Investigation of Antioxidants, Antidiabetic and Antihyperlipidemic Activity of Ficus Racemosa leaves

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Abstract

The purpose of this experiment was to determine whether or not Ficus racemosa has hypoglycemic effects utilizing in-vitro methods. The purpose of this research is to examine the polyphenol content and biological activity of nanofiltered extracts of Ficus racemosa. According to HPLC-MS analysis, the two most abundant phenolic acids in both extracts were chlorogenic acid and rosmarinic acid. Although rutin and isoquercitrin make up the bulk of Ficus racemosa, the extract's primary flavonoid is luteolin.

Keywords: Ficus racemosa, Phytochemical, In-vitro, Antioxidant, Anti-diabetic

Introduction

Type 2 diabetes must be managed with a mix of lifestyle modifications and medication to prevent complications and maintain a high quality of life. Measures done to regulate blood sugar, body mass index, cardiovascular risk factors, comorbidities, and complications fall under this area. This specifically calls for a person-centered method of delivering treatment that is

structured and organized like the chronic care model and that motivates patients to actively participate in their own self-care. Diabetes patients require individualized treatment plans, and these plans must take into account their preferences as well as the social determinants of health. Medical nutrition therapy (MNT), physical activity, emotional support, weight management, and counseling for drug and alcohol addiction are all crucial aspects of diabetes care. Along with DSMES (Diabetes Self-Management Education and Support), this is frequently offered. The number of glucose-lowering strategies accessible to people with diabetes and their healthcare professionals has increased, including behavioral interventions, pharmaceutical interventions, devices, and surgery, but this can also make decision-making more challenging[1]. In high-risk patients with atherosclerotic cardiovascular disease, heart failure, and chronic kidney disease, sodium-glucose cotransporter-2 inhibitors (SGLT2i) and glucagon-like peptide-1 receptor agonists (GLP-1 RA) have showed promise in reducing the progression of diabetes and associated consequences. Even in the absence of lower glucose levels, these benefits still apply. These medications were first created to lower blood sugar levels, but they are now frequently suggested to protect crucial organs from harm. We have assembled a comprehensive body of recent data in this consensus report for practitioners in the United States and Europe with the aim of streamlining clinical decision making and focussing our efforts on providing holistic person-centered care. Achieving prescribed glycaemic objectives has been shown to significantly and sustainably slow the onset and progression of microvascular issues [1,2], which emphasizes the need of early intervention [3]. The largest absolute risk reduction is achieved by lowering very elevated glycaemic levels, while a smaller reduction is achieved by approaching normalization of plasma glucose levels [4]. The impact of glucose control on macrovascular issues is less certain, but it is supported by multiple meta-analyses and epidemiological data. Since the advantages of glucose control take time to manifest, whereas the negative effects can occur right away, people with longer life expectancies stand to benefit more from early intensive glycaemic management. Aim for a HbA1c of 53 mmol/mol (7%) or below in the majority of non-pregnant people with a life expectancy long enough to observe microvascular advantages (usually 10 years) [2]. If achieving an even lower HbA1c level does not materially raise the risk of hypoglycemia or other unfavorable treatment side effects, it might be worthwhile to do so. A lower objective might be reasonable when using pharmacological medications that do not provide a risk of hypoglycemia. It may be preferable to strive higher in situations where the life expectancy is short, there are advanced health issues, poor tolerance levels, or there are other requirements like fragility. As a result, glycaemic treatment goals should be adjusted in accordance with a person's preferences and characteristics, including younger age (i.e., age 40), risk of complications, frailty, and comorbid conditions [5], as well as the effect these traits have on the likelihood of experiencing therapy side effects (such as hypoglycemia and weight gain). People who eat a lot of fats and carbohydrates run the risk of becoming obese and having high cholesterol levels. Hyperlipidemia is characterized by a decrease in high-density lipoprotein (HDL) and an increase in low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) [6]. People with hyperlipidemia have a higher risk of developing cardiovascular disease [2], as this condition is regarded as a traditional risk factor for heart disease. Therefore, reducing hyperlipidemia is essential for the

