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ORIGINAL ARTICLE

In vitro Antidiabetic and Anti-inflammatory activities of fruit peel and root bark of *Pithecellobium dulce*

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ABSTRACT
Pithecellobium dulce, a member of the Leguminosae family, was selected for this study based on medicinal and nutritional importance. The fresh fruits and roots of Pithecellobium dulce were collected and cleaned. The fruit peel and root bark were separated and then dried under shade and the plant materials were ground into powder. About 100 g of dry powder was extracted with different solvents like ethanol, methanol, and diethyl ether. The extracts were used for in vitro antidiabetic and anti-inflammatory studies at various concentrations 50, 100, 150, 200 and 250 µg/ml. The ethanolic, methanolic and diethyl ether extracts of fruit peel showed maximum antidiabetic activity 72.03 ± 1.95%, 76.13 ± 1.70% and 44.16 ± 2.25%, respectively and root bark showed 76.03 ± 1.25%, 84.46 ± 2.13% and 71.93 ± 1.95%, respectively at 250 µg/ml in α-amylase inhibition by 3, 5-dinitrosalicylic acid (DNSA) assay. In α-amylase inhibition by starch-iodine colour assay, the ethanolic, methanolic and diethyl ether extracts of fruit peel showed maximum antidiabetic activity 84.60 ± 2.16%, 84.56 ± 2.40% and 64.60 ± 2.16%, respectively and root bark showed 86.10 ± 1.65%, 85.43 ± 1.20% and 75.56 ± 1.88%, respectively at 250 µg/ml. The ethanolic, methanolic and diethyl ether extracts of fruit peel showed the highest level of anti-inflammatory efficacy 74.73 ± 2.61%, 73.96 ± 1.95% and 63.70 ± 1.96%, respectively and root bark showed 75.10 ± 1.65%, 74.50 ± 2.29% and 64.50 ± 1.50%, respectively at 250 µg/ml. The results showed that all the tested extracts had in vitro antidiabetic and anti-inflammatory activities at various concentrations. The maximum levels of in vitro antidiabetic and anti-inflammatory activities were observed at 250 µg/ml of concentration than other tested concentrations and it is more or less similar to the activities of reference drugs acarbose and diclofenac sodium. From the results, we concluded that the fruit peel and root bark of Pithecellobium dulce have potential in vitro antidiabetic and anti-inflammatory properties and the root bark exhibited more activities than fruit peel.
Key words: Antidiabetic, Anti-inflammatory, fruit peel, root bark, *Pithecellobium dulce*

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INTRODUCTION

In India and other ancient medical systems around the world, plants have been a key source of medications. Herbal medicine has grown exponentially in the last several years, and because of its natural origin and lower side effects, these medications are becoming more and more popular in industrialized nations. Medicinal plants, minerals, and organic materials are the source of many traditional remedies [1]. The world's traditional medical systems heavily rely on plants as a source for all treatments. Herbal remedies have been utilized for medical purposes since ancient times [2]. As per the World Health Organization (WHO) reports that over 80% of the global population depends on conventional medicine for their medical requirements.

India is known as the world's botanical garden and is the world's largestproducer of medicinal herbs [3]. At the moment, a lot of research is being done on plants and herbal medication mixtures used to treat diabetes mellitus and inflammations are serious and debilitating conditions. Research carried out in India over the past few decades has demonstrated that not only is diabetes prevalence high, but it is also rising quickly among the urban population [4]. In India, the number of adults with diabetes is thought to reach

33 million and there will probably be a 57.2 million person rise in the population by 2025. A collection of metabolic changes known as diabetes mellitus is classified as type I and type II. Only 5% of people with diabetes have type I diabetes,often known as juvenile diabetes, which is insulin dependent. Adults over 40 years old are typically diagnosed with type II diabetes, which is non-insulin dependent. Long term harm, dysfunction, and failure of various organs, including the eyes (retinopathy), kidneys (nephropathy), nerves (neuropathy), heart (coronary heart disease), and blood vessels (peripheral vascular diseases), have already been linked to chronic hyperglycemia in diabetes [5].

Living tissue will respond severely to any type of injury by producing inflammation, and there are four main signs of inflammation: discomfort, redness, warmth, fever, and edema. One of the body's nonspecific internal defensive mechanisms is inflammation, and a tissue's reaction to an unintentional cut is comparable to the reaction to other forms of tissue injury brought on by burns from heat, radiation, germs, or viruses [6] Macrophages and neutrophils are involved in an inflammatory response and release several mediators that are in charge of the beginning, continuing, regulating and final resolution of the acute state of inflammation [7]. Inflammation is traditional guarding response to injury in the tissues. This process involves in intricate array of enzyme activation, cell migration, release of mediators, tissue breakdown and repair belching fluid [8]. Inflammatory responses can be suppressed by various medicines such as steroids; non-steroid anti-inflammatory drugs but this medication has inimical effects. Therefore, the goal is to exploit a minimum effective dose by most efficacies with least adverse effect [9, 10].

Herbal preparations used in the Indian traditional health care system contain a number of medicinal herbs known as Rasayana,which have been utilized for over a millennium [11]. One such traditional medicinal plant, *Pithecellobium dulce*, a member of the *Leguminosae* family, was selected for this study based on medicinal value and underwent an extensive scientific investigation into its pharmacological properties. *Pithecellobium dulce*, an evergreen tree with prickly leaves that reaches up to 22 meters in height, is native to tropical America. It is grown on the Andaman Islands and all over India. It has served as a food pod, fence, fodder feed, and tanning material. It is called Vilayati Babul in Hindi, Seema Chinta in Telugu and Kodukkapuli in Tamil. The fragments of pods can be eaten raw or made into a drink for their nutritional and therapeutic properties; nevertheless, most of the chemical components of the pods are still unknown and underutilized. The *Pithecellobium dulce* is commonly called as manila tamarind. Manila tamarind is believed to be utilized as a folk cure for several human ailments, including leprosy, stomach ulcers, toothaches, and earaches [12].

