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ANTI-DIABETIC MEDICINAL PLANT: A REVIEW

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ABSTRACT

Medicinal plants have a vast potential in the treatment of various ailments due to the presence of therapeutically important phytochemicals. Diabetes mellitus is a serious chronic metabolic disorder and several marketed medications are available to alleviate the symptoms of diabetes mellitus. However, these over the counter drugs are expensive and associated with several complications. Herbal medicines are cost-effective and also they are a improved therapeutic effects with minimum side effects. In the present review includes the reports available on medicinal plants such annona squamosa Momordica charantia, ocimum santum, azadriacsta indca, used for treating diabetes mellitus complication. The aim of the review is to categorize and summarize the available information on medicinal plants with anti-diabetic properties and suggesting outlooks for future research. A systematic search was performed on medicinal plants with anti-diabetic properties using several search engines such as science hub, science direct, Google Scholar, PubMed, and other online journals and books. All the plants listed in this review are native to Asian countries and are routinely used by the traditional practitioners for the treatment of various disease. Based on the literature data available, a five medicinal plants with anti-diabetic, anti-hyperglycemic, hypoglycemic, anti-lipidemic and insulin mimetic properties have been compiled in this review. This review provides useful information about the different medicinal plants for treating diabetes-associated complications.

KEYWORDS Diabetes: Herbal drugs, Annona squamosa, Momordica charantia, Ocimum santum, Azadriacsta indica.

INTRODUCTION

Diabetes mellitus is a serious chronic metabolic disorder and It is the third leading cause of death globally, preceded only by cancer and coronary heart disease. Diabetes mellitus of has two types, namely Type I (insulin-dependent) and Type II (non-insulin-independent). Type 2 diabetes is chronic endocrine disorderly caused by lack of insulin and/or reduced the insulin activity and accounts for upwards of 90% of diabetes cases, while Type 1 diabetes accounts for the remaining 5–10% and normally occurs in young adults. The World Health Organization (WHO) estimated that 65-80% of populations from developing countries rely on natural remedies for their health care due to a lower socio-economic status. A WHO survey also reported that more than 422 million people are suffering from diabetes worldwide and 1.6 million death are directly to diabetes. It is estimated that in South Asian countries alone, in the prevalence of diabetes will increase by 151% between the years 2000 and 2030. Diabetes is posing a serious economic burden to both the developed and developing worlds, therefore it is imperative to discover an effective treatment with fewer side effects. Diabetes cannot be cured with allopathic drugs treatment, as these drugs are unable to restore normal glucose balance and carry hosts of negative side effects. There is an urgent need for alternative medicines that are more effective, affordable, and carry fewer side effects. The most frequently used anti-diabetic plants in many countries are Annona squamosa, Azadirachta indica, Momordica charantia L., syzygium cumini, ocimum santum, etc,. Herbal practitioners across the world claim to treat diabetes with medicinal plants, and recently herbs have gained the attention of the medical community as a reliable source of diabetic medicines. Different pharmaceutical products used in the treatment of diabetes are derived from phytocompounds of plant origin. Traditional medicines arepossess strong therapeutic properties and have a markedly lower toxicity than allopathic drugs. The present research was designed to collect detailed ethnomedicinal knowledge of anti-diabetic plants from selected remote regions of India and to review the pharmacological and phytochemical literature in order to provide quantifiable reliability to the long held traditional medicinal knowledge.

