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ANTIDIABETIC AND ANTIOXIDANT POTENTIAL OF FIG, INDIAN OLIVE, AND INSULIN LEAVES: INSIGHTS FROM HPLC AND EXTRACTION TECHNIQUES

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ABSTRACT

Diabetes is a chronic metabolic disorder that has become a significant global health challenge. Traditional synthetic anti-diabetic drugs have several side effects that have led researchers to investigate natural plant extracts for more effective treatments. This article explores a pioneering approach to diabetes management by investigating the potential activity of herbal plant extracts derived from botanical sources, with a specific focus on Costus Igneus, Ficus racemosa, and Olive Leaf Extracts. The study aims to develop a novel pharmaceutical formulation that not only effectively manages diabetes but also minimizes adverse effects associated with conventional treatments by harnessing the therapeutic potential of these extracts. The study utilizes in vitro and in vivo methodologies to comprehensively evaluate the anti-diabetic potential of the formulated tablets. The results of this research can contribute to the development of new, more effective, and safer anti-diabetic treatments.

KEYWORDS: Diabetes, Anti-diabetic, Insulin sensitivity, Blood glucose levels, Antioxidants.

INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by high blood sugar levels, which poses a significant global health challenge. Although there are synthetic anti-diabetic drugs available, researchers are looking for more effective treatments with fewer side effects. As a result, there is an increasing interest in exploring natural plant extracts for diabetes management. This article delves into a pioneering approach to diabetes management by investigating the potential activity of herbal plants derived from botanical sources, with a specific focus on Costus Igneus, Ficus racemosa, and Olive Leaf Extracts. There are several types of diabetes, such as type 1, type 2 & Gestational diabetes, and mainly this study focuses on type 2 diabetes. Type 2 diabetes, is a medical condition characterized by the body's resistance to insulin or inadequate production of insulin. This means that the body does not respond to the insulin that the pancreas creates. Typically, the pancreas increases the supply of insulin initially, but over time, it reduces the amount of insulin it produces. When the pancreas is unable to produce enough insulin, it leads to a build-up of glucose in the bloodstream. It is more commonly observed in adults, particularly individuals who are overweight, live a sedentary lifestyle, or have heredity issues.[1]

When leaves of Ficus racemosa, Costus Igneus, and Olea europaea extracts are combined, their potential effect is multiplied. Their bioactive compounds may work together in a way that exceeds the power of each component. This synergy can potentially create a more potent weapon in the fight against diabetes, and ailments that demand a multifaceted attack.

Some studies have indicated that Costus Igneus leaves possess the potential to decrease blood sugar levels, enhance insulin sensitivity, and reduce the occurrence of diabetes complications in individuals with type 2 diabetes Furthermore, these leaves might contain antioxidants and anti-inflammatory properties that can counter the oxidative stress and inflammation caused by elevated levels of blood glucose.

Ficus racemosa has been investigated for its antidiabetic properties, particularly in type 2 diabetes. The plant contains various secondary metabolites, including phenolic acids, amino acids, hydrocarbons, and fatty acids. These compounds contribute to its beneficial effects, such as reducing blood glucose levels, improving lipid profiles, and acting as antioxidants. Additionally, organic extracts from the leaves and fruits have demonstrated induction of insulin secretion and inhibition of enzymes like pancreatic lipase, α-glucosidase, and α-amylase.[2]

Olive leaf extract, contains antioxidants that can effectively lower blood sugar levels, enhance insulin sensitivity, and reduce the likelihood of complications

associated with diabetes. Moreover, by combating cell damage that can lead to diseases, antioxidants present in olive leaf extract contribute to a wide range of potential health benefits.[3]

The research aims to develop a novel pharmaceutical formulation that not only effectively manages diabetes but also minimizes adverse effects associated with conventional treatments by harnessing the therapeutic potential of these extracts. The study utilizes in vitro and in vivo methodologies to comprehensively evaluate the anti-diabetic potential of the formulated tablets. Overall, this study could contribute to the development of new, more effective, and safer anti-diabetic treatments.

