Chapter 02

Development of Antidiabetic Herbal Formulations Based on *Nigella sativa*, *Silybum marianum* and *Citrullus colocynthis*

Hina Fatima[1], Iqra Arshad[1], Sadia Aslam[1], Mubshara Saadia[*1] and Farzana Shamim[2]

[1]Department of Biochemistry, Government College Women University Faisalabad, 38000, Pakistan
[2]Department of Chemistry, University of Agriculture, Faisalabad, 38000, Pakistan
*Corresponding author: dr.mubsharasaadia@gcwuf.edu.pk

ABSTRACT

Diabetes mellitus is considered a complex metabolic disorder that presents a considerable global health burden owing to its rising prevalence and associated complications. Several plant-based formulations are reported to date to have a significant number of phytochemicals that are helpful in combating diabetic complications. Herbal remedies work synergistically to improve body health, especially by alleviating the overall antioxidant status. Moreover, herbal treatments offer a good alternative for a variety of possible side effects associated with conventional medicines. This chapter focuses on the chemical profile, therapeutic benefits, and the antidiabetic properties of herbal formulations containing specifically *Nigella sativa*, *Silybum marianum*, and *Citrullus colocynthis* extracts.

KEYWORDS
C. colocynthis, Diabetes mellitus, herbal formulations, *N. sativa*, *S. marianum*,

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INTRODUCTION

Diabetes Mellitus (DM) and its Classification

Diabetes Mellitus is a widespread metabolic disorder characterized by chronic high blood sugar levels due to inadequate insulin production, impaired insulin action, or a combination of these factors. Insulin is an important hormone that plays its role and affects the metabolism of proteins, lipids, and carbohydrates in our body (Poznyak et al., 2020). Metabolic imbalances due to insulin resistance primarily impact tissues like fat cells, muscles, and the liver. The intensity of symptoms differs based on the type and duration of diabetes. Hyperglycemia, especially in individuals with low insulin levels, leads to conditions like increased hunger, excessive thirst, difficulty in urination, weight loss, and vision problems. However, certain individuals, particularly those in the initial phases of type 2 diabetes, might not exhibit any noticeable symptoms (Rossi et al., 2019). If left untreated, diabetes can result in serious complications like coma, neuropathy, and in severe cases, fatalities due to untreated ketoacidosis or hyperosmolar syndrome without ketosis (Poznyak et al., 2020).

Diabetes is classified mainly into two types; Type-I diabetes and Type-II diabetes, however, its latest classification is as given in Fig. 1.

Allopathic/Synthetic Approaches to Combat Diabetes

Oral antidiabetic agents treat pathophysiological conditions to control blood sugar levels. Sulfonyl urea's, Dipeptidyl peptidase 4 inhibitors, Sodium Glucose cotransporter 2 inhibitors, and metformin are the most commonly used medicines (Qaseem et al., 2017). To improve the absorption and utilization of glucose thiazolidinedione's are used as insulin sensitizers (Chaudhary et al., 2017). Combining rapid-acting insulin with long-acting basal insulin is effective in glycemic management and mimics physiological insulin formation. Many surgical techniques like Bariatric surgery serve as good alternatives to treat diabetes (Palanisamay et al., 2018). It is important to find out a wide range of alternatives to prevent people from diabetes as it has become a worldwide health problem. Early detection and care are a must to avoid life-threatening consequences (Antea et al., 2022).

Herbal Formulations and their Mode of Action

Using herbal prophylactics, the blood glucose levels are lowered may be due to a rise in the number of β cells of islets of Langerhans to induce more insulin. *Panax ginseng* and *Allium sativum* are known to have cytoprotective properties and protect the functioning of pancreatic β cells (Wickramasinghe et al., 2021). Some other herbal alternatives work by quick uptake and utilization of blood glucose by accelerating the sensitivity of peripheral tissues to insulin. Moreover, many herbal formulations like *Salacia reticulate* and *Phaseolus vulgaris* affects the absorption of sugar in the gut by the inactivation of those enzymes which degrade the sugar content of the food (Tran et al., 2020).

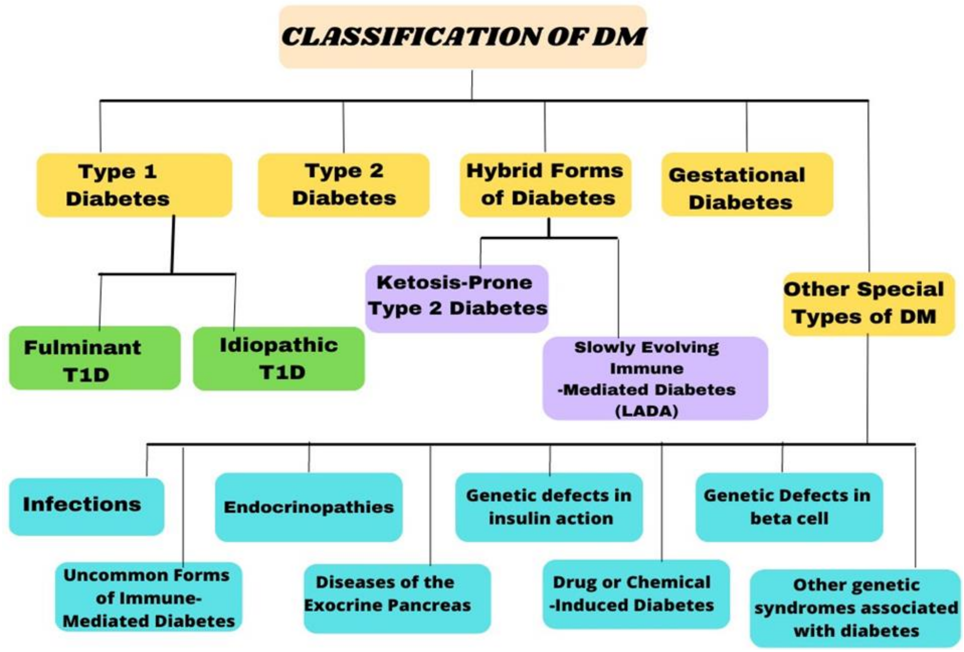


Fig. 1: The latest classification of DM includes various types and subtypes of diabetes (Antar et al., 2023).

Nigella sativa

Dark-colored seeds of black cumin, botanically named *N. sativa* (NS), is a little blooming plant local to southwest Asia. For quite a long time, it has been utilized in conventional medication and as a culinary flavor because of its potential medical advantages. Ongoing logical examination has started to investigate its useful properties, affirming a significant number of its certifiable medicinal purposes and revealing new likely applications (Petruzzello and Melissa, 2024).

