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Phytochemical analysis of few selected medicinal plants used for the treatment of diabetes in Assam, India

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Abstract

Diabetes mellitus has been found to be managed and controlled by various herbal plant extracts. Different herbal products are used as antidiabetic supplements. Various phytochemicals belonging to different classes like flavonoids, alkaloids, phenolic compounds, steroids, tannins, coumarins, terpenoids, etc. have demonstrated bioactivity against high blood glucose level in diabetic patients. Phytochemicals are actually naturally synthesized secondary plant metabolites. In the present study phytochemical screening and Fourier Transform Infrared Spectroscopy (FT-IR) analysis of different parts of some selected antidiabetic medicinal plants was carried out for characterization of the crude extract and detection of functional groups in these plant parts. The FT-IR spectroscopic analysis has shown the presence of various important functional groups. The results of phytochemical screening have confirmed the presence of various secondary plant metabolites viz., steroids, alkaloids, glycosides, flavonoids, terpenoids, carotenoids, tannins and phenolic compounds in the studied plant parts. Hence, the present study gives a strong support and offers a base of using these plant species as herbal alternative for the diabetic patients.

Keywords: FT-IR spectroscopy, phytochemicals, functional groups, secondary metabolites, diabetes.

Introduction

Diabetes is one type of chronic disease. It is one of the major degenerative diseases in the world today. Diabetes is characterized by high blood glucose levels. Various symptoms associated with diabetes are fatigue, frequent urination, blurred vision, mood changes, excessive thirst etc. Diabetes is associated with various long-term complications affecting the heart, retina, kidney, liver and nervous system[1]. Diabetes is a disorder of carbohydrate metabolism in which glucose molecules are not oxidized properly due to lack of insulin[2]. Diabetes affects nearly 5% of the total world population[3]. The accumulation of unused sugar molecules lead to their appearance in the blood and urine. Apart from different synthetic drugs, a large number of herbal drugs have been used for the treatment of diabetic patients. In Indian medicinal system a large number of medicinal plants are used for the treatment of diabetes[4]. There has been an increasing demand of herbal products with antidiabetic activity. The available literature has reported that more than 400 plant species are used in traditional medicine for the treatment of diabetes[5]. People living in different rural areas of Assam have a very long history of traditional knowledge of antidiabetic medicinal plants.

The medicinal plants are found to be the most important source in primary health care system in the developing countries. The therapeutic activity of a plant depends upon the bioactive compounds present in those plant parts used in preparation of the herbal medicine. Plants contain a number of biologically active components referred to as secondary metabolites which are biosynthesized during the normal metabolic processes of plants. Secondary metabolites are mainly responsible for the biological activities which also promote health benefit effects. The medicinal properties of the plants lie in various chemical substances that also produces some physiologic actions on human bodies. The most important of these bioactive constituents are steroids, alkaloids, glycosides, flavonoids, terpenoids, carotenoids, tannins and phenolic compounds[6]. A large numbers of diseases are known to be treated with different herbal medicines throughout the long history of mankind. A large number of purified constituents of these plants have shown beneficial therapeutic effects. Medicinal plants are found to be the richest bio-resources of traditional folk medicines. Natural medicines are gradually becoming a popular part of healthcare system worldwide[7].

Garcinia is a genus of polygamous trees distributed in tropical Asia and Africa. *Garcinia pedunculata* is an evergreen tree with fluted trunk having short spreading branches. The mature fruit can be eaten cooked or raw. It is very commonly available in North Eastern (NE) region of India. In Assam, locally it is known as Borthekera. The ripe fruit is yellow coloured and about 4-6cm in diameter. *Garcinia pedunculata* are known to have different medicinal value and are commonly used in traditional medicinal system for treatment of various diseases[8]. Fruits of *Garcinia pedunculata* are used as an astringent, cardiotonic, antiscorbutic and emollient. It is reported that rural community of Assam are using ripe fruit of *Garcinia*

