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Research Article

Formulation, Phytochemical Estimation of Active principles by Spectroscopic methods and Comparison of the Poly Herbal Anti-diabetic Tablet with the Commercial Tablets

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ABSTRACT

Diabetes mellitus is a group of metabolic disorders characterized by increased glucose levels over a prolonged period. The poly-herbal anti-diabetic tablet was formulated in the laboratory by using herbs of Enicostemma littorale (EL) (Gentianaceae), roots of Aconitum heterophyllum (AH) (Ranunculaceae), rhizomes of Picrorhiza kurroa (PK) (Scrophulariaceae), and fruits of Piper longum (PL) (Piperaceae). Furthermore, Laboratory formulation (LF) and Marketed formulations (MFs) were tested for Phytochemical screening and Qualitative chemical examination. From their result, isolation of active principles, Swertiamarin (EL-1) and Piperine (PN-1), was done and subjected to phytochemical examination by Modern analytical methods like TLC, UV, FTIR. Finally, the quantification and estimation of the afore-mentioned compounds were achieved by HPTLC. SSE of all the four formulations and the chemical tests proved alkaloids, flavonoids, carbohydrates, tannins, phenols and phytosterols. Comparing the data from the melting point, TLC, UV, and IR studies of EL-1 and PN-1 with standard Biomarkers, further calculation of both markers concentrations was done with the help of the calibration equation and the peak area derived from the chromatogram. Study outcomes signify that LF was more potent than MFs for anti-diabetic activity, signifying as a promising natural and safe remedy for the prevention of diabetic complications.

Introduction

Few years ago, there was an increase in the field of herbal medicines and Herbal drugs are gaining popularity both in developing and developed countries because of their natural origin and fewer adverse and side effects. Many herbal formulations which are in use are derived from natural origins.[1] Natural products are rich sources of active chemical constituents.[2] Several herbal plants used for over 1000 years named rasayana are used in many herbal formulations of Indian traditional health care systems.[3] In Ayurvedic systems of medicine, most practitioners formulate and distribute their recipes.[4]

The World Health Organization (WHO) has listed 21,000 plants with medicinal values and are used worldwide. Out of which 2500 herbs are in India, 150 herbs are used commercially on a large scale. India is the largest producer of Traditional herbs and is also known as the botanical garden of the world.[4] The current review is on herbal preparations and medicinal plants used in the treatment of diabetes mellitus, most common disease in the world, leading to huge economic losses.

Diabetes is a chronic disease due to improper carbohydrate, fat, and protein metabolism characterized by increased fasting and post-prandial blood sugar levels. The global prevalence of diabetes is estimated to increase, from 4% in 1995 to 5.4% by 2025. WHO has observed that the major load will occur in developing countries.[5]

Diabetes (DM) is known as Madhumeha in classical Ayurveda with a striking resemblance of its Ayurvedic concepts with the latest knowledge on diabetes mellitus.[6] Diabetes mellitus is a metabolic disease characterized by increased glucose level, known as hyperglycemia resulting

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from a change in insulin secretion, insulin effect, or both. The chronic hyperglycemia of diabetes mellitus is also connected with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, liver, heart, etc. Diabetes will become one of the serious causes during the 21st century, producing many life-threatening health problems.[7]

The common name of EL, is Chota-kirayata or Chota chirayata (Hindi), and Mamejavo (Gujarati), is being used as a medicinal herb for the treatment of diabetes mellitus.[8-9] It is a well-known plant used as a drug in Ayurveda, Unani, Siddha, Allopathic, Homeopathy, Naturopathy and Home Remedies,[10] and it is widely used in Siddha system of medicine under the name "Vellarugu"[11-13] for the treatment of diabetes mellitus, arthritis, rheumatism, constipation, abdominal ulcers, hernia, swelling, fever, skin diseases and insect poisoning.[14-16] The plant AH is used to cure diseases such as hysteria, throat infection, dyspepsia, abdominal pain, diabetes, diarrhea, etc.[17,18] PK (Scrophulariaceae) is a traditional Ayurvedic herb known as Kutki. It is used to cure diabetes.[19-20] PL is found mainly in the Western Ghats and the central Himalayas of India. The fruits of the plant are commonly known as Pippali in Hindi and the roots as Piplamool. The roots and fruits of the plant are used as an antidote to a snake bite, scorpion stings, chronic bronchitis, cough, and cold. It is beneficial for people with diabetes because it can regulate the rate at which glucose is released in the blood. Insulin production is also boosted by PL herb. Hence, regular consumption of long pepper is beneficial for people suffering from diabetics.[21-22]