Phytochemical Profile of *N. sativa*

Till now, *N. sativa* has been adored for its curative properties. In customary medication, it has been utilized to treat different illnesses, including respiratory diseases, for example, asthma and bronchitis, stomach-related messes, erythrogenic conditions, and skin issues and to boost immune functions (Hannan et al., 2021).

Table 1: Biologically active components of NS (Zielinska et al., 2021)

Active Compounds	Contents
Logefoline	1-8%
Carvacrol	6-12%
Nigellicimine, nigellidine, A-hederin and citronellol	<1% (Trace Amounts)
Vitronellol, saponin, Carvone, N-tlenek nigellicimine, nigellicine and limonene	<1% (Trace Amounts)
T-Anethol	1-4%
4-Terpinol	2-7%
Thymohydroquinone, dithymoquinone and p-cymene	7-15%
Thymoquinone	30-48%

N. sativa plant consists of a rich mixture of thymol, omega-3-fatty acids (ώ3fatty acids), omega-6 fatty acids (ώ6fatty acids), saponins, and flavonoids, thymoquinone and thymohydroquinones. All these bioactive mixtures are a rich blend of highly antioxidative properties that contribute to the treatment of different chronic diseases like diabetes. These bioactive compounds lessen the side effects of various inflammatory illnesses like gut sickness, asthma, and joint pain (Eun et al., 2021).

Therapeutic Potential of *N. sativa*

N. sativa can effectively regulate the performance of the immune system, upgrading its capacity to battle against germs and different illnesses. The immunomodulatory properties of *N. sativa* make it flexible against different microbes (Niu et al., 2021). *N. sativa* has antioxidant and anticancer properties which help to protect the cells from oxidative stress and damage induced by free radicals. The healing potential of *N. sativa* interferes with the progress of malicious growth cells in cancerous tissues. The extract of *N. sativa* has strong hepatoprotective properties as it has healing effects on failed liver function caused by oxidative damage. *N. sativa* supplementation has wonderful neuroprotective effects on improved memory function and improved mental capability in older people (Landucci et al., 2021). *N. sativa* is renowned as a powerful tonic with an eminent history of ordinary consumption as a medicinal plant. It has intense antioxidative behavior which presents it as a potent anticancer substance and is known for its immunomodulatory impacts (Ciesielska et al., 2023). Table 1 enlist different compounds reported in *N. sativa* responsible for its biological effects.

Antidiabetic Potential of *N. sativa*

Traditional herbal medications are well known for their antidiabetic properties including *N. sativa*. A strong bioactive compound thymoquinone is responsible for antidiabetic impacts. Moreover, a combination of a variety of bioactive compounds comprising saponins, flavonoids, and omega fatty acids holds pharmacological benefits (Mahomoodally et al., 2022).

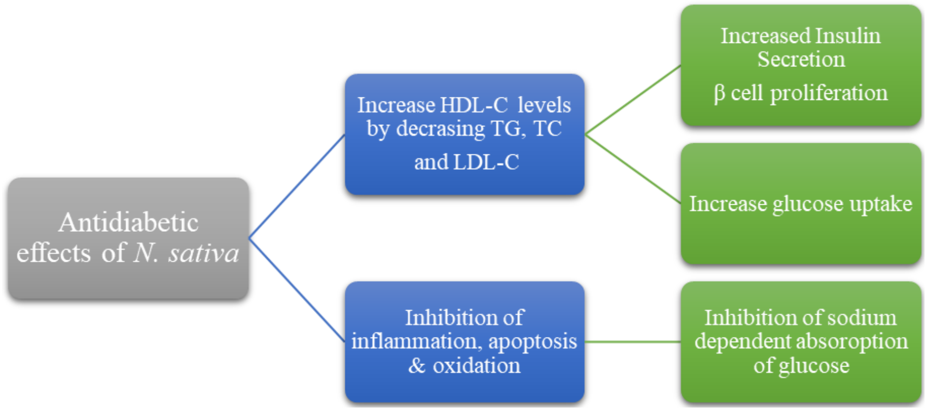


Fig. 2: Proposed antidiabetic effects of NS (Hajar et al., 2022)

The proposed antidiabetic effects of *N. sativa* are shown in Figure 2. Akhtar et al. (2020) evaluated the antidiabetic, hypolipidemic, and antioxidant potential of *N. sativa* seed oil (NSO) and described the significant effects of NSO administration on diabetic rabbits in lowering the serum blood glucose levels and lipid contents with an increase in HDL-C (High-density lipoprotein cholesterol) and vitamin C levels ($p < 0.001$). However, NSO administration has significantly decreased the serum catalase activity along with the decrease in Total Cholesterol (TC), Triglycerides (TGs), Low-Density Lipoproteins (LDL-C), and Very Low-Density Lipoproteins (VLDL-C) levels. *N. sativa* has an important role in lowering blood sugar levels by enhancing insulin sensitivity resulting in the uptake of glucose from peripheral tissues. The shielding effect of NS protects the function of beta cells of islets of Langerhans and their ability to release insulin as it shields the beta cells of the pancreas from damage induced by oxidative stress (Banday et al., 2020). A combination of *N. sativa* with metformin resulted in controlled glycemic levels and reduced insulin barrier. Many studies about the herbal potential of NS on the molecular level affirmed the best antidiabetic effects. A powerful bioactive compound, thymoquinone is known for its astonishing role in the settlement of controlled blood glucose levels and fat digestion by displaying its antidiabetic effects. *N. sativa* has soothing and cell-strengthening properties that moderate the irritation and oxidative stress induced by diabetes and in this way serve as a strong antidiabetic agent. *N. sativa* can be used in combination with other antidiabetic prescriptions to show promising results (Khawandanah, 2019).

Antidiabetic Herbal Formulations Comprising *N. sativa* Extracts

Nigella sativa seeds or extracts have been used commonly in herbal preparations targeted against diabetes. The mechanism of action described so far is the increase in insulin responsiveness, permitting better glucose take-up by pancreatic cells. Table 2 enlists findings of different studies from the supplementation of *N. sativa* on diabetes patients. Saadia et al., (2017) reported the therapeutic effects of *N. sativa* administration to quinine-induced thrombocytopenic rats in elevating the platelet count (PLT) of animals to reduce the risk of thrombocytopenia. Moreover, it was found that *N. sativa* post-treatment in rats elevated the serum catalase, ascorbic acid, and bilirubin levels while the pre-treatment increased the micronutrient levels including iron, nickel, and cobalt. In another study, Saadia et al., (2019) reported that *N. sativa* post-treatment to rabbits was found effective in normalizing the serum alanine transaminase (ALT) levels with a significant increase in catalase levels. However, the *N. sativa* pre-treatment was found effective in maintaining the serum nickel and cobalt concentrations. So, it was suggested that *N. sativa* pre or post-administration to test animals was effective in normalizing the serum concentrations of antioxidants and trace elements.

