



A novel polyherbal formulation for treating prediabetes and diabetes – an *in vitro* antioxidant, antidiabetic activity and phytochemical characterization

Charles Dorni A. Irudaya*, Raamapriya Venkitaraman, Rokesh Srinivasan, Sridhar Moorthy, Umamaheswari Venkatesan, Divya Panneerselvam

Vijayani Nutraceuticals Pvt Ltd, Ambattur, Chennai, Tamil Nadu, India.



*Correspondence: rdscientist@oriensworld.in

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Abstract: Diabetes mellitus is a prevalent disease that is a major concern in the healthcare sector worldwide, despite the availability of chemical agents to control and treat it. However, herbal plants with hypoglycaemic properties can be used as an alternative to synthetic antidiabetic drugs. In this study, a polyherbal formulation called Diacare was prepared using *Tinospora cordifolia*, *Gymnema sylvestre*, *Lagerstroemia speciosa*, *Cinnamomum verum*, *Eclipta alba*, *Trigonella foenum-graecum*, *Murraya koenigii* and *Piper nigrum* was evaluated based on its physico-chemical characteristics, phytochemical studies, and *in vitro* studies. The formulation was found to have greenish-brown colour, a bitter taste, and a characteristic odour, with good solubility, flowability, and disintegration time. Phytochemical analysis showed the presence of phenolic compounds and tannins, and quality control was performed using HPLC profiling and HPTLC fingerprinting. The antioxidant activity of the formulation was found to be 64.7% and 56.52% using water and methanol as solvents, respectively, with an IC₅₀ value of 0.68 and 0.82 mg/mL whereas the IC₅₀ value for α -glucosidase activity was 0.61 mg/mL. This polyherbal formulation is a patent-pending novel remedy that can be used to treat prediabetes and diabetes conditions.

Keywords: Diabetes mellitus, hypoglycaemic, polyherbal, HPTLC fingerprinting, antidiabetic



INTRODUCTION

Pre-diabetes and diabetes are two of the most prevalent metabolic disorders worldwide, affecting millions of people across different age groups and demographics. According to the International Diabetes Federation (IDF), the global prevalence of diabetes was 9.3% in 2020, and is projected to rise to 10.2% by 2030 and 10.9% by 2045. Additionally, around 374 million people worldwide are estimated to have pre-diabetes, which is characterized by higher than normal blood glucose levels but not yet meeting the diagnostic criteria for diabetes. These conditions not only have a significant impact on overall health but also pose a substantial economic burden on healthcare systems and societies worldwide. According to a report by the (IDF, 2019) the global cost of diabetes was estimated to be USD 760 billion, which is equivalent to 10.2% of global healthcare expenditure. Moreover, pre-diabetes and diabetes increase the risk of several complications, such as cardiovascular disease, kidney disease, neuropathy, and blindness, among others. These complications not only reduce quality of life but also contribute significantly to the overall economic burden of these conditions.

The prevalence is estimated to be high in urban areas when compared to rural areas and also high in well-paid countries compared to under-paid countries. Around 50 % of the world's population is still unaware of the potential risk of the disease (Saeedi et al., 2019). Prediabetes is a serious health condition characterised by blood glucose levels that are higher than normal but not high enough to progress to Type II Diabetes. The prevalence of prediabetes has been estimated at 7.3% (352.1 million) of the global adult population, which is predicted to increase to 8.3% (587 million) (Hostalek, 2019). Certain factors, such as an active lifestyle and the use of antidiabetic herbal ingredients that regulate the body's blood glucose level, have the potential to stop the prediabetic state or delay the progression process (Tuso, 2014).

Diabetes mellitus (DM) is a metabolic disorder characterised by abnormal blood glucose levels (chronic hyperglycaemia) as a result of defective insulin action or secretion (Nambirajan & Krishnamoorthy, 2022). Type 1 diabetes is a type of autoimmune disease caused by the autoimmune destruction of pancreatic beta cells through T-cell-mediated and Humoral (B-cell) inflammatory responses, which ultimately results in very little or no insulin in the blood. Type 2 diabetes is the most common form of diabetes mellitus and is characterised by insulin resistance and relative insulin deficiency, caused by the interaction between genetic and lifestyle factors primarily (Olokoba et al., 2012). There are a number of synthetic medications, including oral hypoglycaemic medications and insulin, that can be used to manage blood sugar levels, but the cost, complications, varying degrees of acceptability, and side effects prevent them from being widely accepted. Thus, there is a critical need for alternate medical treatments for this notable refractory condition highlighted by the Indian Council of Medical Research (Balammal et al., 2015).

Phytotherapy, the most popular and economically successful alternative or complementary medicinal practice, has been shown to have a synergistic effect when compared to the individual parts. The use of herbal remedies for the



management of prediabetes and diabetes has gained considerable attention in recent years, as more people are seeking natural and complementary approaches to manage their health conditions. In this study, we have used a polyherbal formulation of eight herbs, namely *Tinospora cordifolia*, *Gymnema sylvestre*, *Lagerstroemia speciosa*, *Cinnamomum verum*, *Eclipta alba*, *Trigonella foenum-graecum*, *Murraya koenigii* and *Piper nigrum* its potential benefits for the management of prediabetes and diabetes. The use of herbal remedies offers several advantages over conventional medications. One of the main advantages is the relatively low risk of adverse effects associated with herbal remedies. Herbal remedies have been used for centuries in traditional medicine systems, and as a result, they are generally considered to be safe when used appropriately. On the other hand, conventional medications may have serious side effects, particularly with long-term use.

Another advantage of using herbal remedies is their potential for synergistic effects when multiple herbs are used in combination. This is because many herbal formulas for diabetes contain multiple herbs, which may work together to produce a greater therapeutic effect than any one herb alone. *Tinospora cordifolia* (Willd.) Miers (*T. cordifolia*), commonly known as Guduchi. The stem of guduchi contains isoquinoline alkaloids such as palmatine, jatrorrhizine, and magnoflorine, which have an insulin-mimicking effect. Berberine, another isoquinoline alkaloid, works well and as effectively as Metformin (an allopathic drug) in humans because of its hypoglycemic activity. In addition to these alkaloids, the stem and root parts contain tinosporin, isocolumbin, palmatine, tinocordiside, cordioside, and sitosterol, which have anti-diabetic, anti-hyperlipidaemic, and antioxidant properties (Sharma et al., 2015). *Gymnema sylvestre* is referred to as "Meshasringa" in the Ayurvedic system. The leaves of *Gymnema sylvestre* contain phytochemicals such as gymnemic acids, gymnema saponins, and gymnemasides, which are responsible for its anti-diabetic activity.

