Phytochemical profiling and *in vitro* assessment of antioxidant and antidiabetic potential of a novel polyherbal formulation of *Catharanthus roseus*, *Mirabilis jalapa*, and *Senna auriculata*

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ABSTRACT

Diabetes mellitus (DM) is a group of metabolic disease of inadequate control of blood glucose level due to the deficiency of insulin. It is classified into 2 types they are type 1 and type 2. It has an impact on both developed and undeveloped countries. It is expected that approximately 25% of the global populace has diabetes. The inhibitors used in the clinical practice of allopathic medicines for the control of diabetes are known to be related with various adverse effects. In recent years, alternative treatment methods have been followed by diabetic patients instead of allopathic medicines. As a result, it is the time to recognize and discover the need for drugs from natural sources without any adverse effects. This study is aimed to identify the preliminary phytochemicals, quantitative analysis of major secondary metabolites such as alkaloids, phenol and flavonoids, *in vitro* antioxidants, and *in-vitro* antidiabetic prospective of polyherbal formulation of *Catharanthus roseus* L., *Mirabilis jalapa* L., and *Senna auriculata* L. The *in vitro* antioxidant assays, such as DPPH, reducing power assay, and ABTS radical scavenging assay, were demonstrated. In our findings the polyherbal formulation exhibited significant increase of free radicals and its scavenging potential. The mean IC_{50} value of the polyherbal formulation for DPPH (172.5 µg/ml), reducing power (90 µg/ml), and ABTS assay (150 µg/ml) were predicted. *In vitro* antidiabetic studies of the α-amylase enzyme inhibitory test revealed excellent inhibitory potential with its mean IC_{50} value of the polyherbal formulation (107.5 µg/ml) was calculated and substantiating that the polyherbal formulation possesses remarkable antioxidant and antidiabetic potential.

Key words: Diabetes, phytochemicals, polyherbal formulation, antioxidants, anti-diabetic, and α-amylase.

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1. INTRODUCTION

Diabetes mellitus (DM) is a severe, persistent, and intricate metabolic disorder of various etiologies with intense consequences, both acute and chronic. DM and its complications influence people from both developed and underdeveloped nations, resulting in a significant socioeconomic problem[1]. As stated by the survey of international diabetes federation (IDF), numeral of individuals with diabetes rises from 380 million to 620 million in 2030. Compared to wealthy nations, developing nations have seen a faster increase in prevalence[2]. There is an increase in the prevalence over 90% of instances of diabetes are type 2. It is a precarious and widespread chronic illness brought on by a difficult inheritance-environment interface in addition to additional risk factors like obesity and sedentary lifestyle. Diabetes is largely influenced by both genetic and environmental factors[3]. Due to insufficient insulin activity on target tissues brought on by insulin insufficiency or insensitivity, the body's cells are unable to adequately digest sugar during the development of diabetes[4]. For the diabetes management contemporary medicines have introduced several synthetic remedies like

insulin, biguanides, metformin, sulfonylureas, glibenclamide and thiazolidinedione but they are causing many adverse effects as well as complications[5]. Diabetic complications are a primary cause of sightlessness, kidney failure, myocardial infarction, cerebrovascular accident (CVA) and lower limb amputation[6]. Type 2 diabetes and associated complications can be prevented or delayed by following a balanced diet, exercising, maintaining a normal body weight, taking medication, getting regular screenings, and receiving treatment[7].

Hence, people prefer ayurvedic medicinal system of plant based medicine as a complementary and alternative treatment approach[8]. Herbal medicine is a traditional healing practice that utilizes plant and plant extracts to prevent, alleviate, or treat various ailments and health conditions. It has been practiced for centuries across cultures worldwide and involves the use of different plant parts like leaves, roots, seeds, and flowers[9]. Herbal remedies can be prepared in various forms such as teas, extracts, formulations, tinctures, and capsules. These remedies contain bioactive substances in the form of secondary metabolites with excellent medicinal properties, including antioxidants, anti-diabetic, anti-inflammatory, and antimicrobial agents etc. The majority of secondary metabolites with curative value for medication development against the most widespread and prevalent diseases, particularly diabetes, originate from plants[10].

Because of the various biological actions of their constituents, more than 1200 plants have been found through experimentation to be valuable in the management of diabetes. Most of the traditional medicinal plants previously reported in ayurvedic literatures as an excellent source for medicines and blood glucose control properties for the development of more secure hypoglycemic medications[11]. Compared to insulin and other oral hypoglycemic medications, there is an emerging need for natural compounds with implicit antidiabetic activity for blood glucose management[12]. Therefore, it is necessary to look for safe and effective medications to manage blood glucose levels (BGL) in diabetes through plant-based therapy. In this study the chosen medicinal plants are ***Catharanthus roseus*** **L.,** ***Mirabilis jalapa*** **L.,** ***and Senna auriculata*** **L.,** commonly known as madagascar periwinkle, four-O-clock, avaram senna has been used for eternity in folklore and established systems of medication as an antidiabetic, antioxidant, anticancer, anti-inflammatory, hepatoprotectivity, antimicrobial, and gastroprotective activity etc. Hence, this study mainly focused on assessing the *in vitro* antioxidants, and antidiabetic potential of polyherbal formulation.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

The studies were conducted using only analytical-grade chemicals. Every chemical was acquired from Sisco Research Laboratories (SRL) Pvt. Ltd. in India. The hydrocarbon diluents were sourced from Merck (Chennai, India). Reagent-grade chemicals were purchased from regional vendors (SRL, Himedia & Biosystems, Chennai, India).

