extract may be associated with the presence of these phytochemical constituents. Furthermore, the detection of genistein provides preliminary phytochemical evidence supporting the traditional use of *Vigna mungo* as a nutritive and vitality-promoting food. Since genistein has been reported to interact with estrogen receptors and influence reproductive physiology under specific conditions, its occurrence in the seed extract may be of interest in future studies investigating

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fertility enhancement and reproductive health. However, direct fertility-related effects were not evaluated in the present investigation.

IV. DISCUSSION

The present investigation evaluated the phytochemical composition and antioxidant potential of the ethanolic seed extract of *Vigna mungo* (L.) Hepper using spectrophotometric methods and HPLC analysis. The results demonstrated that the extract contained measurable quantities of total phenolic compounds, exhibited free radical scavenging activity, and contained the bioactive marker compounds gallic acid and genistein. These observations support the medicinal importance of *Vigna mungo* and provide baseline phytochemical information for future pharmacological investigations [4,31].

The presence of phenolic compounds may also have significance in reproductive health. Oxidative stress is known to adversely affect sperm viability, sperm motility, oocyte quality and hormonal balance. Phenolic antioxidants can neutralize reactive oxygen species and protect reproductive cells from oxidative damage. Therefore, the phenolic constituents detected in *Vigna mungo* may contribute indirectly to fertility enhancement through antioxidant-mediated protection of reproductive tissues [16, 17,39].

➢ *Total Phenolic Content*

Phenolic compounds constitute one of the largest groups of plant secondary metabolites and are well known for their antioxidant properties [20, 31]. The total phenolic content of the ethanolic seed extract of *Vigna mungo* was found to be 3.28 ± 0.04 mg GAE/g extract as determined by the Folin–Ciocalteu method, indicating the presence of appreciable quantities of phenolic constituents.

Phenolic compounds protect biological systems through several mechanisms including hydrogen atom donation, electron transfer, metal ion chelation and inhibition of lipid peroxidation. These compounds are capable of neutralizing reactive oxygen species (ROS), thereby minimizing oxidative damage to proteins, lipids and nucleic acids [20, 32].

The moderate phenolic content observed in the present investigation agrees with previous reports indicating that *Vigna mungo* seeds contain phenolic acids and flavonoids contributing to their antioxidant activity [4, 8, 33]. Environmental conditions, cultivar, geographical origin, maturity stage and extraction solvent may influence the concentration of phenolic compounds in legumes [34].

The presence of phenolic compounds may also have significance in reproductive health. Oxidative stress is known to adversely affect sperm viability, sperm motility, oocyte quality and hormonal balance. Phenolic antioxidants can neutralize reactive oxygen species and protect reproductive cells from oxidative damage. Therefore, the phenolic constituents detected in *Vigna mungo* may contribute indirectly to fertility enhancement through antioxidant-mediated protection of reproductive tissues [16, 17, 39].

➢ *Antioxidant Activity*

The DPPH radical scavenging assay is one of the most widely accepted methods for evaluating antioxidant potential of plant extracts because it is rapid, sensitive and reproducible [22,29].The ethanolic seed extract exhibited 21.43% radical scavenging activity, demonstrating moderate antioxidant potential.

The antioxidant activity observed may be attributed to the presence of phenolic acids and flavonoids capable of donating hydrogen atoms to stabilize DPPH free radicals [20,32].Several investigations have reported a positive relationship between total phenolic content and antioxidant activity in legumes [33,35]. Although the antioxidant activity observed in the present study was moderate, it confirms that *Vigna mungo* seeds possess compounds capable of reducing oxidative stress [4, 35].

Antioxidant activity is closely associated with reproductive health because excessive oxidative stress has been implicated in both male and female infertility. The moderate DPPH radical scavenging activity observed in the present study suggests that *Vigna mungo* seeds possess the ability to reduce oxidative stress. Such antioxidant protection may help maintain sperm membrane integrity, DNA stability, ovarian follicular development and endocrine function, thereby supporting reproductive health and potential fertility enhancement [16, 18, 39].

➢ *HPLC Quantification of Bioactive Compounds*

HPLC analysis confirmed the presence of two important phytochemical marker compounds-Gallic acid (0.04%) and Genistein (0.61%). The identification of these compounds enhances the phytochemical characterization of *Vigna mungo* seeds [23, 24].

- *Gallic Acid*

Gallic acid is an important naturally occurring phenolic acid possessing strong antioxidant, antimicrobial, anti-inflammatory and anticancer properties [10, 12]. It effectively scavenges free radicals and inhibits oxidative damage to cellular components [12, 36].

Although the quantity detected in the present study was relatively low, the presence of gallic acid confirms the occurrence of bioactive phenolic constituents within the ethanolic extract.

- *Genistein*

Genistein is one of the major isoflavones naturally present in legumes. It exhibits numerous pharmacological properties including antioxidant, anti-inflammatory, anti-diabetic and cardioprotective activities [11, 13].

The concentration of genistein (0.61%) was considerably higher than that of gallic acid, suggesting that

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isoflavones may represent one of the major classes of bioactive constituents in *Vigna mungo* [11, 37].