The fruits of *Pithecellobium dulce* possess nephroprotective, antioxidant, and anti-inflammatory qualities [13]. People have been used *Pithecellobium dulce* fruits as nutritional supplements since they are non-toxic, medicinal, and highly nutritious. The fruits of *Pithecellobium dulce* have been utilized as dietary supplements due to their high nutritional content. *Pithecellobium dulce* fruit peels have produced four distinct chemicals: stigmasterol, sitosterol, quercetin, and pinitol, which have been identified as medicinally useful molecules [14] and these chemicals showed that the *Pithecellobium dulce* fruit peel's cardioprotective benefits [15]. Flavonoids and phenolic substances include a hydroxyl functional group that can scavenge radicals and prevent oxidative damage [16, 17]. Fruit peel extracts from *Pithecellobium dulce* have been demonstrated to have antibacterial and antioxidant properties [18] and they have also been proven to have the ability to heal wounds [19]. These days, controlling blood sugar levels using fruits is common and some groups consume the decoction and eat raw fruit peel for the same reason. Fruit peels have been used in ethnomedical research to cure and manage diabetes mellitus, despite the lack of a scientific investigation to support this claim.

The traditional medicine claims that leaves are a larvicide, anodyne, astringent, emollient, abortifacient, antidiabetic, and a treatment for toothaches, earaches, leprosy, peptic ulcers, and venereal diseases [20, 21, 22]. The bark of *Pithecellobium dulce* is reportedly useful as a febrifuge, an astringent for diarrhea, and a remedy for dermatitis, ocular inflammation, and possesses antivenomic and antimicrobial properties [23,24]. Numerous medicinal values of *Pithecellobium*, such as the stem's skin for diarrhea, the leaves for gastrointestinal disorders, and the seeds for ulcers, have also historically been employed as medical remedies [25]. The bark of *Pithecellobium dulce* were used to treat fever, diarrhea, dermatitis, and even inflammation of the eyes [26, 27] and leaves are used to treat gastric ulcers and leprosy [27]. It is used to prevent diabetes related issues by scavenging free radicals and hypoglycemia [14].

The field of plant studies has received increased attention recently from all over the world, and a significant quantity of data has been accumulated to show the immense potential of medicinal plants used in traditional systems like Ayurveda, Siddha, and Unani. Studies have suggested that therapeutic plants could be useful in the treatment of diabetes. *Pithecellobium dulce* fruit peel and root bark's potential therapeutic use for diabetes and inflammation is uncertain. *Pithecellobium dulce* has been employed in

numerous studies, even though a very few studies have focused on fruit peel or root bark. However, there are no studies on *in vitro* antidiabetic and anti-inflammatory activities of *Pithecellobium dulce* fruit peel and root bark. So, the present study is aimed to study the *in vitro* antidiabetic and anti-inflammatory activities of fruit peel and root bark of *Pithecellobium dulce*.

MATERIAL AND METHODS

Collection of Plant Materials

The selected plant parts fruit peel and root bark of *Pithecellobium dulce* was collected from Kollidum, Nagapattinam District, Tamil Nadu, India. The plant was identified by Dr.R.Murugan, Associate Professor and Head, Department of Botany, Government Arts College (Autonomous), Kumbakonam – 612 002, Tamil Nadu, India. The plant materials were washed by pure water and finally with distilled water to remove any dirt particles from it. The plant materials were shade dried at room temperature for 15 days and then it had been ground into powder by using a mechanical grinder. The powdered materials were stored in air tight containers until needed.

Preparation of Plant Extracts

The powdered materials of fruit peel and root bark of *Pithecellobium dulce* were used for extraction. About 100g of powdered materials of fruit peel and root bark were taken separately and extracted by Soxhlet apparatus using different solvents ethanol, methanol, and diethyl ether. A semi-solid mass of the extract was produced by removing the solvents completely through evaporation using hot air oven at 40-50 °C following extraction. The extracts were kept at 4 °C till the time of use.

In vitro Antidiabetic Activity

Determination of α-Amylase Inhibition by 3, 5-Dinitrosalicylic Acid (DNSA) Assay

The *in vitro* antidiabetic activity of extracts of fruit peel and root bark of *Pithecellobium dulce* was determined. For this, 3.5-dinitrosalicylic acid (DNSA) method was employed to study α-amylase inhibition efficacy [28]. The extract of fruit peel and root bark of *Pithecellobium dulce* were dissolved in phosphate buffer and used various concentrations of extracts ranging from 50to 250 µg/ml to study the *in vitro* antidiabetic activity. Mixed 200 µl of theextract with 2 units/ml of α-amylase solution and then the mixture was incubated for 10 minutes at 30 °C. Subsequently, each tube was filled with 200 µl of the 1% starch solution in water (w/v) and allowed to incubate for 3 minutes. 200 µl of DNSA reagent (12 g of sodium potassium tartarate tetrahydrate in 8.0 ml of 2M NaOH and 20 ml of 96mM of 3.5-dinitro salicylic acid solution) was added to end the reaction and then heated for 10 minutes at 85 °C in water bath. After that the mixture was cooled to room temperature and then it was diluted to 5 ml with distilled water and measured the absorbance at540 nm using UV-visible spectrophotometer. To prepare the blank with 100% enzyme activity, 200 µl of the buffer was used in place of plant extract. In the absence of the enzyme solution, a blank reaction was similarly carried out using the plant extract at each concentration. Acarbose was used as a positive control, and the reaction proceeded in a manner akin to the reaction involving plant extract that was previously discussed. The inhibition of α-amylase was determined using the following equation and the results are represented as percentage of inhibition.

