PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND HPTLC FINGER PRINTING ANALYSIS OF AN ANTIDIABETIC POLYHERBAL FORMULATION

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ABSTRACT

Investigating the phytochemical components of an anti-diabetic polyherbal composition was the goal of the current study. *Azadirachta indica* leaves, *Ocimum sanctum* leaves, *Boerhaavia diffusa* flowers, and *Aegle marmelos* fruits were used to make the mixture. Following accepted techniques, the composition's phytochemical qualities were assessed. The composition showed the presence of active phytoconstituents like Quercetin and Ferulic acid as well as carbohydrates, amino acids, proteins, phenols, and flavonoids. At 254 nm, the HPTLC fingerprint analysis identified 18 bioactive substances. The research supports the formulation's presence of numerous phytoconstituents, making continued development of it a viable option.

Keywords: Polyherbal formulation and composition, Antidiabetic activity, Quercetin, Ferulic Acid

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INTRODUCTION

Diabetes is a serious, chronic condition that develops when the pancreas either produces insufficient amounts of the hormone insulin, which controls blood sugar or glucose, or when the body cannot properly utilize the insulin that is produced. One of the four noncommunicable diseases (NCDs) that top the list of priorities for action by world leaders is diabetes, which is a significant public health issue. Over the past few decades, there has been a consistent rise in both the incidence and prevalence of diabetes. All forms of diabetes carry a higher risk of overall early death and can result in problems in various bodily areas. Heart attack, stroke, kidney failure, leg amputation, vision loss, and nerve damage are examples of potential side effects. Poorly managed diabetes during pregnancy raises the chance of fetal death and other problems.[1]

According to estimates, the prevalence of diabetes will rise from 4% in 1995 to 5.4% by 2025. According to WHO, developing nations will shoulder the majority of the burden. Studies carried out in India over the past ten years have shown that not only is the prevalence of diabetes high, but it is also rising quickly among urban residents. In India, there are thought to be 33 million persons who have diabetes. By 2025, this figure is probably going to rise to 57.2 million. [2]

Diabetes requires a multifaceted therapeutic strategy because it is a complex disease that causes several complications. Diabetes patients' cells either do not respond to insulin or do not produce enough of it. Patients receive insulin injections if their bodies are completely insulin-deficient. While many various medications are being researched for those whose cells do not respond to insulin while taking into account potential abnormalities in carbohydrate metabolism. Acarbose, miglitol, and voglibose are a few examples of glucosidase inhibitors that are used to treat post-prandial hyperglycemia at the digestive level. These prevent the breakdown of carbs, which lowers cell absorption of glucose. Metphormine is one type of biguanide that is used to increase glucose absorption by peripheral cells. Glibenclamide, a sulphonylurea, is an insulinotropic secretogogue for pancreatic cells. Despite the fact that many different therapies are used for therapy, there are some restrictions because of their high cost and undesirable side effects, such as the onset of hypoglycemia, weight gain, gastrointestinal problems, liver toxicity, etc. An appropriate anti-diabetic and antioxidant therapy is being sought after in light of current developments and the contribution of oxidative stress to the complications of diabetes mellitus.[3]

Once again, medicinal plants are being researched for the treatment of diabetes. Numerous conventional medications have been developed from model compounds found in therapeutic plants. An effective oral glucose-lowering medication is metformin. The usage of *Galega officinalis* to treat diabetes served as the foundation for its development. The hypoglycemic ingredient, guanidine, is abundant in *Galega officinalis*. More than 400 conventional plant remedies for diabetes have been documented to date, but only a small portion of these have undergone scientific and medical scrutiny to determine their usefulness. Some herbal extracts have been shown to have hypoglycemic effects in type 2 diabetes models in both humans and animals. The World Health Organization's Expert Committee on Diabetes has advised greater research into traditional medicinal herbs. Lack of scientific and clinical evidence demonstrating herbal medicine's efficacy and safety is a major barrier to its incorporation into current medical practices. Clinical studies on herbal medicines are required, as are the creation of straightforward bioassays for biological standardization, pharmacological and toxicological evaluation, and the creation of numerous animal models for testing toxicity and safety. Establishing the active component(s) in these plant extracts is also crucial.[4]