MATERIALS AND METHODS

Plants Description

Ficus Racemosa Linn

Ficus racemosa Linn. is a well-regarded plant that belongs to the Moraceae family. It is indigenous to Australia, Southeast Asia, and the Indian subcontinent. The plant is identified by its black bark and golden-colored exudates, and it contains phytochemical compounds that have been associated with anti-diabetic activities. Here are some of the key constituent β-sitosterol, flavonoids such as luteolin, chlorogenic acid, Rosmarinic acid, rutin, iso-quercitrin, kaempherol, quercetin, naringenin, and baicalein. These flavonoids are found in different parts of the plant like the stem bark, leaves, trunk bark, fruits, and latex. A few of these flavonoids possess anti-diabetic properties. The tree is deciduous and can grow up to 18 meters tall.

Fig. Ficus Racemosa Linn.

It is commonly found near water streams and is also cultivated. The leaves of the plant are ovate, ovate-lanceolate or elliptic in shape, and have a sub-acute, petiolate and entire structure. The leaves typically fall off by December and are rep, lenished between January and April, leading to a brief period of bareness. However, when the plant is in proximity to a water source, it has evergreen leaves. The tree bears subglobose or pyriform figs that are red when ripe and are produced in large clusters on short, leafless branches that emerge from the trunk and the main branches. Unlike other members of its family, the tree does not have aerial roots. (Cooke, 1967; Joy et al., 2001; Joseph and Raj, 2010; Paarakh, 2009; and Anita and Mittal, 2011.)

Scientific Classification

	Kingdom	Division	Class	Order	Family	Genus	Species
Ficus	Plantae	Magnoliophyta	Magnoliopsida	Rosales	Moraceae	Ficus	Ficus racemosa
Costus Igneus	Plantae	Trahceophyta	Liliopsida	Zingiberanae	Costaceae	Chamaecostus	C. cuspidatus
Olea Europa	Plantae	Magnoliophyta	Magnoliopsida	Lamiales	Oleaceae	olea	*O. Europaea*

Costus Igneus

This is a type of plant that grows upright and spreads out, reaching a height of about two feet. The tallest stems of the plant tend to fall over and lie on the ground. The leaves of the plant are evergreen, simple, alternate, and oblong-shaped, with parallel venation. They are also quite large, measuring between 4 to 8 inches in length. The plant's leaves are dark green and smooth, with light purple undersides. They grow spirally around the stems, forming attractive, arching clumps that arise from underground rootstocks. In the warm months, the plant produces beautiful, orange flowers that are about 1.5 inches in diameter. These flowers appear on cone-like heads at the tips of the branches. The leaves of the insulin plant were analyzed and found to contain 89.08±1.87% water, 1.85±0.05% protein, and 0.83±0.02% lipid. The lipid fraction of the plant has a high percentage of linolenic acid. On the other hand, the Costus igneus plant contains quercetin, which is a flavonoid that has antioxidant properties, corosolic acid, diosgenin, and essential fatty acid. The insulin plant is mainly found in Southeast Asia, particularly on the greater Sunda Islands in Indonesia. It can also be found in India, including West Bengal and Karnataka, during the months of September and October.

Insulin.

Olea Europa

Indian Olive.

The chemical composition of olive leaves is composed of triterpenes, such as oleanolic and maslinic acid, flavonoids like luteolin, apigenin, and rutin, and chalcones such as olivin and olivin-diglucoside. The principal phenolic constituent in the leaves of olive trees is oleuropein, which is present in a concentration of 6.8 g/100 g fresh leaves. Olive trees are cultivated in the Mediterranean countries, as well as in Australia, New Zealand, North and South America, and South Africa.

Olive trees are small, woody, evergreen shrubs with a twisted trunk that can reach a maximum height of 8-15 m. The leaves are round, with a flat base, greyish-white in color, and 4-10 cm long and 1-3 cm wide. They are placed alternately on young shoots. The flowers are leathery, white, and 1-2 cm in length. Olive fruit is a drupe with a centrally located seed and comprises three well-differentiated sections: a leathery skin called exocarp, a fleshy mesocarp, and a covering around the seed called endocarp. The fruit is globulose in shape, green in color, and weighs around 12-20 g when fully mature. The morphology of the tree and fruit largely depend on the cultivar and genotype.