Table 1: Some Medicinal Plant used in Diabetes mellitus.[18]

Plant Name	Family	Synonym	Chemical Constituent	Plant used for study	Application
Annona Squamosa linn.	Annonaceae	Custard Sugar, Shitafal	Streptozotocin	Leaves	Antidibetic Agent, Antioxidant Activity
Azadriachta indica	Meliaceae	Neem Tree	Nimbin, Nimbidin	Leaves,& Seeds	Antidibetic Agent, Insecticidal Activity
Momordica charantia	Cucurbitaceae	Bitter guard, balsam pear	Charatin, Charin	Leaves	Antidibedic Activity
Ocimum Sanctum	Lamiaceae	Ram Tulashi, Vishnupriya	Euginol&linanlol	Steam, Leaves and Flower	Antidibedic Activity
Syzygium cumini	Myrtaceae	Jamun	Myrcetin and Jambucin	Fruit & Seed	Antidibetic Agent

A) Ocimum sanctum Kingdom - plantae

Order - Lamials

Family - Lamiaceae

Genus - Ocimum

Species - Sanctum/tenuiflorum, canum, bascilium, gratissinum, kilimandschricum, americanum, camphora, micranthum

Synonym - Ocimumtenuiflorum

Common names - Ram Tulsi, vana tulsi, holy basil, Vishnu priya.

Origin and cultivation

According to ancient literatures, Tulsi is described as a 'queen of herbs'. Universally this plant is familiar for more than 2000 yrs. as a multitalented plant. Basils are native to tropical areas of Asia and are likely to have originated and cultivated in India. It is an annual plant and usually cultivated through seeds. It is found up to an altitude of 1800 m in Himalayas and cultivated in temples and gardens.

Some species of basils readily grow wild in Asian and African areas. On the basis of geographical locations, the varieties of basil plant shows difference in their types and percentage of chem. Constituents. Because of that they possess different pharmacological actions. Some verities like O. sanctum, O. canum, O. basilicum, O. gratissimum are reported as a strong anti-hyperglycemic agent.[1,2,3]

Morphology

It is an upright, branchy, softly adolescent undershrub having 30-60 cm high and red or purple sub-quadrangular branches. Mature plants can grow up to the height of 75 to 90 cm. It is bitter and harsh.

Leaves- They are simple, round, aromatic, opposite, oval shaped, straight, obtuse or acute with entire or semi-zigzag or irregular margins. They having length up to 5 cm.

Flowers- flowers are purplish coloured, present in small compact clusters or cylindrical spikes in close loops, exerted stamens having upper pair with small bearded attachment (calyx tube) at base, hairy flower tubes. Sepal cup is not hairy within. Flowers are longer than 5 mm length.

Fruits- they are small and yellow to reddish coloured. Seeds are yellow in coloured, not sticky when wetted.Stalk- stalks are less, with heart-shaped leaflets at the base of flower cluster.[1,4,5]

Phytochemical constituents

Chem. Constituents of O. sanctum is a complex of many nutrients and some commonly known compounds. Yet non of the study revealed the identity of chem. Constituents which are responsible for the antidiabetic activity of basils.

Gas chromatography-mass spectroscopic study disclosed the presence of differing no. of components in differing percentages. In dried leaf powder 49 components were found. In different extracts different major components were - 1-methyl eugenol (89.20%), 2-eugenol (5.29%). in methanolic extract 1-Stigmast-5-en-3-ol (17.46%), 2-Stigmast-5, 22-dien-3-ol (13.13%), 3-Methyl eugenol (6.19%), in acetonic extract 1- Methyl eugenol (25.31%) and 2-Neophytadiene (7.77%), in Petroleum ether extract 1- Methyl eugenol (20.97%), 2-Octadecane (17.50%), 3-β-caryophylene (8.22%).

Other study of GC-MS screening showed that main components in O. sanctum were linalool (12.63%), eugenol (19.22%), α-bergamotene (3.96%), germacrene D (8.55%), tau-cadinol (15.13%), δ-gurjunene (5.49%) and δ-cadinene (5.04%). Another study showed that methyl chavicol (46.9%), gera- nial (19.1%), neral (15.15%), geraniol (3.0%), nerol (3.0%), caryophyllene (2.4%) are the main components in O. sanctum.