Despite being utilised in Chinese medicine for hundreds of years, herb-herb combinations, often known as polyherbal treatment, lack scientific support for their therapeutic efficacy.[5] When treating diseases, medicine combinations frequently outperform individual medications. In Western medicine, the idea of pharmacological combinations is well-established, and it has seen a lot of success over the years. Drug combination therapy for infectious and cancerous disorders have recently given patients new hope.[6] It has been demonstrated that both naturally occurring herbs and herbal components combined into specific formulas may interact. These include mutual enhancement, mutual assistance, mutual restraint and mutual antagonism.[7], The majority of polyherbal substances are utilised in the Ayurvedic medical system to treat a variety of infections. Ayurvedic doctors frequently recommend Indukantha Ghritha (IG), a polyherbal remedy made up of 17 plant parts, for a variety of diseases.[8] As a result of the incredible clinical efficacy of the formulations, the Unani system of medicine is also gaining recognition on a global scale. Although Unani remedies have been used for a long time, there is little evidence to support either their usefulness or safety. The absence of evaluation has consequently hindered the creation of laws and regulations. Studies on polyherbal formulations revealed that some plants had high concentrations of phenolics and flavonoids, and that their polyherbal combination with green

tea had the highest levels of antioxidant activity of any individual extract.[9] Because of their synergism and reduced side effects, polyherbal combinations of herbs are preferred to single herbs in the majority of traditional systems for managing diabetes[10] .

Neem, or *Azadirachta indica*, has therapeutic benefits and is used in Ayurveda to treat a number of illnesses, including diabetes mellitus. It is well recognized to have a variety of pharmacological activities, including anti-inflammatory, antipyretic, antibacterial, and anti-diabetic effects. However, it is unclear what molecular process causes A. indica to affect insulin signal transduction and glucose homeostasis..[11] Neem leaves contains Quercetin [12] and Ferulic Acid [13]. *Ocimum sanctum* contains polyphenols like ferulic acid [14] and Quercetin[15]. *Boerhaavia diffusa* contains Quercetin [16] and polyphenols like ferulic acid[17] and *Aegle marmelos* also contains Quercetin and ferulic acid.[18].

Quercetin and ferulic acid both have Antidiabetic activity. FA improved the insulin signaling molecules and reduced the negative regulators of insulin signaling. [19,20]. So, for present study we prepare polyherbal extract and perform it's phytochemical screening and fingerprinting analysis.

MATERIAL AND METHODS

Plant material

Plant parts that are leaves of *Azadirachta indica*, leaves of *Ocimum sanctum*, flowers of *Boerhaavia diffusa* and fruits of *Aegle marmelos* collected from local area, then shade dried all procure material then powdered it. By using Soxhlet apparatus extraction procedure perform, for that procedure solvent were used as per polarity basis.

Table 1: Ingredients of the Antidiabetic polyherbal composition

S.No.	Plant Botanical name	Vernacular name	Family	Part used
1.	*Azadirachta indica*	Neem	Meliaceae	Leaves
2.	*Ocimum sanctum*	Tulsi	Lamiaceae	Leaves
3.	*Boerhaavia diffusa*	Punarnava	Nyctaginaceae	Flowers
4.	*Aegle marmelos*	Bael	Rutaceae	Fruit

Table 2: Ingredients proportion in Polyherbal composition

POLYHERBAL FORMULATIONS	CODE
AI-HAE, OS-HAE, BD-HAE and AM-HAE in the ratio of (25:25:25:25)	ISDM-001
AI-HAE, OS-HAE, BD-HAE and AM-HAE in the ratio of (30:20:20:30)	ISDM-002
AI-HAE, OS-HAE, BD-HAE and AM-HAE in the ratio of (20:30:30:20)	ISDM-003
AI-HAE, OS-HAE, BD-HAE and AM-HAE in the ratio of (10:30:30:30)	ISDM-004
AI-HAE, OS-HAE, BD-HAE and AM-HAE in the ratio of (30:10:30:30)	ISDM-005
AI-HAE, OS-HAE, BD-HAE and AM-HAE in the ratio of (30:30:10:30)	ISDM-006
AI-HAE, OS-HAE, BD-HAE and AM-HAE in the ratio of (30:30:30:10)	ISDM-007

