

Phytochemical Screening and Multi-Solvent Extraction of a Synergistic Polyherbal Formulation of *Celosia cristata* and *Clitoria ternatea* Intended for Antidiabetic Research

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ABSTRACT

The growing global prevalence of diabetes mellitus has intensified the search for safer and more effective plant-based therapeutic agents. Polyherbal formulations have gained considerable attention due to their synergistic therapeutic potential, enhanced efficacy, and reduced adverse effects compared to single-herb preparations. The present study focuses on the phytochemical characterization and chromatographic profiling of synergistic polyherbal extracts prepared from *Celosia cristata* and *Clitoria ternatea*, two medicinal plants traditionally recognized for their diverse pharmacological properties. Different solvent extracts of the selected plant materials were prepared using standard extraction procedures, and the extracts were subjected to preliminary phytochemical screening for the identification of major bioactive constituents. The qualitative analysis revealed the presence of flavonoids, alkaloids, phenolic compounds, tannins, glycosides, saponins, and carbohydrates in varying proportions. Among the investigated extracts, the ethanolic extract demonstrated a higher abundance of phytoconstituents and was therefore selected for further chromatographic evaluation. The findings of the present investigation suggest that the synergistic polyherbal combination of *Celosia cristata* and *Clitoria ternatea* possesses significant phytochemical diversity that may contribute to prospective antidiabetic applications. Furthermore, the study provides a scientific basis for the standardization and future biological evaluation of the developed polyherbal formulation.

Keywords: Synergism, polyherbal extract, multi-solvents, phytochemical screening, *Celosia cristata*, *Clitoria ternatea*, herbal standardization, antidiabetic potential.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from impaired insulin secretion, insulin resistance, or both. The increasing incidence of diabetes worldwide has become a major public health concern due to its association with severe complications such as nephropathy, neuropathy, retinopathy, and cardiovascular disorders. Although several synthetic antidiabetic drugs are currently available, long-term therapy is often associated with adverse effects, reduced patient compliance, and economic burden.

Consequently, there has been growing interest in the exploration of medicinal plants and polyherbal formulations as alternative therapeutic approaches for diabetes management. [6-7]

Medicinal plants contain a wide variety of phytochemicals including flavonoids, alkaloids, phenolics, tannins, glycosides, and terpenoids that possess important pharmacological activities [16-17]. In recent years, polyherbal formulations have attracted significant scientific attention because the combination of multiple plant extracts may produce

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synergistic therapeutic effects [18]. Synergism in herbal medicine refers to the enhanced biological activity achieved through the interaction of bioactive compounds from different plants, leading to improved efficacy and broader pharmacological action compared to individual herbal extracts [24-27].

Celosia cristata is a medicinal plant traditionally used in various systems of medicine for its antioxidant, anti-inflammatory, hepatoprotective, and antimicrobial properties. The plant is reported to contain bioactive constituents such as flavonoids, phenolic compounds, saponins, and alkaloids, which may contribute to its therapeutic potential. Similarly, *Clitoria ternatea* is widely recognized for its medicinal importance and has been extensively studied for antioxidant, neuroprotective, antimicrobial, and antidiabetic activities [2-3]. The plant is rich in anthocyanins, flavonoids, tannins, and other secondary metabolites known for their pharmacological significance.

The combination of these two medicinal plants in a synergistic polyherbal formulation may enhance phytochemical diversity and improve therapeutic efficacy through complementary mechanisms of action [9]. However, scientific standardization and phytochemical characterization are essential to ensure the quality, consistency, and reproducibility of herbal formulations. Preliminary phytochemical screening provides valuable information regarding the presence of bioactive constituents [19].

Therefore, the present study was designed to investigate the phytochemical composition of synergistic polyherbal extracts prepared from *Celosia cristata* and *Clitoria ternatea*. The study aims to establish preliminary scientific evidence for the phytochemical richness of the developed polyherbal formulation, thereby providing a foundation for future pharmacological and antidiabetic investigations.