2.2 Collection, identification and authentication of plant materials

As per the literature survey, three plant sources were chosen for the current study: ***Catharanthus roseus*** **L.,** ***Mirabilis jalapa*** **L.,** and ***Senna auriculata*** **L.** The chosen plant sources were collected from within and around Trichy and authenticated **(Authentication numbers: KS0001, KS0002 and KS0003),** with the specimens deposited at the RAPINAT Herbarium, St. Joseph's College, Trichy. The collected leaves and flowers were cleaned, dried, powdered, and used for further studies.

2.3 Preparation of aqueous extract[13]

200 g of leaves powder of *Catharanthus roseus* L.*, Mirabilis jalapa* L., and flowers powder of *Senna auriculata* L., were mixed thoroughly in six parts of water, boiled it until condensed to one-third. The extract was then strained using filter paper and evaporated in a water bath till it reaches a thick paste consistency. Then it was kept in a sealed container and used for further *in vitro* studies.

2.4 Preparation of polyherbal formulation[14]

Aqueous extract of the selected plants **(***Catharanthus roseus* L.*, Mirabilis jalapa* L., and *Senna auriculata* L.,**)** were prepared individually. Each extract was mixed in a proportion of 5:2:3 to make formulation. This formulation was used for *in vitro* antioxidants and antidiabetic study.

2.5 Preliminary phytochemical screening[15]

Initial screening for phytochemicals of the dry plant powders and aqueous extracts of *Catharanthus roseus* L.*, Mirabilis jalapa* L., and *Senna auriculata* L., were performed using standard protocols. Tests for various compounds included terpenoids (Salkowski test), flavonoids (Shinoda test), steroids (Liebermann-Burchard test), reducing sugar (Fehling's test), alkaloids (Dragendroff's test), quinone (Alcoholic KOH test), phenol (Ferric chloride test), tannin (Lead acetate test), saponin (Foam test) and coumarin (NaOH test).

2.6 Quantitative analysis of major secondary metabolites

2.6.1. Estimation of total alkaloids[15]

The aqueous acidified layer that resulted from mixing 10 g of plant extract with 0.1 N HCl was separated using chloroform in a separating funnel. The aqueous layer was basified with NH_4OH to an alkaline pH and separated with $CHCl_3$ in a separating funnel after the chloroform layer was removed. After discarding the aqueous layer and evaporating the chloroform layer, the resulting substance was considered as total alkaloid and the alkaloid was verified using Dragendroff's reagent.

2.6.2. Total flavonoids estimation[16]

5 g of aqueous plant extract was mixed with 10 ml of ethyl acetate solvent. Incubate this mixture at room temperature for 24 hours. The extracts were maintained in a boiling water bath up to being completely dehydrated and getting concentrated. The concentrated filtrate was further diluted with ethyl acetate solution and obtained the extract. Take different aliquots of test solution of the extract. To this 1 ml of 2% aluminium chloride and 1 ml of methanol-acetic acid (1:1 ratio) was added. Then it was kept aside for 30 minutes at room temperature, and the absorbance was read colorimetrically at 390 nm. Additionally, there was a blank also maintained. Quercetin was used as a reference standard. The inference was expressed as mg/g.

2.6.3. Estimation of Phenol[17]

Precisely weighed 1 g of the plant powder was thoroughly ground using 10 times the 80% ethanol volume. The homogenate was centrifuged at 10,000 rpm for 20 minutes. After collecting the supernatant, the filtrate was extracted again using 5 times the 80% ethanol volume. The top layer was obtained after the material was centrifuged once more. After then, it evaporated until it was completely dry. 5 ml of distilled water was used to dissolve the acquired deposit. From this, 0.5 ml of the sample was pipetted into a test tube, and distilled water was added to make the volume up to 3 ml. After 3 minutes, 0.5 ml of Folin's reagent and 2 ml of a 20% $NaHCO_3$ solution were added. After fully mixing the contents, the test tube was submerged in a boiling water bath for 1 minute, allowed to cool, and the color that appeared was measured colorimetrically at 650 nm. Results were reported in mg/g, with gallic acid serving as the benchmark.

2.7 *In vitro* antioxidant assays

2.7.1. DPPH radical scavenging assay[18]

Different concentrations of polyherbal formulation and ascorbic acid (Standard) were taken in sequence of test tubes. Methanol was added to get the volume up to 1 ml. In control experiment without the test compounds but with corresponding amount of methanol was taken and the experiment conducted in an identical manner. Then 0.5 ml of recently prepared DPPH solution was added to all the test tubes and the tubes were incubated at room temperature for 10 minutes. The absorbance was measured at 517 nm using spectrophotometer or colorimeter.

DPPH Scavenging Activity (%) = A Control - A Test /A Control × 100

A Test is the absorbance of test solution (polyherbal formulation). A Control is absorbance of control. The Antioxidant activity of the polyherbal fomulation was expressed as IC_{50}.