HAE: Hydroalcoholic Extract; AI: *Azadirachta indica*; OS: *Ocimum sanctum*; BD: *Boerhaavia diffusa*; AM: *Aegle marmelos*

Chemicals and reagents:

The study's chemicals and reagents were of the analytical variety. Phytochemical investigation: Using the methods outlined by Kokate[21], the phytoconstituents of the anti-diabetic polyherbal formulation were examined. After the reagent was added, the development of colour or precipitate was seen, depending on the test's end point, and the result was indicated as present (+) or absence (-). With the exception of the tests that called for the formulation in powder form, all experiments were conducted using freshly generated stock solutions of the formulation at a concentration of 1 mg/mL.

Tests for the presence of carbohydrates include: a. Molisch's Test, which involves adding a few drops of Molisch's reagent (5% alcoholic -naphthol) to one millilitre of test solution and then coating the inner surface of the test tube with 2 mL of strong sulphuric acid.

Benedict's reagent was added to one millilitre of the test solution and kept boiling in a water bath for 5-7 minutes in order to perform the Benedict's test for decreasing sugar.

c. The Fehling's test for decreasing sugar: 1 mL of the test solution was added to 2 mL of previously combined equal volumes of Fehling's solutions A and B, and the mixture was then placed in a boiling water bath for 5–10 minutes.

d. Selivanoff's test for ketones in sugar: To one millilitre of test solution, Selivanoff's reagent was added, and then one millilitre of strong hydrochloric acid was added. The mixture was then placed in a boiling water bath for 4 to 8 minutes.

One millilitre of the test solution was mixed with a few drops of the 0.25% ninhydrin reagent, and the mixture was then placed in a pot of boiling water for 2–5 minutes to test for amino acids.

Examine your proteins: Heat method: Using a water bath, one millilitre of the test solution was heated for five to ten minutes. Biuret test: 1 mL of the Biuret reagent was added to 1 mL of the test solution.

Phenol test: One millilitre of test solution was mixed with one millilitre of Folin-Ciocalteu reagent and half a millilitre of Na2CO3.

Checking for flavonoids A small amount of strong hydrochloric acid and pieces of magnesium ribbon were added to one millilitre of the test fluid for the Shinoda test.

b. The zinc-hydrochloride test involved adding zinc dust and a few drops of hydrochloric acid to one millilitre of the test fluid.

Test for tannins: A few drops of 5% ferric chloride were added to one millilitre of the test solution.

Test for Terpenoids: Two millilitres of chloroform and three millilitres of concentrated sulphuric acid were added to five millilitres of test solution.

Sulphur powder test: One millilitre of test fluid was mixed well with a small amount of sulphur powder in order to test for steroids.

b. Salkowski's test: A few drops of sulphuric acid were added to one millilitre of test solution and the mixture was thoroughly agitated.

c. The Liberman Burchard test: One millilitre of test solution was mixed with a few drops of acetic anhydride, heated for three to five minutes in boiling water, and then added one millilitre of strong sulfuric acid after cooling.

Glycoside screening: Test generally: Test A: To make the formulation alkaline (as measured by pH paper), 200 mg of the formulation were heated in a water bath with 5 mL of diluted sulphuric acid, filtered, and neutralized with 5% sodium hydroxide solution. Fehling's solutions A and B were added, and 0.1 mL of each was heated in a water bath for two minutes. Test B: The same process as in Test A, but this time using 5 mL of water in place of the diluted sulfuric acid. Whether Test A's intensity of precipitation was greater than Test B's was compared.