This study aimed to prepare and Phytochemical Estimation of an herbal formulation containing active principles EL-1 and PN-1 isolated from EL and PL known for potential anti-diabetic activity. Two plants having sound ethnopharmacological and scientific citation background for anti-diabetic activity were thus selected along with the most bioactive compound. The study encompasses enrichment and authentication of phytoactive compounds, EL-1 and PN-1 development of a standardized formulation compared with the MFs containing both the selected plants along with other herbs.

Thus, the present investigation concluded that the herbs selected will have a prominent effect in the treatment of anti-diabetic activity.

Materials and Methods

Materials

- *Collection and Authentication of Plant Materials:* The entire materials EL herb, roots of AH, rhizomes of PK and fruits of PL were procured from the Ayurvedic drug supplier named M/S Lallubhai Vrajlal Gandhi (LVG), Ahmedabad in December 2009 and authentication was done by Dr. Geetha K. A., Senior Scientist, Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat.
- *Instrumentation:* Determination of UV range, varian UV spectrometer having carry-100 software. For estimation using HPTLC, Silica gel 60F254 Aluminium backed plate was used as:
- *Stationary phase:* Analysis using HPLC was carried out with Simadzu HPLC-MD2010-UV system equipped with the pump and UV/VIS detector.
- *Collection of Market Formulations:* The market formulations of Mamejva ghanvati of different manufacturers 'BAPS Amrut', 'Shree Shanker' and 'Unjha' were procured from the local market of Ahmedabad in December 2009.
- *Formulation of Anti-diabetic Herbal Tablets:* Initially, 25.60 gm leaf powder of EL was taken and water was added, then it was kept for 6 hours with constant stirring. It was then filtered with a cloth and heated until ghan (solid extract) was prepared. Then powders of AH, PK and PL were added in their respective quantities. The ingredients were passed through sieve number 60, and the wet granulation technique did the granulation. 5% starch paste was added as a binding and disintegrating agent.

All the ingredients were mixed properly, and the resulting solid mixture was used to prepare granules with the help of sieve no. 10. The granules were sun-dried and then passed through sieve no. 22 and 44. Granules were collected on sieve no. 44 and were stored in an air-tight pot bottle and used to prepare tablets in the tablet machine.

The die and punches used for making tablets were 7mm. The tablet machine used was RSB-4 Minipress, Rimek India, Single head Rotary tablet compression machine[23] (Table 1).

Preliminary Phytochemical Screening

25 gm powder of each formulation was taken and extracted in soxhlet apparatus with the solvents of increasing polarity, i.e., Petroleum ether, Toluene, Chloroform, Acetone, Methanol, and water.[23-25]

Qualitative Chemical Examination

The extracts obtained in the above procedure were subjected to various Chemical tests for the present or absence of phytoconstituents such as Alkaloids, Flavonoids, Glycosides, Carbohydrates, Phytosterols, Tannins and phenolic compounds, Saponins.[25-28]

Table 1: Composition of formulation of herbal tablets.

Sr No	*Ingridents*	*Quantity*
1	*Enicostemma littorale*	256 mg
2	*Aconitum heterophyllum*	4 mg
3	*Picrorhiza kurroa*	4 mg
4	*Piper longum*	4 mg
5	Starch	2 mg

Weight of each tablet was of 270 mg. A batch size of 100 tablets was prepared.