2. Materials and Methods

2.1 Sample collection and Authentication

The flowers of *Celosia cristata* and *Clitoria ternatea* were selected for the present study based on their

traditional medicinal importance and reported pharmacological activities. *Celosia cristata*, commonly known as Cockscomb (figure-2.1.1), is recognized for its antioxidant, anti-inflammatory, antimicrobial, and potential antidiabetic properties due to the presence of flavonoids, alkaloids, phenolics, and glycosides [21-23]. *Clitoria ternatea*, commonly referred to as Butterfly Pea (figure-2.1.2), is widely known for its rich anthocyanin and flavonoid content and has been traditionally used for antioxidant, neuroprotective, and antidiabetic application [4-5]. Fresh flowers of both plants were collected from local sources and authenticated by the Department of Botany, College of Sciences, Sri Venkateswara University, Tirupati, as per SVU herbarium records. The authenticated specimens were preserved, and voucher numbers **SVUH:1055** (*Clitoria ternatea* L.) and **SVUH:1056** (*Celosia cristata* L.) were assigned for future references.

Figure 2.1.1.



Botanical Information

- a) Scientific name: *Celosia cristata*
- b) Family: Amaranthaceae
- c) Common name: Cockscomb flower
- d) Flower colours: Red, pink, yellow, orange, purple
- e) Characteristic feature: Velvet-like crest resembling a rooster comb

Figure 2.1.2.



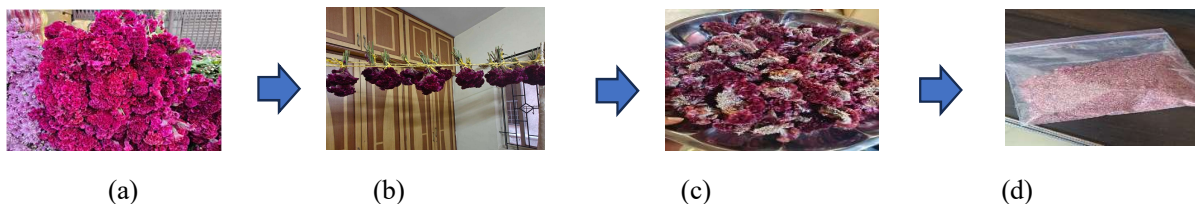
Botanical Information

- a) Scientific name: *Clitoria ternatea*
- b) Family: Fabaceae
- c) Common name: Butterfly pea flower
- d) Flower colour: Bright blue, violet, white, or purple coloured flower
- e) Characteristic feature: Funnel-shaped, resembles the shape of a butterfly

2.2 Sample preparation

The collected flowers were washed thoroughly with distilled water to remove dust and foreign particles, followed by shade drying at room temperature (fig 2.2.1, 2.2.2) for approximately 10–15 days, as represented in (table-2.2.3). The dried flowers were pulverized separately using a mechanical grinder to obtain coarse powder and stored in airtight containers for further experimental studies.

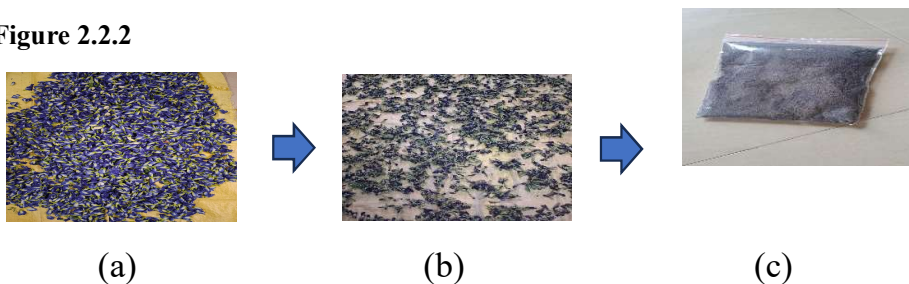
Figure 2.2.1.



Where;

- a) Fresh flowers of *Celosia cristata* were collected.
- b) *Celosia cristata* hung upside down to retain phytoconstituents.
- c) *Celosia cristata* was dried in shade at room temperature.
- d) Triturated and made into the powder.

Figure 2.2.2



Where;

- a) Fresh flowers of *clitoria ternatea* were collected.

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- b) *clitoria ternatea* was dried in shade at room temperature. 70% of moisture got lost without fungal growth.
- c) Triturated and made into the powder.