Gymnemic acid has a similar molecular pattern to glucose, so they compete to inhibit the binding of sugar molecules to the receptor on the external layers of the intestine, preventing glucose absorption and resulting in low blood glucose levels (Rizvi and Mishra, 2013; Kanetkar et al., 2015). *Eclipta alba* (L.) Hassk. (Also known as *Eclipta prostrata* Roxb.) is a member of the *Asteraceae* family, commonly known as bhringoraj or bhringraj in Bangladesh and India. The major phytochemical compounds reported in the leaf extract of *Eclipta alba* are coumestans (coumarin derivatives), alkaloids, saponins, tannins, lignans, sterols, flavonoids, terpenoids and their glycosides, phenolic acids, polypeptides, and volatile oils (Sazia et al., 2015). *Trigonella foenum-graecum* L. (Fenugreek, Methi) belongs to the family Fabaceae and is one of the most important vegetables cum spices in India due to its medicinal potential. The main active constituents responsible for the anti-diabetic activity are saponins, 4-hydroxyisoleucine an amino acid, trigonelline an alkaloid, flavonoids, and galactomannan rich soluble fibres (Qais et al., 2019). These active constituents facilitate pharmacological activities such as carbohydrate digestion enzyme inhibition, lipid accumulation prevention, direct pancreatic -cell stimulation for insulin secretion, -amylase inhibitory activity, slowing glucose digestion and absorption, and delayed gastric emptying (Singh et al., 2021).

Murraya koenigii (*M. koenigii*) (L) Spreng (Family: *Rutaceae*) also known as Curry leaves, has a variety of medicinal properties (Wasnik and Naik, 2016).



Carbazole alkaloids, being the major active constituents, are responsible for increased insulin secretion in the blood plasma, a reduced level of sugar, triglycerides, LDL and VLDL, an increased level of HDL in the blood, decreased oxidative stress, and pancreatic beta cell damage. (Singh et al. 2022) reported as increased glycogen levels in the liver and α -amylase inhibitory activity in diabetic rats treated with the aqueous extract of *Pterospermum acerifolium* leaves, suggesting potential antidiabetic effects of the extract. *Lagerstroemia speciosa* (L.) Pers. or *Lagerstroemia flos-reginae*, commonly known as queen's flower. The active constituents responsible for the anti-diabetic activity are corosolic acid, lagerstroemin and lagertannins. Corosolic acid is the triterpene that facilitates the glucose transport across the cell membrane and hence resulting in low blood glucose level. Lagerstroemin activates the insulin receptor by increasing the phosphorylation of tyrosine present in the insulin receptor subunit. Lagertannins enhance glucose transport and adipocyte differentiation inhibitory activity, leading to weight loss (Chan et al., 2019).

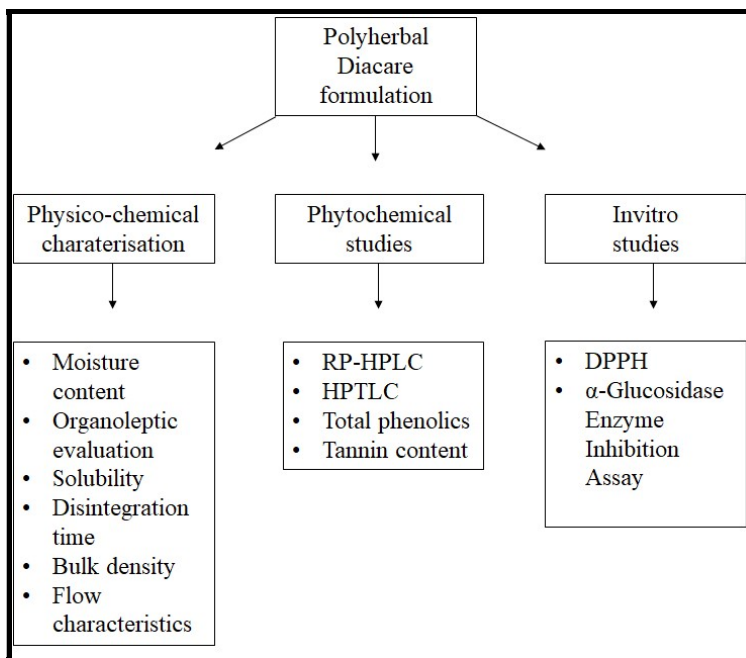
Cinnamomum verum J. Presl (belonging to the family Lauraceae), commonly known as "true cinnamon" or Ceylon cinnamon. Cinnamaldehyde (predominantly), eugenol, caryophyllene, cinnamyl acetate and α -humulene are the major active constituents present. The cinnamaldehyde acts as a chelating agent that activates many signalling pathways, including insulin receptor signalling and glucose transport signalling thereby facilitating low blood glucose levels (Sharifi-Rad et al., 2021). Black pepper (*Piper nigrum* L.), commonly known as the "King of Spices", belonging to the family Piperaceae, is one of the most popular spices in Indian cooking (Takooree et al., 2019). Piperine is well known for its bioenhancer properties, which includes increasing the solubility and absorption of the active compounds, increasing the blood supply in the gastrointestinal tract thereby facilitating increased absorptivity, and increasing the permeability of the intestinal cells to increase the uptake of active compounds (Mashke et al., 2018). The goal of this study was to evaluate and standardise a novel polyherbal formulation in the form of capsules using the selected herbal extracts, effective against prediabetics and diabetics. *In vitro* approaches were performed to investigate the antidiabetic potential of the intended formulation.

METHODS

Materials: The standardised extracts of *Tinospora cordifolia*, *Gymnema sylvestre* and *Lagerstroemia speciosa* were procured from Biomed ingredients, Bangalore; *Cinnamomum verum*, *Salacia reticulata*, *Piper nigrum*, *Eclipta alba* and *Trigonella foenum-graecum* were sourced from Biogen extracts, Bangalore; and *Murraya koenigii* was obtained from Thangam extracts, Sanyasikaradu, Tamil Nadu.

Preparation of the Diacare formulation: The herbal combination in the formulation is designed as mentioned in Table 1. The individual herbal extracts were weighed as per the ratios and homogenized, then stored in an air tight container, labelled and sealed until encapsulation. In order to verify the pharmaceutical properties of the product, the prepared formulation was encapsulated into vegetarian capsules (made of hydroxymethyl cellulose) of size 000. The batch number for Diacare formulation is OGMPL19-TP05 and weight of each capsule around 500 mg manufactured by oriens global marketing, Chennai, India. The standardised

procedure is adopted to prepare the capsules in a semi-automatic capsule vending machine. During the preparation of the formulation, strict hygiene and quality control parameters were adopted as per ISO 22000 food safety and quality standards.



Workflow for polyherbal Diacare formulation studies

Table 1. Diacare standard formulation

Plant	Plant Parts	Amount per capsule	% Composition
<i>Tinospora cordifolia</i>	Plant	100 mg	20
<i>Gymnema sylvestre</i>	Leaf	100 mg	20
<i>Eclipta alba</i>	Plant	75 mg	15
<i>Trigonella foenum-graecum</i>	Seed	75 mg	15
<i>Murraya koenigii</i>	Leaf	75 mg	15
<i>Lagerstroemia speciosa</i>	Leaf	50 mg	10
<i>Cinnamomum verum</i>	Bark	15 mg	3
<i>Piper nigrum</i>	Fruit	10 mg	2

Physico-chemical characterisation: The physicochemical parameters, such as organoleptic evaluation, moisture content, solubility, disintegration time, bulk density and flow characteristics, were determined.

