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QUALITATIVE ANALYSIS OF PHYTOCHEMICALS IN THREE ANTI-DIABETIC MEDICINAL PLANTS OF BRAJ REGION OF UTTAR PRADESH

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Abstract

Hyperglycemia, or elevated blood glucose levels, is a hallmark of diabetes mellitus, a group of chronic metabolic disorders that eventually impairs the metabolism of carbohydrates, fats, and proteins as a result of a corresponding lack of insulin. Bioactive constituents revealed the presence of flavonoids, alkaloids, phenolic compounds, glycosides, sterols and terpenoids for treatment of diabetic activity in selected medicinal plants of Braj region of Uttar Pradesh such as Aloe vera, China rose and Jamun which possess ability to reduce blood glucose, uric acid, urea and creatinine and to increase insulin level, C-peptide and albumin control level. According to International Diabetes Federation (IDF), it is predicted that the number of diabetic patients in the World could reach up to 366 million by the year 2030. Aim of the present study showed the effect of different extracts such as aqueous, acetone, ethanolic, methanolic, chloroform and petroleum ether with various combinations to evaluate the presence of phytochemicals. Strongly presence of flavonoids with aqueous and methanolic leaves extracts according to other solvents in Aloe vera. In an aqueous leaves extracts of China rose, strongly presence of flavonoids and terpenoids according to other phytochemicals and alkaloids, phenolic compounds, flavonoids, terpenoids and glycosides in methanolic and ethanolic leaves extracts. Strongly presence of alkaloids, phenolic compounds, flavonoids, terpenoids and glycosides in an aqueous and methanolic leaves extracts of Jamun but the absence of tannin in all solvents. So, the aqueous, methanolic and ethanolic extracts are better for Aloe vera, China rose and Jamun for extraction of pytochemicals like alkaloids and flavonoids will be an effective tool for the treatment of diabetes and needs more study for drug development.

Keywords : Diabetes, phytochemicals, anti - diabetic plant, qualitative analysis, different extracts

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Introduction

Plants have long been demonstrated to be a source of health and are essential to both conventional and common therapies. The World Health Organisation (WHO) defines therapeutic plants as any plant that includes a substance, such as precursors of chemo-pharmaceutical semi-synthetic novel medications and secondary derivative compounds that can be used for therapeutic purposes (Ali, 2003). The term "phytochemicals" comes from the Greek word "phyto," which means "plant." These naturally occurring, biologically active chemicals are found in plants, and their benefits to human health are related to their macro- and micronutrient content (Hasler & Blumberg, 1999). Different plant sections, such as the roots, stems, leaves, flowers, fruits, or seeds, collect phytochemicals. There are over 45,000 plant species in India, and thousands of them are thought to have medicinal qualities and to be more effective against the symptoms of diabetes. Numerous phytoconstituents derived from a variety of plant sources, including peptidoglycans, alkaloids, glycosides, tannins, terpenoids, phenols, flavonoids, saponins, dietary fibres, polysaccharides, glycolipids, and others, have been identified as potent hypoglycemic agents. Phytochemicals, or phytoconstituents as they are typically known, are abundant in plants. Those are not as it were utilized directly as pharmacological agents but too as beginning materials for synthesis of drugs which is exorbitant and chances of side effects are high (Saxena *et al.*, 2013)

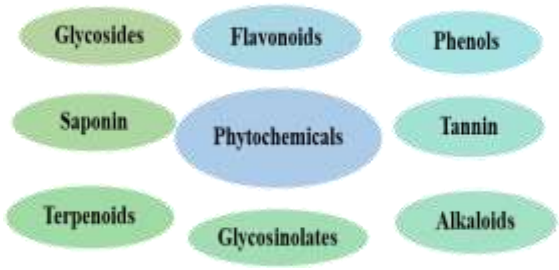

Figure 1: Groups of phytochemicals (Source: Saxena *et al.*, 2013)