management and avoidance of vascular disease and CNS disorders. The typical approach to decreasing LDL cholesterol levels involves statin-based inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. However, statins have been linked to liver dysfunction and muscle myopathy [7]. In clinical practice, fibrates, niacin and its derivatives, ezetimibe, etc. are currently utilized alongside other lipid-lowering drugs. Despite the drug's potent lipid-lowering efficacy, serious side effects such as liver damage, face flushing, hyperglycemia, excessive uric acid or gout, and upper gastrointestinal discomfort have been reported [8]. Therefore, finding lipid-lowering medications that are both safer and more efficient is crucial. For medical and preventative purposes, an increasing number of people prefer to use medications derived from natural sources. The topic of polysaccharides, which are prevalent in nature, is getting more and more attention [7]. More than ten monosaccharide molecules connected by diverse glycosidic connections make up the complicated chemical structure of polysaccharides that have been separated from their natural surroundings. Polysaccharides have been shown to have safe and effective hypolipidemic, antioxidant, liver-protective, and immune-regulatory effects in recent years [8, 9]. Polysaccharides have been demonstrated in numerous studies to considerably improve health by lowering blood lipid levels, and they hold great potential for the future. Research by Rjeibi et al. [9] has demonstrated that polysaccharides from Nitraria retusa fruits can reduce the adverse effects of Triton X-100 on hyperlipidemia, hepatotoxicity, cardiovascular, and coronary diseases. According to study by Zhang et al. [10], normalizing the levels of 15 potential biomarkers, such as glycerol phospholipids, aliphatic acids, propylene alcohol lipids, and sphingolipid metabolism, is associated with the mechanism by which Pleurotus ostreatus polysaccharide regulates dyslipidemia. These techniques necessitate a thorough overview since they involve several cell signaling pathways and a number of targets. Additionally, it is well known that polysaccharides' biological activity is influenced by their molecular weight (MW), monosaccharide composition, glycosidic bond type, and sulfate content [11], and that the outcomes of their actions are inconsistent. The fig tree (Ficus racemosa Linn; Udumbara) serves a number of functions in traditional medicine. Every portion of the plant possesses medicinal qualities. Typically, leaves can be used to treat bile infection. Both constipation and diarrhea can be relieved by eating fruit. The plant's bark can be used to cure a wide range of conditions, including ulcers, diabetes, leucorrhea, diarrhea, and urinary problems. In addition to its various traditional uses, udumbara has biological properties that make it an antitussive, chemopreventive, hepatoprotective, anti-inflammatory, anti-diuretic, and antipyretic. All of the plant's constituent parts contain phytochemicals, which are what give the plant its therapeutic properties. [1] One of the rare plants that has received as much coverage in the Vedas is udumbara. useful for homa (fire ceremonies) and yogna (rituals). The same plant's twigs can be used to make both brushes and brooms. One of the four Nalpamara (ksirivrkas) trees respected in Hinduism and Buddhism, it is suggested that you grow it close to places of worship. A large portion of India is home to the endemic Ficus racemosa Linn (moraceae), an evergreen, spreading, lactiferous, deciduous plant. It can be found in both evergreen and deciduous forests, as well as other wet regions, up to 1800 meters above sea level. It is grown on the Deccan Plateau, the outer Himalayas, the Khasia Mountain, Punjab, Rajasthan, Chota