Isolation of Marker and Its Analysis

Literature survey revealed the presence of EL-1 as a major phytoconstituent in EL plant powder. Therefore it was decided to isolate EL-1 as the marker of this plant.[29,30]

Isolation of Compound EL-1 from EL Plant

50 gm of the dried powdered plant of EL was taken. It was extracted with 250 mL of pure ethanol by cold percolation at room temperature consequently three times. The solution was filtered, and the solvent was removed under reduced pressure. The green viscous mass obtained from the ethanolic extraction was fractionated consequently with ether and ethyl acetate. The ethyl acetate soluble portion yielded extremely bitter, pale yellow amorphous compound EL-1.[29,30]

UV Spectroscopy of Isolated Compound EL-1

UV spectroscopy of isolated compound EL-1 was done to find out its wavelength and it was compared with literature for its proper identification.

1 mg substance was dissolved in 10 mL of methanol and used to determine wavelength.[30]

FTIR Spectrum of Isolated Compound EL-1.

FTIR spectroscopy was done to find out various functional groups present in the isolated compound, and it was compared with the literature.[30]

TLC and Melting Point of Isolated Compound EL-1.

TLC of the isolated sample was carried in different solvent systems, and it should show a single spot with its R_f value ranging from 0.2 to 0.9 in different solvent systems in Table 2.[26]

Melting Point was Determined in Melting Point Apparatus

All the data of melting point, TLC, UV, and IR of EL-1 while comparing with literature revealed its confirmation. Therefore, it can be used as a standard EL-1 of EL.

Isolation of Compound PN-1 from PN Fruits

50 gm of black pepper powder was taken. About 500 mL ethanol was added, and the mixture was used for extraction in a soxhlet apparatus for about 2 hours. It is filtered and concentrated on a water bath at 60°C. The concentrated contents were mixed with 50ml of 10% alcoholic potassium hydroxide with constant stirring. After filtration, the alcoholic solution was kept overnight in the laboratory. Compound PN-1 appeared out in the form of needle-like yellow-colored crystals.[31]

Table 2: TLC of isolated compound EL-1

Solvent system	*R_f of swertiamarin*
A. Ethyl acetate: methanol: water (9:0.8:0.2)	0.32
B. Ethyl acetate: methanol: water (7.7:1.5:0.5)	0.57
C. Ethyl acetate: methanol: water (5:1.5:1)	0.92

UV Spectroscopy of Isolated PN-1

UV spectroscopy of isolated PN-1 was done to find out its wavelength, and it was compared with literature for its proper identification. 1 mg substance was dissolved in 10ml of methanol and used to determine wavelength.[30,31]

FTIR Spectrum of Isolated Compound PN-1.

FTIR spectroscopy was done to find out various functional groups present in the isolated compound and it was compared with the literature.[32]

TLC and Melting Point of Isolated Compound PN-1.

TLC of isolated PN-1 was done. The solvent system taken was toluene: diethyl ether: dioxane (9.4: 3.2: 2.4). Melting point was determined in the Melting point apparatus.

All the data of melting point, TLC, UV and IR of PN-1 while comparing with literature revealed its confirmation as PN-1. Therefore, it can be used as a standard PN-1 form PN fruit.[32]

Estimation of EL-1and PN-1 by HPTLC in Various Formulations

Materials:

Standard: Isolated EL-1 and PN-1

- *Sample:* Extract of MF1, MF2, MF3 and LF
- *Solvents:* Toluene, Ethyl acetate, Methanol and Formic acid
- Selection of chromatographic conditions
- *Stationary phase:* Pre-coated silica gel G_{60}- F_{245}, Aluminium sheet (E. Merck, Germany), 10 x 10 cm, thickness laye r- 0.2 mm
- *Mobile phase: Toluene:* Ethyl acetate: Methanol: Formic acid (6:2:3:1)
- Chamber saturation time – 30 minutes
- Distance run – 8 cm

Preparation of Mobile Phase

A mixture of 6 mL toluene, 2 mL of ethyl acetate, 3 mL of methanol, and 1 mL of formic acid, previously filtered through the filter paper was mixed in a volumetric flask and used as mobile phase.