Anti - diabetic activity

A group of chronic metabolic disorders known as diabetes mellitus are characterised by hyperglycemia, or elevated blood glucose levels. These disorders can interfere with the body's ability to digest carbohydrates, fats, and proteins, which can lead to an overabundance of insulin and subsequent damage to various bodily systems (Baliga *et al.*, 2013). Frequent urine, increased thirst, and increased hunger are symptoms of high blood sugar. As diabetes progress, the body's cells become less capable of metabolising sugar because of insufficient insulin activity. Insulin, a peptide hormone that regulates blood glucose, may be needed or insensitive to certain tissues, and the pancreas fails to

produce enough of it when the body is unable to use the insulin that is produced (Salehi *et al*., 2019). A serious and real difficulty in today's world is diabetes. The modern lifestyle and environment have a significant role in the occurrence of these kinds of major issues (Kumar *et al*., 2020). There are three primary forms of diabetes mellitus that are referred to as:

Type 1 DM : "insulin - dependent diabetes mellitus" (IDDM)
Type 2 DM : "non - insulin - dependent diabetes mellitus" (NIDDM)
Gestational diabetes

Type 1 DM (Diabetes mellitus) or adolescent diabetes

The loss of insulin-producing β-cells in the pancreatic islets of Langerhans causes Type 1 Diabetes Mellitus (DM). This subtype, which used to be known as "insulin - dependent diabetes mellitus" (IDDM) or "adolescent diabetes," once affected about 5% of people with diabetes. The majority of cases of Type 1 diabetes are resistant-mediated, meaning that the immune system attacks T cells, causing β-cell loss (Pozzilli *et al*., 2011; Shivashankar and Mani, 2011).

Type 2 DM (Diabetes mellitus) or adult - onset diabetes

When cells stop responding normally to insulin, a condition known as insulin resistance develops, eventually leading to type 2 diabetes. People over the age of 40 are more likely to develop this kind of diabetes, which is also known as "adult onset diabetes" or "non-insulin dependent diabetes mellitus" (NIDDM). Being overweight and not getting enough exercise is the main reason. Increased risk of cardiovascular disease, stroke, renal failure, and amputation are among long-term consequences of hyperglycemia (Pozzilli and Guglielmi, 2009). Within the fat muscles, non - functioning glycolysis reduced insulin flow and transports the pathogens. So, in this manner beneficial glucose transporter 4 (GLUT4) is over release of fatty acids, adipocytokines and decrease adiponectin from fat tissues and within the liver, glycogenolysis and gluconeogenesis cycle are boost in the presence of insulin resistance. It has an unfavorable impact on carbohydrate, lipid and protein metabolism coming about in chronic hyperglycemia and abnormality of lipid (Eddouks *et al*., 2014; Rashid *et al*., 2022).

Gestational diabetes

One form of diabetes mellitus that can develop in pregnant women is gestational diabetes. With this condition, you can say goodbye to the third trimester and hello to the beginning of the fourth. This usually gets back to normal not long after the baby is born. Because the body's cells use glucose as fuel for growth and energy, the mother and the unborn child are in grave danger if the woman's glucose levels are abnormal during pregnancy (Zhang and Ning, 2011).

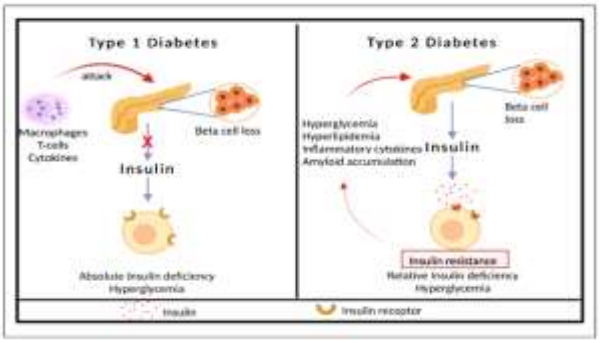

Figure 2: Actions of diabetes (Source: Khin *et al*., 2023)

Anti - diabetic medicinal plants of Braj region:

Aloe vera (*Aloe barbadensis* Miller)

Aloe barbadensis Miller is cactus-like perennial, herbaceous, xerophytes, succulent or juicy and pea-green color plant having a place to the family Liliaceae found in tropical and subtropical regions. Aloe vera derived from the Arabic word 'Alloeh' meaning 'shining bitter substance' whereas 'Vera' Greek word implies 'true'. Interior the leaf is a jelly like substance utilized as cure of minor burns, wounds, skin allergy and leaves to treat constipation, coughs, ulcers, diabetes and hypertension, immune - system deficiency and joint pain etc. (Bozzi *et al*., 2007).