2.7.2. Reducing power assay[19]

Various concentrations of polyherbal formulation and ascorbic acid (standard) were taken in a sequence of test tubes. Phosphate buffer was used to increase the volume to 2 ml and 1 ml of potassium ferric cyanide to the above mixture. A control tube was also treated in the same manner without test solution. Then this mixture was incubated at 50°C for 20 minutes. After incubation, 0.5 ml of recently prepared $FeCl_3$ solution was added to all the tubes and the absorbance was measured colorimetrically at 700 nm.

Ferric reducing antioxidant power (FRAP) (%) = A Test - A Control / A Test × 100

A Test is the absorbance of test solution (polyherbal formulation). A control is absorbance of control. The antioxidant activity of the polyherbal fomulation was expressed as IC_{50}.

2.7.3. ABTS radical scavenging assay[20]

Multiple concentrations of polyherbal formulation and ascorbic acid (standard) solution were taken into a sequence of test tubes. After that, 0.3 ml of ABTS solution was added, and phosphate buffer was used to get the final volume up to 2.5 ml. The control tube was filled with 2.2 ml of phosphate buffer and 0.3 ml of ABTS solution. Then this mixture of solution was read colorimetrically immediately at 734 nm.

ABTS radical scavenging (%) = (A Control - A Test) / A Control × 100

A Control is absorbance of control. A Test is the absorbance of test solution (polyherbal formulation/Standard). The Antioxidant potential of the polyherbal fomulation was expressed as IC_{50}.

Plotting a percent inhibition vs concentration curvature allowed for the determination of the sample concentration is needed for 50% inhibition, or the IC_{50} value. The higher the antioxidant capability of the polyherbal formulation indicates, lower the IC_{50} value.

2.8 *In vitro* antidiabetic assay

2.8.1. Inhibition of alpha amylase enzyme[21]

Different concentration of polyherbal formulation and acarbose (standard drug) was added to 500 µl of 0.02 M PO_4 buffer (pH 6.9) comprising α-amylase (0.5 mg/ml) solution and incubated at 25°C for 10 minutes. Each tube was filled with 500 µl of 1% starch solution in 0.02 M sodium PO_4 buffer (pH 6.9). Following that, the tubes were incubated for 10 minutes at 25°C. Then 1 ml of 3,5 dinitro salicylic acid (DNSA) color reagent was used to stop the reaction. After 5 minutes of incubation in a boiling water bath, the test tubes were allowed to cool at room temperature. A colorimeter was used to assess the reaction mixture absorbance at 540 nm.

Alpha amylase inhibition (%) = (A Control - A Sample) / A Control × 100

A Control is absorbance of control. A Sample is the absorbance of test solution (polyherbal formulation/Standard). The alpha amylase inhibitory potential of the polyherbal fomulation was expressed as IC_{50}.

2.9 STATISTICAL ANALYSIS

Each testing was conducted in triplicate, and the mean ± standard error mean (SEM) was used to express the results.

3. RESULTS AND DISCUSSIONS

3.1. Preliminary phytochemical screening

The health of both individuals and society is greatly impacted by medicinal plants. The secondary metabolites found in any given plant medication determines its therapeutic benefits or medical efficacy[22]. The presence of a group of secondary metabolites named phytoconstituents. They are the prime source of non-enzymatic antioxidants which helps to alleviate oxidative stress when taken as food or medicine[23]. Alkaloids, flavones and phenols are the major group of compounds that play a vital role as antioxidants or free radical scavengers. Pharmacological substances of secondary metabolites obtained from the medicinal plants are of suitable interest in the prevention and treatment of various diseases[24].

Table 1 - Preliminary phytochemical screening of *Catharanthus roseus* L., *Mirabilis jalapa* L., and *Senna auriculata* L. using dry plant powder

Phytoconstituent	***Catharanthus roseus***	***Mirabilis jalapa***	***Senna auriculata***
Terpenoids	+	+	+
Flavones	+	+	+
Steroids	-	-	+
Sugar	-	-	-
Alkaloids	+	+	+
Quinone	-	-	-
Phenols	+	+	+
Tannin	+	+	+
Saponin	-	+	+
Coumarin	+	+	+

Note (-) Absence, (+) Presence

The preliminary phytochemical screening of the test drug of dry plant powders of *Catharanthus roseus* L., *Mirabilis jalapa* L., and *Senna auriculata* L., was tabulated in **Table 1**. The dry plant powder of *Catharanthus roseus* L., revealed the existence of terpenoids, flavones, alkaloids, phenols, tannin, coumarin and the absence of steroids, sugar, quinone, and saponin. In *Mirabilis jalapa* L., dry plant powder exhibited the presence of terpenoids, flavones, alkaloids, phenol, tannin, saponin and coumarin. The other secondary metabolites of steroids, sugar, and quinone were absent. The dry plant powder of *Senna auriculata* L., indicates the presence of secondary metabolites of terpenoids, flavones, steroids, alkaloids, phenol, tannin, saponin, coumarin and the absence of phytochemicals include sugar, and quinone. From the obtained results, *Mirabilis jalapa* L., and *Senna auriculata* L., plant dry powders revealed more number of phytoconstituents compare than *Catharanthus roseus* L. in this study.