A saponins test: Test for the production of froth (foam) by vigorously shaking a 2 mL sample of the test solution.

c. NaOH was added to two millilitres of test solution. A millilitre of the test solution was mixed with a solution of -naphthol and sulphuric acid to perform an insulin test.

High Performance Thin Layer Chromatography (HPTLC) fingerprint examination

At the J.R.D. Tata Foundation for research in Ayurveda and Yoga, Science, Deendayal Research Institute, Chitrakoot, Satna (M.P.), the polyherbal extract's HPTLC fingerprinting was performed. The formulation (2.0 gm) was placed in a conical flask of 100 mL capacity, combined with water and ethyl alcohol (1:1), thoroughly agitated, and then transferred to a funnel of 250 mL capacity. The top layer (ethyl alcohol) was recovered after shaking. Three times, ethyl alcohol was mixed into the aqueous layer. The ethyl alcohol fraction was concentrated to a maximum of 10mL, dried over anhydrous sodium sulphate, and filtered.

A Merck aluminium plate precoated with silica gel 60F254 of 0.2 mm thickness received individual applications of ten and fifteen millilitres of the extract, and the plate was developed in a toluene: ethyl acetate (4:1) solvent system. A photo recording and UV visualization of the plate at 254 wavelengths were performed. At a wavelength of 254 nm, the plate was scanned. After that, the plate was submerged in vanillin-sulfuric acid, heated to 105°C until the spots' colours emerged, and photographs were taken under a white light.

Result and Discussion

The hydroalcoholic extract of the anti-diabetic polyherbal formulation was examined, and it was found to contain carbohydrates, amino acids, proteins, phenols, alkaloids, glycosides, and flavonoids, while Resin, waxes, and starch were not present in the mixture, as shown in Table 3. Strong antioxidants, phenol and flavonoids also have a variety of beneficial biological benefits[22]. It is widely known that a diet and beverages high in phenols may boost

the plasma antioxidant capacity. Diets high in polyphenols offer important defense against the cellular damage that many chronic pathological illnesses, such as cancer, diabetes, cardiovascular issues, and ageing, cause over time.[23] According to a review by Robert et al, flavonoids' therapeutic potential is a result of their antioxidative, anti-atherosclerotic, anticancer, and anti-inflammatory properties. [24]

Table 3: Results of Qualitative Investigation of the Antidiabetic Polyherbal Composition

S. No.	**Constituents Tests**		AI-HAE	OS-HAE	BD-HAE	**AM**-HAE
1	**Carbohydrate and Glycoside**	Molisch's test	+	+	+	+
		Fehling's test	+	+	+	+
		Legal's test	+	+	+	+
		Borntrager's test	+	+	+	+
2	**Protein and aminoacid**	Millon's test	+	+	+	+
		Ninhydrine test	+	+	+	+
		Biuret test	+	+	+	+
3	**Saponins**	Foam test	+	-	-	+
4	**Phenolic compounds and tannins**	FeCl2 test	+	+	+	+
5	**Phytosterol**	Libermann-burchard test	+	+	+	+
		Salkowski test	+	+	+	-
		Sulphur powder test	-	-	-	-
6	**Alkaloids**	Dragendroff's	+	+	+	+
		Mayer's	-	-	-	+
		Wagner's	+	+	+	+
		Hager's	+	+	+	+
7	**Flavonoids**	Aq. NaOH test	+	+	+	-
		Shinoda test	-	-	-	+
		Zinc hydrochloride test	+	+	+	-
8	**Mucilage**	Ppt. With 90% alcohol	+	-	-	-
9	**Resins**		+	-	-	+
10	**Waxes**	Ppt. With alcoholic KOH	-	-	-	+
11	**Starch**	Amylum test	-	-	-	-

Where,+ indicates present − indicates absent

Finger printing analysis of the formulation was carried out using the HPTLC method. The results of the analysis are summarized in Figure 1 to 5. Out of the 7 sample ISDM 007 shows best result that the Rf value is 0.27 for Ferulic acid estimation, Height 72.93 and area covered 2632 and for Quercetin Rf value is 0.16, Height is 17.62 and area covered 319.65.