Figure 3: *Aloe barbadensis* Miller plant

In addition to its many other uses, *Aloe vera* is an excellent natural antiseptic, antimicrobial, antiviral, antitoxic, antibacterial, anti-inflammatory, anti-ulcer, anti-diabetic, anti-tumor, immunological stimulant, and highly effective intracellular anti-oxidant. It can also be used as a purgative, analgesic, tonic, painkiller, anti-asthmatic, anti-leukopenic, anti-pyretic, anti-helmintic, decoagulant, demulcent, diuretic, emollient, worms excellent, hair stimulant, and hypoglycemic (Tiwari and Upadhayay, 2018).

China rose (*Hibiscus rosa-sinensis* L.)

Hibiscus rosa-sinensis L. is ornamental shrub commonly known as 'Chinese Hibiscus' or 'Gurhal' having place to the family Malvaceae a single form with 5 - petaled blood red blooms found in tropical region. In India's conventional pharmaceuticals, its leaves, barks, roots and flowers have been utilized to treat a variety of sicknesses such as anti - oxidant, anti - microbial, anti - diabetic, anti - ulcer, hepatoprotective, anti - fertility, anti - genotoxic and anti - inflammatory etc., (Jadhav *et al*., 2009; Zakaria and Mohd, 2010).

Figure 4: *Hibiscus rosa-sinensis* L. *plant*

Figure 5: *Syzygium cumini* L. plant

Jamun (*Syzygium cumini* L.)

Syzygium cumini L. (synonym *Eugenia jambolana*) is an evergreen tree commonly known as 'Jamun' or 'Java plum' has a place to the family Myrtaceae found in tropical regions in which entire portion contains great nutritional values and it contains different bioactive constituents such as tannins, alkaloids, flavonoids, steroids, terpenoids, phenols, anti - oxidants, fatty acids, minerals, carbohydrates and vitamins, etc., through different extracts showed anti - diabetic, anti - inflammatory, anti - hypertensive, hepatoprotective, diuretic, anti - hyperlipidemic and anti - bacterial activity due to the presence of alkaloids, flavonoids, phenols, tannins and saponins etc., (Margaret *et al*., 2015; Agarwal *et al*., 2019).

Table - 1: Role of phytochemicals presents in *Aloe barbadensis* Miller, *Hibiscus rosa-sinensis* L. and *Syzygium cumini* L. (Jafri *et al*., 2011; Moqbel *et al*., 2011; Sawant *et al*., 2015)

S.No.	Plant	Part	Phytoconstituents	Solvent employed in various studies for extraction	Pharmacologcal effect	Action
1.	*Aloe barbadensis* Miller (Aloe vera)	Leaf	Saponins, lignin, alkaloids, flavonoids anthraquinones	Aqueous	Cardioprotective, anti-tumor, anti-inflammatory, anti-diabetic, hepatoprotective anti-hypertensive	↓Glucose level, ↑Heamoglobin, ↓Weight
2.	*Hibiscus rosa-sinensis* L. (China rose)	Whole plant	Terpenes, alkaloids, flavonoids, saponins	Aqueous, methanol	Anti-diabetic, anti-hypertensive, antioxidant, anti-diarrhetic, anti-microbial, anti-hypertensive	↓Blood glucose level, ↓Blood pressure
3.	*Syzygium cumini* L. (Jamun)	Leaf, stem, bark, flower, root, fruit and seed	Glycosides, alkaloids, flavonoids	Aqueous, alcohol	Anti-diabetic, diuretic, anti-oxidant, anti-inflammatory, anti-bacterial and anti-hypertensive	↓Protein tyrosine phosphataes 1B, ↓Blood glucose level,

Materials and Methods

Research on medicinal plants with anti-diabetic properties began with pre- and post-extraction operations, during which phytochemical analysis of the chosen plant components was carried out. Small manufacturing enterprises (SMEs) and smaller research institutions frequently employed Soxhlet extraction.