Preparation of Sample

Weighed accurately 5 gm of each powdered formulation and extracted with methanol for 2 times with 25 mL methanol each time. For EL-1 quantification, take 10 mL of the filtrate and dilute upto 100 mL. So sample solution for EL-1 10^4 µg/mL concentration. For PN-1 quantification, 25 mL of concentrated filtrate was taken. So the concentration of the sample solution is 200 µg/µL.

Preparation of Standard Solution

Stock solution of isolated EL-1 and PN-1 (1mg/ml) was prepared by dissolving 10mg of reference compound in 10 mL of methanol individually. From this stock solution, 100 µg/mL was prepared by transferring 1 mL stock

solution to 100 mL volumetric flask and adjust the volume with methanol.

HPTLC Analysis (Calibration Curve)

A semiautomatic spotter was used containing a syringe having a capacity of 50 µL. 10 µL standard solution was filled in a syringe and under a nitrogen stream. It was applied in the form of bands of desired concentration range (100 ng/spot to 600 ng/spot) of standard solution on pre-coated plates. For EL-1 and PN-1 individual plates were prepared. Plates were developed using toluene: ethyl acetate: methanol: formic acid (6: 2: 3: 1). The developed plate of EL-1 and PN-1 were subjected to densitometric measurements in absorbance mode at wavelength 235 nm and 345 nm, respectively, using Camag TLC scanner. Plots of peak area vs. concentration of isolated EL-1 and PN-1 were prepared.

Estimation of Marker Compound in Different Formulation

1 µL (for EL-1 quantification) and 5 µL (for PN-1 quantification) of test sample extract of different formulations were injected into chromatographic system. Peak areas of marker compounds in the samples were noted, and the concentration was determined from the calibration curve using peak area.

Results

In the following results

- MF 1 denotes BAPS formulation
- MF 2 denotes SHANKER formulation
- MF 3 denotes UNJHA formulation

LF denotes Laboratory formulation

Successive Solvent Extractive Value and Physical Characteristic

25 gm powder of each formulation was taken and extracted in soxhlet apparatus with the solvents of increasing polarity i.e., Petroleum ether, Toluene, Chloroform, Acetone, Methanol, and water. Successive solvent extractive value and physical characteristics of LF and MFs are given in Table 3.

Qualitative Chemical Examination of all Formulations

The extracts of all formulations were subjected to various Chemical tests for phytoconstituents such as Alkaloids, Flavonoids, Glycosides, Carbohydrates, Phytosterols, Tannins, and phenolic compounds, Saponins (Table 4).

UV Analysis of Isolated Compound EL-1 and PN-1

The UV spectrum of the isolated compound was obtained in methanol. The spectrum was obtained in the 200–400 nm range and showed peak at 235 nm, and the reported peak is at 236.8 nm (Fig. 1).

The UV spectrum of the isolated compound was obtained in methanol. The spectrum was obtained in

Table 3: Successive solvent extractive value and physical characteristic of MF1, MF2, MF3 and LF.

Sample	*Solvent*	*Colour of extract*	*Consistency*	*Yield of residue (%w/w)*
MF 1	Pet.Ether (60–80°C)	Dark green	Solid	2.3%
	Toluene	Greenish black	Solid	3.5%
	Chloroform	Greenish black	Solid	1.2%
	Acetone	Green	Solid	3%
	Methanol	Brown	Sticky	11.7%
	Water	Brownish black	Sticky	8.3%
MF 2	Pet.Ether (60–80°C)	Dark green	Solid	2.1%
	Toluene	Greenish black	Solid	2.7%
	Chloroform	Greenish black	Solid	1.9%
	Acetone	Green	Solid	2.8%
	Methanol	Brown	Sticky	10.9%
	Water	Brownish black	Sticky	7.4%
MF 3	Pet.Ether (60–80°C)	Dark green	Solid	3.4%
	Toluene	Dark green	Solid	3.2%
	Chloroform	Dark green	Solid	2.1%
	Acetone	Green	Solid	3.7%
	Methanol	Dark brown	Sticky	12.2%
LF	Pet. Ether (60–80°C)	Dark green	Solid	5.1%
	Toluene	Dark green	Solid	4.8%
	Chloroform	Dark green	Solid	2.5%
	Acetone	Green	Solid	3.8%
	Methanol	Dark brown	Sticky	13.9%
	Water	Brown	Sticky	10.1%

200-400nm range and showed peak at 342nm and the reported peak is at 340 nm Fig. 2.

