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STUDY ON THE PHYTOCHEMICAL ANALYSIS OF APIUM GRAVEOLEN L. SEEDS

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Abstract

Introduction: The world is blessed with an abundance of healing herbs. Since ancient times, India has relied primarily on herbal remedies to treat illness, and this practise is gaining popularity across the developed world as people seek ways to maintain their health in the face of increasing exposure to environmental hazards like pollution and stress.

Aim of the study: The main aim of the study is the phytochemical analysis of *Apium graveolen L.* Seeds.

Material and method: There are four parts to the study. Phase 1 involves testing the hypoglycemic effects of extracts in type 1 and type 2 diabetes animals. In phase 3, researchers investigate how exactly the medicine is working to prevent diabetes complications. In Phase 4 of the study, the extract is analysed for its phytochemical composition using a battery of established assays and procedures.

Conclusion: Results from the study demonstrate the anti-diabetic impact of *Apium graveolans L.* seed extracts on diabetic animal models.

Keywords: Phytochemical, *Apium graveolans L.*, Herbal remedies, Plant based drugs.

Introduction

1.1 Herbal Medicine

The world is blessed with an abundance of healing herbs. Since ancient times, India has relied primarily on herbal remedies to treat illness, and this practise is gaining popularity across the developed world as people seek ways to maintain their health in the face of increasing exposure to environmental hazards like pollution and stress. The globe over, people are increasingly turning to herbal remedies for their purported therapeutic benefits. Modern pharmacological methods may enhance the efficacy of herbal medicines while also having fewer negative effects than synthetic molecules.

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In botany, a herb is a plant that has a fleshy rather than a woody stem that dies back to the ground after flowering and producing seeds. However, the term "herb" may also refer to other things, which broadens the scope of the idea. The root of the term means "to eat" in ancient Sanskrit. Herbs are defined as any plant or plant component that is used for its perceived health benefits, flavour, or scent. Herbs may be defined as either the whole plant or a specific component of it, whether they are fresh or dried.

1.2 Traditional Knowledge and Drug Discovery

The development of the contemporary pharmaceutical business may be traced back in large part to traditional remedies derived from plants. Traditional ethnobotanical medicine is an important part of basic health care prehistoric practice but their potential role in modern healthcare is a recent propaganda in many developed and under developed countries. Traditional and folk medicine are responsible for providing an estimated 75% of the world's plant-based medicinal substances (Sen and Chakraborty, 2017). Therefore, use of ethnomedicinal plants in therapeutic purpose can be justified with the extended use in modern health care.

1.3 Importance of Plant Based Drugs:

It is believed that 80% of the population in underdeveloped nations still relies on traditional medicines, and most of these medications are derived from higher plants. It was formerly believed that plants used for therapeutic purposes are no longer as important as they once were. This, however, was disproved. From 1959 to 1973, researchers found that 41 percent of all prescriptions filled at community pharmacies included natural goods as therapeutic agents. Twenty-five percent came from lower plants, thirteen percent from microorganisms, and three percent came from animals. Synthetic medications accounted for the remaining 59%. A worldwide investigation indicated that at least 130 different plants were being employed in contemporary medicine.

Literature Review

Hariharan *et al.* (2018) researchers in India's Kancheepuram region looked studied the prevalence of diabetes and hypertension among persons who visited health clinics. Out of a total sample size of 825, they identified 215 men and 610 women, with a prevalence of diabetes and hypertension of 10.7% and 6.4%, respectively. There was a 5.7% incidence of simultaneous diabetes and hypertension.

De Sousa (2018) Throughout their article, they discussed how different forms of physical exercise (PE) like resistance training (RT), aerobic training (AT), and high-intensity interval training (HIIT) can lead to an increase in insulin receptor phosphorylation, which in turn boosts insulin pathway activity both during and after exercise. He concluded that moderate AT delivers the most advantages to those with T2DM, surpassing the benefits provided by RT and HIIT.