Stem and fresh leaves of basil extract yield some phenolic components like cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid, and marked quantities of eugeno. The leaves contain 0.7% volatile oil encompassing 71% eugenol and 20% methyl eugenol, carvacrol and sesquiterpine hydrocarbon caryophyllene. Two flavonoids orientin and andvicenin have been also extracted from aqueous leaf extract. Ocimumoside A, ocimumoside B, and ocimarin, α-copaene, α-cubebene, α-thujene, α-pinene, α-guaiene, α-amorphene, α-humulene, α-selinene, α-guaiol, α-bisbolol, β-selinene, β-elemene, β-farnesene, β-pinene, β-cubebene, β-bourbonene, cis- β-ocimene, trans- β-ocimene, citronellal, camphene, sabinene, myrecene, limonene, terpiniolene, geraniol, linlool, eugenol, carvacrol, eugenol methylether, luteolin-5-glucoside were also found in leaves.

Ursolic acid, rosmarinic acid, caffiec acid, vallinin, procatechuic acid, gallic acid, galuteolin, chlorgenic acid, aesculin, aesculectin, isovitexin, vitexin, stigmasterol, isorientin, orientin, apgenin, luteolin are obtained from seeds. Eugenol and ocimumoside A structures.[3,4,6,7]

Table 2: Chemical test Ocimum Sanctum.[4]

Phytochemicals	Tests	Observation	Inferences
Alkaloids	Wagner's test	Red ppt	+
Fixed oil and fatty acids	Spot test	Presence of spots	+
Tannins and phenolic comp.	Lead test	Green color	+
Terpenoids and phytosterols	Salkowaski's test	Reddish brown color	+
Test for proteins	Biuret's test	-	-

Presence (+), absence (-).

Method of extraction

Air dry the aerial parts of O. sanctum i.e. stem, leaves and flowers and the powder it. Then pack 10 gm powder from it into the thimble of soxhlet apparatus and extract it by using 150 ml solvent like methanol, hexane. Reflux it for 24 hrs. Until the dark green extract is obtained. Filter the extract. Evaporate the hexane extract under pressure at 50^0 C until dryness. Concentrate the methanolic extract by rotatory vaccume evaporator.[6]

Animal study

Ethanol, butanol, aq. And ethyl acetate fraction of leaves of O. sanctum stimulate insulin secretion in the damaged pancreas, secluded islets and in clonal pancreatic β cells of experimental rats is seen in the study. In normal and STZ induced dose dependent diabetic rat, a 70% methanolic extract of O. sanctum leaves decreases the blood sugar level. The activity of methanolic extracts was 91.55 and 70.43% of that of tolbutamide in

hyperglycemic rats respectively.

Various animal studies revealed that- hyperglycemia was reduced in alloxan diabetic rats after administration of methanolic extract of O. sanctum for long and short term feeding. Triterpenoids withdrawn from hydroalcoholic extracts of O. sanctum shows remarkable antidiabetic activity at the dose of 20 mg/kg in alloxan induced diabetic rats. 200 mg/kg plant extract for 30 days decreases the plasma glucose level by 26.4% in STZ-diabetic rats. Diet carrying 1% leaf powder fed to normal and diabetic rats for 30 days reduces fasting blood sugar, uronic acid, total amino acids, total cholesterol, triglycerides and total lipids.

Trasina is an ayurvedic herbal formulation which contains O. sanctum as an ingredient shows slight effect on blood sugar concentration and islet superoxide dismutase (SOD) action in euglycemic rats in 100 and 200 mg/kg/day dose for 28 days.[1,2,3,6,7].

B) Momordica Charantia

Kingdom- plantae **Division**- magnoliphyta **Class**- Magnolipsida **Order**- violales **Family**- cucurbitaceae **Genus**- Momordica **Species**- charantia.

Common names- bitter guard, bitter mellon, balsam pear, karela.

Origin and cultivation

It is native of tropics. Grows in tropical areas including parts of Amazon, east Africa, Asia and Caribbean. It is widely grown in India and other parts of Indian subcontinent, southeast Asia, China, Africa and Caribbean. It is an annual or everlasting climbers found throughout.