Collection and storage of plant

For conducting the present study, fresh leaves of Aloe vera (*Aloe barbadensis* Miller), China rose (*Hibiscus rosa-sinensis* L.) and Jamun (*Syzygium cumini* L.) were collected from Botanical Garden of Department of Botany, SLS Khandari Campus, Dr. Bhimrao Ambedkar University, Agra (U. P.) during February 2021 kept in polythene bags and protect from dust or microbes. We rinsed the stored material with tap water, let it dry in the shade, and then ground it into a powder for future experiments.

Methods of plant extraction

Preparation of aqueous extract

To separate the leaf powder, sterile distilled water was added in a 1:8 weight-to-volume ratio using a pestle and mortar. The mixture was then filtered using Whatman No. 1 filter paper. Everything was done at ambient temperature throughout the extraction. (Zore *et al*., 2004).

Preparation of organic extract

Different solvents were used for extraction are as follow:

i. Methanol
ii. Ethanol
iii. Acetone
iv. Chloroform
v. Petroleum ether

The organic compounds were extracted using the Soxhlet technique. Whatman No. 1 filter paper was used to prepare a thimble. A packed thimble was used to evenly run approximately 50 grammes of powdered material. The extraction process was repeated 22 times over the course of 48 hours until the solvent in the syphon tube lost its hue. The next step was to use filter paper to collect the extracts, and then a rotary evaporator to remove the solvent. The resulting syrup was then stored in a refrigerator at 4 ºC. (Okeke *et al*., 2001).

Qualitative analysis of phytochemicals

The different qualitative chemical tests were performed for establishing profile using different solvents extracts for its chemical composition.

Table - 2: Qualitative analysis of some phytochemicals present in Aloe vera, China rose and Jamun leaf using different solvents extraction (Brain and Turner 1975)

S. No.	Phytochemicals	Tests	Solvent extracts
1.	Alkaloids	**Mayer test** (A small amount of Mayer's reagent interacted with one millilitre of plant extract.)	Aqueous, Methanol, Ethanol, Acetone, Chloroform and Petroleum ether
2.	Flavonoids	**Sodium hydroxide test** (One millilitre of plant extract was subjected to a 10% sodium hydroxide solution.)	
3.	Phenolic compounds	**Lead acetate test** (The reaction between a 10% lead acetate solution and 1 millilitre of plant extract)	
4.	Terpenoids	**Salkowski test** (Two millilitres of chloroform and three millilitres of concentrated sulphuric acid were used to react one millilitre of plant extract.)	
5.	Glycosides	**Keller Kiliani test** (Two millilitres of plant extract, one millilitre of glacial acetic acid, a few drops of ferric chloride, and one drop of concentrated sulphuric acid)	

6.	Tannin	**Ferric chloride test** (A few drops of 5% ferric chloride, 10 millilitres of boiling distilled water, and 1 millilitre of plant extract)	

Results and Discussion

Selected anti - diabetic medicinal plants of Braj region:

In the present study one herb, one shrub and one tree species have been selected the three anti - diabetic medicinal plants of Braj region of Uttar Pradesh. Phytochemical analysis of selected anti - diabetic plants Aloe vera (*Aloe barbadensis* Miller), China rose (*Hibiscus rosa-sinensis* L.), Jamun (*Syzygium cumini* L.) and) were done in different solvents leaves extraction.

Inferences of leaves extraction of selected anti – diabetic medicinal plants

Aloe vera (*Aloe barbadensis* Miller)

Colour: Green
Odour: Pungent and oniony
Taste: Bitter

China rose (*Hibiscus rosa-sinensis* L.)

Colour: Green
Odour: Odourless
Taste: Mucilaginous

Jamun (*Syzygium cumini* L.)

Colour: Green
Odour: Pleasant
Taste: Mildy sour and astringent

Table - 3: Phytochemical analysis with different solvents leaves extraction of selected plant