Elbarsha*et al.* (2018) evaluated the safety of fasting during Ramadan for people with T1D aged 18 and above, and characterised the incidence of acute complications. Ninety-two percent of the total number of fasting days were completed by 73 patients. Patients broke their fast due to hypoglycemia 2.36 percent of the time and 2.7 percent of the time, respectively.

Mendoza et al. (2018) research on the link between tuberculosis and Type 2 diabetes has been undertaken in Mexico for the last 17 years, using both observational and experimental methods. They found that those with uncontrolled diabetes were more likely to get tuberculosis.

Singh *et al.* (2017) analysed the rate of T2DM in the over-25 rural population of Etawah district, Uttar Pradesh, India, and the surrounding regions. Results showed that among rural residents, the prevalence was 8.03 percent; among women, 9.91 percent compared to 6.79 percent among men; the peak incidence occurred among those aged 50 to 59; 10.04 percent of participants were classified as prediabetic; 35.77 percent of diabetics were newly diagnosed.

Methodology

3.1 Method and Materials

In the process of complex formation, molecular docking is used to determine the most favourable binding configuration between two molecules. Docking may occur between several types of molecules, including proteins and small molecules, proteins and nucleic acids, proteins and lipids, proteins and sugars, polymers, and ligands, and so on. In the instance of protein-ligand interaction, the focus of this chapter, a search algorithm often predicts that the ligand will fit within the protein cavity.

3.2 Experimental Design

There are four parts to the study. Phase 1 involves testing the hypoglycemic effects of extracts in type 1 and type 2 diabetes animals. In phase 3, researchers investigate how exactly the medicine is working to prevent diabetes complications. In Phase 4 of the study, the extract is analysed for its phytochemical composition using a battery of established assays and procedures.

3.3 Study Design

The research process was methodical. In the first and second stages of the experiment, three female rats were given a 300mg/kg dosage of TA (Aq) and TA (Ethanol) by an oral feeding needle. Three to four hours before to medication, and two hours thereafter, the rats fasted. Drinking water was free-flowing. After the first dose, the animals were monitored for a total of 14 days. Three female rats were given the test materials several times at the dosages. After the first dose, the animals were monitored for a total of 14 days.

3.4 Phytochemical Analysis of *Apium graveolen L.* Seeds

3.4.1 Preparation of celery extract

The acquisition of celery seeds was made from a nearby marketplace, and subsequently, a sample was dispatched to the Botanical Survey of India for the purpose of authentication. The seeds were pulverised to a fine powder using a mortar and pestle, with each batch weighing 1 kg. Subsequently, the powder was subjected to soxhlet extraction for a duration of 30 hours, utilising 7 L of 96% ethanol as the solvent. The extract underwent filtration and subsequent concentration to dryness at a temperature of 40°C, followed by reduction under pressure, resulting in a yield of 6.5%. The desiccated substance served as the primary compound for all subsequent investigations.

3.4.2 Biochemical constituents in seeds

Biochemical constituents of carbohydrate, protein and lipid in seeds of celery was estimated.

- **Estimation of carbohydrate**

Hugget and Nixon's (1957) (Huggett and Nixon 1957) technique for estimating carbohydrate content was adapted for use with crustaceans in the manual of research methodologies for crustacean biochemistry and physiology authored by Saravanan and Ravindranath (1981).

- **Principle:**

To create gluconic acid and H2O2, glucose oxidase oxidises the sugar. Hydrogen peroxide reacts with peroxidase to produce chromogenic oxidation products by oxidising ortho-dianisidine or another oxygen acceptor. The amount of glucose in the mixture determines how bright the coloured chemical will become.

- **Reagent:**
    - Glucostat: A coupled glucose oxidase-peroxidase enzyme preparation. Dissolved the reagents in 80 ml of distilled water.
    - Standard: A solution of 100 mg of glucose and 100 cc of saturated benzoic acid was made.
    - 80% Ethanol: We mixed 80 millilitres of pure ethanol with 100 millilitres of water.