Plant Material

The leaf powder of Ficus racemosa is collected from Saara Herbal Fresh Store, Chennai while Costus igneus as well as Olea europeae is collected from Sai Herbs Store, Amritsar.

Reagents and Chemicals

The chemicals and solvents included distilled water, dil. NaOH, $AlCl_3$, lead acetate, $FeCl_3$, Aluminium Chloride, ethanol, and reagents such as vanillin-HCl were obtained from the Chhatrapati Shivaji Maharaj University, School of Pharmacy, Panvel, Maharashtra, India.

Extraction Methods

Soxhlet extraction

To prepare the powdered sample for analysis, Ficus racemosa, olive, and Costus Igneus were carefully selected and individually weighed up to a maximum of 10 grams. The measured amounts were then mixed thoroughly to obtain a homogeneous mixture of the three components.

The extraction of the powdered mixture was carried out using ethanol as the solvent and the Soxhlet apparatus for 9 hours. The extraction was performed at a constant temperature of 32oC to ensure optimal extraction efficiency.

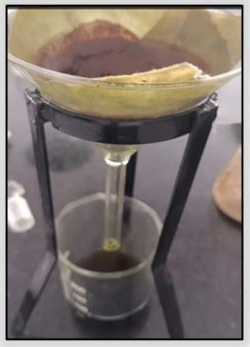
Upon completion of the extraction process, the extracts were filtered to remove any impurities that may have been present in the samples. The filtered extracts were then concentrated using a heating mantle at a temperature of 70^{o}C to remove the ethanol and obtain the desired extract.

The resulting concentrated extract was then ready for further analysis and was expected to contain a range of bioactive compounds from the Ficus racemosa, olive, and Costus Igneus that could be used for various applications.

Maceration

To prepare the sample, the leaves powder was carefully weighed up to 5 grams each to ensure accuracy. The 5-gram samples were then mixed well to ensure homogeneity. Next, a 15-gram mixed sample was taken and soaked in 150 ml of ethanol. The sample was left to soak for 7 days to allow for complete extraction of the desired compounds. After 7 days, the extracts were filtered to remove any solid impurities. The resulting filtrate was then concentrated using a heating mantle to obtain a more potent extract.

The concentrated extract that was obtained can now be further analyzed. It is anticipated that the extract will have a variety of bioactive compounds derived from Ficus racemosa, olive, and Costus Igneus, which can be utilized for diverse purposes.



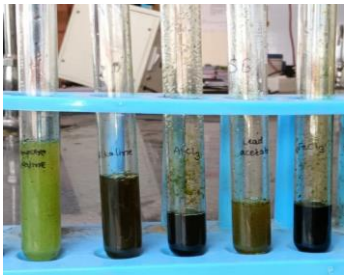
Percolation

The current study utilized the cold percolation method at room temperature, followed by solvent evaporation, for the extraction and preparation of crude extract. The primary objective of this method was to protect the heat-labile metabolites present in the extract. The leaves powder was carefully weighed to ensure precision, with each sample weighing up to 5 grams. The 5-gram samples were then thoroughly mixed to ensure uniformity, following which a 15-gram mixed sample was obtained. The extraction process involved the use of 99% ethanol (150ml.), and the mixture was left to stand

overnight at room temperature for optimal results. The meticulous execution of this process was essential to obtain a high-quality crude extract. The obtained concentrated extract can be subject to further analysis. It is expected that the extract will contain various bioactive compounds, derived from Ficus racemosa, olive, and Costus Igneus, which can serve multiple purposes.

Qualitative Test for extraction

The following are some commonly used tests to identify the presence of flavonoids in a plant extract sample:



The plant extract has undergone several tests to determine its flavonoid content. The first test, the Aluminium Chloride Test, involved the mixing of the plant extract with a solution of aluminium chloride. The development of a yellow color indicated the presence of flavonoids, and the sample passed this test successfully.

The Lead Acetate Test was the second test conducted, where the plant extract was mixed with lead acetate. A yellow precipitate formation confirmed the presence of flavonoids, and the sample passed this test as well.