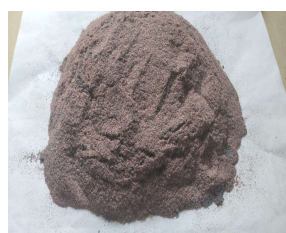
Table- 2.2.3

DAY	<i>CELOSIA CRISTATA</i> + PROCESS OBSERVATION	<i>CLITORIS TERNATEA</i> + PROCESS OBSERVATION	CHANGES OBSERVED
1-5	Fresh flowers hung upside down[1000g] – 50% moisture loss[520g]	Fresh petals spread on cloth[1000g] – 50% moisture loss[560g]	- High moisture with shade drying. - Noticeable shrinkage, 40-55% water loss
6-10	70% dry[400g-280g]	70% dry[430g-320g]	No fungal growth
11-15	Final dry weight: 200g	Final dry weight: 240g	Stable dry matter retained

2.3 Solvents used for Extraction: Analytical grade solvents including petroleum ether, ethanol, ethyl acetate, methanol, and distilled water were used for extraction procedures. Standard reagents required for phytochemical screening were procured from authorized laboratory suppliers.

2.4 Method used

The extraction of powdered flower materials was carried out individually using the **maceration** technique with five different solvents of varying polarity, namely petroleum ether, ethanol, ethyl acetate, methanol, and distilled water [10-12]. This maceration is done both individually each and on combination by using multi-solvents.



flower powder



Maceration process



filtering the extracted residue

Natural

2.5 Procedure

Approximately 100 g of dried powdered flower material from each plant was mixed separately with the respective solvent in a ratio of 1:5 (drug: solvent ratio), where 100 g of powder was immersed in 500 mL of

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solvent in clean, airtight conical flasks. The mixtures were kept at room temperature for 72 hours with intermittent shaking to ensure maximum penetration of solvent into the plant material and effective extraction of phytoconstituents.

After completion of maceration, the extracts were filtered using muslin cloth followed by Whatman No.1 filter paper to remove plant residues. The concentrated extracts were dried, weighed, and stored in airtight containers under refrigerated conditions for further analysis. The percentage yield of each extract was represented in table 2.5.1. and graphically represented in 2.5.2., and calculated using the following formula:

$$\text{Percentage Yield} = \frac{\text{Weight of Dried Extract}}{\text{Weight of Powdered Drug}} \times 100$$

Preparation of Synergistic Polyherbal Extract: The synergistic polyherbal extract was prepared by combining the individual extracts of *Celosia cristata* and *Clitoria ternatea* in equal proportions (1:1 ratio) in 500ml of solvent. The combination was prepared separately for each solvent extract to evaluate the synergistic phytochemical influence of the polyherbal formulation [8-9].

The prepared polyherbal mixtures were homogenized thoroughly to ensure uniform distribution of phytoconstituents. The combined extracts were then subjected to phytochemical screening along with the individual extracts for comparative evaluation.

3. Qualitative Phytochemical screening

Qualitative phytochemical screening was carried out to identify the presence of major secondary metabolites in the extracts obtained using different solvents [13-14]. The analysis helps in determining the bioactive constituents responsible for pharmacological activities, particularly antidiabetic potential [15].

The extracts prepared from petroleum ether, ethyl acetate, methanol, ethanol, and aqueous solvents were subjected to standard phytochemical tests for alkaloids, flavonoids, phenolics, tannins, glycosides, saponins, terpenoids, steroids, carbohydrates, and proteins [20,25] are represented in (Table 3.1,3.2,3.3)

3.1 Preparation of Test solution

Here the extract was dissolved in its respective solvent and filtered (i.e.; for example, 100mg ethanol crude extract powder dissolved in 10ml ethanol. Then filter the residue and from that use 1ml of the filtrate for phytochemical test). The filtrate obtained was used for phytochemical analysis.

Test for Alkaloids

Mayer's Test

Procedure

- To 1 mL of extract, a few drops of Mayer's reagent were added carefully along the sides of the test tube.

Observation

- Formation of a cream or pale-yellow precipitate indicates the presence of alkaloids.

Wagner's Test

Procedure

- To 1 mL of extract, few drops of Wagner's reagent were added.

Observation

- Formation of reddish-brown precipitate confirms the presence of alkaloids.

Test for Flavonoids

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Shinoda Test

Procedure

- To 1 mL of extract, a small quantity of magnesium ribbon was added followed by a few drops of concentrated hydrochloric acid.

Observation

- Development of pink, orange, or red coloration confirms the presence of flavonoids.

Alkaline Reagent Test

Procedure

- To 1 mL of extract, 2 mL of 2% sodium hydroxide solution was added. Subsequently, dilute hydrochloric acid was added.