% Inhibition =Abs (Control) – Abs (Extract) / Abs (Control) × 100

Determination of α-Amylase Inhibition by Starch-Iodine Colour Assay

The *in vitro* antidiabetic activity of extracts of fruit peel and root bark of *Pithecellobium dulce* was determined through α-amylase inhibition by starch-iodine colour assay [29]. In this assay, 20 µl of α-amylase solution (1 mg/ml) was combined with 390 µl of phosphate buffer (0.2M phosphate buffer containing 0.06M sodium chloride) at pH 7.0 containing varying concentrations 50 to 250µg/ml of plant extracts. The samples mentioned above were incubated for 10 minutes at 37°C. After the incubation, 100µl of 1% starch solution was added and the mixture was then allowed to stand for one hour. 5 ml of distilled water and 0.1 ml of 1% iodine solution were added to the end product. At 565 nm, the absorbance was measured. The same reaction conditions were followed for blank determinations. Using the below mentioned formula, for the determination of percentage inhibition of enzyme activity was calculated.

% inhibition = (OD control – OD sample) / OD control × 100

In vitro Anti-Inflammatory Activity

The protein denaturation inhibition assay [30] was carried out to assess the *in vitro* anti-inflammatory activities of ethanol, methanol, and diethyl ether extracts of fruit peel and root bark of *Pithecellobium dulce*. The 5 ml of reaction mixture contains 2 ml of plant extract at different concentrations 50 to 250 µg/ml, 2.8 ml of phosphate-buffered saline (PBS, pH 6.4), and 0.2 ml of egg albumin (from fresh hen's egg). As a control, a comparable volume of dimethyl sulfoxide (DMSO) was taken. The mixtures were heated for five minutes at 70°C after being incubated for fifteen minutes at 37 °C. Using a spectrophotometer, the absorbance was measured at 660 nm after cooling. To determine the anti-

inflammatory activity of diclofenac sodium, which was employed as a reference medication at a concentration of 100 µg/ml, it was treated in the same manner as previously and compared with plant extracts at different concentrations. The following formula was used to calculate the percentage inhibition of protein denaturation and the results are expressed as a percentage of inhibition.

% Inhibition = 100 × (Vt / Vc - 1)

Where, Vt = absorbance of test sample, Vc = absorbance of control

Statistical Analysis

The results were subjected to statistical analysis and the values were expressed as mean± SD of triplicates.

RESULTS AND DISCUSSION

Diabetes mellitus is a chronic, diverse, and potentially fatal disease that affects people all over the world. By 2025, there will be 300 million people with diabetes worldwide, and its prevalence will be 5.4%. Southeast Asia is the most impacted region of all the WHO regions (1985), with the highest global burden of illness by 2025. Given the pandemic development of type II diabetes and the estimated 80 million people who will have the disease in the region, finding novel therapeutic approaches to address all pathological elements of the illness continues to be a significant issue for biological medical research [31]. An inadequacy in insulin secretion and the action of insulin on its target tissues is a characteristics of diabetes mellitus [32] with hyperglycemia, a clinical feature of diabetes, causing disruption in the metabolism of fat, protein, and carbohydrates [33].

Globally, there must be a sharp rise in the number of diabetes patients due to the aging population, calorie-rich diet intake, obesity, and sedentary life styles [33]. In addition to hyperglycemia, this condition is frequently linked to a significant rise incardiovascular risk factors such as dyslipidemia and triglyceridemia, which are implicated in the emergence of microvascular complications of diabetes that result in morbidity and mortality [34]. Diabetes related cardiovascular problems are exacerbated by hyperlipidemia [35] and drug therapy has been used to treat diabetes in recent years. Sulfonylureas are among the common synthetic medications used to treat diabetes. Adverse effects from these medications include nausea, vomiting, diarrhea, and stomach pain, headache, unusual weight gain, allergic reaction, hypoglycemia, dark urine, retention of fluids, or edema. As an alternative to synthetic pharmaceuticals, active research has been conducted on traditionally used medicinal herbs to find new antidiabetic medications. Repaglinide, metformin, glibenclamide, pioglitazone, sitagliptin, and acarbose are the most often used medications for the treatment of type 2 diabetes [36]. Though not all diabetic issues may be avoided, using these oral hypoglycemic medications may be beneficial in regulating blood glucose levels.

The present investigation employed the α-amylase inhibition by 3, 5-dinitrosalicylic acid (DNSA) assay to ascertain the *in vitro* antidiabetic activity of ethanolic, methanolic and diethyl ether extracts of *Pithecellobium dulce* fruit peel and root bark and the results are presented in Table 1. The findings indicated that all of the extracts examined were able to suppress the activity of α-amylase enzyme at various concentrations. The α-amylase inhibition activity of ethanolic extracts of fruit peel of *Pithecellobium dulce* at different concentrations 50, 100, 150, 200 and 250 µg/ml showed 48.23 ± 1.66%, 56.83 ± 3.32%, 64.76 ± 2.44%, 68.46 ± 1.84% and 72.03 ± 1.95%, respectively, the methanolic extracts of fruit peel showed 28.16 ± 3.25%, 36.00 ± 2.5%, 60.00 ± 2.0%, 68.50 ± 2.29% and 76.13 ± 1.70%, respectively and the diethyl ether extract of fruit peel showed 16.36 ± 1.62%, 20.93 ± 2.72%, 24.80 ± 1.74%, 31.83 ±2.75% and 44.16 ± 2.25%, respectively. The α-amylase inhibition activity of ethanolic extract of root bark of *Pithecellobium dulce* at tested concentrations 50, 100, 150, 200 and 250 µg/ml showed 51.66 ± 3.51%, 55.43 ± 2.69%, 63.86 ± 2.00%, 73.13 ± 1.90% and 76.03 ± 1.25%, respectively, the methanolic extract of root bark showed 43.40 ± 2.16%, 55.76 ± 2.15%, 68.26 ± 1.91%, 76.40 ± 1.83% and 84.46 ± 2.13%, respectively and the diethyl ether extract of root bark showed 47.96 ± 2.45%, 51.8 ± 2.20%, 55.93 ± 2.70%, 63.66 ± 2.51% and 71.93 ± 1.95%, respectively.