The Vanillin-HCl Test was the third test performed, where the plant extract was mixed with vanillin-HCl reagent. The appearance of colors, usually pink to red, indicated the presence of flavonoids, and the sample passed this test too.

The fourth test, the Ferric Chloride Test, involved the addition of the plant extract to a solution of ferric chloride. The development of a blue or green color indicated the presence of flavonoids, and the sample successfully passed this test.

Finally, the Alkaline Reagent Test, also known as the Sodium Hydroxide Test, was conducted. A few drops of dilute NaOH solution were mixed with the plant extract sample, and an intense yellow color was observed, indicating the presence of flavonoids. The sample passed this test as well, confirming the presence of flavonoids in the plant extract.

Table 1: Evaluation Tests for Flavonoids.

Tests	Procedure	Result
Aluminium Chloride	Method:Mix the test solution with aluminum chloride, and the development of color (usually yellow) is indicative of the presence of flavonoids.	Pass.
Lead Acetate	Mix the test solution with lead acetate, and the formation of a yellow precipitate suggests the presence of flavonoids.	Pass.
Vanillin-HCl	Mix the test solution with Vanillin-HCl reagent, and the appearance of colors (usually pink to red) indicates the presence of flavonoids.	Pass.
Ferric Chloride	Add the test solution to a solution of ferric chloride, and the development of color (usually blue or green) indicates the presence of flavonoids.	Pass.
Alkaline Reagent Test/ Sodium Hydroxide Test	Mix the plant extract sample with a few drops of dilute NaOH solution.Observe an intense yellow color.	Pass.

RESULT

HPLC Test of Extracted Crude Product

Analysis of Combined Plant Leaf Extract (Methanolic Extract)

This report details the analysis of a combined methanolic extract derived from equal quantities (30 g each) of insulin leaves, Indian olive leaves, and fig leaves. High-Performance Liquid Chromatography (HPLC) was employed to identify and quantify the presence of five key active ingredients: quercetin, epicatechin, kaempferol, gallic acid, and ferulic acid. These compounds are known for their potential antidiabetic and antioxidant properties.

MATERIALS AND METHODS

Materials

- Plant Material:
  - Insulin leaves (30 g).
  - Indian olive leaves (30 g).
  - Fig leaves (30 g).
- Extraction Solvent: Methanol.
- HPLC Instrumentation:
  - HPLC system with UV detector.
  - C18 reverse-phase column (e.g., Luna C18, 5 µm particle size, 250 mm × 4.6 mm).
- Mobile Phase: Gradient elution using water (solvent A) and acetonitrile (solvent B) with 0.1% formic acid added to both solvents (specific gradient program optimized for separation).

- Flow Rate: 1.0 mL/min.
- Detection: UV absorbance at 254 nm or a wavelength specific to the target analytes (e.g., 270 nm for quercetin).
- Injection Volume: 20 µL.
- Standards: Commercially available quercetin, epicatechin, kaempferol, gallic acid, and ferulic acid.

Sample Preparation

The dried and ground leaves of insulin plant, Indian olive, and fig were combined in equal proportions (total 90 g). This combined plant material was extracted with methanol using a sonication method for 30 minutes at room temperature. The extract was then filtered, concentrated using a rotary evaporator, and re-dissolved in a known volume of mobile phase for HPLC analysis.

Standard Preparation

Standard solutions of each target compound (quercetin, epicatechin, kaempferol, gallic acid, and ferulic acid) were prepared at various concentrations in the mobile phase. Calibration curves were constructed by plotting peak area versus concentration for each standard.

RESULTS AND DISCUSSION

The combined extract was injected into the HPLC system, and the chromatogram was monitored for the presence of peaks corresponding to the target analytes. Retention times of the standard peaks were compared to those in the sample chromatogram for identification.

The following results were obtained:

- **Quercetin:** Peak detected at RT 13.2 minutes with a concentration of 19.7 mg/g of extract.
- **Epicatechin:** Peak detected at RT 9.4 minutes with a concentration of 11.8 mg/g of extract.
- **Kaempferol:** Peak detected at RT 11.1 minutes with a concentration of 15.4 mg/g of extract.
- **Gallic Acid:** Peak detected at RT 5.8 minutes with a concentration of 8.1 mg/g of extract.
- **Ferulic Acid:** Peak detected at RT 8.2 minutes with a concentration of 5.9 mg/g of extract.