pedunculata for the treatment of diabetes mellitus[9]. *Musa paradisiacal* is commonly known as Banana in English. It is one kind of perennial tree like herb generally grown indigenously throughout the tropical regions. Fruits are golden yellow colour on ripening. Plantains provide a very good source of vitamin. Plantains are used in the folklore management of various diseases like diabetes mellitus, ulcer and wound healing[10]. It grows 5-9m in height, with tuberous rhizome and long pseudostem. The ripe fruits are sweet and juicy. Mature unripe fruit of *musaparadisiaca* are reported to be used in the treatment of diabetes[11]. *Aegle marmelos* is a sacred tree of India. It is a medium size tree of Rutaceae family. This tree is naturally grown and also cultivated all over India. This plant is known as Bael tree. Leaves are green and trifoliate. The leaflets are generally oval shaped. This tree is found all over India. It is found in the sub mountainous regions and also in plains almost throughout India. Mature leaves emit a special type of fragrance. The flowers are generally greenish white coloured with a peculiar fragrance. Fruit is spherical and oval in shape. Fruits are yellowish green. These are good source of vitamin C and protein. There are various medicinal uses of this plant in curing diarrhea, poor absorption, bleeding, vomiting, nausea with blood and bronchitis. Almost all parts of this plant are used in traditional folk medicine against various human ailments. It is reported that *Aegle marmelos* leaf are used for the treatment of diabetes in herbal folk medicines[12,13]. Bael fruits are used in health problems like diarrhea, dysentery etc. The leaf extracts are reported to have antispermatogenic effect and can be used in fertility control.

Three antidiabetic plants namely *Garcinia pedunculata*, *Musa paradisica* and *Aegle marmelos*have been selected for the present study. The present investigation was carried to analyze the phytochemical constituents of *Garcinia pedunculata* ripe fruit pulp, *Musa paradisiaca* unripe fruit pulp and *Aegle mermelos* leaves. The present study was also aimed to identify the functional groups present in crude ethanolic extract of *Garcinia pedunculata* ripe fruit pulp, *Musa paradisiaca* unripe fruit pulp and *Aegle mermelos* leaves through FT-IR spectroscopy.

Materials and methods

Plant materials: The fresh ripe fruit of *Garcinia pedunculata,* unripe fruit of *Musa paradisiaca and* leaves of *Aegle mermelos* were collected. The plant parts were washed thoroughly with clean water in order to remove dust, soil, sand particles, dirt and other different solid contaminants. After washing the collected plant parts were cut into small pieces and thoroughly air dried under shade at room temperature. The dried plant materials are then cut into small pieces and were ground into powder and stored carefully in closed sample bottle until further extraction.

Sampling area: The plant parts were collected from Nalbari district of Assam, India. These plant parts were collected from various local village area of Barbhag Revenue Circle of Nalbari district. The plant parts were authenticated from the department of Botany, Royal Global University, Guwahati, Assam. Nalbari district is situated between 91°E and 91°47′E longitude and 26°N and 27°N latitude.

Preparation of plant extracts: The dried powdered plant parts were mixed with the solvents and stirred magnetically in magnetic stirrer for 48 hours at room temperature. Solvents like hexane, dichloromethane, ethyl acetate and ethanol are used for extract preparation. The solvent extracts were filtered using Buchner funnel; Whatman filter paper and a vacuum pump. The solid residues were removed by filtration and these were once again extracted as above and filtered. The combined filtrates were evaporated to dryness at under reduced pressure in a rotary evaporator to get the crude solvent extracts. The crude plant extracts were then used for further investigations.

Chemicals: Analytical grade chemicals and solvents collected from S.D. Fine Chemicals, Sisco Research Laboratories, Qualigens, E. Merck and Sigma Chemicals (U.S.A.) were used.

Qualitative phytochemical analysis: Preliminary phytochemical analysis of various crude plant extracts were carried out using standard procedures and tests[14-16].

Identification of functional groups: The FT-IR spectral analysis was used to identify and characterize the functional groups of the phytochemical components present in the plant samples. The scan range was taken from 400 to 4000cm^{-1}.

Preparation of IR sample: Oven-dried powder of ethanol extracts of each plant materials were used for FTIR analysis.10 mg of the sample of plant extract was mixed with 100mg KBr and then compressed to prepare translucent sample discs. The sample discs of each plant specimen were loaded in FTIR spectroscope and spectra were recorded in the absorption range between 400 and 4000cm^{-1}. All the analysis were carried out using Shimadzu FT-IR spectrometer.

Results and discussion

Table-1: Percentage yields of various plant extracts (%).