S. No.	**Solvent**						
	Pytochemical	**Alkaloids**	**Flavonoids**	**Phenolic compounds**	**Terpenoids**	**Glycosides**	**Tannin**
	Test	Mayer	Sodium hydroxide	Lead acetate	Salkowski	Keller - Kiliani	Ferric chloride
	Observation	Cream color	Yellow color	Reddish yellow color	Reddish brown color	Brown color ring	Brownish green color
	Aloe vera (*Aloe barbadensis* Miller)						
1.	Aqueous	---	+++	---	++	++	---
2.	Methanol	+	+++	+	++	++	---
3.	Ethanol	++	++	---	---	---	+
4.	Acetone	---	---	---	+	---	---
5.	Chloroform	+	---	---	---	---	---
6.	Petroleum ether	---	+	---	---	---	---
	China rose (*Hibiscus rosa-sinensis* L.)						
1.	Aqueous	++	+++	+	+++	++	++
2.	Methanol	+++	+++	+++	+++	+++	++
3.	Ethanol	+++	+++	+++	+++	+++	++
4.	Acetone	---	---	+	---	---	+
5.	Chloroform	+	---	---	---	---	---
6.	Petroleum ether	---	---	---	+	+	---
	Jamun (*Syzygium cumini* L.)						
1.	Aqueous	+++	+++	+++	+++	+++	---
2.	Methanol	+++	+++	+++	+++	+++	---
3.	Ethanol	++	++	+	++	++	---
4.	Acetone	---	---	+	---	---	---
5.	Chloroform	+	---	---	+	+	---
6.	Petroleum ether	---	---	---	+	+	---

Where, (+++): Strongly present; (++): Present; (---): Absent; (+): Lightly present

Due to its high incidence, morbidity, and mortality, diabetes is quickly overtaking cancer and cardiovascular diseases as the third leading cause of death for humans. The oral hypoglycemic agents nowadays utilized in anti - diabetic treatment which is related with genuine side effects so, there is most prerequisite to investigated more current to the anti - diabetic agents that hold therapeutic efficacy and no side effects. Phytochemical analysis was done used the different solvents in selected anti - diabetic medicinal plants. Above mentioned the table - 3 strongly presence of flavonoids with aqueous and methanolic leaves extracts of *Aloe vera* observed yellow color with sodium hydroxide test while terpenoid and glycosides also observed with reddish brown ring and brown color ring. According to Paul *et al.* (2014) reported that dynamic components such as polysaccharides, monosaccharides, organic compounds, anthraquinones, vitamins, inorganic compounds and proteins etc., displayed in *Aloe vera* which have responsible for anti - bacterial, anti - fungal, anti - oxidant, anti - diabetic and anti - hypertensive activity. The ethanolic leaf extracts of Aloe vera revealed a cream colour with the Mayer test and a yellow colour with the sodium hydroxide test, indicating the presence of alkaloids and flavonoids, as shown in table 3. Other solvents also confirmed this. The ability of aloe vera gel to lower blood sugar levels is well-known. Regardless, the results might vary depending on the differences in the mucilaginous layer's separation from anthraquinones. This compound improves glucose metabolism, which in turn lowers peroxide levels and oxidative damage, as well as hepatic transaminases, cholesterol (both plasma and tissue), triglycerides, free fatty acids, and phospholipids (Sharma *et al*., 2014). Various disorders, including diabetes mellitus (DM), obstruction, infection, cancer, sclerosis, hepatitis, haemorrhoids, glaucoma, depression, and alopecia, can be improved with chemotherapy by ingesting aloe vera gel, latex, or leaves (Saleem *et al*., 2021). An potent anti-hyperglycemic medication against type 2 diabetes, aloe vera leaf gel extract lowers blood glucose levels without affecting normal liver and kidney function. The outer, verdant part of an aloe leaf contains vascular bundles, while the inner, white part contains the parenchyma, which is where the main powdery components of the plant are found. These include amino acids, flavonoids, alkaloids, anthraquinones, proteins, minerals, vitamins, lignins, monosaccharides, polysaccharides, salicylic acid, saponins, and many more. The aloe vera leaf also has haemoglobin levels that are lower than normal (Labban and Malek, 2019). Table 3 shows that there were abundant flavonoids in the aqueous, methanolic, and ethanolic leaf extracts of the Chinese rose, as detected by the sodium hydroxide test (yellow colour) and terpenoids in the Salkowski test (reddish brown colour ring). Because of its safety and efficacy in enhancing insulin sensitivity and proper carbohydrate, lipid, and protein digestion, as well as its anti-diabetic and hypoglycemic effects, *Hibiscus rosa-sinensis* L. is a potentially useful medicinal plant with a broad variety of pharmacological activities used in a number of medical