Asia and also cultivated up to an altitude of 1500 m. It is cultivated during April to July using 2-3 seeds in a large hole. Holes are prepared half meter away from each other and provided with manures. Only one plant is retained and seedlings are watered one or two times in a week. After 30 to 35 days of sowing plant starts flowering and after 15 to 20 days of flowering fruits can be harvested.[8,9]

Morphology

It is a flowering Vine and comes in a variety of shapes and sizes. The plant grows up to 5 m in height.

Plant - Lean climber, 2-4 m height, hardly and thickly younger and unisexual.

Stem -12 to 15 cm long. round, internodes after every 5 to 6 cm, delicate tendrils.

Leaves- Alternate, reniform to orbicular or sub- orbicular margin, hand shaped, heart-shaped at base, 5-9 oval shaped lobes, acute or needle-like at the top of leaf and narrowed at base.

Flowers- plant bears separate male and female flowers. Male flower stalks slim with leaflets green coloured, reniform, at midway or toward base, peduncle 2-5 cm length and 5-11 mm width, pedicle 2-6 cm long, Sepals ovate-elliptic, 4-6×2-3 mm, pale green, petals obovate. Female flower peduncle 1-6 cm long, pedicel 1-8 cm long, Sepals narrow, oblong V-shaped, 2-5 mm long, petals smaller or equal as in male, 7-10 mm long; ovary spindle shaped, style 2 rare long.

Fruit- hanging, disc-shaped, swollen, ellipsoid to straight or blocky, frequently narrowed at ends, at times finely rostrate, 3-20×2-5 cm, white or green in color and orange on maturity, soft tuberculate with 8-10 broken or continuous ridges, dividing from base into 3 non-uniform valves. Marked warty looking exterior and an oval shape, hollow in cross-section, thin layer of flesh surrounding a central seed cavity. Crunchy and watery flesh like cucumber.

Seed- 5-30, 8-13 mm, squarish rectangular, squeezed faces, carved, 5-9×3-6 rarely, grooved margins, brown or black seed coat.[8,9,11]

Phytochemical constituents

M. charantia fruits consists glycosides, saponins, alkaloids, reducing sugars, resins, phenolic constituents, fixed oil and free acids. M. Charantia consists the following chemical constituents those are Alkaloids, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins, guanylate cyclase inhibitors, gypsogenin, hydroxytryptamines, karounidiols, lanosterol, lauric acid, linoleic acid, linolenic acid, momorcharasides, momorcharins, momordenol, momordicilin, momordicins, momordicinin, momordicosides, momordin, momordolo, multiflorenol, myristic acid, nerolidol, oleanolic acid, oleic acid, oxalic acid, pentadecans, peptides, petroselinic acid, polypeptides, proteins, ribosome-inactivating proteins, rosmarinic acid, rubixanthin, spinasterol, steroidal glycosides, stigmasta-diols, stigmasterol, taraxerol, trehalose, trypsin inhibitors, uracil, vacine, v-insulin, verbascoside, vicine, zeatin, zeatin riboside, zeaxanthin, zeinoxanthin Amino acids-aspartic acid, serine, glutamic acid, thscinne, alanine, g-amino butyric acid and pipecolic acid,

ascorbigen, b-sitosterol-d-glucoside, citrulline, elasterol, flavochrome, lutein, lycopene, pipecolic acid. The fruit pulp has soluble pectin but no free pectic acid. Research has found that the leaves are nutritious sources of calcium, magnesium, potassium, phosphorus and iron; both the edible fruit and the leaves are great sources of the B vitamins.[9,10,11]

Method of extraction

Methanolic extraction

Air dry the leaves of M. charantia and powder it. Add methanol in it and keep it in thimble loaded with soxhlet vessel. Extract the powder using soxhlet extractor. Stop the process at the boiling temperature of solvent.

Hydro-alcoholic extraction

Simple maceration process

Soak the dried powder sample in distilled water and methanol in a flask. Keep it for 7 days by covering it with cotton plug and aluminum foil and daily shake it vigorously. After 7 days, shake the crude extract vigorously and filter it. Evaporate the filter paper to dryness using thermal evaporator. Store the concentrated extract in airtight container for further use.[14]

Phytochemical analysis

Table 3: Chemical test of Momordica Charantia.