Observation

- Formation of intense yellow colour that becomes colourless after addition of acid indicates the presence of flavonoids.

Test for Phenolic compounds

Ferric chloride Test

Procedure

- A few drops of 5% ferric chloride solution were added to 1 mL of extract.

Observation

- Appearance of dark blue, green, or black coloration indicates phenolic compounds.

Test for Tannins

Procedure

Lead Acetate Test

Procedure

- To 1 mL of extract solution, few drops of 10% lead acetate solution were added.

Observation

- Formation of white or yellow precipitate indicates the presence of tannins.

Test for Saponins

Foam Test

Procedure

- The extract was diluted with distilled water and shaken vigorously for 2–3 minutes.

Observation

- Persistent froth formation indicates the presence of saponins.

Test for Glycosides

Keller–Killiani Test

Procedure

- To the extract, glacial acetic acid containing ferric chloride was added followed by concentrated sulfuric acid carefully along the wall of the test tube.

Observation

- Formation of a brown ring at the interface confirms glycosides.

Test for Terpenoids

Salkowski Test

Procedure

- The extract was mixed with chloroform followed by careful addition of concentrated sulfuric acid.

Observation

- A reddish-brown coloration at the interface indicates terpenoids.

Test for Steroids

Liebermann–Burchard Test

Procedure

- To 1 mL of extract, 2 mL of acetic anhydride and few drops of concentrated sulfuric acid were added.

Observation

Appearance of bluish-green coloration indicates the presence of steroids.

Test for Carbohydrates

Benedict's Test

Procedure

- Benedict's reagent was added to the extract and heated in a water bath.

Observation

- Formation of reddish-brown precipitate indicates carbohydrates.

Test for Proteins

Biuret Test

Procedure

- To the extract, Biuret reagent was added and mixed thoroughly.

Observation

- Appearance of violet or purple coloration indicates proteins.

Quantitative Phytochemical screening

The quantitative phytochemical screening was performed by determining total phenolic content (TPC) and total flavonoid content (TFC) of the extracts [41]. The TPC and TFC of the extracts were expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of extracts and milligrams quercetin equivalent per gram (mg QE/g) of extracts, respectively.

Determination of TPC Content

Folin–Ciocalteu reagent was used for the determination of total phenolic content. 1 ml of each extract, Folin–Ciocalteu reagent (5 ml) and aqueous sodium carbonate (4 ml) solution were mixed together. The mixture was allowed to stand in the dark for 15 min at room temperature, and the absorbance at 765 nm was measured with the help of ultraviolet (UV-visible) spectrophotometer. Then, the total polyphenolic content was determined in terms of mg GAE/g of dry weight of the extract with the help of a calibration curve prepared with a series of gallic acid standards (10-80 µg/ml).

Determination of TFC Content

1 ml of each extract was separately mixed with 1.5 ml methanol and 0.1 ml aluminium trichloride (10%). Then, 0.1 ml of 1 M potassium acetate and 2.8 ml distilled water was added into each test tube. Then, absorbance at 415 nm was measured after it was allowed to stand in the dark for 30 min using a UV-visible spectrophotometer. Finally, a calibration curve was prepared with a series of quercetin standards and the total flavonoid compound concentration was determined in terms of mg QE/g of the extract.