Silybum marianum

Milk thistle, with the scientific name *Silybum marianum* (L.) Gaertn., is a medicinally significant plant from the Asteraceae family, originally found in the Mediterranean Basin. It thrives in warm, arid soil across various regions including North and South America, southern Australia, Europe, Central and Western Asia, and North Africa. Owing to its competitive nature, milk thistle can grow well in light soils with insufficient water supply. Milk thistle is a plant that can be either annual or biennial and grows up to 2.0 meters tall, with stems ranging from 40 to 200 cm in height (Figure 3). Its leaves are arranged alternately possess a shiny appearance, and possess spiny edges and prominent white veins, typically measuring 50-60 cm long and 20-30 cm wide. The flower heads have an average diameter of about 5 cm (Simora et al., 2020).

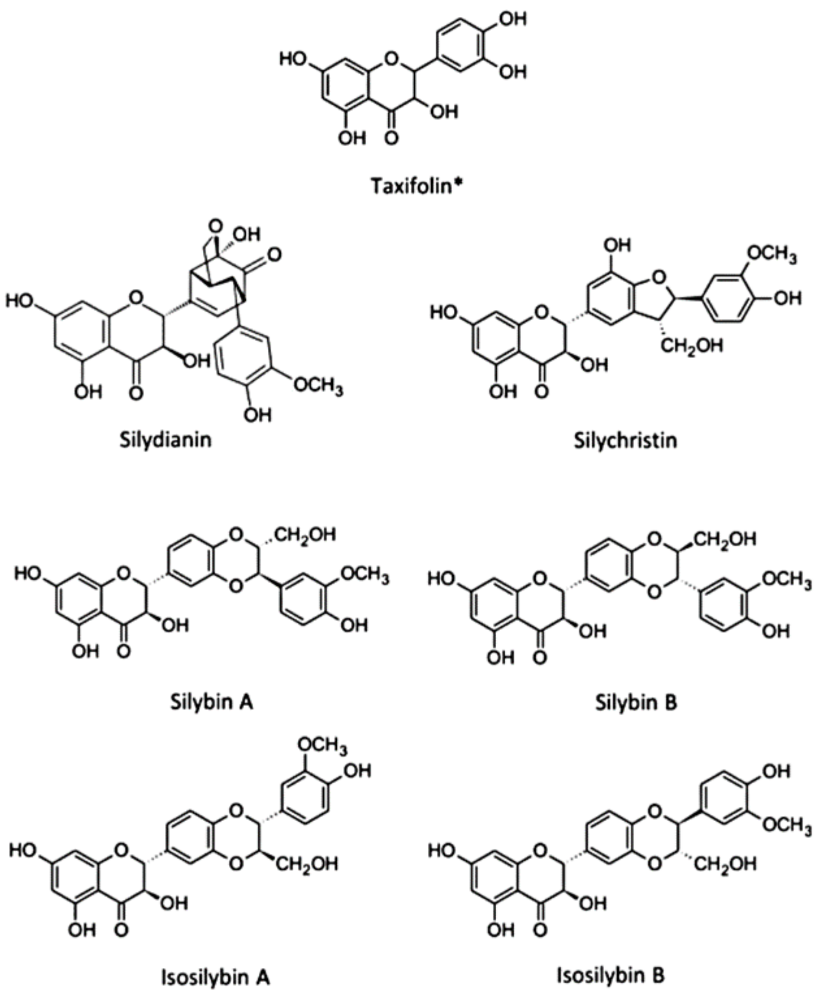
Table 2: Effect of *N. sativa* supplements on patients with diabetes (Tavakkoli et al., 2017)

Sr.	Study Design	Dose	Result
1	(RDBP) Random double-blind placebo Trial(control)	N.S oil (3g per day)/forty days	Decrease in body mass index, insulin, and insulin resistance including HDL C (compared with baseline)
2	Prospective study	N.S oil (5mL per day)/six weeks	Decrease in LDL cholesterol and fasting blood sugar (FBS)
3	(RDBP) Random double-blind placebo Clinical trial (Control)	N.S oil extract (1g per day)/ Eight weeks	Decrease in triglycerides, LDL-cholesterol, Total count

(a) (b) (c)

Fig. 3: *S. marianum* (a and b) plant, c) Fructus silybi mariani (Simora et al., 2020).

Fig. 4: Chemical structure of silymarin marker compounds (Drouet et al., 2018).other Ingredients



Chemical Constituents of Milk Thistle Extract

The key active compound found in milk thistle is silymarin, which is extracted from the seeds (achenes). Silymarin contains various flavonolignans (flavonolignan isomers), flavonoids, and the most important flavonolignans encompass silybins A and silybins B, silychristin A, isosilybins A and isosilybins B, silydianin and isosilychristin (Fig. 4). Of these compounds, silybin is the most prevalent and biologically active compound, comprising around 60-70%, while silychristin accounts for approximately 20%, silydianin for 10%, and isosilybin for 5%. Additionally, silymarin contains the flavonoid taxifolin (Simora et al., 2020). Apart from flavonolignans and flavonoids, milk thistle also contains sugars (such as rhamnose, arabinose, glucose, and xylose) proteins (25-30 %), sterols (including sitosterol, cholesterol, stigmasterol, and campesterol), tocopherol, and lipids (15-30 %) in the form of triglycerides. Notably, the lipid content consists primarily of palmitic acid (9 %), oleic acid (30 %), and linoleic acid (60 %). Although the lipid-rich oil obtained from milk thistle fruits is nutritionally valuable, it is often regarded as an unwanted byproduct of silymarin extraction. It must be separated from the fruits before silymarin extraction (Aziz et al., 2020). Chemical compound analysis of different parts of *S. marianum* was performed using GC-MS procedures, and the findings are outlined in Table 3.

Table 3: Chemical composition of extracts from seeds, stem, and leaves of *S. marianum* (Javeed et al., 2022).