Table 2 - Preliminary phytochemical screening of *Catharanthus roseus* L., *Mirabilis jalapa* L., and *Senna auriculata* L. using aqueous extract

Phytoconstituent	***Catharanthus roseus***	***Mirabilis jalapa***	***Senna auriculata***
Terpenoids	+	-	+
Flavones	+	+	+
Steroids	-	-	-
Sugar	-	-	-
Alkaloids	+	+	+

Quinone	+	-	-
Phenols	+	+	+
Tannin	+	+	+
Saponin	+	+	+
Coumarin	+	+	+

Note (-) Absence, (+) Presence

The preliminary phytochemical screening of aqueous extract of *Catharanthus roseus* L., *Mirabilis jalapa* L., and *Senna auriculata* L., was tabulated in **Table 2**. *Catharanthus roseus* L., aqueous extract revealed the presence of terpenoids, flavones, alkaloids, quinone, phenols, tannin, saponin, coumarin and the absence of steroids, and sugar. The aqueous extract of *Mirabilis jalapa* L., indicates the presence of flavones, alkaloid, phenols, tannin, saponin, coumarin and the absence of terpenoid, steroid, sugar, and quinone, were observed. In *Senna auriculata* L., aqueous extract exhibited the existence of preliminary phytochemicals such as terpenoids, flavones, alkaloids, phenol, tannin, saponin, coumarin and the absence of steroid, sugar, and quinone were noted. From the obtained results *Catharanthus roseus* L., and *Senna auriculata* L., plant aqueous extract revealed more number of phytoconstituents compare than *Mirabilis jalapa* L., plant in this study.

The phytochemical screening is primarily related with the identification of secondary metabolites[25]. This phytoconsitutents have rich medicinal values and physiological properties of the whole plant and their various parts used for curing of many diseases[26]. The obtained results from the dry plant powders and aqueous extracts of *Catharanthus roseus* L., *Mirabilis jalapa* L., and *Senna auriculata* L., revealed the presence of various major phytochemical substances. The existence of this phytochemicals may be the prime role to enhance the biological effect for the control of post prandial BGL through delay in absorption of glucose in the small intestine by various hypoglycemic mechanisms[11].

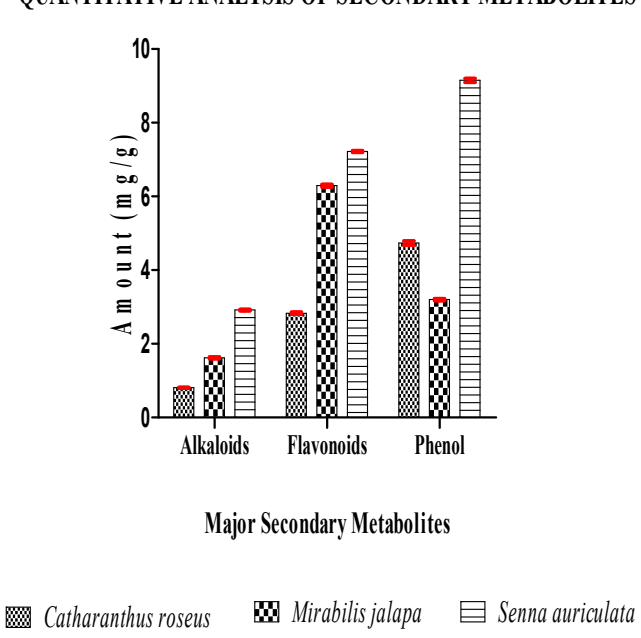

Figure 1 - Quantitative analysis of major secondary metabolites

Quantitative analysis of chief secondary metabolites were carried out for the aqueous extract of *Catharanthus roseus* L., *Mirabilis jalapa* L., and *Senna auriculata* L. The results were represented in **figure 1.** In *Catharanthus roseus* L., aqueous extract indicates rich amount of phenolic content compare than falvonoids and alkaloids. The plant aqueous extract of *Mirabilis jalapa* L., revealed higher amount of flavonoids compare than alkaloids and phenols. The aqueous extract of *Senna auriculata* L., plant showed higher content of phenols compare than flavonoids and alkaloids. The major secondary metabolites of alkaloids, flavonoids and phenol possesses higher medicinal values and pharmacological activity of curing diseases especially diabetes[27]. Most of the studies strongly reported previously that alkaloids, flavonoids and phenols have rich antidiabetic potential as well as rich antioxidant capacity[28].