Part of plant	Percentage yields of plant extracts (%)			
	Hexane extract	Dichloro methane extract	Ethyl acetate extract	Ethanol extract
Garcinia pedunculata ripe fruit pulp	0.92	1.87	1.21	2.11
Musa paradisiaca unripe fruit pulp	1.13	2.21	1.97	2.46
Aegle mermelos leaves	0.95	1.79	1.63	2.37

Table-2: Preliminary phytochemical screening of crude extract of *Garcinea pedunculata* ripe fruit pulp.

Phytochemicals	Hexane extract	Dichloromethane extract	Ethyl acetate extract	Ethanol extract
Alkaloids	– –	– –	– –	– –
Glycosides	++	++	– –	++
Cardiac glycosides	– –	– –	– –	– –
Tannins and phenolic compounds	++	++	++	++
Flavonoids	++	++	++	++
Saponins	– –	++	++	++
Phytosterols	– –	– –	– –	– –
Carbohydrates	++	++	++	++
Protein and Amino acids	– –	– –	– –	– –
Phlobatannins	– –	– –	– –	– –
Coumarins	– –	– –	– –	– –

Key: + + = Present; - - = Absent

Table-3: Preliminary phytochemical screening of crude extract of *Musa paradisiacal* unripe fruit pulp.

Phytochemicals	Hexane extract	Dichloromethane extract	Ethyl acetate extract	Ethanol extract
Alkaloids	– –	– –	++	++
Glycosides	– –	– –	– –	– –
Cardiac glycosides	– –	– –	– –	– –
Tannins and phenolic compounds	++	++	++	++
Flavonoids	++	++	++	++
Saponins	– –	++	++	++
Phytosterols	– –	– –	– –	– –
Carbohydrates	++	++	++	++
Protein and Amino acids	– –	– –	– –	– –
Phlobatannins	– –	– –	– –	– –
Coumarins	– –	– –	– –	– –

Key: + + = Present; - - = Absent

Table-4: Preliminary phytochemical screening of crude extract of *Aegle mermelos* leaves.

Phytochemicals	Hexane extract	Dichloromethane extract	Ethyl acetate extract	Ethanol extract
Alkaloids	++	++	++	++
Glycosides	_ _	_ _	_ _	_ _
Cardiac glycosides	_ _	_ _	_ _	_ _
Tannins and phenolic compounds	++	++	++	++
Flavonoids	_ _	++	++	++
Saponins	++	_ _	++	++
Phytosterols	_ _	_ _	_ _	_ _
Carbohydrates	_ _	_ _	_ _	_ _
Protein and Amino acids	_ _	_ _	_ _	_ _
Phlobatannins	++	++	++	++
Coumarins	_ _	++	_ _	_ _

Key: + + = Present; - - = Absent

Table-5: FT-IR peak values and functional groups of crude ethanol extract of ripe fruit pulp of *Garcinia pedunculata*

Wave number in cm-1 (of the test sample)	Bond stretching	Corresponding functionality
856.43	C-H	Aromatic C-H bending
1400.38	C=C aromatic	Aromatic compound
1633.78	C=O	Amide C=O stretching
1736.01	C=O	Ketone C=O stretching
2940.61	C-H	Methylene C-H stretching
722.37	C-X	Alkyl halides
609.53	C-X	Alkyl halides

Table-6: FT-IR peak values and functional groups of crude ethanol extract of *Musa paradisiaca* unripe fruit pulp.

Wave number in cm-1 (of the test sample)	Bond stretching	Corresponding functionality
736.84	C-H	Aromatic C-H bending
1628.95	C=C	Aromatic C=C stretching
2866.34	C-H	Alkyl C-H stretching
2928.07	C-H	Methylene C-H stretching
3432.48	O-H	Alcoholic O-H stretching