Nagpur, Odisha, Karnataka, and Kerala. It is a medium-sized deciduous tree with a 10–16 meter height range. It is also farmed and frequently seen close to flowing water. The plant's leaves, which can be ovate or elliptical, are typical of this species. These are green in color and range in length from 7 to 10 cm. The leaves drop off in December, but from January through April they are in full flower. The blossoms of the plant are hidden within the fruits because they are not immediately visible. You can consume the pears-shaped fruits. They grow in bunches from the primary stem. Sizes for this range from 2 to 5 cm. The fruit is drab green when it is unripe, but when it is fully grown, it is a vivid orange or a rich crimson. Fruit that is ripe emits fragrant scents. Although the seeds look like tiny grains, there are actually quite a few of them. The thickness of the bark, which ranges from 0.5 to 1.8 cm, is greyish brown. The surface is cracked and squishy. The inner surface of the bark has fibers and a little brown colour. mucilage-like in flavor but without any smell. Plants can be reproduced sexually (through seeds) or vegetatively (by cuttings). Both the tree and its fruit are referred to as "atti" in southern India and "gular" in northern India. This plant is used by traditional medicinal systems around the world to cure a variety of diseases. It is effective as a treatment for overeating[12]. Once more, no extensive research on the isolated flavonoids from the stem bark of FR has been done in diabetic rats induced by STZ. The goal of this study was to determine whether or whether the isolated flavonoids from FR's stem bark might be used to reverse diabetes. Four flavonoids were isolated from FR stem bark and tested on rats that had received STZ for their anti-diabetic, hypolipidemic, and toxicological effects to get there. Docking studies were utilized to calculate the affinities of the compounds to PPAR and GLUT1 receptors in order to understand more about the mechanism of action of the isolated materials. For the first time, we suggested that the flavonoids' antidiabetic effect was induced by their binding to GLUT1 and PPAR[13].

Material & Methods

Collection of plants

The Leaves of Ficus racemosa were collected from a local village of Nanital, Uttarakhand, India. And authonicate in DDGU, Gorakhpur.

Preparation of Extract

About 180 g of powdered material was soaked in 500 ml of 95% ethanol for 7 days while being occasionally shaken and stirred in a clean, flat-bottomed glass container. The entire mixture was filtered using successive layers of Whatman filter paper (Bibby RE200, Sterilin Ltd., UK) and a piece of clean, white cotton fabric. The resultant filtrate (ethanol extract) was evaporated using a rotary evaporator (STUART RF3022C, UK). A sticky reddish-black concentration was the result.

Phytochemical Screening

Carbohydrate, saponin, alkaloid, flavonoid, fixed oil and fat, phenolic and tannin, glycoside, phytosterol, and triterpenoids assays, as well as preliminary qualitative phytochemical analyses, were performed on the extracts using established protocols.

Qualitative Phyto-chemical Screening

Initial or qualitative analyses of phytochemicals in plant components (leaves) were conducted in accordance with established norms in the field.

Alkaloids

1% hydrochloric acid solution was heated for two minutes in water before being used to extract two grammes of plant material. After being mixed, the substance was strained through a filter. It was determined that there were alkaloids present by adding a few drops of Dragendroff's reagent, which caused the solution to turn a reddish-brown colour and become cloudy.

Flavonoids

In order to dissolve 2 grammes of the extracts, 10% NaOH and HCl were both required. The presence of flavonoids can be determined by observing what happens to an initially yellow solution after HCl is added to it. The solution becomes clear.

Glycosides

Two grammes of unprocessed extract were mixed with two millilitres of an aqueous solution containing 5% iron chloride chloride ($FeCl_3$) and two millilitres of diluted sulfuric acid. The oxidation of anthraquinones, which takes place when the solution is heated for five minutes, allows the glycoside content to be determined.

Tanins

After heating up ten millilitres of water, two grammes of extract powder were added to the mixture. Following the filtration of the solution, ferric chloride was added to the concentration that was produced as a result. When a blue-black, blue-green, or green precipitate was observed, tannins may be deduced from the sample.

Steroids

Two grammes of the extract were mixed with some chloroform, and then a few drops of concentrated hydrosulfuric acid were added while the combination was being thoroughly stirred. After this, the mixture was allowed to sit for a while before being analysed. The colour red indicated the presence of anabolic steroids.

In vitro Antioxidant

DPPH Assay

An almost exact replica of the previous methodology was employed to carry out the DPPH assays on the Ficus racemosa sample [74]. In order to make a DPPH solution (0.1mM), methanol was utilized. A well-plate was first filled with 190 L of prepared DPPH, 10 L of the obtained

sample extract, and positive controls. In order to measure the combination's absorbance at 517 nm, a multi-purpose 96-well plate reader (Tecan, Infinite M20PRO, Switzerland) was used after the mixture had grown in the dark for 30 minutes. The baseline was corrected using the methanol-filled blank. There were three different tests run. The percentage of DPPH scavenging was calculated and is shown as follows: DPPH activity (percent) = [(CO-C1/CO)] 100% where Co is the absorbance of the control and C1 is the sample extract's absorbance in relation to the control.