- **Procedure:**
  - Fifty milligrammes of the powdered substance was deposited into a clean test tube. It is hydrolyzed in 5 ml of 2.5 N-HCI in a water bath for 3 hours, and then cooled to room temperature. The fizzing was stopped by adding solid sodium carbonate and waiting. Before centrifugation, the final volume was brought up to 100 ml by adding distilled water. The supernatant is collected for analysis. The supernatant was collected via centrifugation after 1.8 ml of 80% ethanol was added to 0.2 ml of supernatant. Two millilitres of glucostat reagent, one millilitre of supernatant, one millilitre of standard, and one millilitre of distilled water were added to each test tube. After waiting 10 minutes, we added 2 drops of 4N HCL to each tube. After the colours were created, the 450 nm absorbance was determined.

- **Calculation**
  - Carbohydrate (mg/100 g) = mg of Glucose x 100
    Volume of Test sample

3.4.3 Estimation of Total Protein

The quantification of total protein was conducted utilising the modified Lowry et al (1951) method, as outlined in the manual of research methods for crustacean biochemistry and physiology edited by Subhashini and Ravindranath (1981).

- **Principle**

This technique has a two-stage principle. The reagent forms a blue copper potassium biuret complex when it combines with the carbamyl group of protein molecules. The protein's tyrosine and phenolic compounds work together with this combination to inhibit the folin reagent's phosphomolybdate and thereby boost the solution's colour.

Results

4.1 Phytochemical Analysis of *Apium Graveolen L.* Seeds

The plant exhibits a variety of medicinal components, this prompted us to study the biochemical components like carbohydrates, protein and lipid from the seeds of celery plant. Those results are tabulated in table 4.1. Celery showed high concentration of carbohydrate from the seeds 100 g sample respectively.

Table 4.1: Biochemical constituents of *Apium graveolen L. Seeds (per 100 mg)*

	Carbohydrate (mg)	Protein (mg)	Lipid (mg)
Seeds	156	142	16

HPTLC fingerprinting analysis

The HPTLC analysis of seed extract shown many peaks with varying Rf values, as tabulated in 5.2. The Rf value of 0.12 for a peak in the seed extract was found to be identical to the standard tannin Gallic acid. Flavonoids were identified using quercetin. In addition to Gallic acid, many additional notable peaks showed up, suggesting that SE contains several other compounds in significant quantities as well.

Table 4.2: *Apium graveolen L.* extracts chromatogram at 254 nm: peak list and relative retention times.

Peak No	Maximum Rf	Peak Area	% Area
1	0.12	3081.7	39.02
2	0.26	1445.4	18.30
3	0.38	2349.7	29.75
4	0.51	1020.8	12.93