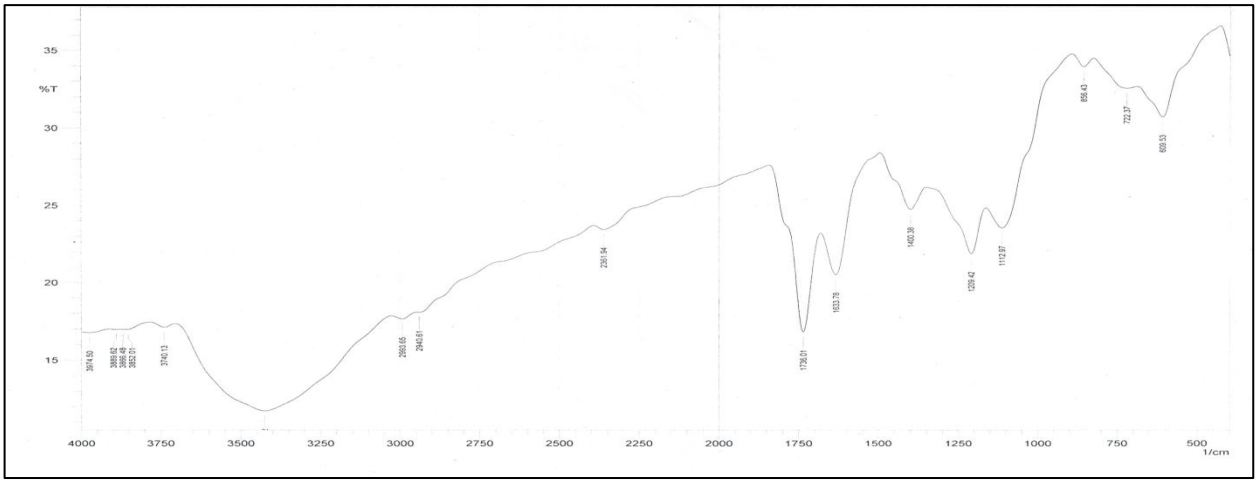

Figure-1: FT-IR Spectrum of ethanol extract of Garcinea pedunculata ripe fruit pulp.

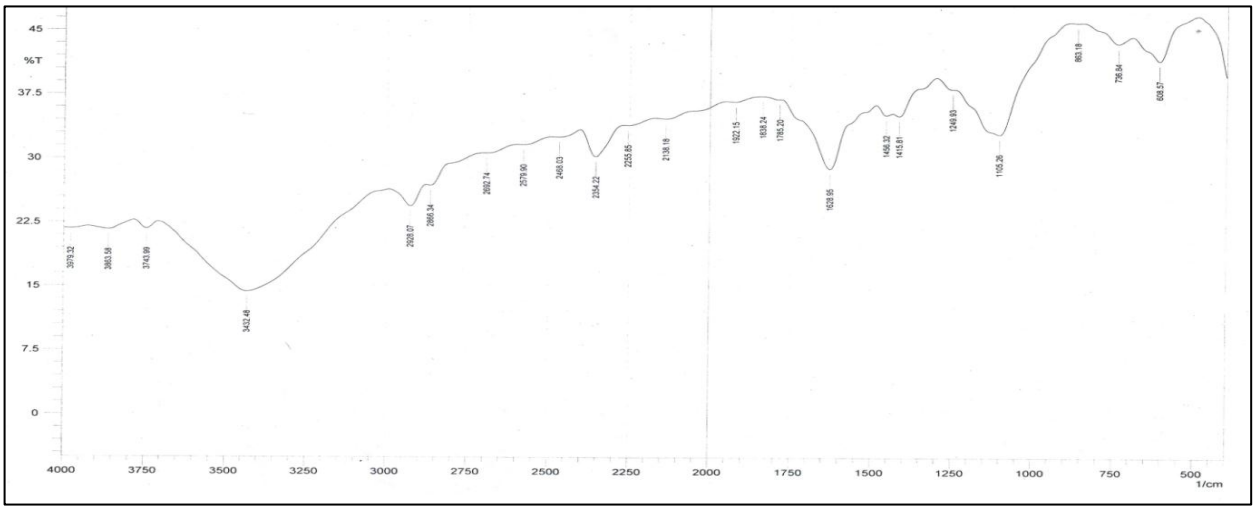

Figure-2: FT-IR Spectrum of ethanol extract of Musa paradisiaca unripe fruit pulp.