Organoleptic evaluation: The colour, taste and odour of the polyherbal formulation were evaluated by (AOAC, 1997) method. The taste of the formulation was characterised as either bitter, salty, sour, astringent, sweet, pungent or umami. The formulations were placed in beakers and examined for their odour by slow and repeated inhalation of the air above the material.

Moisture content: A thermo gravimetric analyser (PerkinElmer, TGA 8000, India) with integrated scales was used to estimate the moisture content. About 2.0 g of



the sample formulation was placed in the sample pan and dried at 85°C by infrared drying. The analysis stops automatically when there is no change in mass detected by the instrument.

Solubility: One g of sample was weighed into a glass tube. 1 ml of water was added and mixed, and the procedure continued until complete solubility of the sample was achieved. The solubility of the formulation is described as very soluble (<1 part), freely soluble (1-10 parts), soluble (10-30 parts), sparingly soluble (30-100 parts), slightly soluble (100-1000 parts), very slightly soluble (1000-10000 parts), and practically insoluble (>10,000 parts) (Savjani et al., 2017).

Density and flow characteristics: The graduated cylinder provided with the tapped density apparatus was tared, and the sample powder was transferred into the cylinder in a free-flowing manner until it occupied a volume of 20 ml. The mass was noted, and the cylinder was secured into the holder of the tapped density apparatus and tapped 100 times (Rashid et al., 2013). The corresponding volume to the nearest graduated unit was noted. The bulk and tapped densities along with the flow characteristics such as Carr's index and Hausner's ratio were calculated using the formulas given below:

$$\text{Bulk density } (\rho_B) = [\text{Mass of the sample in g} / V_B]$$

$$\text{Tapped density } (\rho_T) = [\text{Mass of the sample in g} / V_T]$$

$$\text{Carr's Index} = [100 * (V_B - V_T)] / V_B$$

$$\text{Hausner's ratio} = \rho_B / \rho_T$$

Whereas, V_B - Free volume of the sample, V_T - Tapped volume of the sample, ρ_B - Free bulk density and ρ_T - Tapped density

The interparticle interactions are less significant in a free-flowing sample, to which the bulk and tapped densities will be closer. A Carr's Index value of more than 25 indicates poor flowability, and lower than 15 indicates good flowability. A Hausner's ratio greater than 1.25 indicates poor flowability.

Disintegration time test: Disintegration time for Diacare capsule was determined using a digital microprocessor- (DISSOTEST DT 100, Labindia Instruments, India) based disintegration apparatus. One capsule was placed in each tube, a disc was added to each tube of the basket, and the apparatus was operated using water as the release medium maintained at $37 \pm 2^\circ\text{C}$. The setup was suspended in water in a 1000 ml beaker. The capsules were observed, and the time taken for complete disintegration of the capsules was determined (Silva et al., 2018).

Phytochemical studies: The Diacare formulation was qualitatively evaluated for phytochemical profile and fingerprinting by RP-HPLC and HPTLC methods. The total phenolics and tannin content were also evaluated quantitatively.

RP-HPLC profiling: The RP-HPLC system (MODEL: Shimadzu Prominence-i LC-2060, India), consisting of pump (LC-20AD), UV detector (SPD-20 A), autosampler (SIL-20AC) with Column Solar C-18 (250 × 4.6 mm ID, 5 μm) was used. The flow rate (1 ml/min), sample injection of 10 μL and the oven temperature (40°C) were kept standard for the sample and the standards.



For berberine standard, mobile phase A consisted of 1.36 g of KH_2PO_4 in 1000 ml of water and mobile phase B consists of ACN with pH adjusted to 2.6 was used. For gymnemic acid standard, mobile phase consisted of 0.1 % acetic acid and ACN (25:75 v/v) was used. For piperine standard, mobile phase consisted of water and methanol (25:75 v/v) was used. The sample preparation and extraction process for the standards and the sample were followed by the methods adopted from literature review (Setyaningsih et al. 2021).

HPTLC Fingerprinting: The HPTLC fingerprinting was performed by dissolving 10 mg of the sample in methanol. 4-10 μL of the sample extract were spotted on a HPTLC Silica Gel 60 F 254 (Merck) plate as bands of length 6 mm at a distance of 10 mm. The plates were created in a previously saturated CAMAG twin trough glass chamber for 30 mins. Toluene: ethyl acetate: methanol in the ratio of 4:4:1 was used as the mobile phase. The plates were dried at 60°C and scanned using a CAMAG TLC scanner in absorbance mode. The plates were also derivatized with 10 % methanolic sulphuric acid. The compounds were scanned at 254 nm and 366 nm.

Total phenolic content: The total phenolic content of the sample formulated was determined using the Folin-Ciocalteu method. The powdered formulation from the unsealed capsules was made up to a 1mg/ml stock solution, and 200 μL of this solution was taken in a test tube. For the standard curve, gallic acid was made up to a 1mg/ml stock solution, and 200 μL of this solution was taken in a test tube at various concentrations (10 to 500 $\mu\text{g}/\text{mL}$). 800 μL of Folin-Ciocalteu's reagent (1:1 dilution) and 2 mL of 7.5% sodium carbonate were added to the sample solution and incubated at 37°C for 4 hours in dark place. The absorbance was measured at 765 nm using a UV spectrophotometer against a suitable blank (Prabhavathi et al., 2016).

Total tannin content: The total tannin content of the formulations was determined using the Folin-Ciocalteu method. Tannic acid was made up to a 1mg/ml stock solution, and 500 μL of this solution was taken in a test tube at various concentrations (10 to 500 $\mu\text{g}/\text{mL}$). The sample from the unsealed capsule was made up to a 1mg/ml stock solution, and 500 μL of this solution was taken in a test tube. 3.75 ml of distilled water, 0.75 ml of Folin-Ciocalteu reagent, and 0.5 ml of 35% sodium carbonate were added to the sample solution and incubated at 37°C for 1 hour in dark place after which the absorbance was measured at 765 nm using a UV Spectrophotometer (Prabhavathi et al., 2016).

DPPH Radical scavenging method for antioxidant activity: The sample was made up to a 1mg/ml stock solution, and the solution was taken at different concentrations (10 to 1000 $\mu\text{g}/\text{mL}$), with which an 800 μL solution of DPPH in methanol was mixed. The solution was allowed to stand at room temperature for 30 minutes, after which the absorbance was measured at 517 nm (Hossain et al., 2013). The percentage inhibition was calculated using the following equation

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] * 100$$

Where, A_0 was the absorbance of control reaction and A_1 was the absorbance of the sample. The results were expressed in terms of IC_{50} value.



α-Glucosidase enzyme inhibition assay: Elya et al., 2016 described a method for optimising the inhibition activity of α -glucosidase. 20 μ L of the stock solution was taken and placed in the wells of a microplate at different concentrations (10 to 1000 μ g/mL). 50 μ L of 20 mM phosphate buffer and 10 μ L of α -glucosidase were used in the reaction. The reaction was incubated at 37 °C for 5 minutes before adding 20 μ L of 1 mM PNPG was added and incubated at 37 °C for 30 minutes. 50 μ L of 1 mM sodium carbonate was used to stop the reaction, and the change in absorbance was read at 405 nm using a microplate reader. Acarbose was used as the standard. The percentage inhibition was calculated using equation (5) and the results were expressed in terms of the IC₅₀ value.

