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EVALUATION OF PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF *AMOMUM SUBULATUM, ELETTARIA CARDAMOMUM* AND *MOMORDICA CHIRANTIA* AND THEIR COMBINATION

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ABSTRACT

This study investigates the phytochemical composition and in vitro antidiabetic activity of ethanolic extracts of *Amomum subulatum*, *Elettaria cardamomum*, and *Momordica charantia*, along with their combination. The percentage yield of each extract was recorded, and phytochemical screening revealed the presence of various bioactive constituents, including alkaloids, flavonoids, phenolics, saponins, and proteins. In vitro α-amylase inhibition assays were performed to assess antidiabetic potential. Among the individual extracts, *Momordica charantia* showed the highest inhibitory activity. Notably, the combination of all three extracts in a 1:1:1 ratio demonstrated significantly enhanced α-amylase inhibition, indicating a synergistic effect. These results support the potential use of these plants, individually and in combination, as natural antidiabetic agents. Further in vivo studies and pharmacological evaluations are needed to confirm their therapeutic applicability.

Keywords: *Amomum subulatum, Elettaria cardamomum, Momordica charantia*, Phytochemical screening; α-Amylase inhibition, Antidiabetic activity, Polyherbal formulation.

INTRODUCTION

Diabetes mellitus is a prevalent metabolic disorder characterized by chronic hyperglycemia due to either inadequate insulin secretion or poor cellular response to insulin. The disease can lead to serious complications, including cardiovascular issues, kidney failure, and neuropathy (Srinivasan *et al.*, 2015). Current pharmaceutical therapies for managing diabetes include oral hypoglycemic agents, insulin therapy, and lifestyle modifications. However, the side effects and long-term efficacy of synthetic drugs have led to the exploration of alternative treatments, particularly from natural plant sources (Nimse *et al.*, 2016).

Amomum subulatum (cardamom), *Elettaria cardamomum* (green cardamom), and *Momordica charantia* (bitter melon) are three plants traditionally used in various parts of the world for their medicinal properties. Cardamom is known for its antioxidant, anti-inflammatory, and digestive properties (Liu *et al.*, 2014). *Momordica charantia* has been reported to possess antidiabetic activity through multiple mechanisms, including enhancing insulin sensitivity and increasing glucose uptake (Sharma *et al.*, 2015). *Amomum subulatum*, a close relative of cardamom, has also demonstrated various biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects (Kumar *et al.*, 2019).

Despite the potential of these plants individually, limited studies have explored the synergistic effects of their combination for managing diabetes. Combining bioactive compounds from different plants might offer enhanced therapeutic effects due to synergistic interactions. Therefore, the current study aims to evaluate the phytochemical composition, antidiabetic activity, and the synergistic potential of *Amomum subulatum*, *Elettaria cardamomum*, and *Momordica charantia* through in vitro screening. Phytochemical analysis will identify the presence of bioactive compounds, while the anti-diabetic potential will be assessed using well-established assays such as α-amylase inhibition, α-glucosidase inhibition, and glucose uptake assay in cell lines.

This study will contribute to the understanding of the pharmacological efficacy of these plants, both individually and in combination, as potential natural alternatives for managing diabetes and related complications.

MATERIALS AND METHODS

Collection of Plant materials

The plants have been selected on the basis of its availability and folk use of the plant. Fruits of *Amomum subulatum, Elettaria cardamomum,* and *Momordica charantia* were collected from local area of Bhopal.

Drying

Drying of fresh plant parts was carried out in sun but under the shade.

Storage

Dried *Amomum subulatum, Elettaria cardamomum,* and *Momordica charantia* were preserved in plastic bags, closed tightly and powdered as per the requirments.

Extraction by microwave assisted extraction technique

The shade dried plant material was coarsely powdered and subjected to extraction maceration. 50 gram of each dried powdered of *Amomum subulatum, Elettaria cardamomum,* and *Momordica charantia* has been extracted with ethanol solvent using microwave assisted extraction technique, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007).

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

1. Detection of alkaloids: Extract were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's Test: Filtrates was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extract was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

4.5 *In vitro* anti diabetic activity of *Amomum subulatum, Elettaria cardamomum,* and *Momordica charantia* by alpha amylase enzyme

10 mg acarbose was dissolved in 10 ml methanol, and various aliquots of 100-1000µg/ml were prepared in methanol (Ademiluyi and Oboh, 2013). 10 mg of dried extract was extracted with 10 ml methanol. 500 µl of this extract solution was used for the estimation of enzyme inhibition. A total of 500 µl of test samples and standard drug (100-500µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent.