The ethanolic extract of fruit peel showed lower level of α-amylase inhibition 48.23 ± 1.66% at 50 µg/ml and higher level of inhibition 72.03 ± 1.95% at 250 µg/ml, the methanolic extract of fruit peel showed lower level of inhibition 28.16 ± 3.25% at 50 µg/ml and higher level of inhibition 76.13 ± 1.70% at 250 µg/ml and the diethyl ether extract of fruit peel showed the lowest inhibition 16.36 ± 1.62% at 50 µg/ml and the highest inhibition 44.16 ± 2.25% at 250 µg/ml. The ethanolic extract of root bark of *Pithecellobium dulce* exhibited the lowest inhibition 51.66 ± 3.51% at 50 µg/ml and the maximum inhibition 76.03 ± 1.25% at 250 µg/ml. The lower level of inhibition 43.40 ± 2.16% was observed in the methanolic extract of root bark at 50 µg/ml whereas the higher level of inhibition 84.46 ± 2.13% was observed at 250 µg/ml. The diethyl ether extract of root bark of *Pithecellobium dulce* revealed the lowest inhibition 47.96 ± 2.45% at 50 µg/ml and the highest inhibition 71.93 ± 1.95% at 250 µg/ml.

In the present investigation was also employed the α-amylase inhibition by starch-iodine colour assay and the results of the *in vitro* antidiabetic activity of fruit peel and root bark of *Pithecellobium dulce* at different concentrations are represented in Table 2. The *in vitro* antidiabetic activity of ethanolic extract of fruit peel at various concentrations 50, 100, 150, 200 and 250 µg/ml showed 68.73 ± 1.44%, 75.6 ± 1.93%, 71.6 ± 1.83%, 76.06 ± 2.30% and 84.6 ± 2.16%, respectively, the methanolic extract of fruit peel showed 17.50 ± 1.37%, 55.80 ± 2.55%, 64.10 ± 1.65%, 68.36 ± 2.86% and 84.56 ± 2.40%, respectively and the diethyl ether extract of fruit peelshowed 35.66 ± 1.72%, 47.83 ±2.35%, 52.03 ± 2.45%, 60.33 ± 2.31% and 64.60 ± 2.16%, respectively. The ethanolic extract of root bark of *Pithecellobium dulce* at various concentrations 50, 100, 150, 200 and 250 µg/ml revealed 67.56 ± 2.37%, 71.70 ± 2.36%, 76.06 ± 1.90%, 77.90 ± 1.55% and 86.10 ± 1.65% of inhibition, respectively, the methanolic extract of root bark showed 56.93 ± 1.79%, 60.60 ± 1.21%, 72.46 ± 1.26%, 76.46 ± 1.07% and 85.43 ± 1.20% of inhibition, respectively and the diethyl ether extract of root bark of *Pithecellobium dulce* showed 39.60 ± 1.72%, 47.63 ± 2.27%, 59.76 ± 1.56%, 72.13 ± 1.20% and 75.56 ± 1.88% of inhibition, respectively.

The ethanolic extract of the fruit peel showed that it had the maximum inhibition 84.60 ± 2.16% at 250 µg/ml and the lowest inhibition 68.73 ± 1.44% at 50 µg/ml, the methanolic extractof *Pithecellobium dulce* fruit peel exhibited a maximum inhibition 84.56 ± 2.40% at 250 µg/ml and a minimum inhibition 17.50 ± 1.37% at 50 µg/ml and the diethyl ether extract of fruit peel of *Pithecellobium dulce* exhibited the highest level of inhibition 64.60 ± 2.16% at 250 µg/ml and the lowest level of inhibition 35.66 ± 1.72% at 50 µg/ml. The ethanolic extract of root bark of *Pithecellobium dulce* showed the highest inhibitory activity 86.10 ± 1.65% at 250 µg/ml and the lowest inhibition 67.56 ± 2.37% at 50 µg/ml, the methanolic extract of root bark showed the highest inhibition 85.43 ± 1.20% at 250 µg/ml and the lowest inhibition 56.93 ± 1.79% at 50 µg/ml and the diethyl ether extract of root bark of *Pithecellobium dulce* showed the highest inhibition 75.56 ± 1.88% at 250 µg/ml and the lowest inhibition 39.60 ± 1.72% at 50 µg/ml. These results are confirmed that the *in vitro* antidiabetic activity of fruit peel and root bark of *Pithecellobium dulce* and the activity is concentration dependent, because the 250 µg/ml extract showed maximum activity and 50 µg/ml showed minimum activity.