RESULTS

Physico-chemical characterization: The organoleptic properties such as colour, taste, and odour of the polyherbal formulations were evaluated, and the results were represented in Table 2.

Other parameters include the moisture content, solubility, bulk, and tapper density. Table 3. presents the results of the Carr's and Hausner's ratio tests and capsule disintegration time. The moisture content of the formulated capsule was below 5%, indicating effective capsule formation. The solubility of the capsule formulation is crucial for its absorption and effectiveness, and the Diacare formulation was found to be sufficiently soluble. The Carr's Index and Hausner's Ratio are used to assess the flowability of the capsule formulation. A Carr's Index value above 25 indicates poor flowability, while a value below 15 indicates good flowability. A Hausner's Ratio greater than 1.25 also suggests poor flowability. The Diacare capsule formulation demonstrated a Carr's index value between 25 and 15 and a Hausner's ratio value of 1.25, which indicates acceptable flowability. Additionally, the capsule disintegration time was evaluated, and within 30 minutes, the capsule fully disintegrated as intended. These results demonstrate the quality and effectiveness of the Diacare capsule formulations.

Phytochemical studies: The RP-HPLC analysis of the Diacare formulation, which is a mixture of several herbs, showed that it contains a variety of different phytochemicals. The presence of polyphenols, terpenoids, alkaloids, and flavonoids in the formulation contributed to the appearance of 34 distinct peaks in the chromatogram. These peaks corresponded to different compounds in the mixture, which were separated and identified using the HPLC technique. Out of the many compounds detected in the Diacare formulation, berberine, gymnemic acid and piperine were found to be the most abundant. To confirm their presence, the primary and secondary phytochemical standards were used, which are chemical compounds known to produce specific signals in the HPLC instrument. By comparing the signals produced by the Diacare formulation with those of the standards, were able to identify berberine, gymnemic acid and piperine in the mixture and also quantified the amount of each compound in the Diacare formulation. Each 500 mg capsule of Diacare contained 0.29 mg of berberine, 77.20 mg of gymnemic acid and 10.04 mg of piperine. This information is important because it can help determine the appropriate dosage of the formulation for patients. The results of the RP-HPLC analysis in (Fig 1-3), which show the chromatograms of the Diacare formulation and the standards for berberine, gymnemic acid, and piperine.



In this study, we conducted a preliminary phytochemical screening of the Diacare formulation using HPTLC fingerprinting. HPTLC, or high-performance thin-layer chromatography, is a powerful analytical technique that can be used to separate and identify complex mixtures of compounds. In this case, we used HPTLC to identify the different phytochemicals present in the Diacare formulation. To perform the HPTLC analysis, we applied a sample of the Diacare formulation to a thin layer of silica gel on a glass plate. Under 366 nm, we observed three major spots in the HPTLC plate at R_f values of 0.8 (blue), 0.73 (red), and 0.58 (blue). When we visualized the plate under white light, we saw two spots at a R_f value of 0.58 (green). Finally, under 254 nm, we observed a single spot at R_f value of 0.58. To confirm the identity of these spots, we compared them to phytochemical standards. Phytochemical standards are pure chemical compounds that are known to produce specific spots on an HPTLC plate. By comparing the spots in the Diacare formulation to the standards, we were able to identify the different phytochemicals present in the formulation. The HPTLC profiling of the Diacare formulation is shown in Figure 4. The figure illustrates the different spots we observed in the HPTLC plate and their corresponding R_f values of the Diacare formulation.

Polyphenols are the secondary metabolites of plants that act as a defence mechanism against ultraviolet radiation, oxidants and pathogens with natural phytochemical compounds. Dietary polyphenols modulate carbohydrate metabolism, attenuate hyperglycaemia, and insulin resistance, and alleviate oxidative stress. Phenolic compounds carry out antidiabetic activities by modifying enzymatic and transcriptional activities (Saeedi et al., 2019). Tannins are antioxidants that act as free radical scavengers, activate antioxidant enzymes, and enhance glucose uptake. Tannins act as anti-nutritional factors that prove beneficial in diabetes as they inhibit intestinal glucose absorption and reduce food intake by promoting satiety (Hostalek, 2019).

The total phenolic and tannin content present in the formulated sample was represented in Table 4. The total phenolic and tannin content are important indicators of the antioxidant and therapeutic potential of natural products. In our study, the total phenolic content of the formulated sample was found to be 82.19 ± 7.50 mg/g, while the total tannin content was 61.9 ± 5.18 mg/g. The high total phenolic content observed in the formulated sample may be attributed to the presence of various polyphenolic compounds, such as flavonoids, phenolic acids, and tannins, which are known to exhibit potent antioxidant and anti-inflammatory activities. These compounds scavenge free radicals and reactive oxygen species, thereby reducing oxidative stress and protecting against cellular damage. Similarly, the high total tannin content observed in the sample may contribute to its therapeutic potential.

The experiments were conducted in duplicate, and the values were represented as mean \pm SD. The antioxidant activity of the sample was assessed to determine its potential efficacy in the prevention of β -cell degeneration and the formation of advanced glycation end products (AGEs) (Bahadoran et al., 2018). The antioxidant analysis performed revealed that the formulated sample with water as the solvent has an antioxidant activity of 8.18% higher than the sample with methanol as solvent. The antioxidant activity is represented in the (Fig. 5).



The corresponding IC₅₀ (mg/ml) values of the samples were represented in Table 5.

The primary intestinal enzyme α -glucosidase converts complex carbohydrates such as starches into their simpler form, glucose. Inhibition of this enzyme lowers the breakdown of complex carbohydrates, which in turn lowers the formation and absorption of glucose and results in lowering the blood glucose levels (Bahadoran et al., 2018). The α -glucosidase enzyme inhibitory activity of the Diacare formulation was found to be nearly equal to that of standard antidiabetic drug, acarbose, which may be attributed to the formulation's higher tannin content (Huang et al., 2019). The percentage inhibition of α -Glucosidase activity by the sample and acarbose (standard) were illustrated in the (Fig. 6). The IC₅₀ value of the Diacare is represented in the Table 5. The antioxidant and α -glucosidase inhibitory activity results obtained from the present study show the potential therapeutic value of the Diacare polyherbal formulation in the management of prediabetes and diabetes. The polyherbal formulation was found to possess significant antioxidant activity, which could be attributed to the presence of polyphenols, flavonoids, and other phytochemicals in the herbs used. The antioxidant activity of the formulation could play a crucial role in reducing oxidative stress in diabetic patients, which is known to contribute to the development and progression of diabetes complications.