Extract	Chemical Compounds	Molecular Formula	Molecular Weight
	Methyl stearate	$C_{19}H_{38}O_2$	298.5
	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270.5
Stem	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	268.5
	1,2-Benzenedicorboxylic acid, butyl decyl ester	$C_{22}H_{34}O_4$	362.50
	Carbamic acid, (3-methylphenyl)-, methyl ester	$C_9H_{11}NO_2$	165.2
	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5
	Benzene, 1-isocyanato-3-methoxy-	$C_8H_7NO_2$	149.1
	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34
	Tetradecanoic acid, 12-methyl-, methyl ester	$C_{16}H_{32}O_2$	256.4
	Dibutyl phthalate	$C_{18}H_{36}O$	268.5
	7-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5
Leaves	2-(2-Methoxy-5-methyl-phenyl)-propionaldehyd	$C_{11}H_{14}O_2$	178.2
	2-Undecanone, 6,10-dimethyl-	$C_{13}H_{26}O$	198.3
	Hexadecanoic acid, methyl ester	$C_{13}H_{22}OSi$	222.4
	Silane, (1,1-dimethylethyl dimethyl (phenylmethoxy)-	$C_9H_{10}O_3$	166.2
	Methyl stearate	$C_{19}H_{38}O_2$	298.5
	2-Pentadecanone, 6,10,14-trimethyl	$C_{16}H_{22}O_4$	278.34
	Heptadecanoic acid, 14-methyl-, methyl ester (+/-)	$C_{18}H_{36}O_2$	284.4
Seeds	Silane, (1,1 dimethylethyl) dimethyl (phenylmethoxy)-	$C_{13}H_{22}OSi$	222.4
	Benzene, 1-isocyanato-2-methoxy	$C_8H_7NO_2$	149.1
	Hexadecanoic acid, methyl ester	$C_{18}H_{36}O$	268.5
	8-Octadecenoicacid, methyl ester	$C_{19}H_{36}O_2$	296.5
	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.3

The investigation identified eight, nine, and six chemical compounds, which collectively constituted 99.96%, 99.89%, and 99.95% of the total extract from the stems, leaves, and seeds respectively. Dibutyl phthalate was identified as the primary compound in all extracts. Conversely, Hexadecanoic acid methyl ester and Silane, (1,1-dimethylethyl) dimethyl (phenylmethoxy), were not found in the stem extract. Benzene, 1-isocyanato-2-methoxy, an important compound, appeared in the seed and stem extracts but was absent in the leaf extract. Additionally, Methyl stearate was detected in all extracts except those from the seeds. Several minor compounds were also observed in the extracts, though they exhibited low peak areas. (Javeed et al., 2022).

Therapeutic Properties of *S. marianum*

Silymarin, the active component of milk thistle, is a lipophilic extract derived from the seeds and contains three flavonolignan isomers: silychristin, silydianin, and silybin. Standardized extracts of milk thistle usually consist of 70% to 80% silymarin.

Silymarin, (Fig. 5) with silybin being the predominant physiologically active component (Sapthasri, 2021). These constituents have been employed in treating various conditions such as cancer, inflammatory ailments like arthritis, cardiovascular diseases, autoimmune disorders, and ophthalmological issues (Porwal et al., 2019). The growing interest in plant-based extracts and nutraceuticals arises from their therapeutic potential. Recent studies have revealed milk thistle's effectiveness in addressing diverse disorders. Silybin, a constituent of silymarin, modulates oxidative stress, hepatic fat storage, and blood insulin levels, thereby enhancing hepatic function and mitigating hepatotoxicity (Tajmohammadi et al., 2018). Furthermore, silymarin demonstrates anti-inflammatory properties, particularly beneficial for individuals with arthritis (Shavandi et al., 2022).

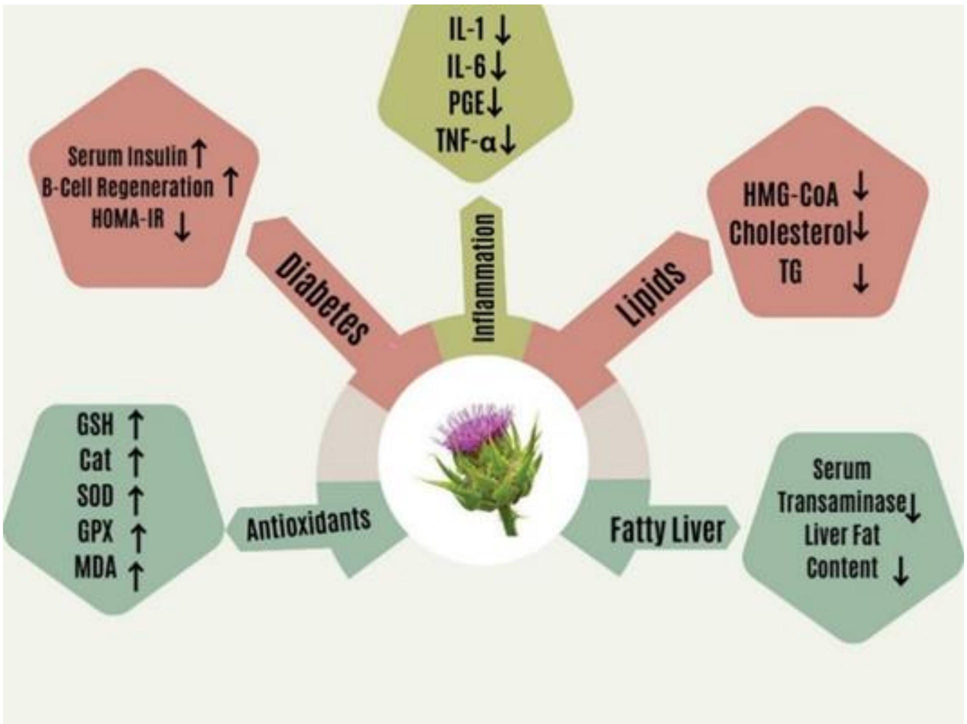


Fig. 5: Pharmacological properties of silymarin (Mohammadi et al., 2020).

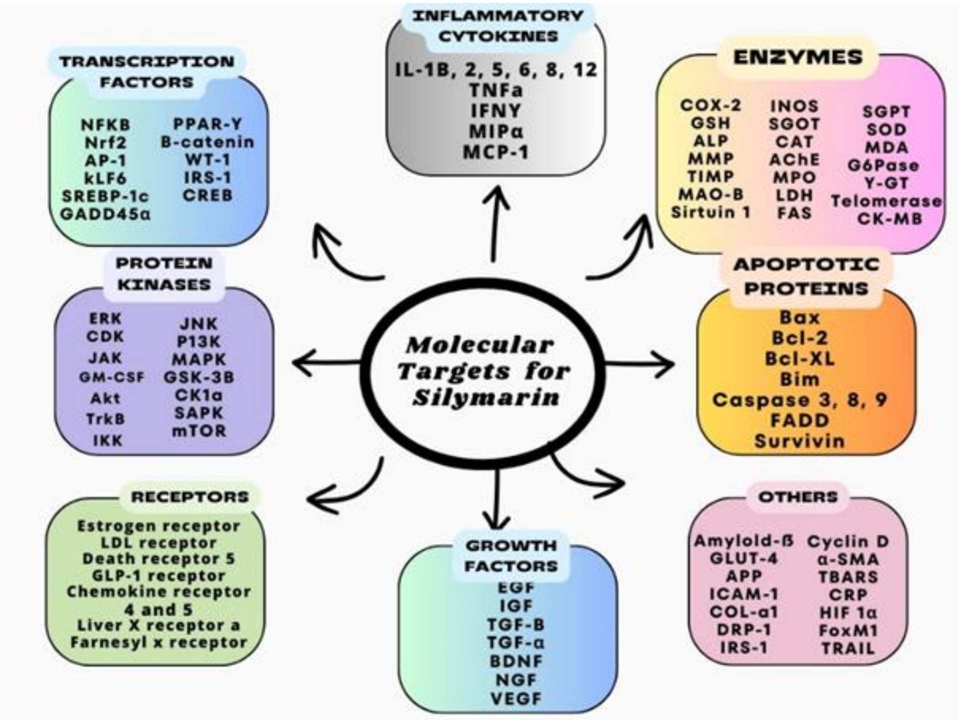


Fig. 6: Various molecular targets for silymarin (Wadhwa et al., 2022)