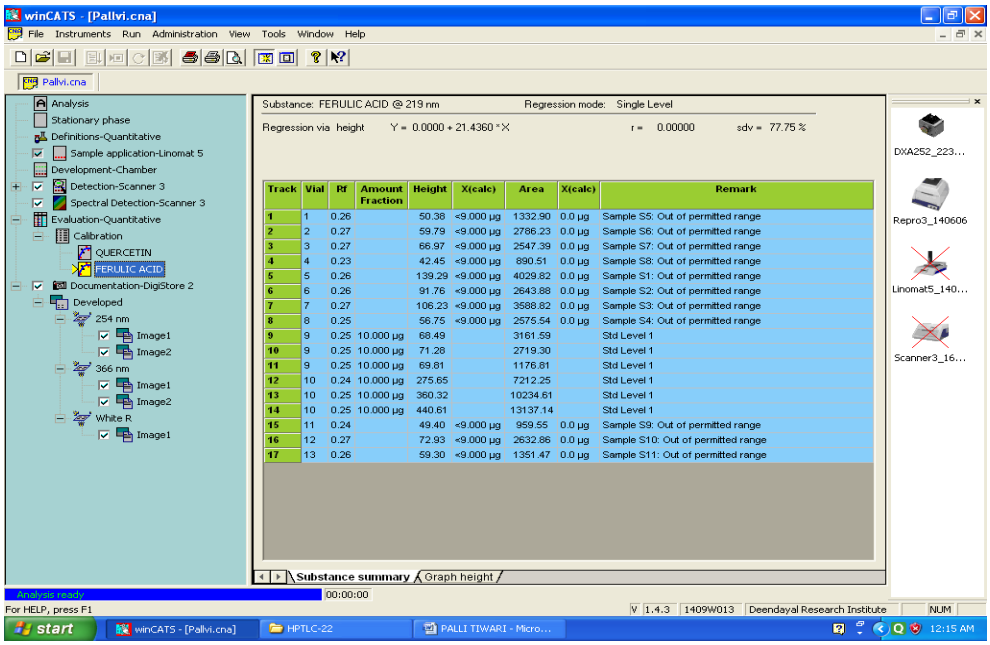
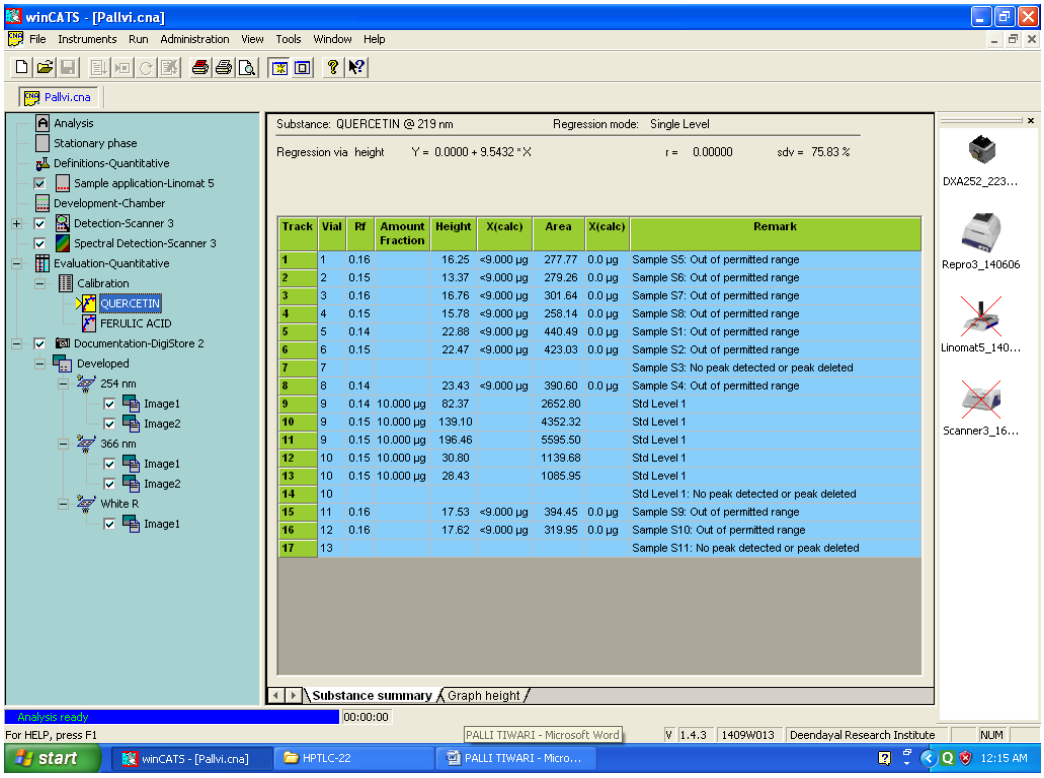