4. Results and Discussion

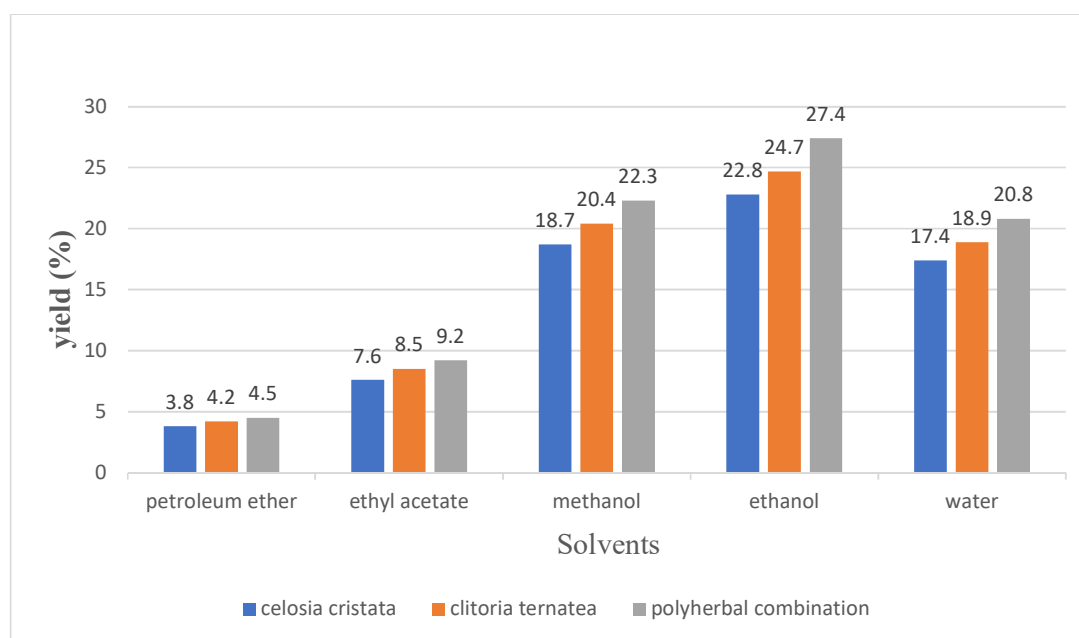
Table 2.5.1. Extraction yield percentage for CC And CT (Individual vs Polyherbal) by using different solvents

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Solvent	<i>Celosia cristata</i> (%)	<i>Clitoria ternatea</i> (%)	Polyherbal Combination (%)
Petroleum ether	3.8	4.2	4.5
Ethyl acetate	7.6	8.5	9.2
Methanol	18.7	20.4	22.3
Ethanol	22.8	24.7	27.4
Water	17.4	18.9	20.8

2.5.2. Graphical Representation

Extraction yield (%) of *Celosia cristata*, *Clitoria ternatea* and Polyherbal Extracts (Different Solvents)



Among all the solvents investigated, ethanol produced the highest extraction yield for both individual and polyherbal extracts. The ethanolic extract of *Celosia cristata* showed a yield of 22.8%, whereas *Clitoria ternatea* exhibited 24.7% yield. The synergistic polyherbal ethanolic extract demonstrated the maximum yield of approximately 27.4%, indicating enhanced extraction efficiency when both plant materials were combined. This observation suggests that ethanol possesses superior ability to dissolve a wide spectrum of phytoconstituents including flavonoids, phenolics, alkaloids, glycosides, and tannins.

The higher extraction yield observed in the polyherbal formulation compared to the individual extracts may be attributed to synergistic interaction between phytoconstituents of both plants, resulting in improved solubility and extraction of bioactive compounds. Overall, the findings indicate that ethanol is the most suitable solvent for obtaining phytochemically rich extracts from the selected medicinal flowers.

Qualitative Phytochemical representation of CC and CT individually each and on combination

3.1. *Celosia Cristata* (individual extract)

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Phytochemical	Pet Ether	Ethyl Acetate	Methanol	Ethanol	Water
Alkaloids	–	+	++	+++	++
Flavonoids	–	++	++	+++	++
Phenols	–	+	++	+++	++
Tannins	–	+	++	+++	++
Saponins	–	–	+	++	+++
Glycosides	–	+	++	+++	++
Terpenoids	+	++	+	+	–
Steroids	+	+	–	–	–
Carbohydrates	–	+	++	++	+++
Proteins	–	–	+	+	++

3.2. *Clitoris ternatea* (individual extract)

Phytochemical	Pet Ether	Ethyl Acetate	Methanol	Ethanol	Water
Alkaloids	–	+	++	+++	++
Flavonoids	–	++	+++	+++	++
Phenols	–	+	++	+++	++
Tannins	–	+	++	+++	++
Saponins	–	–	+	++	+++
Glycosides	–	+	++	+++	++
Terpenoids	+	++	+	+	–
Steroids	+	+	–	–	–

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Carbohydrates	–	+	++	++	+++
Proteins	–	–	+	+	++

3.3 Polyherbal Extract (Combination of both the plants)

Phytochemical	Pet Ether	Ethyl Acetate	Methanol	Ethanol	Water
Alkaloids	+	++	+++	+++	+++
Flavonoids	+	++	+++	+++	+++
Phenols	+	++	+++	+++	+++
Tannins	+	++	+++	+++	+++
Saponins	–	+	++	+++	+++
Glycosides	+	++	+++	+++	+++
Terpenoids	++	++	+	+	–
Steroids	++	+	–	–	–
Carbohydrates	+	++	+++	+++	+++
Proteins	–	–	+	+	++