Anti-diabetic Activity of *S. marianum*

The growing occurrence of diabetes mellitus (DM) poses a significant worldwide health issue. DM is a metabolic condition marked by chronic high blood sugar levels, insulin resistance, and impaired insulin production, often accompanied by increased glucose production in the liver. Silymarin and its components have shown potential hypoglycemic effects, effectively reducing blood glucose levels and enhancing insulin secretion (Sharma et al., 2021). Several experimental investigations of the anti-diabetic activity of silymarin are given in Table 4.

In rat models of diabetes induced by streptozotocin (STZ), administering silymarin orally lowers HbA1c levels and fasting blood sugar. Additionally, it inhibits the activity of glucose-6-phosphatase (G6Pase) and gluconeogenesis. Chronic hyperglycemia damages mitochondria, leading to oxidative stress (Rahimi et al., 2018). Silychristin A, a component of silymarin, protects against ROS-induced apoptosis in pancreatic cells. Inflammation exacerbates diabetes complications. Silymarin reduces the expression of inflammatory cytokines and NF-κB target genes, preserving pancreatic β-cell function (Xu et al., 2018). Ashraf et al., (2020) comparatively evaluated the protective effect of seed oil extracts of *S. marianum* and *N. sativa* administration in cisplatin-induced nephrotoxic mice. *S. marianum* oil extract significantly reduced the elevated blood glucose and blood urea nitrogen (BUN) levels in comparison to *N. sativa* oil extract. SM Oil extract administration also ameliorated the cisplatin-induced lowering of triglyceride levels. Moreover, the histological study showed improvement in kidney cells with slight signs of cellular damage in mice treated with SM oil extract. Figure 7 explains the different antidiabetic mechanisms of silymarin.

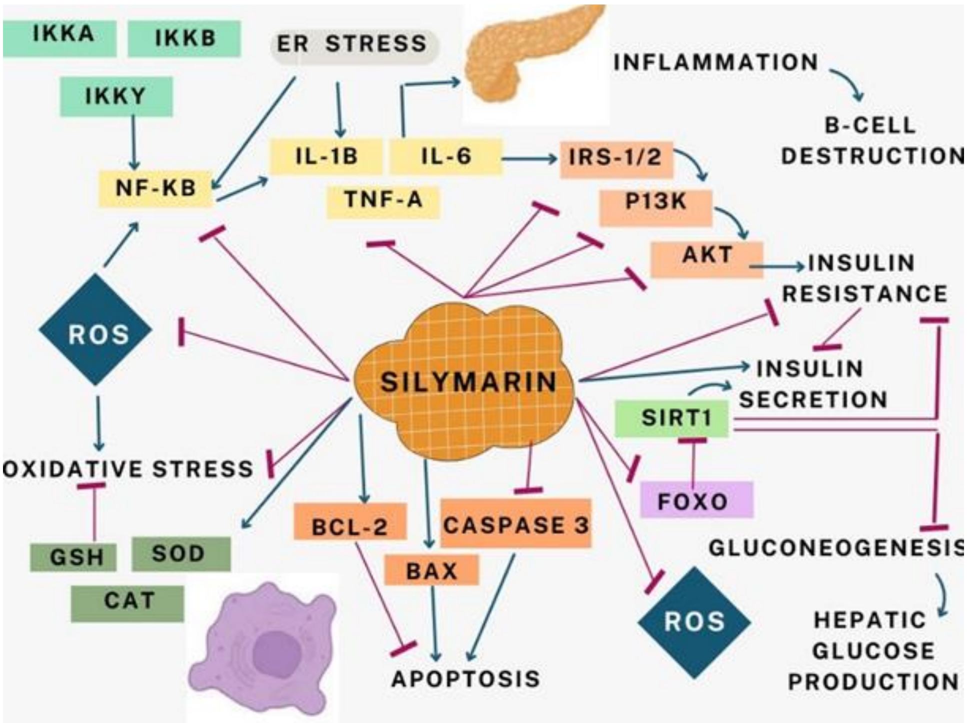


Fig. 7: Various anti-diabetic mechanisms of silymarin (Wadhwa et al., 2022).

Table 4: Experimental investigation of the anti-diabetic activity of silymarin (Wadhwa et al., 2022).

Study Model	Possible Target Site/Mechanism of Action	Dose/Concentration Used
Obesity-induced insulin resistance model and HepG2 cells	Increase in SIRT1 expression Reduction in phosphorylation of FOXO1 and Akt Enhancement in SIRT1 enzymatic activity	30 mg/kg/day p.o. for one month
HFD-induced insulin resistance	Decrease in levels of TNF-a, IL-6, and IL-18 Lowering of SGOT, SGPT, CH, TG, and LDL levels Improvement in insulin sensitivity	30 and 60 mg/kg p.o.
HFD model	Improvement in insulin sensitivity Lowering of hepatic NADPH oxidase expression and NF-kB activity Decrease in CAT, GSH, and SOD activity Reduction in levels of IL-6, TNF-a, NO, and iNOS	30 mg/kg/day p.o. for one month
Pancreatectomy model	Rise in serum insulin levels Enhancement in β cell proliferation Increase in expression of Pdx1 and insulin genes	200 mg/kg p.o.
HFD-induced insulin resistance model and HEK293T cells	Decrease in fasting blood sugar (FBS) levels Suppression of NF-κB signaling pathway Stimulation of Farnesoid X receptor (FXR)	40 µg/mL 50 µM
STZ-and HFD-induced diabetes	Reduction in liver glucose output Enhancement in GLP-1 receptor expression in the duodenum	100 and 300 mg/kg p.o.
STZ-induced diabetes and INSI cells	Reduction in fasting blood sugar (FBS) levels and increase in insulin secretion Increase in protein levels of Bax and cleaved-caspase-3 Decrease in gene expression of Bcl-2 and pro-caspase-3	50 µg/mL 25-100 µ.M
STZ-induced diabetes	Decrease in HbA1C levels Lowering of MDA, SGOT, SGPT, LDH, and CK-MB levels in the heart Reduction in CH, TG, and LDL levels Increased Bcl-2 and decreased Bax levels prevent apoptosis	80 mg/kg p.o. for 21 days
STZ-induced diabetes	Decrease in urotensin II gene expression Lowering of fasting blood sugar (FBS), CK-MB, LDH, MDA, cholesterol (CH), LDL, and nitric oxide (NO) levels	60 and 120 mg/kg/day p.o. for 2 months