Figure 1: Qualitative Analysis for Ferulic acid in polyherbal composition in different ratio.



Track	Vial	Rf	Amount Fraction	Height	X(calc)	Area	X(calc)	Remark
1	1	0.16		16.25	<9.000 µg	277.77	0.0 µg	Sample S5: Out of permitted range
2	2	0.15		13.37	<9.000 µg	279.26	0.0 µg	Sample S6: Out of permitted range
3	3	0.16		16.76	<9.000 µg	301.64	0.0 µg	Sample S7: Out of permitted range
4	4	0.15		15.78	<9.000 µg	258.14	0.0 µg	Sample S8: Out of permitted range
5	5	0.14		22.88	<9.000 µg	440.49	0.0 µg	Sample S1: Out of permitted range
6	6	0.15		22.47	<9.000 µg	423.03	0.0 µg	Sample S2: Out of permitted range
7	7							Sample S3: No peak detected or peak deleted
8	8	0.14		23.43	<9.000 µg	390.60	0.0 µg	Sample S4: Out of permitted range
9	9	0.14	10.000 µg	82.37		2652.80		Std Level 1
10	9	0.15	10.000 µg	139.10		4352.32		Std Level 1
11	9	0.15	10.000 µg	196.46		5595.50		Std Level 1
12	10	0.15	10.000 µg	30.80		1139.68		Std Level 1
13	10	0.15	10.000 µg	28.43		1085.95		Std Level 1
14	10							Std Level 1: No peak detected or peak deleted
15	11	0.16		17.53	<9.000 µg	394.45	0.0 µg	Sample S9: Out of permitted range
16	12	0.16		17.62	<9.000 µg	319.95	0.0 µg	Sample S10: Out of permitted range
17	13							Sample S11: No peak detected or peak deleted

Figure 2: Qualitative Analysis for Quercetin in polyherbal composition in different ratio.