Phytochemicals	Tests	Observation	Inferences
Carbohydrates	Molish's test	+	Present
Alkaloids	Mayer's test	+	Present
Terpenoids	Test using chloroform	+	Present
Steroids	Test using acetic anhydride	+	Present
Saponins	Test with water	+	Present

Animal study

Until now, more than 100 laboratory studies have explained the blood sugar-lowering effect of this bitter fruit. Charantin rich extract of M. charantia (CEMC) at a 200 mg/kg/day causes a significant decline in body wt. And non-fasting blood glucose level with decreased insulin resistance in high fat diet induced diabetic mice is observed in a studyof type 1 and type 2 diabetic animal model. Oral administration of M. charantia juice to STZ induced diabetic rats was found to regulate the uptake of glucose into the vesicles of jejunum and stimulate the uptake of glucose into muscle cell. Oral administration of saponin fractions of fruit shows notable antidiabetic activity by enhancing biochem. Parameters in db/db mice at 150 mg/kg. 500 mg/kg saponin dose from the aq. Extract of bitter melon shows remarkable hypoglycemic

effect in hyperglycemic and normal mice. M. charantia improves glucose tolerance and suppresses postprandial hyperglycemia in rats. Acetone extract of whole fruit powder in the doses of 0.25, 0.50 and 0.75 mg/kg of body wt. lowers blood glucose level from 13.3% to 50.0% after 8 to 30 days treatment in alloxan diabetic albino rats. One study states that there was a remarkable increase in the number of pancreatic cells of streptozotocin-induced diabetic rats after 8 weeks treatment of bitter gourd fruit juice. Aq. Extract of this fruit might have a notable role in reducing kidney damage in the streptozotocin- induced diabetic rats. Lipid and saponin extracts of melon are more potent in lowering glycated haemoglobin levels and overindulgent body weight gain in mice than the hydrophilic extract or the whole fruit is observed in a study. Daily administration of 4 gm/kg/day M. charantia fruit extract for 2 months to alloxanized diabetic rats (120 mg/kg) delayed development of cataract. The Dihar is a polyherbal formulation containing bitter fruit as an ingredient showed markable antidiabetic and antihyperlipidemic acivity in streptozotocin induced diabetic rats.[8,9,10,11]

C) Syzygium Cumini

Synoname- syzygium cumini, jamun.

familly- Myrtaceae

Biological Name- syzygium cumini

General Information

Ayurveda suggest jamun as a highly effective fruit while fight against diabetes mellitus.[14] The seeds of the fruit have active ingredient called jamboline and jambosine that slow down the rate of sugar release in to blood and increase the insulin level in body.[13]

Extraction method

The syzygium cumini fruit first washed. Pulp was removed from the Seeds and they are washed several time with distilled water to remove the traces of pulp from the seeds. Seeds were dried at room temperature and coarsely powdered. The powder are extracted with hexane to remove lipids and It was then filter and filtrate was discard. The residue was successively extracted with ethyl acetate and methanol using cold percolation method. The percentage yield were 1.81% in ethyl acetate and 10.36% in methanol.[13,14]

History of plant

Syzygium cumini has been spread overseas from India by Indian emigrants and at present is common in tropical former British colonies the trees was introduced to Florida in 1911 by the

USDA, and is also now commonly grown in Suriname Guyana, and Trinidad and Tobago.[14] syzygium cumini, commonly known as Malabar plum, java plum, black plum is an evergreen tropical tree in the flowering plant. It was found in Indian sub-constituents, Adjoining region of southeast Asia, China and Queensland.[15,16]

Chemical constituents

It contains Anthocyanine, Glycoside, elagic acid, Isoquercetin, Kaemferol, myrecetin, Alkoloid, jambosine.[17]

Chemical Test

1) Detection of alkaloids

Extracts were dissolved individually in dil. Hydrochloric acid and filtered. The filtered acidified extracts were then subjected to the following tests:

i) Mayer's Test

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide) Formation of a yellow coloured precipitate indicates the presence of alkaloids.

ii) Wagner's Test

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

iii) Hager's Test

Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of a yellow coloured precipitate.

2) Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were then used to test for the presence of carbohydrates.

i) Benedict's test

Filtrates were treated with Benedict's reagent and heated gently. Orange-red precipitate indicates the presence of reducing sugars.

ii) Fehling's Test

Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A

& B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3) Detection of glycosides

(Coumarin glycosides) Alcoholic extract when made alkaline, shows blue or green fluorescence indicating the presence of glycosides.

4) Detection of saponins

i) Froth Test

Were diluted with distilled water to makeup 20ml of volume and was then shaken in a graduated cylinder for 15 minutes. Formation of the foam layer of 1cm indicates the presence of saponins.

ii) Foam Test

0.5 g of the extract was shaken with 2 ml of water. Persistence of foam for ten minutes indicates the presence of saponins. Detection of phyto sterol.

iii) Salkowski's Test

Extracts were treated with chloroform and filter.

5) Detection of phenols

i) Ferric Chloride Test

Extracts were treated with 3-4 drops of 10% ferric chloride solution. Development of a bluish black colour shows the presence of phenols.

6) Detection of tannins

i) Gelatin Test

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white colour precipitate shows the presence of tannins in the extract.

7) Detection of flavonoids i) Alkaline Reagent Test

Extracts were treated with few drops of sodium hydroxide solution. Development of deep yellow colour, which becomes colourless on the addition of dilute acid, shows the presence of flavonoids.

ii) Lead acetate Test

Extracts were treated with few drops of lead acetate solution. Development of yellow colour

precipitate shows the presence of Flavonoids.

8) Ninhydrin Test

To the extract, 0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Development of blue colour demonstrates the presence of amino acid.[17]

Animal study

Syzygium cumini (L.) SKEELS (Myrtaceae) against diabetes--125 years of research. After the Second World War, research was concentrated on animal studies. Not all, but many of them reported some success in reducing type 2 diabetes symptoms.[15] Animal experiments Most reports rely on animal experiments done by Graeserat the university of Bonn, Germany, already published in1889 (Graeser 1889). He induced experimental diabetes in three dogs by administration of 1 g phloridzin/kg bodyweight. The dogs weighing 2.75–4.57 kg then received upto 18 g Syzygium fruit extract a day, which led to a de-crease in urine glucose excretion by almost 90% in enhance. The dogs received a carbohydrate reduced diet com-prised of meat and some milk. Minkowski (1893) firstly tried Syzygium in two pancrea-tectomized animals and reported the results as an appendixto a large study on diabetes following pancreatectomy. He used two different fluid extracts from Syzygium fruits andcould not find any benefit. Colasanti (1895) criticized Minkowksis studies as occasional observations and re-peated the experiments systematically. He observed a significant decrease in urine sugar content of pancreatecto-mized dogs having received 3–4 ml Syzygium fluid extracter kg body weight. The plant part the extract was derived from was not further specified. When drug treatment was stopped, sugar excretion increased and decreased again after restarting Syzygium administration. As an additional result, it was noted that dogs survived longer than without treatment (three vs. two months).In a research program to explore the value of anti-diabetic medicinal plants, Kaufmann (1928) administered Syzygiumcortex extracts intraveniously to rabbits and guinea pigs. The animals immediately died under convulsions. Surpris-ingly, the animals suffered from hyperglycemia, most prob-ably due to a release of glycogen from the liver. In cases the injection did not lead to death, initial hyperglycemia was followed by a decrease in blood sugar levels.[13,15]

D) Annona squamosa linn.

Annona squamosa linn. Belongs to family annonaceae traditionally its used in the various diseases such as antioxidant activity, anti-tupor activity, anti-diabitic activity, antimalarial activity, antiulcer activity, hepatoprotective activity, stress and depression, analgesic activity,

etc.