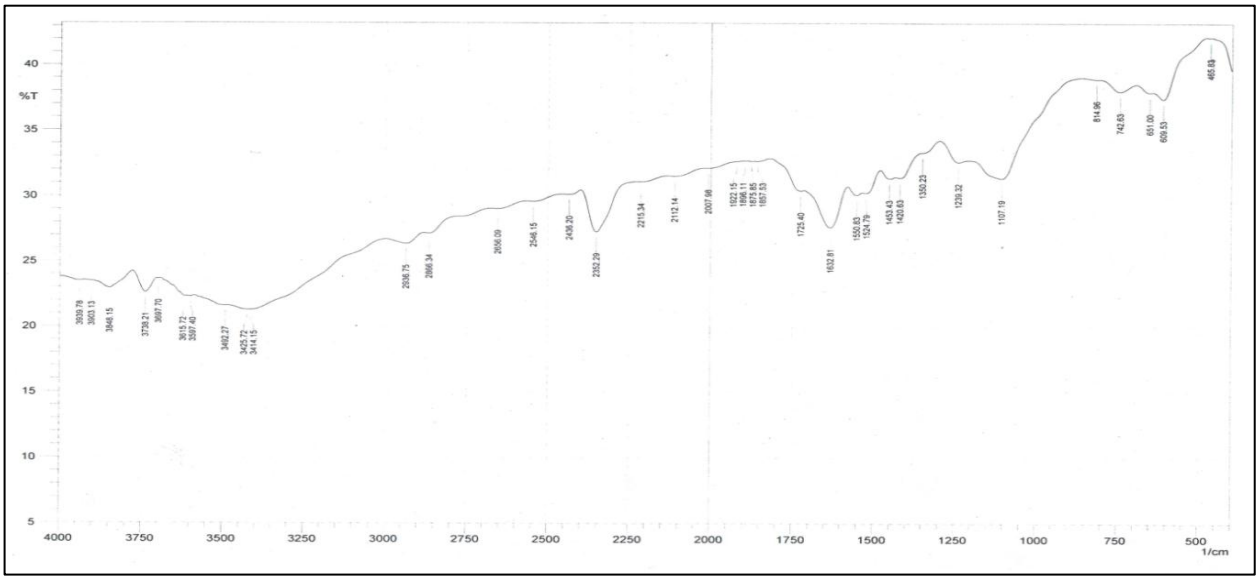

Figure-3: FT-IR Spectrum of ethanol extract of Aegle mermelos leaves.

Table-7: FT-IR peak values and functional groups of crude ethanol extract of *Aegle marmelos* leaves.

Wave number in cm-1 (of the test sample)	Bond stretching	Corresponding functionality
742.63	C-H	Alkene C-H
814.96	C-H	Alkene C-H
1239.32	C-O	C-O of alcohol group
1524.79	C=C	Aromatic C=C

The efficacy of medicinal plants depends on their different phytochemical constituents. A large number of plant species are reported to have potential antidiabetic activity[17]. Various herbal drugs are used as antidiabetic agents. These plant derived constituents, commonly known as phytochemicals, belonging to various classes of compounds like tannins, flavonoids, alkaloids, glycosides, coumarins, polysaccharides, phenolic compounds, steroids etc. These are the main effective compounds of different medicinal plants. Many phytochemical constituents have demonstrated antidiabetic activities[18].

Flavonoids are found to be exhibit a broad range of biological activities such as antimicrobial, antiviral, anti-inflammatory, anticancer, anti-tumour and anti-allergic properties[19]. Flavonoids are also reported to be highly water-soluble antioxidant as well as free radical scavengers, which prevent oxidative damage of cells. Flavonoids can reduce the risk of heart disease and it provides significant anti-inflammatory activity[20]. Flavonoids prevent diabetes complicacy by their antioxidant effect[21]. Dietary intake of flavonoid reduces the risk of diabetes. In plant kingdom various phenolic compounds are present. They have also pharmacological activities including antioxidant property. Polyphenol compounds protect the organs from free radicals damage. Their high level of antioxidant activities ultimately help to prevent diabetes. Tannins are polyphenol compounds. They are generally anti nutritional compounds. Tannins are reported to have different physiological effects like antisecretolytic, antimicrobial and antiparasitic effects. A large number of flavonoid and tannin compounds are reported to have anti-diabetic properties. Alkaloids are generally toxic substances but they often possess various pharmacological effects. Alkaloids mainly show antidiabetic activity by inhibiting alpha-glucosidase[22]. Saponins are found to be heterogeneous groups of natural products. Triterpenoid and steroidal are mainly two types of major saponins. Saponins have different pharmalogical properties including hypoglycemic activity.