3.2. *In vitro* antioxidants and antidiabetic assays

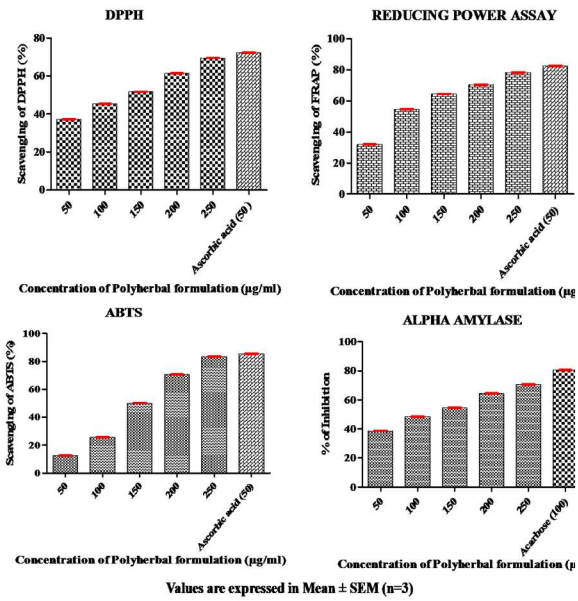

Figure 2 – *In vitro* antioxidants and antidiabetic assays

The result depicted an increased antioxidant effect on DPPH (diphenyl-2- picrylhydrazyl) assay, reducing power assay and ABTS assay (2,2"-azinobis-3-ethyl- benzothiozoline-6-sulphonic acid) with increased concentration of the polyherbal formulation (50-250 µg/ml). The maximum percentage inhibition of the DPPH, reducing power and ABTS radicals were exhibited at highest concentration (250 µg/ml). The free radical scavenging potential of antioxidant assays were represented in **figure 2.** The mean IC_{50} value of the polyherbal formulation for the DPPH assay was found to be 172.5 µg/ml, for the reducing power assay was found to be 90 µg/ml, for the ABTS assay was found to be 150 µg/ml respectively. The percentage inhibition of free radical scavenging potential strongly depends on the concentration of polyherbal formulation. The inferences were compared with that of standard ascorbic acid. The obtained results for the antioxidant study suggested that, this polyherbal formulation has rich antioxidant potential due to its combination effect as well as rich free radical scavenging ability as a promising natural antioxidant source.

Numerous clinical conditions, including diabetes mellitus, cancer, Alzheimer's, Parkinson's, arthritis, etc., have been shown to be significantly influenced by free radicals[29]. Its overloading has numerous detrimental effects on biological systems. Biological substances like proteins, lipids, and carbohydrates can be combined and oxidized by it. Generally, the free radicals cause damage to cells, tissues and organs, leading to the severity of the disease condition[30]. Most of the studies reported that extreme production of reactive oxygen species (ROS) causes oxidative injuries to the cells and tissues respectively. The overloaded free radicals in the body cannot destroy gradually, but their accumulation in the body creates oxidative stress (OS)[31]. The superoxide anion radical (O_2•−), hydroxyl radical (OH•), nitric oxide (NO), and hydrogen peroxide (H_2O_2) are among the prominent ROS that particularly significant because of their capacity to harm cellular structures, including lipid membranes, proteins, and DNA[32]. High levels of free radicals are related to diabetes, and many plants contain antihyperglycemic properties because of their antioxidant capacity[33]. Research on potent antioxidants or scavengers thus contributes to the prevention and progression of diseases by preventing the development of the free radical substances and to reduce its detrimental impacts. Plants contain rich amount of antioxidants that can effectively scavenge the free radicals and improve the prevention of cellular damage. Green leaves and flowers from plants have enormous source of natural antioxidants that are important for preventing free radical damage. Consuming plant green leaves, flowers and vegetables that contain phenolic and flavonoid chemicals with strong antioxidant activity has been linked in several epidemiological studies to a decreased risk of diabetes, cardiovascular disease, cancer, and neurological disorders[16]. It is believed that dietary antioxidants derived from plants are essential for maintaining human health[34]. Vitamins C and E, beta-carotene, flavonoids, and polyphenols are common antioxidants that are widely present in various parts of the plants[35]. Plants contain rich amount of natural substances especially secondary metabolites. It provides a crucial role in free radical scavenging potential due to its antioxidant effect. Some of the previous studies reported that phenols, alkaloids, and flavonoids have strong antioxidant potential as an effective agent of natural metabolites[36].

3.3. Alpha amylase inhibition assay

The inhibitory effect of the polyherbal formulation of *Catharanthus roseus* L., *Mirabilis jalapa* L., and *Senna auriculata* L., on alpha amylase enzyme inhibition assay was represented in **figure 2.** The obtained result of the polyherbal formulation revealed increased inhibition of the alpha amylase enzyme with augmented concentration and exhibited its highest activity at 250 µg/ml. The mean IC_{50} value of the polyherbal formulation for the alpha amylase assay was found to be 107.5 µg/ml. In the current study, the improved ability of the polyherbal formulation to inhibit the amylase enzyme, it may be due to the existence of secondary metabolites such as alkaloids, phenols and flavonoids. These secondary metabolites present in the polyherbal formulation are rich that is responsible for the antidiabetic potential. This polyherbal formulation showed as a better

inhibitory potential on carbohydrate hydrolyzing enzyme.