The α-amylase enzyme hydrolyse α-1, 4-glucosidic linkages found in starch and other oligosaccharides, converting them into maltose, maltotriose, and other simple sugars[37]. By reducing the absorption of glucose that is broken down fromstarch by this enzyme and the suppression of α-amylase activity in the human digestive system arethought to manage diabetes [38]. Analogous to α-amylase, the α-glucosidase catalyzes conversion of oligosaccharides and disaccharides into monosaccharides. The inhibitors of these enzymes cause a significant decrease in the rate of glucose absorption, which blunts the postprandial plasma glucose spike [39]. They also delay and lengthen the overall period during which carbohydrates are digested. Therefore, to maintaincontrol over their glucose levels, people with diabetes typically have low α-amylase levels.

The findings of present study demonstrated that *Pithecellobium dulce* fruit peel and root bark had an inhibitory effect on α-amylase activity and that this activity was comparable to that of the reference medication acarbose. Fruit peel and root bark extracts' ability to suppress α-amylase activity may be due to the presence of certain antidiabetic phytocompounds. The α- amylaseinhibitors are also employed by plants as a defense mechanism, such as against insects. These inhibitors interfere with the way that proteinases and α-amylases function in the guts of insects, preventing them from feeding normally. Thus, α-amylase inhibitors may play a part in both crop protection and blood sugar regulation [40].

A therapeutic strategy that lowers postprandial hyperglycemia may be helpful in the treatment of diabetes. This can be accomplished by inhibiting the digestive tract enzymes that hydrolyze carbohydrates, which will delay the absorption of glucose. In the present study, the acarbose at 100 µg/ml of concentration was used as reference drug for comparing the results of the extracts of fruit peel and root bark of *Pithecellobium dulce* at various tested concentrations. Acarbose, an antidiabetic medication was utilized as the reference medication and it showed 81.21 ± 4.58% suppression of α-amylase activity at 100 µg/ml. The α-amylase inhibition assay is a method by which a plant may demonstrate its antidiabetic efficacy and which assesses the plant extract's inhibitory potency against the enzyme [41].

Table 1: *In vitro* antidiabetic activity of ethanolic, methanolic and diethyl ether extracts of fruit peel and root bark of *Pithecellobium dulce* by determination of α-amylase inhibition by 3, 5-dinitrosalicylic acid (DNSA) assay

Concentration of plant Extract (µg/ml)	*In vitro* antidiabetic activity (% of inhibition)					
	Ethanolic extract		Methanolic extract		Diethyl ether extract	
	Fruit peel	Root bark	Fruit peel	Root bark	Fruit peel	Root bark
50	48.23 ± 1.66	51.66 ±3.51	28.16 ± 3.25	43.40 ± 2.16	16.36 ± 1.62	47.96 ±2.45
100	56.83 ± 3.32	55.43 ±2.69	36.00 ± 2.50	55.76 ± 2.15	20.93 ± 2.72	51.80 ±2.20
150	64.76 ± 2.44	63.86 ±2.00	60.00 ± 2.00	68.26 ± 1.91	24.80 ± 1.74	55.93 ±2.70
200	68.46 ± 1.84	73.13 ± .90	68.50 ± 2.29	76.40 ± 1.83	31.83 ±2.75	63.66 ±2.51
250	72.03 ± 1.95	76.03 ±1.25	76.13 ± 1.70	84.46 ± 2.13	44.16 ± 2.25	71.93 ±1.95
Acarbose (100 µg/ml)	85.66 ± 1.20					

Values are expressed as mean ± standard deviation of triplicates

Table 2: *In vitro* antidiabetic activity of ethanolic, methanolic and diethyl ether extracts of fruit peel and root bark of *Pithecellobium dulce* by determination of α-amylase inhibition by starch-iodine colour assay

Concentration of plant extract (µg/ml)	*In vitro* antidiabetic activity (% of inhibition)					
	Ethanolic extract		Methanolic extract		Diethyl ether extract	
	Fruit peel	Root bark	Fruit peel	Root bark	Fruit peel	Root bark
50	68.73 ± 1.44	67.56 ± 2.37	17.50 ± 1.37	56.93 ± 1.79	35.66 ±1.72	39.60 ± 1.72
100	71.60 ± 1.93	71.70 ± 2.36	55.80 ± 2.55	60.60 ± 1.21	47.83 ±2.35	47.63 ± 2.27
150	75.60 ± 1.83	76.06 ± 1.90	64.10 ± 1.65	72.46 ± 1.26	52.03 ±2.45	59.76 ± 1.56
200	76.06 ± 2.30	77.90 ± 1.55	68.36 ± 2.86	76.46 ± 1.07	60.33 ±2.31	72.13 ± 1.20
250	84.60 ± 2.16	86.10 ± 1.65	84.56 ± 2.40	85.43 ± 1.20	64.60 ±2.16	75.56 ± 1.88
Acarbose (100 µg/ml)	89.30 ± 1.20					

Values are expressed as mean ± standard deviation of triplicates

Table 3: *In vitro* anti-inflammatory activity of ethanolic, methanolic and diethyl ether extracts of fruit peel and root bark of *Pithecellobium dulce*

Concentration of Plant extract (µg/ml)	*In vitro* anti-inflammatory activity (% of inhibition)					
	Ethanolic extract		Methanolic extract		Diethyl ether extract	
	Fruit peel	Root bark	Fruit peel	Root bark	Fruit peel	Root bark
50	21.66 ± 2.31	17.50 ± 2.29	26.63 ± 2.47	21.60 ± 1.83	18.40 ± 2.22	17.60 ± 1.80
100	27.86 ± 2.10	29.13 ± 1.80	35.56 ± 2.18	26.73 ± 1.61	27.00 ± 2.50	35.73 ± 1.90
150	46.16 ± 2.25	37.16 ± 2.25	42.11 ± 2.00	36.00 ± 1.80	45.40 ± 2.12	46.00 ± 1.85
200	55.03 ± 1.95	55.40 ± 2.42	55.40 ± 2.32	50.90 ± 1.55	54.93 ± 2.10	53.90 ± 1.11
250	74.73 ± 2.61	75.10 ± 1.65	73.96 ± 1.95	74.50 ± 2.29	63.70 ± 1.96	64.50 ± 1.50
Diclofenac sodium (100 µg/ml)	81.36 ± 2.47					