Taxonomy

Kingdom	**Plantae**
Order	Magnoliales
Family	Annonaceae
Genus	Annona
Species	Squamosa

History of the Name Sitaphal

This fruit has an interesting history attached to it in respect to its name i.e. Sitaphal. Myth logically it is said that Sita, wife of Lord Rama during her vanvaas used to eat this fruit. While some texts says that when Ravana was abducting Sita, at that time the drops of tears from her eyes and nose fell on to the ground and they gave birth to Sitaphal trees in the wilderness. Although, many people believe that sitaphal has nothing to do with Sita. Its origin is in Sanskrit i.e. *"sheet"* in hindi means cold and *"phal*" is fruit and having excess of it can give you cold and also it has a cooling effect on your body so hence the name is Sitafal.[19]

Extraction

Collection & Extraction of Plant Materials

Preparation of custard apple leaf extract the aqueous extract prepared by cold maceration process. The fully matured fresh leaves and seeds of the fruit annona squamosa was collected. The leaf was washed thoroughly with tap water followed with sterilized distilled water and shade dried for four to six days and then powered with the help of blender. For aqueous extraction, 50 gm. of the plant powder was taken in 1000 ml distilled water and allowed to stand overnight and boiled in water bath at 70-80°C for five to ten hours till the volume half of its original volume the solution was then cooled. Then It was then filtered by using whatmann number one filter paper. This procedure was repeated twice with an interval of two hours. After six hours the filtrate was centrifuged at 10000 rpm for 15 minutes. The supernatant was collected and autoclaved at 121°C under 15 lbs. pressure the yield of extract is 7 gm. then this extracts were then stored in screw capped bottles in refrigerator for further use. The dried plant powder of *Annona squamosa* was extracted with methanol, petroleum ether, chloroform and hexane separately. 1000 ml of each solvent is mixed with 50 grams of plant powder and kept in mechanical shaker for 48 hours at room temperature. Extracts were then filtered by using Whatman filter paper No. 1. Extracts were concentrated in rotary evaporator and dried. All the extracts were stored in the refrigerator at 4°C for future use. The

extracted powder was dissolved in 10 % dimethyl sulfoxide (DMSO) for the further use.[21]

Observation table

Table 4: Chemical Test of Annona squamosal.[17,20]

Compound	Test	Result	
		Positive	Negative
Test for alkaloid	1) Wagner test	Positive	-
	2) Mayer test	positive	-
	3) Hager test	-	Negative
Test for carbohydrate	1) Fehling test	Positive	-
	2) Bar ford test	Positive	-
	3) Benedict test	-	Negative
Test for glycoside	1) Borntrager test	Positive	-
	2) Legal test	Positive	-
	3) Killer killiani test	-	Negative
Test for saponin	1) Saponin test	Positive	-
Test for protein	1) Biuret test	Positive	-
	2) Ninhydrin test	Positive	-
Test for tannin	1) Lead acetate test	Positive	-
Test for phenol	1) Ferric chloride test	Positive	-
Test for flavonoid	1) Flavonoid test	Positive	-

Chemical Constituent

Leaf of custard sugar yielded 59 compounds. Main components were ß-caryophyllene, (natural bicyclic sesquiterpene) δ-cadinene (6.7%), α-muurolene (5.5%), T- and α-cadinol (4.3%). Leaves gaveisoquinoline alkaloids Two acetogenins, annoreticuin and isoannoreticuin isolated from the leaves, were found to be selectively cytotoxic to certain human tumours. The leaves and stems also gave alkaloids dopamine, salsolinol andcoclaurine. Others are anonaine, aporphine, coryeline, isocorydine, norcorydine.[19,20]

CONCLUSION

Medicinal plant extracts are commonly used for anti-diabetic treatment. Use of these products is not only motivated by more difficult access to conventional anti-diabetic drugs and their high cost, but also to socio cultural belief in the efficacy of herbal medicine.

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