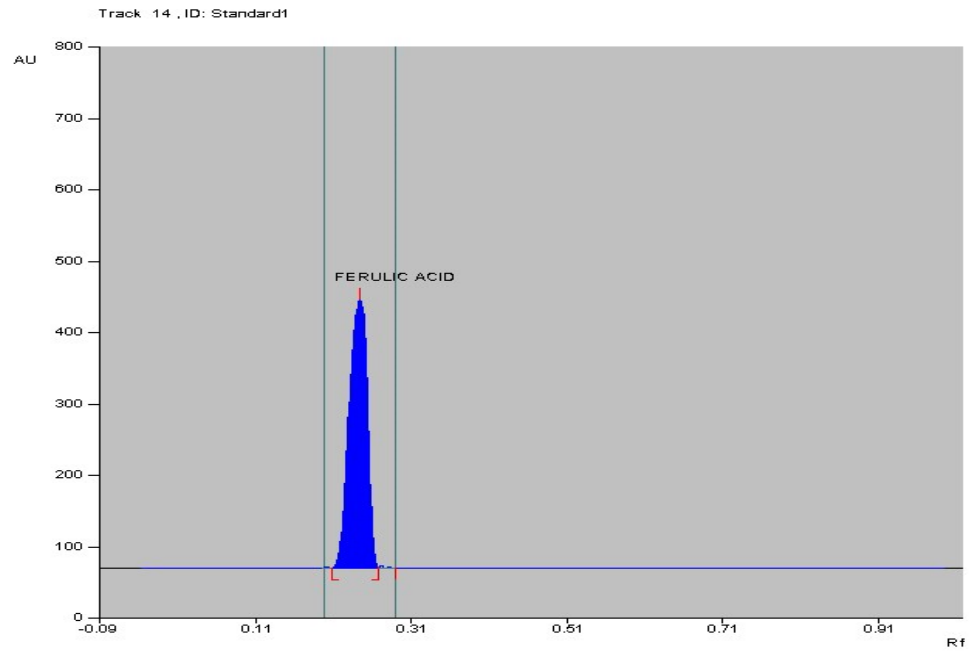


Figure 3: Ferulic Acid Biomarker estimation.

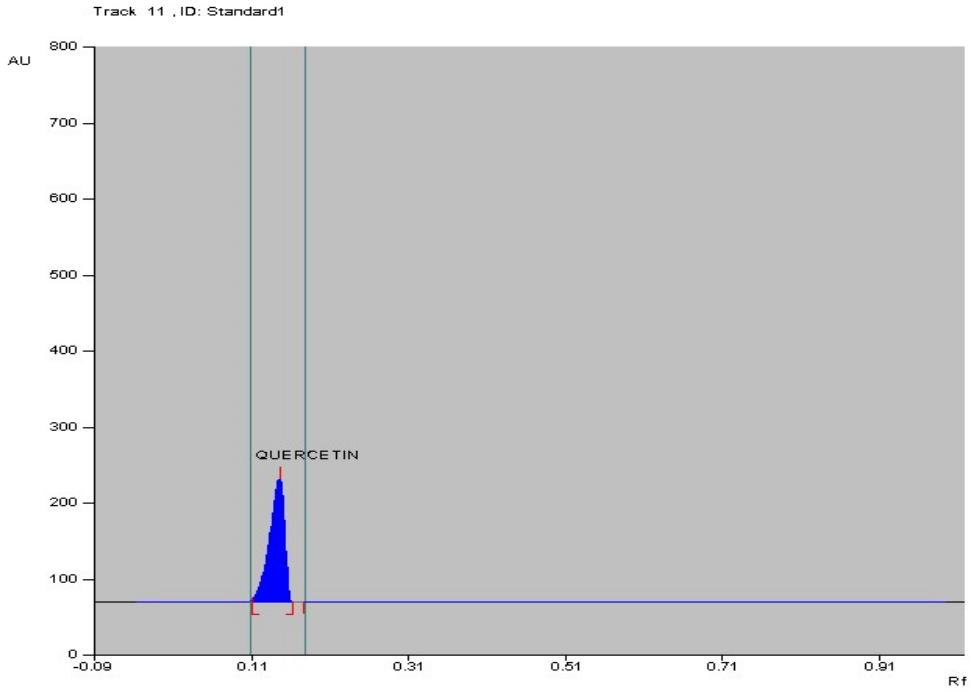


Figure 4: Quercetin Biomarker estimation.