Values are expressed as mean ± standard deviation of triplicates

The *in vitro* anti-inflammatory activity of ethanolic, methanolic and diethyl ether extracts of fruit peel and root bark of *Pithecellobium dulce* was examined in the present study using the suppression of protein denaturation assay and the results are displayed in Table 3. The ethanolic extracts of fruit peel of *Pithecellobium dulce* at different concentrations 50, 100, 150, 200, and 250 µg/ml exhibited *in vitro* anti-inflammatory activity as follows: 21.66 ± 2.31%, 27.86 ± 2.10%, 46.16 ± 2.25%, 55.03 ± 1.95% and 74.73 ± 2.61%, correspondingly, the methanolic extracts of fruit peel showed 26.63 ± 2.47%, 35.56 ± 2.18%, 42.11 ± 2.00%, 55.40 ± 2.32% and 73.96 ± 1.95%, respectively and the diethyl ether extract of fruit peel showed 18.40 ± 2.22%, 27.00 ± 2.50%, 45.40 ± 2.12%, 54.93 ± 2.10% and 63.70 ± 1.96%, respectively. The ethanolic extract of root bark of *Pithecellobium dulce* at different concentrations 50, 100, 150, 200, and 250 µg/ml revealed anti-inflammatory activity 17.5 ± 2.29%, 29.13 ± 1.80%, 37.16 ± 2.25%, 55.4 ± 2.42% and 75.10 ± 1.65%, respectively, the methanolic extract of root bark showed 21.60 ± 1.83%, 26.73 ± 1.61%, 36.00 ± 1.80%, 50.90 ± 1.55% and 74.50 ± 2.29%, respectively and the diethyl ether extract of root bark showed 17.60 ± 1.80%, 35.73 ± 1.90%, 46.00 ± 1.85%, 53.90 ± 1.11% and 64.50 ± 1.5%, respectively.

The ethanolic extract of fruit peel of *Pithecellobium dulce* had the highest inhibitory activity 74.73 ± 2.61% at 250µg/ml of concentration and the lowest activity 21.66 ± 2.31% at 50µg/ml, the methanolic extract had the highest inhibitory activity 73.96 ± 1.95% at 250µg/ml and the lowest activity 26.63 ± 2.47% at 50 µg/ml and the diethyl ether extract showed the highest inhibitory activity 63.70 ± 1.96% at 250µg/ml and the lowestinhibitory activity 18.40 ± 2.22% at 50µg/ml. The ethanolic extract of *Pithecellobium dulce* root bark had the highest inhibitory activity 75.10 ± 1.65% at 250µg/ml and the lowest inhibitory activity 17.50 ± 2.29% at 50µg/ml, the methanolic extract of root bark showed the highest inhibitory activity 74.50 ± 2.29% at 250µg/ml and the lowest inhibitory activity 21.60±1.83% at 50µg/ml and the diethyl ether extract showed the highest inhibitory activity 64.50 ± 1.50% at 250µg/ml and the lowest inhibitory activity 17.60 ± 1.80% at 50µg/ml of concentration. The concentrations of extract may play an important role in the inhibitory activity, because the highest inhibitory activity was observed at maximum level of tested concentration 250 µg/ml and the lowest inhibitory activity was observed at minimum level of tested concentration at 50 µg/ml. As a reference medication, diclofenac sodium, an anti-inflammatory medication revealed 81.36 ± 2.47 % of inhibitory activity at 100µg/ml of concentration. All the solvent extracts were showed inhibitory activity at all the tested concentrations and the percentage of inhibitory activity increased along with the increased level of concentrations.

Inflammation is a response of living tissue to injury and there are four primary indicators of inflammation such as pain, redness, heat or warmness and swelling and also inflammation is the defense mechanism of body. Cellular and molecular processes lesser damage or infection during an acute inflammatory response. The resolution of acute inflammation and the restoration of tissue homeostasis are both aided by this mitigation mechanism. However, unchecked acute inflammation has the potential to turn chronic and contribute to a range of chronic inflammatory illnesses [42].

Leukocytes release proteases and other lysosome enzymes during inflammation as part of their defense functions, which further damages tissue and fuels inflammation [43]. The release of lysosomal enzymes during inflammation causes a range of disorders that damage macromolecules and cause lipid peroxidation of membranes, which is thought to be the cause of certain pathological conditions like rheumatoid arthritis, heart attacks, and septic shocks, among others. These enzymes' extracellular activity is thought to be connected to either acute or chronic inflammation. Preventing the release of lysosomal components of activated neutrophils, such as bactericidal enzymes and proteases, which further exacerbate tissue inflammation and damage upon extracellular release, or by stabilizing the lysosomal membrane, stabilization of the lysosomal membrane plays a crucial role in reducing the inflammatory response [44].

Disruption to the cell membrane will also increase the cell's vulnerability to secondary damage caused by lipid peroxidation brought on by free radicals [45]. The process of denaturing proteins involves applying an external stressor or compound such as heat, an organic solvent, a concentrated inorganic salt, or a strong acid or base that causes the proteins to lose their secondary and tertiary structures. Inflammation is known to be caused by protein denaturation, when denatured the majority of biological proteins cease to function biologically. Non steroid anti-inflammatory medications are used to treat inflammation; however, they have several side effects, including gastrointestinal and skin irritation that can result in rashes and ulcers [46]. The present study's findings also supported the *Pithecellobium dulce* fruit peel and root bark's *in vitro* anti- inflammatory properties and these outcomes matched those of the reference medication. Thus, by acting as an anti-inflammatory synthetic medicine, extracts from the fruit peel and root bark of *Pithecellobium dulce* may perform one or more of the aforementioned actions to keep tissues from inflammation.