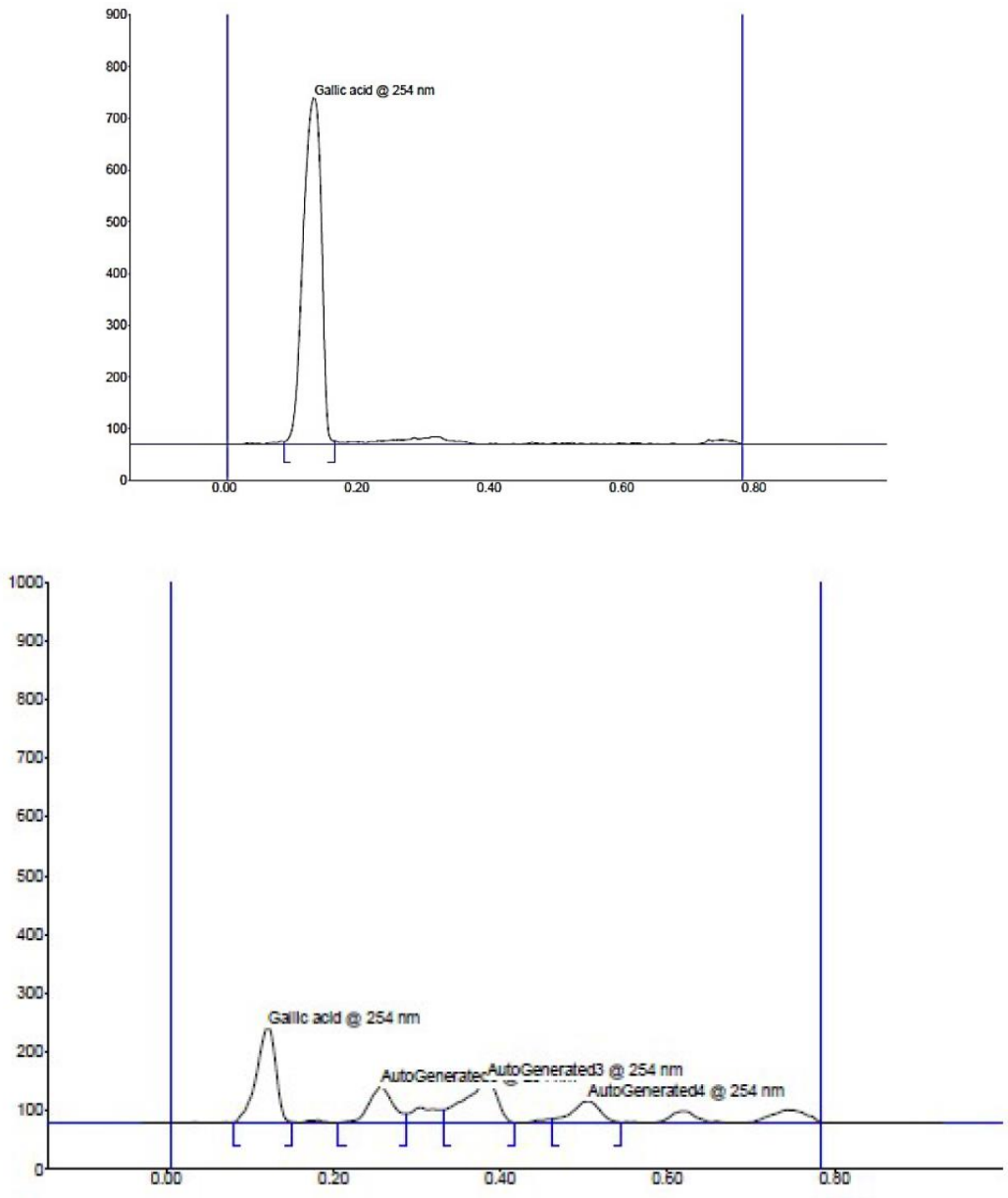

Fig 4.1: HPTLC chromatogram of Gallic acid and seed extract

The extracts' residual solvent content was determined using gas chromatography.

GC evaluation of the seed extracts showed that ethanol residue was 1526.39 ppm for seed extract respectively, which was under USP limit of 3000 ppm.

4.2 Characterization of DM Models by Detecting Insulin Sensitivity Using Intravenous Insulin Tolerance Test

Animals with DM induced by STZ injection (50 mg/kg) had KITT 2.189 ±0.35 %/min while those with fructose + STZ induced DM had KITT of 0.644 ±0.23 %/min. The slope (KITT) of the lines obtained, if more than If your insulin sensitivity is 2.0%/min or more, you're in the normal range, and if it's less than 1.5%/min, you have insulin resistance. This indicates that the STZ injected animals had normal insulin sensitivity, while fructose + STZ animals had reduced insulin sensitivity or insulin resistance. Therefore, STZ injected animals were used as model for T1DM and fructose + STZ administered rats were used as T2DM model. Figure 4.2 indicates reduction in plasma glucose levels against time in both types of diabetic animals.