Invitro Hypoglycemic Activity

Hypoglycemic activity Glucose tolerance test:

A glucose tolerance test is necessary for determining how quickly glucose is flushed out of the system. After fasting (without eating or drinking for 10-16 hours), the mice were subjected to a battery of tests. The mice were fed glucose after the initial blood sample was taken. The mice were given the high glucose solution, and their blood was resampled 30, 1, 2, and 3 hours later.

Statistical Analysis

Variations in the means that were found to be statistically significant were highlighted by the standard error of the mean. One-Way Analysis of Variance was used to help us make the comparison between the means of our experimental data and those of the vehicle-control group (ANOVA). The Dunnett's test and the Tukey's multiple comparison test were both utilised in the subsequent study that was carried out. The analysis was carried out with Graph Pad Prism Version 4. p-value of less than 0.5 was regarded as being statistically significant

Result & Discussion

The findings of a qualitative phytochemical analysis of ethanolic extracts from Ficus racemosa are shown in Table 1. Phenols, flavonoids, and steroids were found in all of the analyzed plant extracts.

Phytochemical Group	**Ficus racemosa (Leaves)**
Alkaloids	+
Flavonoids	+
Steroids	-
Triterpenoids	+
Phenolic	+
Tanins	+
Carbohydrates	+

In vitro Antioxidant

DPPH Assay

The ELISA reader and the spectrophotometer both detected decreased levels of DPPH RSA activity, with IC50 values for the FR extract being 31.87 and 334.95 g/ml, respectively. In this investigation, the reference standard was ascorbic acid (10ug/ml).