Gallic acid has been reported to protect reproductive tissues against oxidative injury and may improve cellular defense mechanisms through its antioxidant activity. Previous studies suggest that gallic acid can reduce lipid peroxidation and improve antioxidant enzyme status, which may be beneficial in maintaining reproductive function [12, 36].

- *Relationship between Phenolic Content, Antioxidant Activity and HPLC Findings*

The three experimental approaches employed in the present investigation complement one another.

The Folin–Ciocalteu assay demonstrated the presence of phenolic compounds.
↓
The DPPH assay confirmed antioxidant potential.
↓
HPLC identified specific phytochemical markers responsible for these biological activities.

Thus, the combined analytical approach provides stronger evidence regarding the phytochemical quality of *Vigna mungo* than any single analytical technique alone [23, 24, 38].

From a reproductive health perspective, the combined occurrence of phenolic compounds, antioxidant activity and fertility-related phytochemicals such as genistein provides a scientific basis for the traditional belief that *Vigna mungo* contributes to vitality and reproductive wellness. The antioxidant protection offered by these compounds may help reduce oxidative stress-associated reproductive dysfunction [6, 7, 16].

- *Possible Relevance to Reproductive Health*

Oxidative stress is recognized as an important factor contributing to reproductive dysfunction in both males and females [16,17]. Excessive production of reactive oxygen species may impair sperm membrane integrity, reduce sperm motility, damage DNA, disturb ovarian follicular development and alter endocrine regulation [18,39].Natural antioxidants have therefore attracted considerable attention as supportive agents for maintaining reproductive health [16,39].

The present investigation demonstrated that *Vigna mungo* possesses Phenolic compounds, Antioxidant activity, Gallic acid and Genistein. These phytochemicals have been investigated by other researchers for their potential biological roles. Gallic acid has been reported to reduce oxidative stress and protect cells against oxidative injury [12,36].

Genistein is a naturally occurring phytoestrogen that has been studied for its interaction with estrogen receptors and its possible influence on reproductive physiology [11,14]. Experimental studies have suggested that genistein may affect hormone signaling and reproductive tissues under specific conditions [15, 40]. However, these effects depend on dose, exposure duration, species, and physiological context.

Collectively, the presence of phenolic antioxidants, measurable radical scavenging activity, gallic acid and genistein suggests that *Vigna mungo* may possess fertility-supportive properties through mechanisms involving oxidative stress reduction and modulation of reproductive physiology. While the present study does not provide direct evidence of fertility enhancement, the phytochemical profile obtained offers a strong scientific rationale for future in vivo investigations focusing on reproductive performance, hormonal regulation and fertility-related biomarkers [11, 16, 39, 40].

Nevertheless, the presence of antioxidant phytochemicals together with measurable antioxidant activity indicates that *Vigna mungo* deserves further investigation using animal models and clinical studies to determine whether these phytochemicals contribute to reproductive health [4,11,40].

V. CONCLUSION & FUTURE SCOPE

The present study demonstrated that the ethanolic seed extract of *Vigna mungo* possesses measurable antioxidant potential supported by its phenolic composition and HPLC-based phytochemical profile.

The total phenolic content was found to be 3.28 mg GAE/g extract, while the DPPH assay revealed 21.43% radical scavenging activity. HPLC analysis confirmed the presence of gallic acid (0.04%) and genistein (0.61%), indicating that the seeds contain biologically important phenolic and isoflavonoid compounds.

These findings support the nutritional and medicinal value of *Vigna mungo* and provide baseline phytochemical information for future quality control and pharmacological research. Based on published literature, the identified antioxidant compounds may have relevance to reproductive health through reduction of oxidative stress; however, dedicated in vivo and clinical studies are necessary before any fertility-enhancing effect can be established.

The detection of genistein together with antioxidant phenolic compounds provides preliminary scientific support for the traditional use of *Vigna mungo* in promoting vitality and reproductive health, warranting further fertility-oriented investigations.

- *Future Scope*

The findings of the present study provide a foundation for further research on the phytochemical and pharmacological potential of *Vigna mungo*. Future investigations may focus on the isolation and purification of individual bioactive compounds responsible for the observed antioxidant activity. Advanced analytical techniques such as LC–MS/MS can be employed for

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comprehensive characterization and identification of phytochemical constituents. Further studies should also evaluate the anti-inflammatory, antimicrobial, and other therapeutic properties of the identified compounds. In vivo antioxidant studies are necessary to validate the biological efficacy of the extract under physiological conditions.

Additionally, toxicological assessments should be conducted to establish the safety profile of the extract for long-term use. Since the presence of genistein and other antioxidant phytochemicals suggests possible relevance to reproductive health, well-designed experimental studies involving animal models and clinical investigations are required to evaluate their effects on fertility-related parameters. Furthermore, the development of standardized herbal formulations based on *Vigna mungo* seed extract may contribute to the utilization of this nutritionally and medicinally valuable legume in pharmaceutical and nutraceutical applications.

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- *Conflict of Interest*

The author declares that there is no conflict of interest regarding the publication of this research.

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