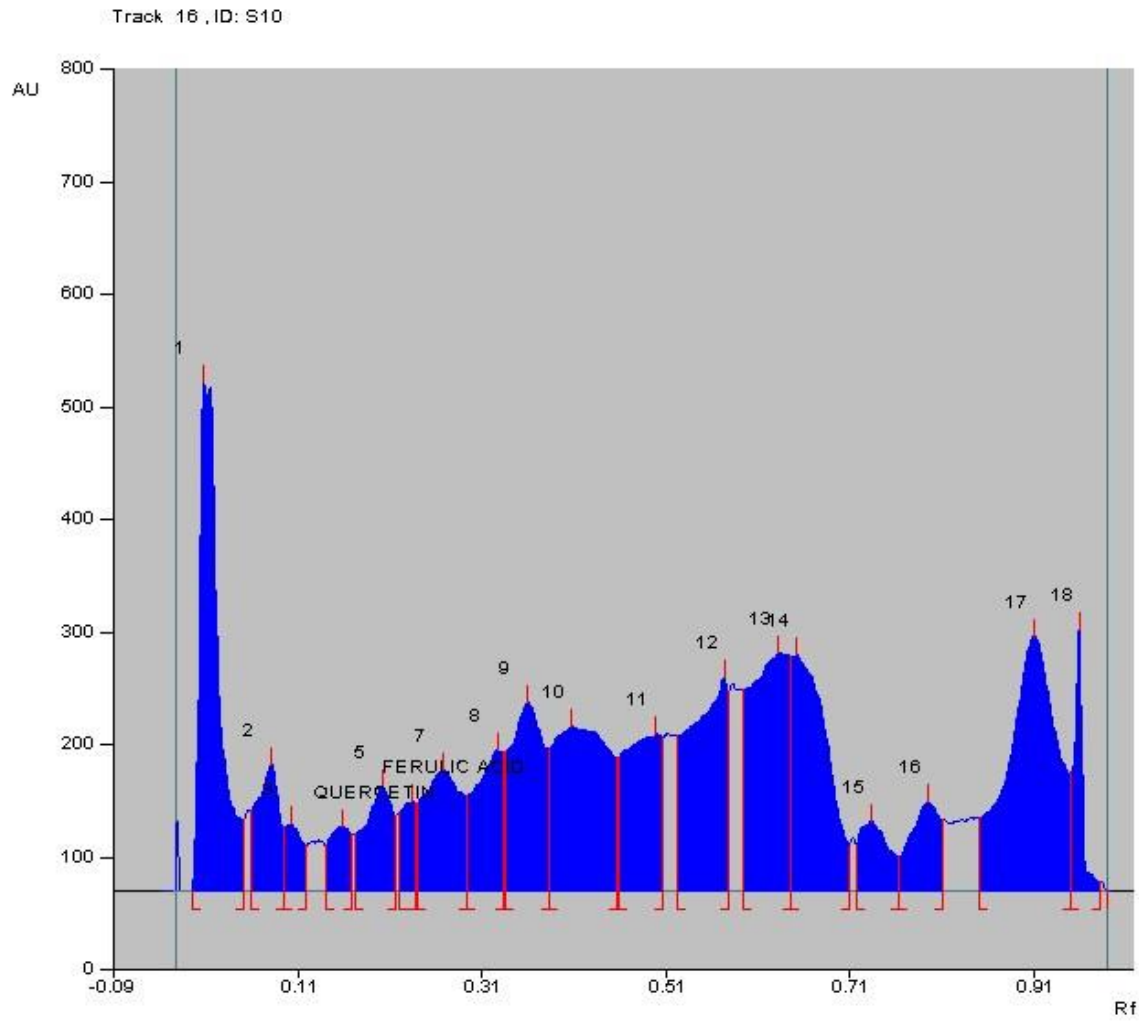


Figure 5: HPTLC Chromatograph of the Antidiabetic Polyherbal Composition- scanning at 254 nm: Polyherbal composition ISDM 007 shows quantitative analysis of both secondary metabolites, Quercetin and Ferulic Acid.

CONCLUSIONS: The current study found that the polyherbal anti-diabetic formulation contains bioactive substances such as phenols, flavonoids, tannins, terpenoids, steroids, and insulin. The formulation has several phytoconstituents, especially quercetin and ferulic acid, according to the HPTLC fingerprint study. A prospective contender for the treatment of diabetes and other metabolic disorders, the polyherbal formulation is regarded as a potential source of natural antioxidants. Utilizing in vitro and in vivo models, more research is required to examine the formulation's pharmacological profile.

CONFLICT OF INTERESTS: The writers affirmed that they do not have any conflicts of interest.

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