Citrullus colocynthis

C. colocynthis, a member of the Cucurbitaceae family, is abundantly found in desert areas around the world including Pakistan (Ahmed et al., 2019). The plant is famous for its nutraceutical and medicinal properties. The fruit of *C. colocynthis* has several names in different languages, Bitter apple or Colocynth in English, in Urdu as Hanjal, Pcitummatti in Tamil, Indrayan in Hindi, and Rakhal in Bengali (Banjo et al., 2021). The plant is famous for its traditional properties and is broadly used for the treatment of different ailments like diabetes, asthma, and jaundice. Each gourd of *C. colocynthis* typically contains 200–300 seeds, and the fruit has a bitter taste. Figure 8. The plants are Perennial vines that provide small, fragrant flowers and display seed-fruit ratio, density, and mesocarp thickness (Aggarwal et al., 2020; Rashid et al., 2021).

A B C

Fig. 8: A) The whole plant of *C. colocynthis* B) Fruit C) Seeds

Chemical Composition and Nutritional Profile of *C. colocynthis*

Despite use of different parts *C. colocynthis* in food and pharmaceutical industries, there is a lack of nutritional information available to readers worldwide. Such variations in characteristics can have resulted from national variations in farming practices and agricultural conditions (Berwal et al., 2022). The seed components which appear golden yellow constitute about 23-25% oil. The seeds contain 70% of unsaturated fatty acids and 51% of polyunsaturated fatty acids. The matured fruit contains a high moisture level, constituting more than 90% of its overall weight. In another study, the seeds are reported to contain 4.91g of moisture content per 100g, while 13.19g and 2.00 g of protein and ash content per 100 g respectively (Banjo et al., 2021). Tryptophan, arginine, and methionine are abundant amino acids found in *C. colocynthis* (Hameed et al., 2020). The protein also contained aspartic acid, glutamic acid, glycine, and serine. Another category of equally significant micronutrients that the body needs is minerals. It is thought that all minerals are helpful for preserving the alkalinity of the body's different fluids as well as the correct electrolyte balance (Khan et al., 2023). Table 5: A variety of bioactive substances extracted from *C. colocynthis* together with an explanation of how they work.

Phytochemical Composition of *C. colocynthis*

The therapeutic potential of *C. colocynthis* is contributed by a wide array of bioactive compounds: polyphenols, flavonoids, and cucurbitacin. The crude extract of *C. colocynthis* contains a wide array of phytochemicals that includes saponins, flavones, tannins, phenols, glycosides, flavonoids, alkaloids, steroids, and terpenoids (Bhasin et al., 2020). Different parts of *C. colocynthis* revealed the presence of different types of cucurbitacins A, B, C, D, E, J, and L, different phytochemicals like terpenoids, glycosides, alkaloids, phenolics, steroids, tannins, proteins, carbohydrates, and specific amino acids (Dhakad, 2017). Different compounds reported from *C. colocynthis* are listed in Table 6.

Phenolic compounds are considered among the largest and widest distributed groups of plant metabolites (Ahmed et al., 2019). They exert various *in vitro* and *in vivo* biological actions including cell proliferation, inhibition of angiogenesis, improving vascular endothelial function, causing cardiovascular benefits, and acting against atherosclerosis, including anti-aging effects (Mazher et al., 2020).

Flavonoids represent a class of phenolic compounds produced by plants in response to microbial infection. They have been shown to exhibit *in vitro* antimicrobial activity against a range of microorganisms. They also have good antioxidant and cytotoxic properties. Due to their antioxidant and radical scavenging abilities, flavonoids offer benefits to humans as promising anticancer agents. These compounds are beneficial and provide resistance against diseases (Al-Nablsi et al., 2022).

Table 5: Bioactive compounds isolated from *C. colocynthis* and their mechanism of action (Li et al., 2022)

Sr no	Class	Mechanism of action
1	Flavonoids	They make complexes with cell walls, bind to adhesions, and prevent prostaglandins and autacoid release
2	Alkaloids	They possess antioxidant properties and prevent the release of prostaglandins and autacoids.
3	Saponins	Hold membrane permeabilizing properties, prevent *in vitro* release of histamine
4	Glycosides	Prevent prostaglandin and autacoid release.
5	Lectins	Forms disulfide bridges, blocks viral adsorption or fusion
6	Steroids	Improves sodium and water absorption in the intestine
7	Terpenoids	Prevent autacoids and prostaglandins release, membrane disruption

Cucurbitacins are natural triterpenoid compounds well known for their bitter taste and toxicity, they have significant cytotoxic effects, thus playing a crucial part in drug exploration, notably for the development of anticancer drugs (Li et al., 2021; Marzouk et al., 2022).

Alkaloids have long been recognized for their role in structural and biochemical functions within biological organisms. *C. colocynthis* contains a high concentration of alkaloids (Ponsankar et al., 2020). These compounds serve as defensive molecules within living organisms and have been utilized in healthcare, most importantly steroidal alkaloids which form the majority of beneficial substances. Moreover, phytochemicals, with significant trypanocidal activity were also reported such as phenolics and flavonoids, etc. (Tabani et al., 2018).

Saponins: *C. colocynthis* extract was reported to contain saponins with significant anti-inflammatory activity. Saponins are also used in medicinal formulation and pharmaceutical industries for foaming and frothing effects (Elgerwi et al., 2017). Although saponins are used in medicine from ancient times, still they are among widely recognized bioactive elements in cytotoxicity. Saponins are made up of sterol glycosides and triterpene, which have a pharmaceutical application as an emulsifier and expectorant. Saponins are thus classified as a carbohydrate derivative and can either take the form of tri-terpenoids or steroids (Afzal et al., 2023). They are formed from the combination of phenylpropanoids and acetate derivatives as precursors. Several studies have proven the effectiveness and activity of steroidal saponins in community pharmacies (Alzarah et al., 2021).