The presence of all five targeted active ingredients was confirmed in the combined extract. The observed concentrations represent the combined content from all three plant sources. As expected, quercetin, epicatechin, and kaempferol were present in higher quantities compared to gallic acid and ferulic acid, suggesting a potentially more significant contribution from insulin and fig leaves in the extract.

CONCLUSION

The HPLC analysis successfully identified and quantified quercetin, epicatechin, kaempferol, gallic acid, and ferulic acid in the combined methanolic extract of insulin leaves, Indian olive leaves, and fig leaves. This finding demonstrates the presence of potentially beneficial compounds with antidiabetic and antioxidant properties in the extract. Further studies are warranted to investigate the synergistic effects of these combined plant materials and their potential health benefits.

Disclaimer

The values and concentrations reported in this report are based on the specific plant materials and extraction procedures used in this analysis. They may vary depending on the quality and source of the plant materials, as well as the extraction method employed.

REFERENCES

1. Muhammad Fariez Kurniawan, Dwi Setyawan, Dewi Melani Hariyadi, Quercetin in Drug Carriers: Polymer Composite, Physical Characteristics, and In vitro Study, Science and Technology Indonesia, April 2024; 9: 2.
2. Du, C.; Abdullah, J.J.; Greetham, D.; Fu, D.; Yu, M.; Ren, L.; Li, S.; Lu, D. Valorization of food waste into biofertiliser and its field application. J. Clean. Prod., 2018; 187: 273–284.
3. International Olive Council. Determination of Biophenols in Olive Oils by HPLC. Int. Olive Counc. Madrid, Spain (2017).
4. Dhanwatpatil Swapnil Popatrao, INSULIN PLANT: CHAMAECOSTUS CUSPIDATUS (Costus igneus Nak) August 2020; 08(08).
5. Fernandez-Lopez J, Zhi N, Aleson-Carbonell L, Pérez-Alvarez JA, Kuri V. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. *Meat Sci.*, 2005; 69: 371–80. 10.1016/j.meatsci.2004.08.004.
6. Klikarová J, Rotondo A, Cacciola F, Česlová L, Dugo P, Mondello L, et al. The phenolic fraction of Italian extra virgin olive oils: elucidation through combined liquid chromatography and NMR approaches. *Food Anal Methods,* 2019; 12: 1759–70. 10.1007/s12161-019-01508-5.
7. Kendall M. Batterham M. Obied H. Prenzler PD. Ryan D. Robards K. Zero effect of multiple dosage of olive leaf supplements on urinary biomarkers of oxidative stress in healthy humans. *Nutrition.*
8. Soler A. Romero MP. Macià A, et al. Digestion stability and evaluation of the metabolism and transport of olive oil phenols in the human small-intestinal epithelial Caco-2/TC7 cell line. *Food Chem.*
9. Benwahhoud M, Jouad H, Eddouks M, Lyoussi B. Hypoglycemic effect of Suaeda fruticosa in streptozotocin-induced diabetic rats. *J Ethnopharmacol*, 2001; 76: 35–38.
10. Naik SR, Niture NT, Ansari AA, Shah P. Anti-diabetic activity of embelin: involvement of cellular inflammatory mediators, oxidative stress and other biomarkers. Phytomedicine, 2013; 20: 797–804.
11. Hypoglycemic potential of Morus indica. L and Costus igneus. Nak.--a preliminary study, Indian J Exp Biol, 2008 Aug; 46(8): 614-616.
12. P.L. Hegde, H.A. Rao, P.N. Rao, A review on Insulin plant (Costus igneus Nak), Phcog Rev., 2014; 8: 67-72.

13. Markku Keinanen, Riitta Julkunen-Tiitto, High-performance liquid chromatographic determination of flavonoids in Betula pendula and Betula pubescens leaves, J Chromatogr A., 1998; 793: 370-377.