The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

RESULTS AND DISCUSSION

The ethanolic extraction of *Amomum subulatum*, *Elettaria cardamomum*, and *Momordica charantia* yielded different percentages, indicating variability in extractable phytoconstituents. *Elettaria cardamomum* exhibited the highest yield at 7.34%, followed by *Momordica charantia* at 6.99%, and *Amomum subulatum* at 5.60%. These variations could be attributed to differences in the solubility of bioactive compounds in ethanol, the nature of plant material, and extraction efficiency.

Phytochemical screening revealed the presence of various classes of secondary metabolites with therapeutic relevance. *Amomum subulatum* and *Momordica charantia* tested positive for alkaloids, while *Elettaria cardamomum* lacked them. Flavonoids were found in *Elettaria cardamomum* and *Momordica charantia*, both of which are known for their antioxidant and anti-inflammatory activities. Phenolic compounds were universally present, suggesting antioxidant potential in all three extracts. Proteins were detected in *Amomum subulatum* and *Momordica charantia*, but not in *Elettaria cardamomum*. Interestingly, only *Elettaria cardamomum* showed the presence of carbohydrates. All three extracts tested positive for saponins, compounds associated with antidiabetic and cholesterol-lowering effects. None of the extracts showed the presence of diterpenes.

The in vitro antidiabetic activity, assessed by the α-amylase inhibition assay, showed that *Momordica charantia* had the highest individual activity (IC_{50} = 82.7 ± 1.8 µg/mL), followed by *Amomum subulatum* (IC_{50} = 89.0 ± 1.0 µg/mL) and *Elettaria cardamomum* (IC_{50} = 96.5 ± 1.2 µg/mL). Notably, the combination of all three extracts in a 1:1:1 ratio resulted in significantly improved activity with an IC_{50} value of 58.3 ± 1.7 µg/mL, indicating a synergistic effect. However, the standard antidiabetic drug Acarbose remained the most potent (IC_{50} = 45.2 ± 1.5 µg/mL). The enhanced effect of the combination supports the rationale of polyherbal formulations and suggests that these extracts, when combined, may offer promising natural alternatives for managing diabetes. Further studies, including in vivo validation and mechanistic insights, are warranted to confirm these findings.

Table 1: % yield of ethanolic extract of *Amomum subulatum, Elettaria cardamomum, Momordica charantia*

S. No.	Extract	% Yield
1	*Amomum subulatum*	5.60
2	*Elettaria cardamomum*	7.34
3	*Momordica charantia*	6.99

Table 2: Phytochemical screening of ethanolic extract of *Amomum subulatum, Elettaria cardamomum, Momordica charantia*

S. No.	Constituents	*Amomum subulatum*	*Elettaria cardamomum*	*Momordica charantia*
1.	**Alkaloids** Hager's test	 +ve	 -ve	 +ve
2.	**Flavonoids** Lead acetate Alkaline test	 -ve +ve	 +ve -ve	 +ve +ve
3.	**Phenol** Ferric chloride test	+ve	+ve	+ve
4.	**Proteins** Xanthoproteic test	+ve	-ve	+ve
5.	**Carbohydrates** Fehling's test	-ve	+ve	-ve
6.	**Saponins** Foam test	+ve	+ve	+ve
7.	**Diterpenes** Copper acetate test	-ve	-ve	-ve

Table 3: *In vitro* Antidiabetic Activity by α-Amylase assay

Sample	α-Amylase (IC_{50} µg/mL)
A. subulatum	89.0 ± 1.0
E. cardamomum	96.5± 1.2
M. charantia	82.7 ± 1.8
Combination (1:1:1 ratio)	58.3 ± 1.7
Acarbose (Std)	45.2 ± 1.5

CONCLUSION

The present study demonstrates that the ethanolic extracts of *Amomum subulatum*, *Elettaria cardamomum*, and *Momordica charantia* possess notable phytochemical constituents and exhibit promising in vitro antidiabetic activity through α-amylase inhibition. Among the individual extracts, *Momordica charantia* showed the strongest enzyme inhibitory effect. However, the combination of all three plant extracts in equal proportion significantly enhanced the antidiabetic potential, suggesting a synergistic interaction between their bioactive compounds. These findings support the traditional use of these plants in managing diabetes and highlight the potential of polyherbal formulations as effective natural

alternatives to synthetic drugs. Further in vivo studies, toxicity assessments, and mechanistic investigations are necessary to validate their safety and efficacy for future therapeutic applications.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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