The increased absorbance in the extracts with respect to control indicates the protein stabilizing activity (denaturation is inhibited) with increased concentration of extract. Denaturation of tissue proteins may be the cause behind the production of autoantigens in certain arthritic diseases. So it may be said that tissue protein denaturation is a marker for inflammatory and arthritic diseases [30]. Agents that can prevent protein denaturation, therefore, would be possible candidate for anti-inflammatory drug development. The *in vitro* test was done as a preliminary screen to check presence of antidiabetic and anti-inflammatory activities before doing the *in vivo* experiment.

CONCLUSION

The present study's findings validated and concluded that the ethanolic, methanolic, and diethyl ether extracts of *Pithecellobium dulce* fruit peel and root bark's *in vitro* antidiabetic and anti-inflammatory properties. Further study is needed to find the active principles present in the *Pithecellobium dulce* fruit peel and root bark with exact mechanism of action for their antidiabetic and anti-inflammatory effects.

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CONFLICTS OF INTEREST
The authors declare that they have no conflict of interests regarding the publication of this work.

REFERENCES
1. Grover JK, Yadav S, Vats V. (2002). Medicinal plants of India with antidiabetic potential. Journal of Ethnopharmacology. 81(1):81-100
2. Dragland S, Senoo H, Wake K, Holte K, Blomhoff R. (2003). Several culinary and medicinal herbs are important sources of dietary antioxidants. The Journal of Nutrition. 133(5):1286-1290
3. Seth SD, Sharma B. (2004). Medicinal plants in India. Indian Journal of Medical Research. 120(1):9-11
4. Ramachandran A, Snehalatha C, Viswanathan V. (2002). Burden of type 2 diabetes and its complications - The Indian scenario. Current Science. 83(12):1471-1476.
5. Huang TH, Peng G, Kota BP, Li GQ, Yamahara J, Roufogalis BD, Li Y. (2005). Antidiabetic action of *Punica granatum* flower extract: activation of PPAR-γ and identification of an active component. Toxicology and Applied Pharmacology. 207(2):160-169
6. Barnes PJ. (2009). Targeting the epigenome in the treatment of asthma and chronic obstructive pulmonary disease. Proceedings of the American Thoracic Society. 6(8):693-696
7. Ahmed AU. (2011). An overview of inflammation: mechanism and consequences. Frontiers in Biology. 6(4):274-281
8. Vane JR, Botting RM. (1995). New insights into the mode of action of anti-inflammatory drugs. Inflammation Research. 44(1):1-10
9. Bagad AS, Joseph JA, Bhaskaran N, Agarwal A. (2013). Comparative evaluation of anti- inflammatory activity of curcuminoids, turmerones and aqueous extract of *Curcuma longa*. Advances in Pharmacological Sciences. 2013(Article ID 805756):1-7
10. Ghasemian M, Owlia MB. (2015). A different look at pulsed glucocorticoid protocols; is high dose oral prednisolone really necessary just after initiation of pulse therapy?. Journal of Case Reports in Practice. 3(1):1-3
11. Scartezzini P, Speroni E. (2000). Review on some plants of Indian traditional medicine with antioxidant activity. Journal of Ethnopharmacology. 71(1-2):23-43
12. Sivakumar A, Murugesan M. (2005). Ethnobotanical studies on the wild edible plants used by the tribals of Anaimalai Hills, the Western Ghats. Ancient Science of Life. 25(2):69
13. Pal PB, Pal S, Manna P, Sil PC. (2012). Traditional extract of *Pithecellobium dulce* fruits protects mice against CCl_4 induced renal oxidative impairments and necrotic cell death. Pathophysiology 19(2):101-114
14. Sukantha TA, Sripathi SK. (2015). Isolation and characterization of secondary metabolites from *Pithecellobium dulce* Benth fruit peel. International Journal of Pharmacy and Pharmaceutical Sciences. 7(11):199-203
15. Pakutharivu T, Anitha A, Usha V, Sharmila S, Chitra S. (2015). Cardioprotective activity of *Pithecellobium dulce* fruit peel on isoproterenol induced myocardial infarction in rats. Int. J. Pharm. Sci. 30(1):133-136
16. Katekhaye SD, Kale MS. (2012). Antioxidant and free radical scavenging activity of *Pithecellobium dulce* (Roxb.) Benth wood bark and leaves. Free Radicals and Antioxidants. 2(3):47-57
17. Bagchi S, Kumar KJ. (2016). Studies on water-soluble polysaccharides from *Pithecellobium dulce* (Roxb.) Benth. seeds. Carbohydrate Polymers. 138:215-221
18. Sukantha TA, Subashini KS, Ravindran NT, Balashanmugam P. (2011). Evaluation of *in vitro* antioxidant and antibacterial activity of *Pithecellobium dulce* Benth fruit peel. Int J Curr Res. 3(11):378-382
19. Sukantha TA, Subashini KS, Ravindran NT. (2014). Antibacterial activity of selected medicinal plant in traditional treatment of wound infection in Southeast India. Int J Pharm Pharm Sci. 6(11):511-3
20. Sugumaran M, Vetrichelvan T, Quine SD. (2009). Antidiabetic potential of aqueous and alcoholic leaf extracts of *Pithecellobium dulce*. Asian Journal of Research in Chemistry. 2(1):83-85
21. Olivares-Pérez J, Avilés-Nova F, Albarrán-Portillo B, Castelán-Ortega OA, Rojas-Hernández S. (2013). Nutritional quality of *Pithecellobium dulce* and *Acacia cochliacantha* fruits, and its evaluation in goats. Livestock Science. 154(1-3):74-81
22. Pradeepa S, Subramanian S, Kaviyarasan V. (2014). Evaluation of antimicrobial activity of *Pithecellobium dulce* pod pulp extract. Asian Journal of Pharmaceutical and Clinical Research. 7(1):32-37
23. Pithayanukul P, Ruenraroengsak P, Bavovada R, Pakmanee N, Suttisri R, Saen-oon S. (2005). Inhibition of *Naja kaouthia* venom activities by plant polyphenols. Journal of Ethnopharmacology. 97(3):527-533
24. Singh M, Khatoon S, Singh S, Kumar V, Rawat AKS, Mehrotra S. (2010). Antimicrobial screening of ethnobotanically important stem bark of medicinal plants. Pharmacognosy Research. 2(4):254
25. Sotelo A, Lucas B, Garza L, Giral F. (1990). Characteristics and fatty acids content of the fat of seeds of nine wild Mexican plants. Journal of Agricultural and Food Chemistry. 38(7):1503-1505
26. Duke JA. (2002). Handbook of medicinal herbs. CRC press
27. Duke JA. (2017). Handbook of nuts: herbal reference library. CRC press