Table 6: A comprehensive list of the chemical constituents derived from *C. colocynthis* (Cheng et al., 2023)

Sr.	Class	Compounds
1	Cucurbitacin and its glucosides	Cucurbitacin A, B, C, D, E, I, J, K, L, Iso-cucurbitacin B, Cucurbitacin L 2-O-β-D-glucoside, Cucurbitacin K 2-O-β-D-glucoside, Cucurbitacin J 2-O-β-D-glucoside, Cucurbitacin I 2-O-β-D-glucoside, Cucurbitacin E 2-O-β-D-glucopyranoside, Deoxocucurbitoside B, Dihydrocucurbitacin E, Colocynthosides A, Colocynthosides B, Norcolocynthenins B, Norcolocynthenins A, 16-(2-prop-1-enyl)-25-O-acetyl-2-O-β-D-glucopyranosyl cucurbitacin I, 16-(2-prop-1-enyl)-2-O-β-D-glucopyranosyl cucurbitacin I, 25-p-coumaroyl-3'-acetyl-2-O-β-D-glucocucurbitacin I, 6'-acetyl-2-O-β-D-glucocucurbitacin E, Dihydroisocucurbitacin B-25-acaetate, Dihydro-epi-iso-cucurbitacin D, Khekadaengoside E, Hexanocucurbitacin I 2-O-β-D-glucopyranoside,
2	Phenolic acids	Gallic acid, Syringic acid, P-coumaric acid, Vanillic acid, Sinapic acid, Hydroxycaffeic acid, Protocatechuic acid, Caffeic acid, Chlorogenic acid, Ferulic acid, Gallic acid monohydrate, 3,4-dihydroxyphenylacetic acid, P-hydroxy benzoic acid
3	Alkaloids	8-methylquinoline, 7,8-benzoquinoline, 6-methylquinoline, 6-hydroxyquinoline, 4-methylquinoline, 4-hydroxyquinoline, 2-methylquinoline, 2-hydroxyquinoline, Uracil, Quinoline, Nicotinamide,
4	Flavonoids	Catechin, 6-C-p-methylbenzoylvitexin, Quercetin, Kaempferol, Isovitexin, Isoorientin, Isosaponarin,3-O-methyl ether, Myricetin
5	Steroid and its saponins	22,23-dihydrospinasterol, β-sitosterol, α-spinasterone, α-spinasterol-3-O-β-D-glucopyranoside,
6	Aromatic rings	4-(β-D-glucopyranosyloxy)-benzal alcohol, 4-(β-D-glucopyranosyloxy)-benzaldehyde, Benzyl β-D-glucopyranoside, 4-hydroxybenzyl β-D-glucopyranoside
7	Tocopherols	α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol
8	Coumarin	6-hydroxy-4-methyl coumarin

Therapeutic Potential of *C. colocynthis*

Medicinal plant extracts have been utilized since ancient times as an ingredient in herbal formulations. Herbal drugs are formulated using parts of plants that have applications in the food industry, too. The medicinal plant remedies employ powders, electroactive, liniments, emulsions, and decoctions (Zheng et al., 2020). Bitter apple is used in various diseases like cancer, mastitis, bronchitis, leprosy, and for joint pain. It is also used in complementary medicines for the treatment of inflammatory conditions (Karimabad et al., 2020). Several phytochemicals belonging to different classes have been reported to show inhibitory action against microorganisms. These constituents are also used in a variety of industries, viz. food industry, cosmetics and perfumery. The active ingredients of bitter apples are extracted by using different techniques and then used in various formulations of functional foods and pharmaceuticals. Egusi seed kernels have been in use for a long time in cooking, across Africa (Hameed et al., 2020).

Antidiabetic Potential of *C. colocynthis*

The bitter apple has been in wide usage as an anti-diabetic agent in many countries, thus attracting substantial research studies both in animals and human beings. *C. colocynthis* is a powerful antidiabetic plant, and ethnopharmacological studies have documented and validated its antidiabetic potential in many countries, including India, Nigeria, Morocco, Iran, the Mediterranean region, and Algeria (Sharma et al., 2020). Various dosage forms, delivery methods, and forms of *C. colocynthis* are used to treat diabetes mellitus. For instance, in Algeria, *C. colocynthis* is used under the feet during bathing. There is also a recipe where fruits need to be crushed with the soles of the feet in the morning and then roasted seeds are ingested on an empty stomach (Meybodi, 2020). A few studies conducted on animals have documented the impact of *C. colocynthis* and its complications against diabetes, using various types of extracts, such as methanolic, ethanolic, aqueous, hydroethanolic, hydroalcoholic, etc. Among these, an aqueous extract was most commonly used. Besides this, other forms of plant administration, such as a suspension, oil, powder, etc., have also been used. Several studies have been reported regarding the antidiabetic activity of *C. colocynthis*. According to recent reports, *C. colocynthis* has anti-inflammatory, antioxidant, and hypolipidemic potential, improving the condition of diabetic animals. Kamran et al. (2021) developed the antidiabetic potential of *C. colocynthis in vitro* by an α-glucosidase inhibition assay and *in vivo* by STZ-induced diabetes in rats. Results showed a hypoglycemic effect that was significant at two different doses: 150 mg/kg and 300 mg/kg. The regular administration of *C. colocynthis* extract for 14 days significantly lowered serum glucose. Besides, it showed a remarkable decrease in serum cholesterol and triglyceride levels in diabetic rats compared to the negative control. The hydroethanolic extract of *C. colocynthis* may exert the hypoglycemic effect due to its potential for inhibiting α-glucosidase (Ghauri et al., 2020).

The aqueous extract of *C. colocynthis* in rats with type 2 diabetes revealed its effect on gene expression, particularly in relation to anti-hyperlipidemic and antidiabetic mechanisms. The ability of the *C. colocynthis* extract to decrease the fasting blood glucose, and hepatic, and serum triglycerides both in early and late type 2 diabetes revealed its potential to act via various mechanisms (Afshari et al., 2021). Herbalists in Iran also use *C. colocynthis* fruit in treating diabetes. In a two-month clinical trial conducted on 50 patients with diabetes mellitus, there was a remarkable reduction in HbA1c and fasting blood glucose in patients treated with bitter apple (Mariod and Jarret, 2022).