FTIR Analysis of Isolated Compound EL-1 and PN-1

FTIR analysis of isolated compound EL-1 in (Fig. 3) and FTIR comparison of standard peaks with isolated EL-1 in Table 5.

FTIR analysis of isolated compound PN-1 in Fig. 4 and FTIR comparison of standard peaks with isolated PN-1 in Table 5.

TLC and Melting Point of Isolated Compound EL-1 and PN-1

TLC of the isolated EL-1 carried out in different solvent systems given in literature showed a single spot with R_f value same as given in standard literature (Fig. 5). Melting point was reported it was reported to be 108°C which matches the standard mp range, i.e., from 108°C to 110°C. All the data of melting point, TLC, UV, and IR studies of EL-1 while comparing with the literature revealed the confirmation of it as standard swertiamarin and can be considered as a marker of EL.

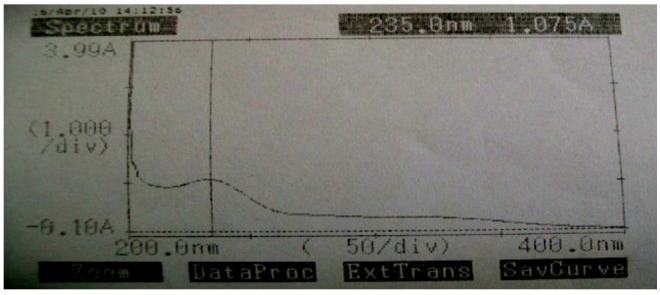


Fig. 1: UV Spectrum of isolated EL-1.