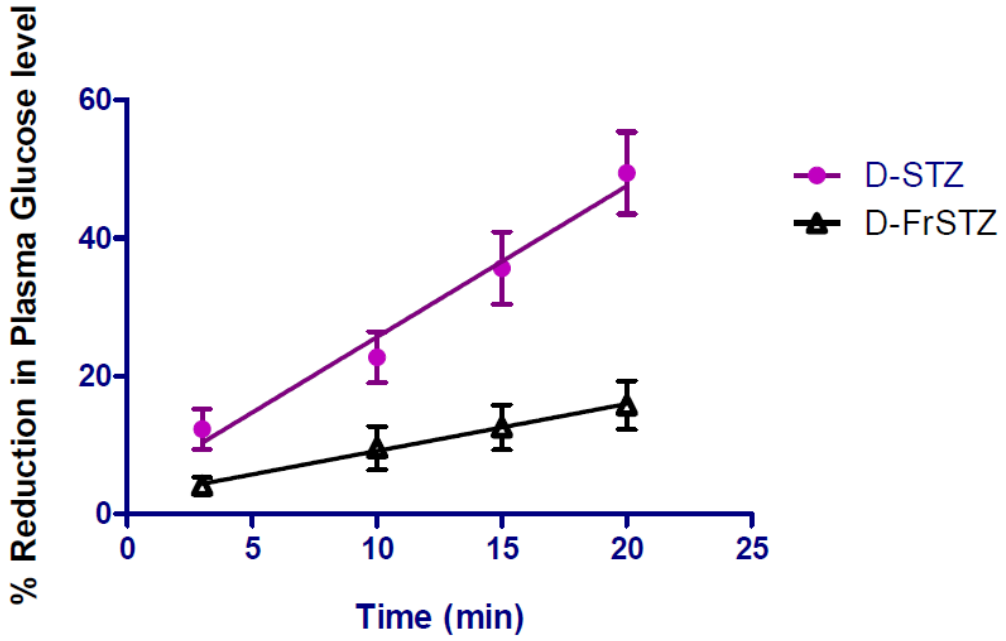

Figure 4.2: Insulin Sensitivity Test for two models of diabetes.

Analyzed using GraphPad Prism 5.03. Linear regression analysis. Each point represents Mean ± SEM of 6experimentsD-STZ: Animals with diabetes induced by 50 mg/kg streptozotocin, i.p. onceD-FrSTZ: Animals with diabetes induced by 10% fructose for 3 weeks + 40 mg/kg streptozotocin, i.p. once

Conclusion

Results from the study demonstrate the anti-diabetic impact of Apium graveolans L. seed extracts on diabetic animal models. Parameters of diabetic neuropathy and nephropathy revealed a positive response to extracts, suggesting reduced neuronal and renal damage from DM.

Ethanolic extracts also exhibited beneficial effects on cardiac functionalparameters like, mean blood pressure, heart rate, hypertrophy indices, lipid levels, serumLDH and CK-MB levels, oxidative stress status and insulin resistance. This indicates thatSE has beneficial effect in preventing cardiovascular complications associated withdiabetes mellitus.

Ethanolic extracts possess potent antioxidant action, which may be an importantmechanism of its protective effect on diabetic complications. Also, consideringthe PTP1B inhibitory results of extracts, we can conclude that it has ability to reduceincrease glucose uptake in tissue andinsulin resistance. These effects may be due toits diverse flavonoid and tannin contents. Also, as discussed earlier, various chemical constituents present in SE have demonstrated varied actions like hypolipidemic,analgesic, insulinsecretogogue, hypotensive, neuroprotective,anti-inflammatory, TNF α inhibitory, prevention of AGE formation, COX inhibitory, and beta cell protectiveactions, which all together may provide an protection against diseasedevelopment and progression of complications as observed in the study.

Recommendations of the Study

- Medicinal plants should be studied for various diseases.
- different methods should be used to evaluate the pharmacokinetic interaction of celery seed extracts.
- Because of its many applications in medicine, cooking, and even as a decorative plant, celery has a high market value. Since the seeds, leaves, and stems of celery may be combined in so many ways to create medicines, there is a pressing need to learn more about the plant's potential benefits.

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