DM is the major health setback and continues to be one of the major causes of death all over the world. Various remedial agents are offered in medication to treat diabetes, but they are exclusive and related with many side effects[37]. Carbohydrate metabolizing inhibitors like α-amylase, α-glucosidase and sucrase slow down the carbohydrate digestion, which reduces the rate of glucose absorption and, as a result, blunts the rise in postprandial plasma glucose[38]. Alpha-amylase is a key enzyme and ubiquitous in nature due to its involvement in carbohydrate metabolism[39]. It plays a vital role in hydrolyzing alpha-bonds of glycosidic linkages of polysaccharide starch to yield glucose and maltose of structural components[40]. The inhibition of the alpha amylase enzyme has been recommended as a better approach to control the BGL for the management of diabetes[41]. The polyherbal formulation used to determine the inhibitory potential of the digestive enzyme alpha amylase due to the presence of secondary metabolites, which has rich pharmacognostic potential of regulating blood sugar level[42]. The α-amylase inhibitors, which impede the digestion and absorption of carbohydrates is due to the antidiabetic effect of polyherbal formulation[38]. Suppression of this digestive enzyme α-amylase activity would hindrance the digestion of starch and oligosaccharides, which in turn lowers the absorption of glucose as a result reduces the post prandial blood sugar level[43].

4. CONCLUSION

In the world, diabetes mellitus is among the most widespread non-communicable diseases. Diabetes management in India is tricky by a number of factors, such as the disease's increasing prevalence in both urban and rural regions, the public's lack of awareness of the condition, the scarcity of healthcare facilities, the high cost of treatment, inadequate glycemic control, and the augmented occurrence of complications from diabetes. Therefore, it is the time to recognize and discover the need for drugs from raw materials without any adverse effects. The preliminary phytochemical studies of dry plant powders and aqueous extracts of *Catharanthus roseus* L., *Mirabilis jalapa* L., and *Senna auriculata* L. revealed the existence of a variety of secondary metabolites with rich pharmacological potential. It has a crucial role in medicinal property of the secondary metabolites and antioxidant status for controlling the oxidative damage of cells from the oxidative stress during various ailments. The major secondary metabolites analysis exhibited the quantification of alkaloids, flavonoids, and phenols. The rich content of alkaloids and phenols are responsible for the hypoglycemic effect of this polyherbal formulation. The *in vitro* antioxidants of DPPH, FRAP, and ABTS showed the highest free radical scavenging potential based on the concentration of polyherbal formulations, and their inhibitory effect was predicted. The rich antioxidant potential and its free radical scavenging action in the polyherbal formulation play a significant role in this study due to the existence of diverse secondary metabolites.

Antidiabetic potential of inhibiting the α-amylase enzyme is thought to be a better approach for managing diabetes because it plays a significant role in starch digestion. Thus, *in vitro* studies of polyherbal formulation exhibited excellent antioxidants and antidiabetic potential. Hence, this study shows a link between antioxidant and antidiabetic potential, which may be very essential in minimizing the negative consequences of oxidative stress and its related diabetes. This study validating the polyherbal formulations of *Catharanthus roseus* L., *Mirabilis jalapa* L., and *Senna auriculata* L. for diabetes. The combination of the three medicinal plants was found to have excellent potential of antioxidants, which may be due to the combined activity of each plants and its rich availability of antioxidants and secondary metabolites. Taking these findings into account, it is promising that the polyherbal formulation might be a useful and different kind of management regimen for the control of free radicals and diabetes. This polyherbal formulation was strongly confirmed as a suitable traditional medicine for diabetes as well as a valuable source for the therapeutic approach as a novel strategy. However, further in-depth *in vivo* studies that lead to molecular diagnosis would help to identify a unique drug for treating diabetes mellitus.

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AUTHOR CONTRIBUTIONS

Conceptualization, methodology, experimentation, writing - original draft preparation: JA and GH; Methodology: GH, JA and SK; Conceptualization, formal analysis, review and editing: GH, SK, PE, KS and KV; Conceptualization, supervision, writing - review and editing: JA and GH. All authors agreed the final version and approved the submission.

DATA AVAILABILITY STATEMENT

All relevant data are available within the manuscript.

COMPETING INTERESTS

The authors declare that they have no potential conflicts of interest.