Furthermore, the α -glucosidase inhibitory activity of the formulation indicates its potential in controlling postprandial hyperglycaemia, which is a critical factor in the pathogenesis of diabetes. The inhibition of α -glucosidase by the formulation could reduce the absorption of glucose from the intestine, thus preventing the sudden rise in blood glucose levels after a meal. In comparison to other antidiabetic drugs and natural remedies, the Diacare polyherbal formulation's antioxidant and α -glucosidase inhibitory activity is encouraging. Several studies have reported the antioxidant and α -glucosidase inhibitory activity of various plant extracts and their isolated compounds (Singh et al. 2022). However, most of the studies have reported these activities individually, and very few studies have reported the combined antioxidant and α -glucosidase inhibitory activity of plant extracts or formulations. Gautam et al. (2020) showed that a polyherbal formulation containing several herbs, including *Gymnema sylvestre*, *Tinospora cordifolia*, and *Cinnamomum verum*, was effective in reducing blood glucose levels in patients with type 2 diabetes. (Nair et al. 2021) showed that a polyherbal formulation containing Fenugreek, *Salacia reticulata*, and *Cinnamomum cassia* was effective in reducing blood glucose levels in patients with prediabetes.

In addition to their safety and potential for synergistic effects, herbal remedies may offer unique benefits for the management of prediabetes and diabetes. Herbs like *Gymnema sylvestre* and Fenugreek, have been shown to improve insulin sensitivity and reduce blood glucose levels. Other herbs, such as *Cinnamomum verum* and *Murraya koenigii*, have antioxidant and anti-inflammatory properties, which may help to reduce the risk of complications associated with diabetes.



Table 2. Organoleptic evaluation of the formulated capsule (Diacare)

Organoleptic Evaluation	
Organoleptic parameters	Results
Colour / Appearance	Greenish brown colour
Taste	Bitter
Odour	Characteristic

Table 3. Physico-chemical characteristics of the formulated capsule (Diacare)

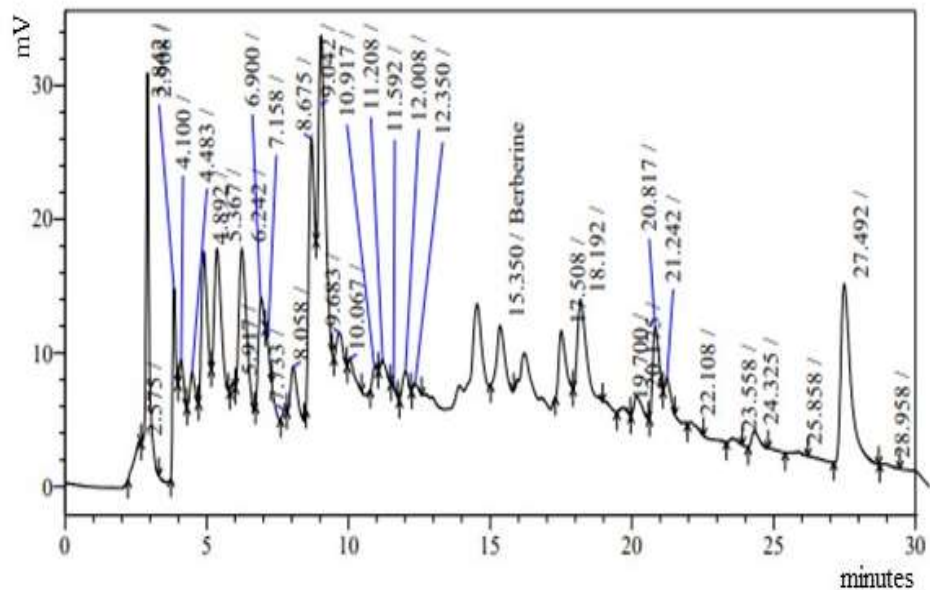
Parameters	Diacare Formulation
Moisture Content (%)	2.9
Solubility (in water)	Soluble
Bulk density (g/ml)	0.54
Tapped density (g/ml)	0.68
Carr's Index	20
Hausner's Ratio	1.25
Capsule Disintegration Time Test	18 min 30 sec

Table 4. Total phenolic and tannin content in the formulated sample

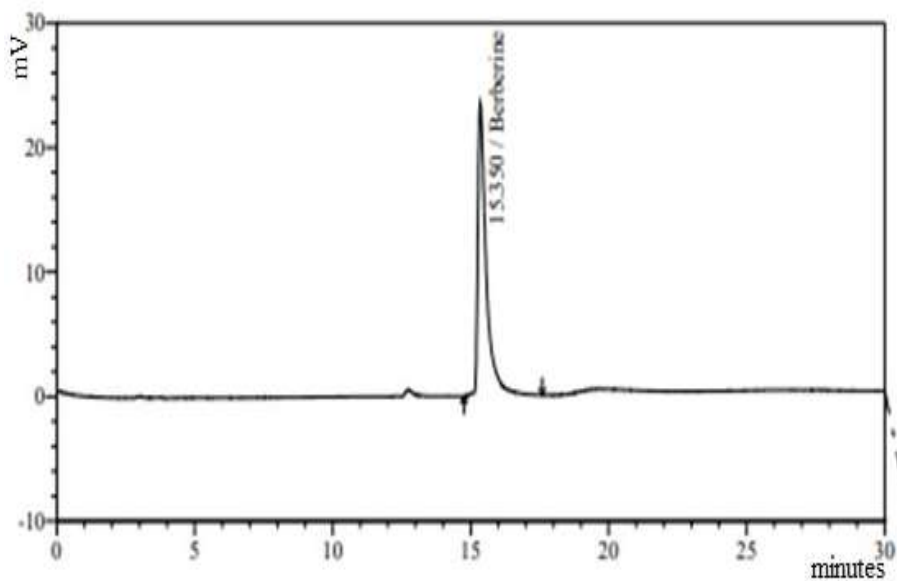
Content	Value (mg/g)
Total phenolic content	82.19 ± 7.50
Total tannin content	61.9 ± 5.18