28. Lorenz MG. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal Chem. 31(3):426–8
29. Xiao Z, Storms R, Tsang A. (2006). A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. Anal Biochem. 351(1):146–8
30. Dey P, Chatterjee P, Chandra S, Bhattacharya S. (2011). Comparative *in vitro* evaluation of anti-inflammatory effects of aerial parts and roots from *Mikania scandens*. Journal of Advanced Pharmacy Education & Research. 1(6):271-7
31. Semwal DK, Bamola A, Rawat U. (2007). Chemical constituents of some antidiabetic plants. Univ J Phytochem Ayur Heig. 2(3):40-48
32. Ahmad F, Azevedo JL, Cortright R, Dohm GL, Goldstein BJ. (1997). Alterations in skeletal muscle protein-tyrosine phosphatase activity and expression in insulin-resistant human obesity and diabetes. The Journal of Clinical Investigation. 100(2):449-458
33. Ghosh R, Sharatchandra KH, Rita S, Thokchom IS. (2004). Hypoglycemic activity of *Ficus hispida* (bark) in normal and diabetic albino rats. Indian Journal of Pharmacology. 36(4):222
34. Taskinen MR. (2002). Diabetes Dyslipidemia. Atheroscler Suppl. 3(1):47-51
35. Nabel EG. (2003). Cardiovascular disease. New England Journal of Medicine. 349(1):60-72
36. Bodmer M, Meier C, Krahenbuhl S, Jick SS, Meier CR. (2008). Metformin, sulfonylureas, or other antidiabetes drugs and the risk of lactic acidosis or hypoglycemia: a nested case-control analysis. Diabetes Care. 31(11):2086-2091
37. Dineshkumar B, Mitra A, Manjunatha M. (2010). A comparative study of alpha-amylase inhibitory activities of common antidiabetic plants at Kharagpur 1 block. International Journal of Green Pharmacy. 4(2).
38. He Q, Lv Y, Yao K. (2007). Effects of tea polyphenols on the activities of α-amylase, pepsin, trypsin, and lipase. Food Chemistry. 101(3):1178-1182
39. Reddy GJ, Reddy KB, Reddy GS. (2019). In vitro α-Amylase and α-Glucosidase inhibitory activity of extracts of *Feronia elephantum* fruit and *Paspalum scrobiculatum* Grains. Asian J. Pharm. Pharmacol. 5(S1):42-47
40. Kumanan R, Manimaran S, Saleemulla K, Dhanabal SP, Nanjan MJ. (2010). Screening of bark of *Cinnamomum tamala* (Lauraceae) by using α-amylase inhibition assay for antidiabetic activity. International Journal of Pharmaceutical and Biomedical Research. 1(2):69-72
41. Oboh G, Ademiluyi AO, Akinyemi AJ, Henle T, Saliu JA, Schwarzenbolz U. (2012). Inhibitory effect of polyphenol rich extracts of jute leaf (*Corchorus olitorius*) on key enzyme linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin I converting) *in vitro*. Journal of Functional Foods. 4(2):450-458
42. Zhou Y, Hong Y, Huang H. (2016). Triptolide attenuates inflammatory response in membranous glomerulonephritis rat via down regulation of the NF-κB signaling pathway. Kidney and Blood Pressure Research. 41(6):901-910
43. Okoli CO, Akah PA, Onuoha NJ, Okoye TC, Nwoye AC, Nworu CS. (2008). *Acanthus montanus*: An experimental evaluation of the antimicrobial, anti-inflammatory, and immunological properties of a traditional remedy for furuncles. BMC Complementary and Alternative Medicine. 8:1-11
44. Vadivu R, Lakshmi KS. (2008). *In vitro* and *in vivo* anti-inflammatory activity of leaves of *Symplocos cochinchinensis* (Lour) Moore ssp laurina. Bangladesh Journal of Pharmacology. 3(2):121-124
45. Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziwa P, Nkeh-Chungag BN, Iputo JE. (2010). An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation, and white blood cell migration during acute inflammation. J Med Plants Res. 4(9):789-795
46. Leela Prakash G, Dass SM. (2011). In vitro anti-inflammatory activity of methanol extract of *Enicostemma axillare*. International Journal of Drug Development and Research. 3(3):189-196