The study of these three plant species namely *Garcinia pedunculata*, *Musa paradisiaca* and *Aegle mermelos* for screening of phytochemical components were performed using generally accepted standard laboratory technique of qualitative analysis. Solvent extracts of *Garcinia pedunculata* ripe fruit pulp contains glycosides, flavonoids, saponins, carbohydrates, tannins and phenolic compounds. Solvent extracts of *Musa paradisiaca* unripe fruit pulpcontains alkaloids, flavonoids, saponins, carbohydrates, tannins and phenolic compounds. Solvent extracts of *Aegle mermelos* leaves contains alkaloids, flavonoids, saponins, coumarins, phlobatannins, tannins and phenolic compounds.

FT-IR spectral analysis is useful for identification of compounds. IR spectroscopy is used for the identification of various functional groups like hydroxyl groups, carboxylic acid, amines, aromatic compounds, hydrocarbons, aliphatic bromo compounds, aryl disulfides, ester compounds, alkenyl groups, ether groups, nitro compounds, amide compounds etc. in the molecules. Such functional groups can be easily identified by their respective absorption bands. In the present analysis, the crude ethanolic extract of *Garcinia pedunculata* ripe fruit pulp, *Musa paradisiaca* unripe fruit pulp and *Aegle mermelos* leaves were subjected to FT-IR analysis. The results of FT-IR peak values and functional groups were represented in Tables- 5, 6 and 7. The FT-IR spectroscopic analysis of ethanolic extract of *Garcinia pedunculata* ripe fruit pulp indicated the presence of 856.43cm^{-1} (aromatic C-H bending), 1400.38cm^{-1} (C=C of aromatic compounds), 1633.78 cm^{-1} (amide C=O), 1736.01cm^{-1} (ketone C=O), 2940.61cm^{-1} (methylene C-H) and 722.37cm^{-1} (alkyl halides) as represented in Table-5. FT-IR spectral analysis of ethanolic extract of *Musa paradisiaca* unripe fruit pulp indicated the presence of 736.84cm^{-1} (aromatic C-H bending), 1628.95cm^{-1} (aromatic C=C stretching), 2866.34cm^{-1} (alkyl C-H stretching), 2928.07cm^{-1} (methylene C-H stretching), and 3432.48cm^{-1} (alcoholic O-H stretching)as represented in Table-6. FT-IR analysis of ethanolic extract of of*Aegle mermelos* leaves indicated the presence of 742.63cm^{-1} (alkene C-H), 1239.32cm^{-1} (C-O of alcohol group) and 1524.79cm^{-1} (aromatic C=C) as represented in Table-7.

The phytochemical screening in the study has indicated the presence of different biomolecules such as flavonoids, alkaloids, tannins, triterpenoids, carbohydrate, glycosides, saponins etc. Out of all these phytochemicals mainly flavonoids, tannins and saponins may be responsible for the antidiabetic activities of these plants. The information of these phytochemical constituents has provided a strong justification of the traditional use of these plants for the treatment and management of diabetes. The FT-IR spectrum was used to identify different

functional groups of the phytochemicals based on the characteristic peak value in the region of infrared radiation. Based on the functional group analysis in IR spectrum, it can be concluded that alkanes, alkenes, hydroxyl group, ketone, aromatic rings, alkyl halides and amides might be present in different phytochemicals.

Conclusion

The results of the present study has confirmed that the investigated plant species namely *Garcinia pedunculata*, *Musa paradisiaca* and *Aegle mermelos* can be considered as wealthy resource of phyto-constituents which can be isolated as well as examined for bio-efficacies and different pharmacological activities. The Fourier transform infrared (FT-IR) spectra of all plant extracts revealed the presence of various functional groups ranging from O-H stretching for hydroxyl group, alkenes (C=C), alkanes (C-H), ketone (C=O), aromatic rings (C=C), alkyl halides (C-X) and amides (C=O). This type of spectral analyses and phytochemical screening is the first step towards understanding the nature and types of active principles in these medicinal plants which may be helpful for further detailed analysis. The presence of various biologically important secondary plant metabolites detected in the present investigation has clearly proved the usefulness of these plant extracts in diabetes treatment. Further studies are needed with these plants to identify the unknown functional groups, isolate, purify, characterize and elucidate the exact structure of the bioactive compounds which are mainly responsible for the antidiabetic activity and other medicinal values.

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