Table 5. IC₅₀ values (mg/ml) of the sample with water and methanol as solvent

Sample	IC₅₀ value (mg/ml)
Water	0.68
Methanol	0.82
Diacare	0.61

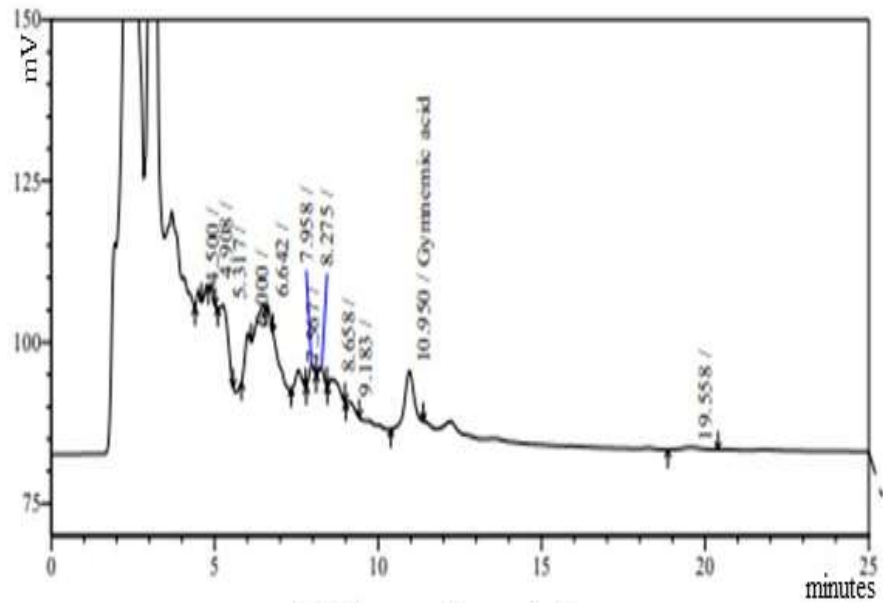


(a) Diacare formulation

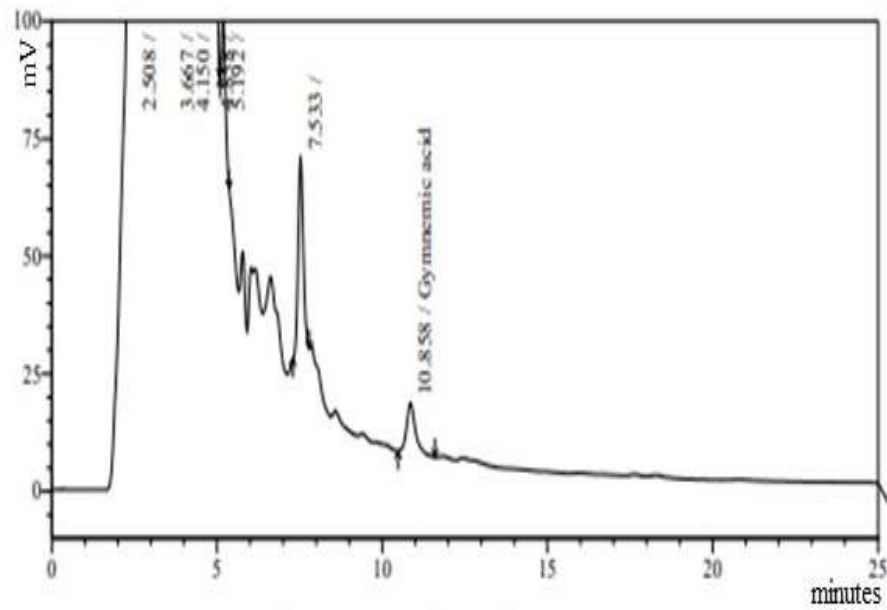


(b) Berberine standard

Fig. 1. HPLC representing the peaks of Berberine in (a) Diacare formulation and (b) Standard

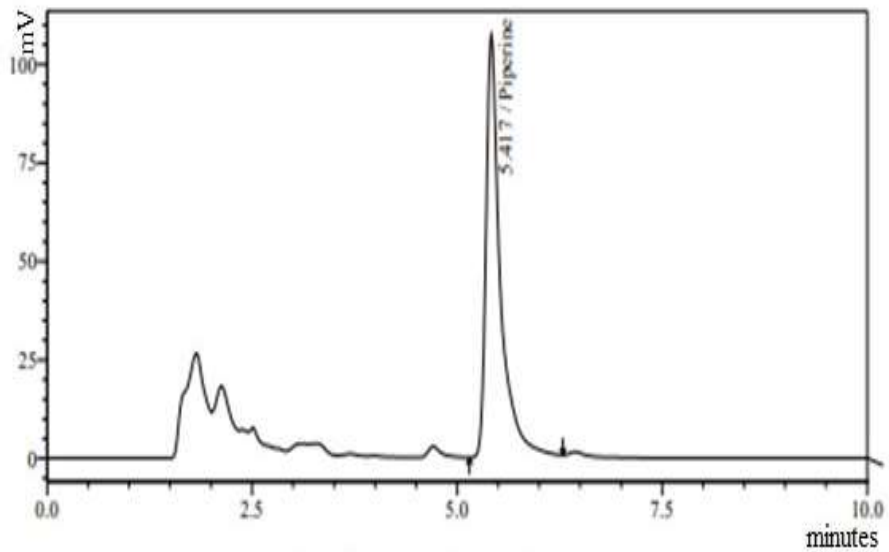


(a) Diacare formulation

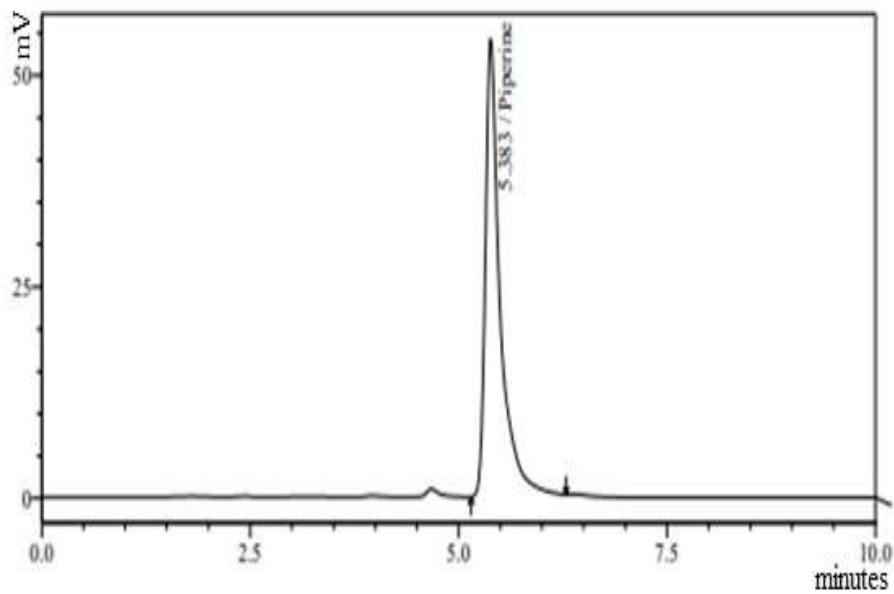


(b) Gymnemic acid standard

Fig. 2. HPLC representing the peaks of Gymnemic acid in (a) Diacare formulation and (b) Standard

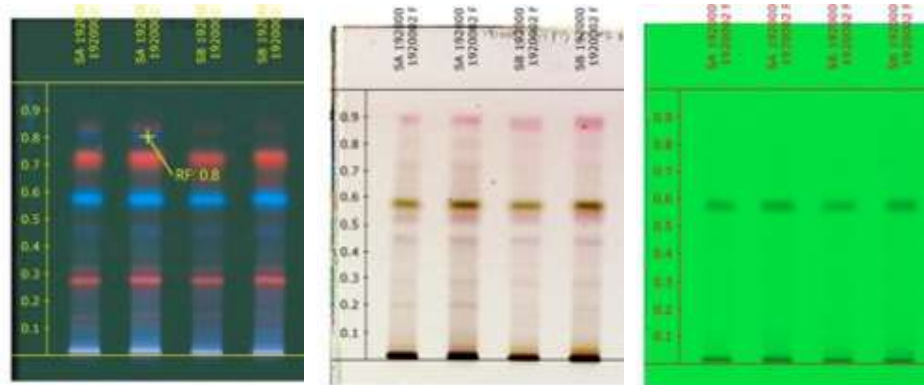


(a) Diacare formulation



(b) Piperine standard

Fig. 3. HPLC representing the peaks of Piperine in (a) Diacare formulation and (b) Standard