REFERENCES

1. Hossain MJ, Al-Mamun M, Islam MR. Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. *Health Science Reports*. 2024; 7(3): e2004. https://doi.org/10.1002/hsr2.2004
2. Khan J, Shaw S. Risk of cataract and glaucoma among older persons with diabetes in India: A cross-sectional study based on LASI, Wave-1. *Scientific Reports*. 2023; 13: 11973. https://doi.org/10.1038/s41598-023-38229-z
3. Singh S, Kriti M, Anamika KS, Sarma DK, Verma V, Nagpal R, Mohania D, Tiwari R, Kumar M. Deciphering the complex interplay of risk factors in type 2 diabetes mellitus: A comprehensive review. *Metabolism Open*. 2024; 22: 100287. https://doi.org/10.1016/j.metop.2024.100287
4. Mohd S, Kumar LL, Harish V, Kumar R, Chaudhary A, Sharma V. Diabetes mellitus: Complications, emerging therapeutic targets, and evolving treatment approaches. *Obesity Medicine*. 2025; 58: 100652. https://doi.org/10.1016/j.obmed.2025.100652
5. Padhi S, Nayak AK, Behera A. Type II diabetes mellitus: A review on recent drug based therapeutics. *Biomedicine & Pharmacotherapy*. 2020; 131: 110708. https://doi.org/10.1016/j.biopha.2020.110708
6. Hippisley-Cox J, Coupland C. Diabetes treatments and risk of amputation, blindness, severe kidney failure, hyperglycaemia, and hypoglycaemia: open cohort study in primary care. *BMJ*. 2016; 352: i1450. https://doi.org/10.1136/bmj.i1450
7. Asif M. The prevention and control the type-2 diabetes by changing lifestyle and dietary pattern. *Journal of Education and Health Promotion*. 2014; 3: 1. https://doi.org/10.4103/2277-9531.127541
8. Hariharan G, Suthanthirapandian P, Jothi G. *In vitro* evaluation of antidiabetic potential of *Vitiveria zizoniodes* L. [Nash] root extracts. *Journal of Stress Physiology and Biochemistry*. 2024; 20(3): 81-89. http://www.jspb.ru/issues/2024/N3/JSPB_2024_3_81-89.pdf
9. Balkrishna A, Sharma N, Srivastava D, Kukreti A, Srivastava S, Arya, V. Exploring the safety, efficacy, and bioactivity of herbal medicines: Bridging traditional wisdom and modern science in healthcare. *Future Integrative Medicine*. 2024; 3(1): 35-49. https://doi.org/10.14218/FIM.2023.00086
10. Mangle AP, Hatwar PR, Bakal RL. Pharmacological and pharmaceutical aspects of herbal medicines: A review. *International Journal of Herbal Medicine*. 2025; 13(4): 06-11. https://doi.org/10.22271/flora.2025.v13.i4a.991
11. Alam S, Sarker MMR, Sultana TN, Chowdhury, NR, Rashid MA, Chaity NI, Zhao C, Xiao J, Hafez EE, Khan SH, Mohamed IN. Antidiabetic phytochemicals from medicinal plants: Prospective candidates for new drug discovery and development. *Frontiers in Endocrinology*. 2022; 13: 800714. https://doi.org/10.3389/fendo.2022.800714
12. Tran N, Pham B, Le L. Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. *Biology (Basel)*. 2020; 9(9): 252. https://doi.org/10.3390/biology9090252
13. Keerthana K, Jothi G. Determination of *in vitro* antidiabetic potential of aqueous and ethanol extracts and isolated fraction of *Zanthoxylum armatum* DC stem bark. *Research Journal of Pharmacy and Technology*. 2020; 13(8): 3681-3684. https://doi.org/10.5958/0974-360X.2020.00651.4
14. Kumar R, Kumar DS, Suresh D. Polyherbal formulation: A promising approach for determining pharmacological activities and therapeutic uses. *Anveshana's International Journal of Research in Pharmacy and Life Sciences*. 2023; 8(4): 1-8. http://publications.anveshanaindia.com/wp-content/uploads/2023/10/POLYHERBAL FORMULATION-A-PROMISING-APPROACH-FOR-DETERMINING PHARMACOLOGICAL-ACTIVITIES-AND-THERAPEUTIC-USES.pdf
15. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. Springer Science & Business Media; 1998. https://link.springer.com/book/10.1007/978-94-009-5570-7
16. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants (Basel)*. 2019; 8(4): 96. https://doi.org/10.3390/plants8040096
17. Senthilkumar M, Amaresan N, Sankaranarayanan A. Estimation of total phenol content of plant tissues. *Plant-Microbe Interactions: Laboratory*