applications (Pethe *et al*., 2017). In a study conducted by Pillai and Mini (2016), it was found that diabetic rats administered a standard drug called metformin at a dosage of 25 mg/kg as per body weight along with an ethyl acetate extract of *Hibiscus rosa-sinensis* L. petals. The rats' elevated levels of glycated haemoglobin and serum glucose levels decreased as a result of the drug's effect on glycogen enzyme activity. Table 3 shows that both the ethanolic and methanolic extracts of the Chinese rose leaves contained abundant amounts of glycosides, alkaloids, and phenolic chemicals. The Mayer, lead acetate, and Keller-Kiliani tests revealed cream, reddish-yellow, and brown hues, respectively. After twenty-eight days, the size, number, and diameter of islets, as well as decay and necrosis, progress, according to Al-Snafi *et al.* (2019). The researchers also found that the aqueous extract of *Hibiscus rosa-sinensis* L. flower had antioxidant, hyperlipidimic, and anti-diabetic potential when given orally to alloxan-actuated diabetic rats at dosage levels of 50–200 mg/kg. The insulin secreting activity of *H. rosa-sinensis* L. leaf extracts in Alloxan-induced diabetic rats was found to boost blood insulin level and therapeutic efficacy in recovering type I diabetes, according to Shandilya and Pathak (2020). Bala *et al*. (2022) recorded that streptozotocin, when given orally to diabetic rats at a dose of 500 mg/kg for four weeks, significantly reduced elevated levels of blood glucose, creatinine, uric acid, and urea. Treatment also restored the levels of marker enzymes and increased the activities of albumin, insulin, and C-peptide. Results from the Mayer, sodium hydroxide, lead acetate, Salkowski, and Kellar-Kiliani tests, as well as observations of cream, yellow, reddish yellow, reddish brown, and brown colour rings, were presented in table 3, which demonstrated the presence of alkaloids, flavonoids, phenolic compounds, terpenoids, and glycosides in the aqueous and methanolic leaf extracts of jamun. Researchers Swami *et al.* (2012) found that primary adipocytes showed better insulin-mediated glucose uptake after being tested for lipogenic, anti-lipolytic, glucose uptake, block epinephrine, and induced lipolysis using methanolic leaf extract of S. cumini L. S. cumini L. leaves have rich phytoconstituents such as kaempferol, quercetin, myricetin, isoquercetin (quercetin - 3 - glucoside), myricetin - 3 - L - arabinoside, quercetin - 3 - D - galactoside, oleanolic acid and acetyl oleanolic acid and also in fruit was rich in raffinose, glucose, fructose, citric acid, mallic acid, gallic acid, anthocyanin, petunidin - 3 - gentiobioside, cyanidin diglycoside, petunidin and malvidin and sourness of fruits may be due to presence of gallic acid (Kumawat *et al*., 2018). In their study, Franco *et al.* (2020) proposed that ethanolic extracts of *S. cumini* L. leaves could inhibit the enzymes α-amylase and lipase, in addition to having antioxidant, anti-diabetic, and anti-hypertensive properties.

Conclusion

A large percentage of the global population lives with diabetes, a chronic metabolic condition. The International Diabetes Federation (IDF) projects that by 2030, there could be as many as 366 million people living with diabetes worldwide (Whiting *et al*., 2011; Agrahari and Dwivedi, 2022). The present study revealed that the aqueous, methanolic and ethanolic better solvents extraction efficiency favors the highly polar solvents so therefore sustained limit in hyperglycemia will be decrease the possibility of developing microvascular diseases and reduce their complications. Alkaloids, flavonoid, rich extracts from selected Braj region of Uttar Pradesh anti - diabetic medicinal plants according to herb, shrub and tree category such as Aloe vera (*Aloe barbadensis* Miller), China rose (*Hibiscus rosa-sinensis* L.) and Jamun (*Syzygium cumini* L.) having potential to cure and management of Diabetes mellitus. The presence of bioactive compounds especially flavonoids and alkaloids that could be served as anti - diabetic which may provide a rationale use. The leaves

extract of Aloe vera, China rose and Jamun might hold potential as an active agent in the treatment of diabetes. Commercial value of phytoconstituent may generate considerable interest in drug companies for the manufacture of the new drugs for curing of various diseases.

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