(a) Diacare derivatized (366nm) (b) Diacare (White light) (c) Diacare (254nm)

Fig. 4. HPTLC profiling (a) Diacare derivatized (366nm), (b) Diacare (White light) and (c) Diacare (254nm)

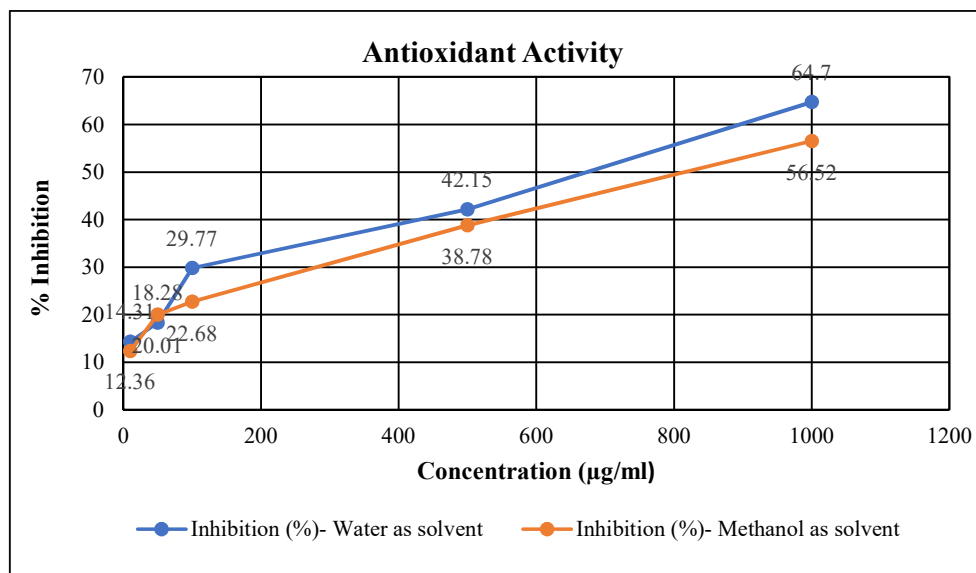


Fig. 5. Percentage inhibition of antioxidant activity of the formulated sample in the presence of water and methanol as solvent

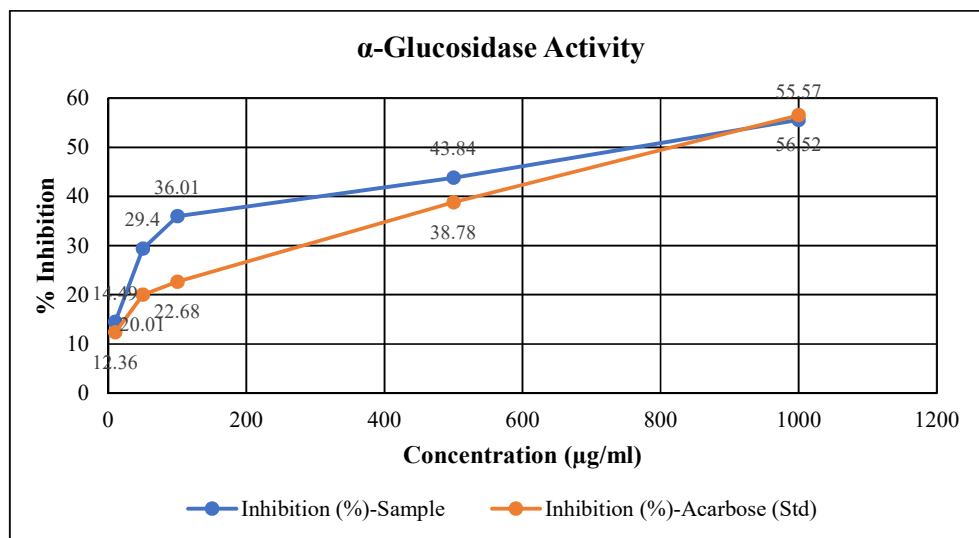


Fig. 6. Percentage inhibition of α -Glucosidase activity by the sample and acarbose (standard)

CONCLUSION

The increase in the prevalence of prediabetes and diabetes, along with the late complications, has been the driving force behind the continuous development of anti-diabetic formulations from herbal sources. Herbal formulations have the advantage of fewer adverse effects with prolonged pharmacological actions. The present study has validated the antidiabetic activity of the polyherbal formulation Diacare based on the selected plants: *Tinospora cordifolia*, *Gymnema sylvestre*, *Lagerstroemia speciosa*, *Cinnamomum verum*, *Eclipta alba*, *Trigonella foenum-graecum*, *Murraya koenigii* and *Piper nigrum*. The phytochemical evaluation of the Diacare formulation revealed a possible mechanism for regulating blood glucose levels by inhibiting α -glucosidase and exhibiting antioxidant activity.

Many synthetic antidiabetic drugs can cause adverse effects such as hypoglycaemia, weight gain, and gastrointestinal disturbances. Additionally, patients may struggle with adhering to treatment regimens, which can lead to poor glycaemic control and increased risk of complications. The polyherbal formulation of Diacare may address some of these limitations. The herbs used in the formulation have been traditionally used for their hypoglycemic properties and have a low risk of adverse effects. Furthermore, the use of multiple herbs in the formulation may provide a synergistic effect, resulting in better glycaemic control and fewer side effects. The use of a polyherbal formulation may also be more acceptable to patients, potentially improving adherence to treatment regimens. This research concludes that the use of the polyherbal formulation of Diacare may be a promising alternative for the management of prediabetes and diabetes, potentially addressing some of the limitations of current treatments. Further studies are needed to evaluate the safety and efficacy of this formulation in human subjects.



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PATENT

The patent registration number of the Diacare formulation is **202141006934**.

DISCLOSURE OF STATEMENT

Charles Dorni A. Irudaya*, Raamapriya Venkitaraman, Rokesh Srinivasan, Sridhar Moorthy, Umamaheswari Venkatesan and Divya Panneerselvam are the employees of Oriens Global Marketing Pvt Ltd, Ambattur, Chennai, Tamil Nadu, India.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available from the corresponding author upon reasonable request.

DECLARATION OF CONFLICT OF INTEREST

Authors declare that they have no conflicts of interest.