Techniques. Springer US; 2021: 153-154. https://doi.org/10.1007/978-1-0716-1080-0_41
18. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical Analysis of *Ficus religiosa*. *Molecules*. 2022; 27(4): 1326. https://doi.org/10.3390/molecules27041326
19. Vijayalakshmi M, Ruckmani K. Ferric reducing anti-oxidant power assay in plant extract. *Bangladesh Journal of Pharmacology*. 2016; 11(3): 570. https://doi.org/10.3329/bjp.v11i3.27663
20. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*. 1999; 26(9-10): 1231-1237. https://doi.org/10.1016/s0891-5849(98)00315-3
21. Kazeem MI, Adamson JO, Ogunwande IA. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of *Morinda lucida* Benth Leaf. *Biomed Research International*. 2013; 2013: 527570. https://doi.org/10.1155/2013/527570
22. Wink M. Modes of action of herbal medicines and plant secondary metabolites. *Medicines (Basel)*. 2015; 2(3): 251-286. https://doi.org/10.3390/medicines2030251
23. Muscolo A, Mariateresa O, Giulio T, Mariateresa R. Oxidative stress: The role of antioxidant phytochemicals in the prevention and treatment of diseases. *International Journal of Molecular Sciences*. 2024; 25(6): 3264. https://doi.org/10.3390/ijms25063264
24. Parham S, Kharazi AZ, Bakhsheshi-Rad HR, Nur H, Ismail AF, Sharif S, Ramakrishna S, Berto F. Antioxidant, antimicrobial and antiviral properties of herbal materials. *Antioxidants (Basel)*. 2020; 9(12): 1309. https://doi.org/10.3390/antiox9121309
25. Abirami J, Jothi G, Brindha P. Microscopic, physicochemical and phytochemical screening of *Crateva magna* (Lour) dc. (leaf). *International Journal of Current Pharmaceutical Research*. 2017; 9(5): 201-204. https://doi.org/10.22159/ijcpr.2017v9i5.22564
26. Babulal PS, Shaikh AA, Ahad A, Asema SUK. Phytochemistry, phytochemical screening of medicinal plants and exraction methods of phytochemical. *International Journal of Creative Research Thoughts.* 2025; 13(1): f655-f676. https://ijcrt.org/papers/IJCRT2501648.pdf
27. Singh S, Bansal A, Singh V, Chopra T, Poddar J. Flavonoids, alkaloids and terpenoids: A new hope for the treatment of diabetes mellitus. *Journal of Diabetes and Metabolic Disorders*. 2022; 21(1): 941-950. https://doi.org/10.1007/s40200-021-00943-8
28. Dangani HA, Salihu AS, Maigoro AL. Comparative phytochemical screening, antioxidant, and antidiabetic activities of selected medicinal plant leaves extracts. *Discover Chemistry*. 2026; 3(1): 232. https://doi.org/10.1007/s44371-026-00696-4
29. Martemucci G, Costagliola C, Mariano M, D'andrea L, Napolitano P, D'Alessandro AG. Free radical properties, source and targets, antioxidant consumption and health. *Oxygen*. 2022; 2(2): 48-78. https://doi.org/10.3390/oxygen2020006
30. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative stress: Harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*. 2017; 2017: 8416763. https://doi.org/10.1155/2017/8416763
31. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science*. 2008; 4(2): 89-96. https://pmc.ncbi.nlm.nih.gov/articles/PMC3614697/
32. Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valco M. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. *Archives of Toxicology*. 2023; 97(10): 2499-2574. https://pmc.ncbi.nlm.nih.gov/articles/PMC10475008/
33. Co A, Ovobovwori SU, Etatuvie SO. Phytochemical and antioxidant activities of a polyherbal formulation from selected medicinal herbs. *International Journal of Clinical Studies and Medical Case Reports*. 2023; 25(5): 1-8. https://doi.org/10.46998/IJCMCR.2023.25.000623
34. Ayoka TO, Ezema BO, Eze CN, Nnadi CO. Antioxidants for the prevention and treatment of non-communicable diseases. *Journal of Exploratory Research in Pharmacology*. 2022; 7(3): 179-189. https://doi.org/10.14218/JERP.2022.00028
35. Mukherjee B, Al Hoque A, Hota SH, Gope S, Ray M, Barman M, Bhattacharya S, Chakraborty S, Das L. Antioxidants and their physiological role in free radical scavenging. *Dietary Supplements and Nutraceuticals*. Springer, 2025: 397-440. https://doi.org/10.1007/978-981-96-8622-3_12
36. Rahman MM, Dhar PS, Sumaia, Anika F, Ahmed L, Islam MR, Sultana NA, Cavalu S, Pop O, Rauf A. Exploring the plant-derived bioactive

substances as antidiabetic agent: An extensive review. *Biomedicine & Pharmacotherapy*. 2022; 152: 113217. https://doi.org/10.1016/j.biopha.2022.113217

37. Aloke C, Egwu CO, Aja PM, Obasi NA, Chukwu J, Akumadu BO, Ogbu PN, Achilonu I. Current advances in the management of diabetes mellitus. *Biomedicines*. 2022; 10(10): 2436. https://doi.org/10.3390/biomedicines10102436
38. Gong L, Feng D, Wang T, Ren Y, Liu Y, Wang J. Inhibitors of α-amylase and α-glucosidase: Potential linkage for whole cereal foods on prevention of hyperglycemia. *Food Science and Nutrition*. 2020; 8(12): 6320-6337. https://doi.org/10.1002/fsn3.1987
39. Hariharan G, Manjula B, Jothi G. Determination of the role of ethanol extract of *Parmelia perlata* on glucose adsorption and diffusion – An *in vitro* study. *Journal of Stress Physiology and Biochemistry*. 2023; 19(3): 47-57. http://www.jspb.ru/issues/2023/N3/JSPB_2023_3_47-57.pdf
40. Kalinovskii AP, Sintsova OV, Gladkikh IN, Leychenko EV. Natural inhibitors of mammalian α-amylases as promising drugs for the treatment of metabolic diseases. *International Journal of Molecular Sciences*. 2023; 24(22): 16514. https://doi.org/10.3390/ijms242216514
41. Kaur N, Kumar V, Nayak SK, Wadhwa P, Kaur P, Sahu SK. Alpha-amylase as molecular target for treatment of diabetes mellitus: A comprehensive review. *Chemical Biology and Drug Design*. 2021; 98(4): 539-560. https://doi.org/10.1111/cbdd.13909
42. Kour L, Shabnam, Sutee A, Singh R. Alpha amylase inhibitor as an anti-diabetic potential from medicinal plants: A Review. *International Journal of Ayurvedic Medicine*. 2023; 13: 925-932. https://doi.org/10.47552/ijam.v13i4.2836
43. Freitas D, Lazaridou A, Duijsens D, Kotsiou K, Corbin KR, Alongi M, Perez-Moral N, Simsek S, El SN, Gwala S, Karakaya S, Feunteun SL, Grauwet T, Mario M, Martinez, Edwards CH. Starch digestion: A comprehensive update on the underlying modulation mechanisms and its *in vitro* assessment methodologies. *Trends in Food Science and Technology*. 2025; 159: 104969. https://doi.org/10.1016/j.tifs.2025.104969