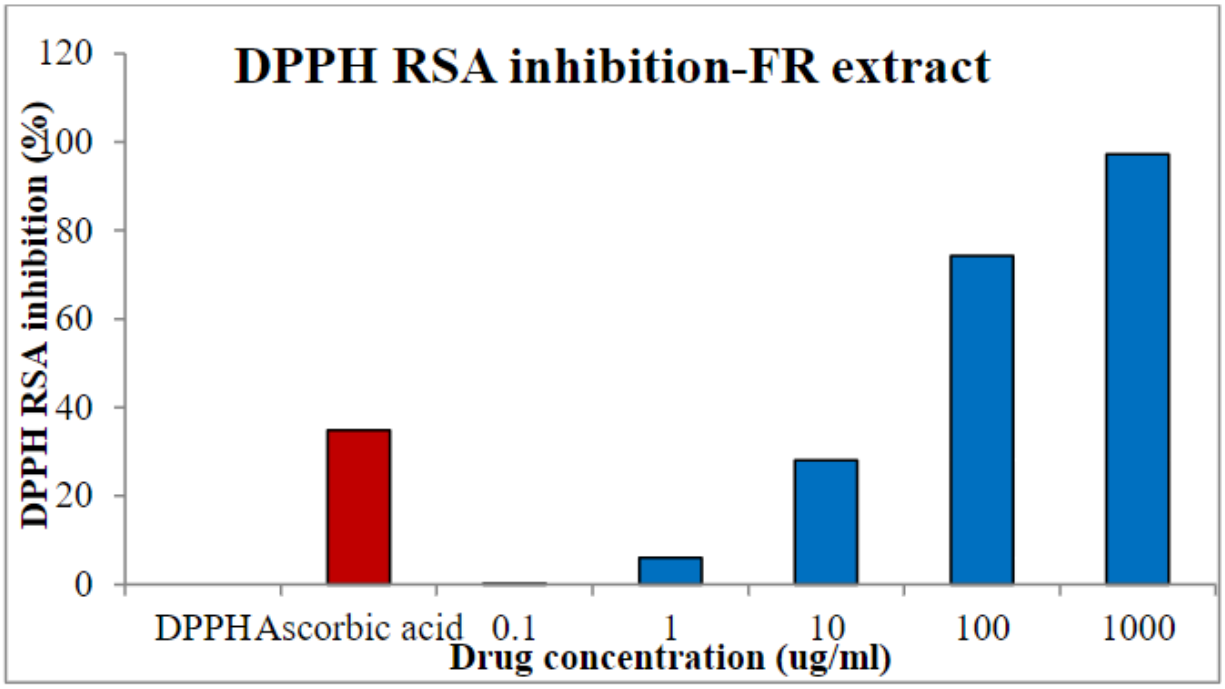

Fig: 1 *Ficus racemosa*(leaves) ethanolic extract DPPH-RSA assay

Hypoglycemic activity Glucose tolerance test:

The results of the chosen plant extracts' ability to adsorb glucose are shown in Figure 2. Ficus racemosa efficiently removes bound glucose, according to experiments on glucose adsorption. It was shown that the extracts' capacity to absorb glucose increased with glucose concentration. Both plant extracts were successful at adsorbing glucose at the experiment's low (5 mmol L-1) and high (100 mmol L-1) concentrations. It was shown that the capacity of the chosen plant extracts to bind glucose directly linked with the molar concentration of glucose, with a greater amount of glucose being bound at higher glucose concentrations. There were no statistically significant changes in Ficus racemosa's adsorption capacities ($p>0.05$). Glucose can be successfully bound by Ficus racemosa extracts at concentrations as low as 5 mM, which lowers the amount of glucose present and slows down the rate at which it is transported across the intestinal lumen.

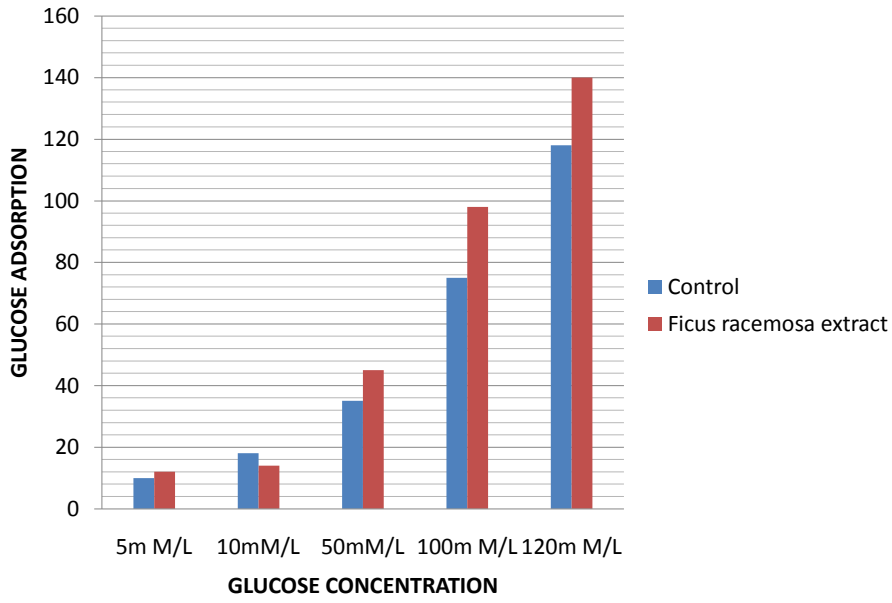

Fig: 2 Ficus racemosa's ability to bind glucose at various glucose concentrations. Mean + standard deviation of three independent measurements.

Conclusion

In conclusion, our findings are consistent with the hypothesis that Ficus racemosa exerts its hypoglycemic impact by preserving or increasing beta-cell and pancreatic health and function, a mechanism independent of insulin. Ficus racemosa may exert its antidiabetic effects through interactions with insulin receptors, strong proliferative and antioxidant effects, activation of the MAPK and P13K pathways, and translocation of glucose transporters, among other potential mechanisms. The phytochemical components of this extract are thought to have an anti-diabetic action. The traditional usage of Ficus racemosa to treat diabetes is supported by this study. However, due to its potential toxicity, care should be taken when recommending the plant extract.

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