REFERENCES

- AOAC (1997). Official Methods of analysis. 18th edition. Association of official Analytical Chemists.
- Bahadoran, Z., Mirmiran, P., Azizi, F., 2018. Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. *J. Diabetes Metab. Disord.* 12, 1-9.
- Balammal, G., Sekar, B.M., Reddy, J.P., 2015. Analysis of Herbal Medicines by Modern Chromatographic Techniques. *Int. J. Pre. Pharm. Res.* 3, 50-63.
- Bishnu, C., 2015. *Lagerstroemia speciosa* – A Review. *Int. J. of Allied Med. Sci. and Clin. Res.* 3, 521-524.
- Chan, E.W., Tan, L.N., Wong, S.K., 2019. Phytochemistry and pharmacology of *Lagerstroemia speciosa*: A natural remedy for diabetes. *Int. J. Herbal. Med.* 2, 100–5.
- Elya, B., Basah, K., Mun'im, A., Yulastuti, W., Bangun, A., Septiana, E.K., 2016. Screening of α -glucosidase inhibitory activity from some plants of *Apocynaceae*, *Clusiaceae*, *Euphorbiaceae*, and *Rubiaceae*. *J. Biomed. Biotechnol.* 2012, 1-6.
- Falzon, C.C., Balabanova, A., 2017. Phytotherapy: An Introduction to Herbal Medicine. *Prim. Care.* 44, 217-227.
- Gautam R, Srivastava S, Sharma N, et al. Evaluation of a polyherbal formulation in patients with type 2 diabetes mellitus: a randomized controlled trial. *J Ayurveda Integr Med.* 2020;11(3):287-293.
- Hossain, H., Karmakar, U.K., Biswas, S.K., Shahid-Ud-Daula, A.F., Jahan, I.A., Adnan, T., Chowdhury, A., 2013. Antinociceptive and antioxidant potential of the crude ethanol extract of the leaves of *Ageratum conyzoides* grown in Bangladesh. *Pharm. Biol.* 51, 893-898.
- Hostalek, U., 2019. Global epidemiology of prediabetes - present and future perspectives. *Clin. Diabetes Endocrinol.* 5, 1-5.
- Huang, Q., Chai, W.M., Ma, Z.Y., Ou-Yang, C., Wei, Q.M., Song, S., Zou, Z.R., Peng, Y.Y. 2019. Inhibition of α -glucosidase activity and non-enzymatic glycation by tannic acid: Inhibitory activity and molecular mechanism. *Int. J. Biol. Macromol.* 1, 358-368.
- International Diabetes Federation. *IDF Diabetes Atlas*, 9th edn. Brussels, Belgium: International Diabetes Federation, 2019. Available at: <https://www.diabetesatlas.org>.



- Kanetkar, P., Singhal, R., Kamat, M., 2015. *Gymnema sylvestre*: A Memoir. J. Clin. Biochem. Nutr. 41, 77–81.
- Mashke, D.B., Sreedharan, S., Mahadik, K.R., 2018. Role of Piperine as an Effective Bioenhancer in Drug Absorption. Pharm. Anal. Acta. 09, 1-4.
- Nair AK, Sasidharan S, Sreejith R, et al. Efficacy of a polyherbal formulation in prediabetes: a randomized, double-blind, placebo-controlled study. Complement Ther Med. 2021; 59:102694.
- Olokoba, A.B., Obateru, O.A., Olokoba, L.B., 2012. Type 2 diabetes mellitus: a review of current trends. Oman Med. J. 27, 269-73.
- Prabhavathi, R.M., Prasad, M.P., Jayaramu, M., 2016. Studies on Qualitative and Quantitative Phytochemical Analysis of *Cissus quadrangularis*. Adv. Appl. Sci. Res. 7, 11-17.
- Qais, F.A., Khan, M.S., Althubiani, A.S., Al-Ghamdi, S.B., Ahmad, I., 2019. Chapter 13: Understanding Biochemical and Molecular Mechanism of Complications of Glycation and Its Management by Herbal Medicine. New Look to Phytomedicine, 331–366.
- Rashid, N.S., Muhammad, A., Abdul, M., Muhammad, N., Hassali, K.A., Azmi, M., 2013. Formulation development of metformin tablet and its comparative in-vitro study with different brands in Pakistan. Int. J. Pharm. Sci. Rev. Res. 19, 12–17.
- Rizvi, S.I., Mishra, N., 2013. Traditional Indian Medicines Used for the Management of Diabetes Mellitus. Journal of Diabetes Research, 2013, 1–11.
- Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A.A., Ogurtsova, K., Shaw, J.E., Bright, D., Williams, R., 2019. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res. Clin. Pract. 157, e107843.
- Savjani, K.T., Gajjar, A.K., Savjani, J.K., 2017. Drug solubility: importance and enhancement techniques. ISRN Pharm. 2012, 1-10.
- Sazia, Singh, S., Shankar, P., Nath, R., Sachan, A.K., Dixit, R.K., 2015. Effect of *Eclipta Alba* against blood glucose level in diabetic patients. Int. J. Biomed. 6, 210-213.
- Setyaningsih, D., Santoso, Y.A., Hartini, Y.S., Murti, Y.B., Hinrichs, W.L.J., Patramurti, C., 2021. Isocratic high-performance liquid chromatography (HPLC) for simultaneous quantification of curcumin and piperine in a microparticle formulation containing *Curcuma longa* and *Piper nigrum*. Heliyon. 7, e06541.
- Sharifi-Rad, J., Dey, A., Koirala, N., Shaheen, S., El Omari, N., Salehi, B., Goloshvili, T., Cirone Silva, N.C., Bouyahya, A., Vitalini, S., Varoni, E.M., Martorell, M., Abdolshahi, A., Docea, A.O., Iriti, M., Calina, D., Les, F., López, V., Caruntu, C., 2021. Cinnamomum Species: Bridging Phytochemistry Knowledge, Pharmacological Properties and Toxicological Safety for Health Benefits. Front. Pharmacol. 12, e600139.
- Sharma, R., Amin, H., Prajapati, P.K. 2015. Antidiabetic claims of *Tinospora cordifolia* (Willd.) Miers: critical appraisal and role in therapy. Asian Pac. J. Trop. Biomed. 5, 68-78.
- Silva, D.A., Webster, G.K., Bou-Chacra, N., Löbenberg, R., 2018. The Significance of Disintegration Testing in Pharmaceutical Development. Dissolution Technol. 25, 30-38.
- Singh, B., Kumar, R., Singh, V., Singh, S. P., & Banerjee, S. (2022). Comparative evaluation of in vitro antioxidant and α -glucosidase inhibitory activities of *Momordica charantia* and *Syzygium cumini* extracts and their potential as antidiabetic agents. Journal of Food Biochemistry, e13908.
- Singh, V.I., Sharma, R.K., Kumar, Y., Saqulain, S., 2021. Pharmacological aspects & medicinal uses of *Trigonella foenum-graecum*: A Current Review. Int. J. Ayurveda Res. 12, 776–786.
- Takooree, H., Aumeeruddy, M.Z., Rengasamy, K.R.R., Venugopala, K.N., Jeewon, R., Zengin, G., Mahomoodally, M.F. 2019. A systematic review on black pepper (*Piper nigrum* L.): from folk uses to pharmacological applications. Crit. Rev. Food Sci. Nutr. 59, 210-243.
- Tuso, P., 2014. Prediabetes and lifestyle modification: time to prevent a preventable disease. Perm J. 18, 88-93.
- Wasnik, S.V., Naik, T.M., 2016. A review on role of *Murraya koenigii* (curry leaf) in (diabetes mellitus – type ii) prameha. Int. J. Dev. Res. 06, 7468-7469