The present investigation demonstrated that solvent polarity plays a crucial role in the extraction efficiency and phytochemical composition of herbal extracts. Polar solvents such as ethanol and methanol extracted higher amounts of phytoconstituents compared to non-polar solvents like petroleum ether. Among all the extracts studied, the ethanolic polyherbal extract showed the highest extraction yield and strongest phytochemical positivity, indicating its suitability for further chromatographic profiling and future antidiabetic studies.

The abundant presence of flavonoids, phenolic compounds, tannins, alkaloids, and glycosides in the polyherbal formulation suggests possible synergistic therapeutic potential. These phytoconstituents are well known for antioxidant activity, free radical scavenging ability, pancreatic β -cell protection, inhibition of carbohydrate metabolizing enzymes, and enhancement of glucose uptake, which collectively may contribute toward antidiabetic effects.

Quantitative phytochemical screening

TPC of the ethanolic polyherbal combination yields 90.8 ± 1.4 mg GAE/g and TFC of the ethanolic polyherbal combination yields 83.5 ± 1.5 mg QE/g. These concentrations are marked higher than the values recorded for

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individual *Celosia cristata* (TPC: 65.7 ± 1.5 ; TFC: 54.2 ± 1.4) and *Clitoria ternatea* (TPC: 73.5 ± 1.3 ; TFC: 63.8 ± 1.6) extracts.

Therefore, the present study scientifically supports the use of the synergistic polyherbal combination of *Celosia cristata* and *Clitoria ternatea* as a promising candidate for further chromatographic characterization, and pharmacological evaluation in antidiabetic research.

5. Conclusion

The present study successfully established the phytochemical standardization and preliminary characterization of a synergistic polyherbal formulation prepared from *Celosia cristata* and *Clitoria ternatea* using different solvents of varying polarity. Previous studies have reported that medicinal plants rich in flavonoids, phenolics, alkaloids, tannins, and glycosides possess significant antioxidant and antidiabetic potential, thereby supporting the therapeutic relevance of the selected medicinal flowers. The extraction study demonstrated that solvent polarity plays a major role in the recovery of phytoconstituents. Among all solvents used, ethanol exhibited the highest extraction yield in both individual and polyherbal extracts, indicating its superior efficiency in extracting bioactive secondary metabolites. Similar findings have been reported in earlier studies where ethanolic extracts showed enhanced phytochemical recovery and biological activity due to their ability to dissolve both moderately polar and polar compounds.

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins, carbohydrates, terpenoids, steroids, and proteins in varying intensities. The ethanolic and methanolic extracts demonstrated stronger phytochemical positivity compared to petroleum ether extracts, suggesting that polar solvents are more effective for extraction of therapeutically active compounds. Similar phytochemical profiles have been previously reported for *Clitoria ternatea* and related medicinal plants possessing antioxidant and antidiabetic properties. The synergistic polyherbal formulation showed comparatively enhanced phytochemical richness when compared with the individual extracts. The increased intensity of flavonoids, phenolics, alkaloids, tannins, and glycosides in the combined extract suggests possible synergistic interactions between phytoconstituents of both plants. Such

synergism in polyherbal formulations has been widely recognized for improving therapeutic efficacy and broadening pharmacological action. The abundant presence of flavonoids and phenolic compounds in the polyherbal extract may contribute to antioxidant activity, free radical scavenging ability, and protection against oxidative stress associated with diabetes mellitus. Similarly, alkaloids and glycosides may support glucose-lowering mechanisms through multiple complementary pathways. Previous investigations on *Clitoria ternatea* have demonstrated significant antioxidant and antidiabetic activity associated with these phytoconstituents.

Overall, the findings of the present investigation provide scientific evidence supporting the phytochemical richness and synergistic potential of the developed polyherbal formulation. The study establishes ethanol as the most suitable solvent for extraction and provides a strong foundation for future chromatographic profiling, antioxidant evaluation, and antidiabetic pharmacological studies of the synergistic formulation. Therefore, the polyherbal combination of *Celosia cristata* and *Clitoria ternatea* may be considered a promising herbal candidate for future antidiabetic drug development and herbal standardization research.

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