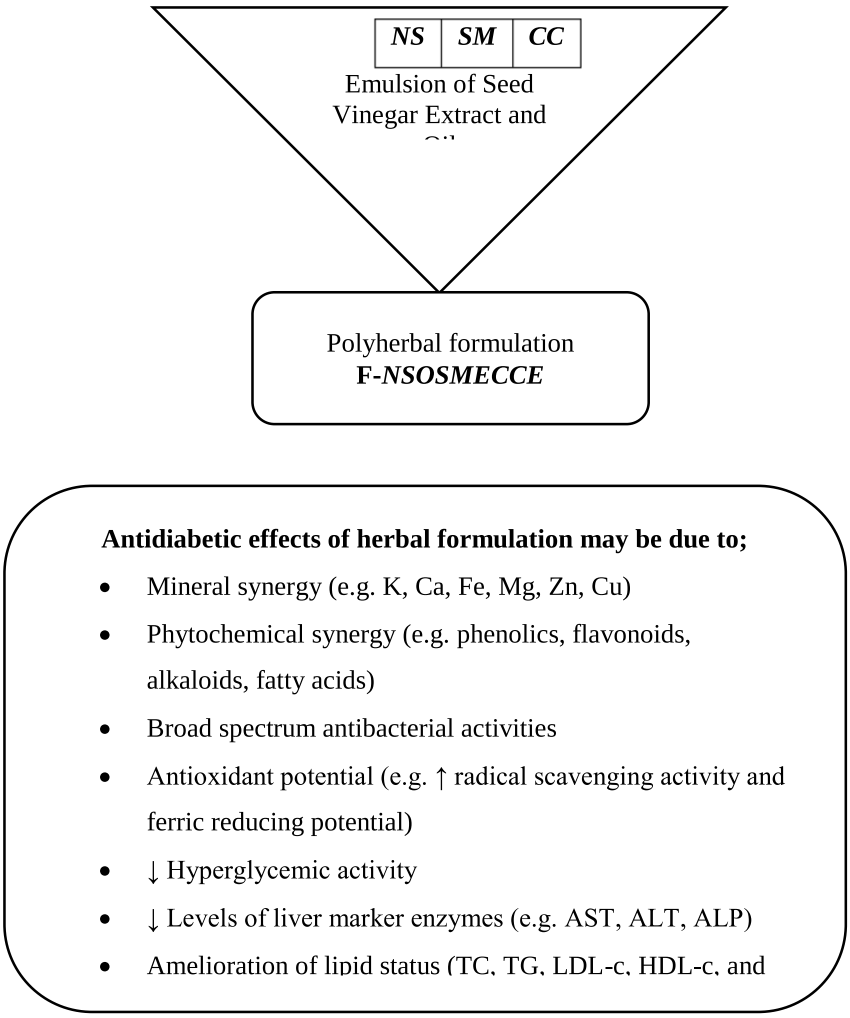


Fig. 9: Schematic mechanism of action of formulated polyherbal emulsion (F-NSOSMECCE) to manage diabetes and its complications.

Antidiabetic Herbal Formulations Comprising *N. sativa*, *S. marianum,* and *C. colocynthis*

Herbal materials are the whole plants or parts of medicinal plants in their natural state, including dried herbs powder, herbs, gums, fresh juices, resins, essential oils and fixed oils. Various methods of processing are adopted in different regions such as steaming, roasting, or stir-baking, which may involve ingredients that include alcoholic beverages, honey, or any other substance depending on the regional factors (Tran et al., 2020). Low solubility is one of the major barriers to the therapeutic utilization of herbal medications. Additionally, despite their potential efficacy, herbal medicinal products often encounter criticism due to deficient standardization and perceived deficiencies in quality (Wu et al., 2021). Herbal extracts face the challenge of compound degradation within the stomach's highly acidic pH, and certain ingredients may undergo liver metabolism before reaching systemic circulation (Lian et al., 2021). With the considerable potential of herbal drugs, numerous researchers are actively exploring the development of innovative drug delivery systems including nanoparticles, microcapsules, microparticles, mucoadhesive systems, sustained and extended-release formulations, solid dispersion, and fast-dissolving tablets (Ahda et al., 2023). The antidiabetic potential of a polyherbal formulation consisting of *C. colocynthis*, *Cinnamomum tamala*, *Asparagus racemomusin*, and *Piper nigrum* in alloxan-induced diabetic rats at two different doses of 200 mg/kg b.w. and 400 mg/kg b.w significantly improve the blood glucose, total glycerides, total cholesterol, SGOT (Serum Glutamic-Oxaloacetic Transaminase), SGPT (Serum Glutamic-Pyruvic Transaminase), VLDL and LDL and HDL level (Folane et al., 2020).

Gardezi et al., (2022) investigated the antidiabetic effects of a polyherbal extract (PHE) containing *N. sativa*, *Cicer arietinum*, *S. marianum*, *C. colocynthis* and *Zingiber officinale* extract. PHE showed a hypoglycemic effect in diabetic rats by lowering the blood glucose level. Akhtar et al., (2022) comparatively evaluated the antidiabetic potential of *N. sativa* seed methanolic extract (NSSE) and *N. sativa* seed oil (NSO) extract in alloxan-induced diabetic rabbits. The finding exhibited that the administration of *N. sativa* seed oil has significantly normalized the serum catalase, ascorbic acid, and total bilirubin levels as compared to the *N. sativa* methanolic extract, proposing that NSO may be used as nutraceutical adjuvant to normal antidiabetic drugs remedies. Akhtar et al. (2023) developed an herbal formulation 'F6- SMONSECCE', prepared from a combination of seeds extract from *S. marianum* oil (SMO), *N. sativa* extract (NSE), and *C. colocynthis* extract (CCE). Figure 9 describes the proposed mechanism of action of herbal formulation developed in the study. The formulation showed the presence of significant amount of total phenolic and flavonoid contents exhibiting the antioxidant potential determined through DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays. The polyherbal formulation was also administered to diabetic rats which significantly attenuated the blood glucose levels, total cholesterol, total glycerides, LDL-C, and VLDL-C levels with an increase in HDL-C levels. The administration of herbal dose also showed significant normalization of pancreatic and kidney cells in histological study. The formulation was therefore proposed as an antioxidant, antilipidemic, and hypoglycemic remedy against diabetes.

Safety Concerns

It is important to consider the safety aspects of herbal medications though it is used to treat diabetes but a complete information guide should be available about antidiabetic drugs including information about its hidden side effects and toxic effects whenever prescribed in combination (Ekor, 2014). All medical conditions like hepatotoxicity, nephrotoxicity, GIT disturbances, and interaction with other drugs by the use of antidiabetics should be taken into serious consideration and strict supervision by medical professionals is a must (Zhang et al., 2015). Although herbal medications serve as natural remedial options to treat diabetes but still more investigation and research are need of the time to fully explore their medicinal potential (Temitope and Santwana 2021). It is also important to find their diverse mode of action, efficacy, safety profiles, and ideal dosage requirements for better treatment (Kumar et al., 2021). Furthermore, due to the increasing demand for these herbal formulations, it is also important to collaborate among researchers, traditional medical practitioners, and healthcare professionals to revolutionize the discipline of herbal medication to combat different diseases (Hassen et al., 2022).

Wellbeing and Future Contemplations

Despite of miraculous effects of natural antidiabetics, it is important to consult medical experts before using any herbal antidiabetic therapy to avoid possible side effects and complications. Antidiabetic formulations are promising because of their potential benefits to cure diabetes.

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