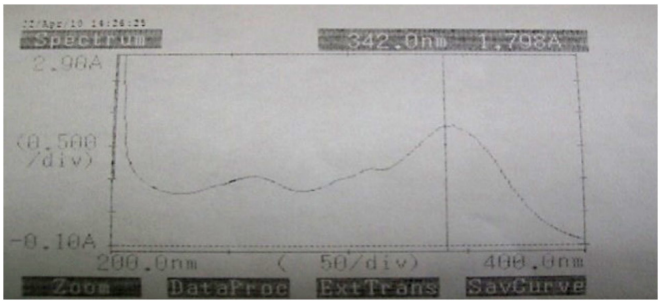


Fig. 2: UV spectrum of isolated PN-1

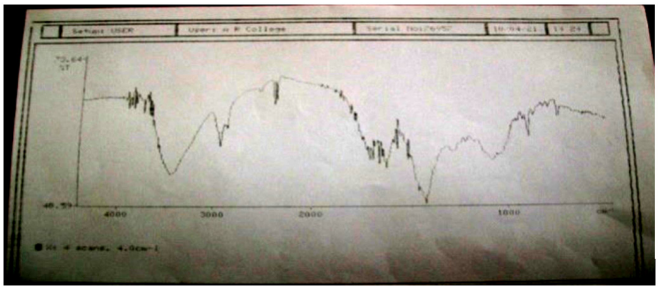


Fig. 3: FTIR analysis of isolated compound EL-1

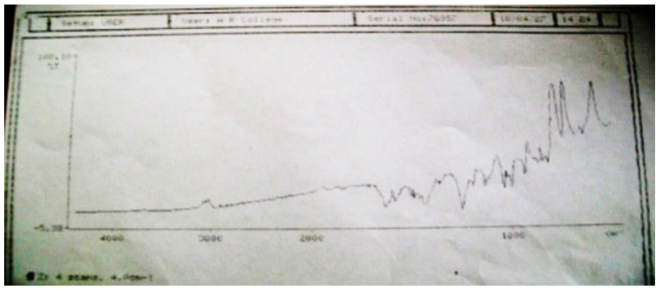


Fig. 4: FTIR analysis of isolated compound PN -1

Table 4: Qualitative chemical examination of all formulations

Sr. No	*Chemical Test*		*Observation*	*Result*
1	Dragendorff's test	Tests of Alkaloids	Orange brown precipitates	Alkaloids were present
2	Mayer's reagent		cream precipitate	Alkaloids were present
3	Wagner's reagent		reddish brown precipitate	Alkaloids were present
4	Shinoda test	Tests of Flavanoids	Formation of pink colour	Flavonoids were present.
5	fluorescence test		greenish fluorescence under UV light in the ethereal layer	Flavonoids were present.
6	Borntrager's test	Tests of Glycosides	The ammoniacal layer was colourless	anthraquinone glycosides were absent.
7	Modified Borntrager's test		The ammoniacal layer was colourless	anthraquinone glycosides were absent.
8	Fehling's test	Tests of Carbohaydrates	First yellow, then brick red precipitate	Carbohydrates were present.
9	Benedict's test		red precipitates	Carbohydrates were present.
10	Lieberman Burchard test	Tests of Phytosterols	Green precipitates	Phytosterols were present.
11	Salkowski test		chloroform layer appeared red	Phytosterols were present.
12	lead acetate test	Tests of Tannins and phenols	white precipitate	Tannins and phenols were present.
13	ferric chloride test		deep blue-black colour	Tannins and phenols were present.
14	Forth Test	Test of Saponins	Persistent froth was not produced	Saponins were absent.

Table 5: FTIR comparison of peaks of standard with isolated EL-1 and PN-1.

Compound	*Wavelength cm^{-1} (STANDARD)*	*Wavelength cm^{-1} (ISOLATED)*	*Assigned grouping*
EL-1	3300-3500 cm^{-1}	3420.6-3587.4 cm^{-1}	-OH stretching
	1697 cm^{-1}	1684.5 cm^{-1}	C=O stretching
	1618 cm^{-1}	1617.4 cm^{-1}	CH=CH stretching
	1235; 1273; 1275 cm^{-1}	1068 cm^{-1}	C-O stretching
	3000 cm^{-1}	2939 cm^{-1}	Aromatic C-H stretching
	1635; 1608 cm^{-1}	1635.7 cm^{-1}	Symmetric and asymmetric stretching of C=C(diene)
	1580; 1495 cm^{-1}	1586.8; 1490.1 cm^{-1}	Aromatic stretching of C=C (benzene ring)
	1250; 1190 cm^{-1}	1252.6; 1194.2 cm^{-1}	Asymmetrical stretching =C-O-C
PN-1	1030 cm^{-1}	1036.2 cm^{-1}	Symmetrical stretching =C-O-C
	930 cm^{-1}	929 cm^{-1}	C-O stretching
	1132 cm^{-1}	1134.3 cm^{-1}	In-plane bending of phenyl C-H
	995 cm^{-1}	997.4 cm^{-1}	C-H bending of trans –CH=CH-
	850; 830; 805 cm^{-1}	848.2; 829.8; 804	Out of plane C-H bending 1,2,4 trisubstituted phenyl

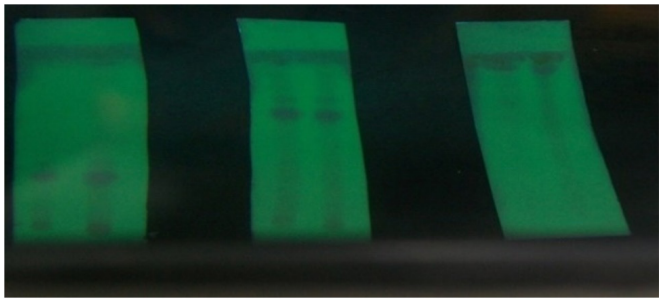


Fig. 5: TLC and Melting point of isolated EL-1.

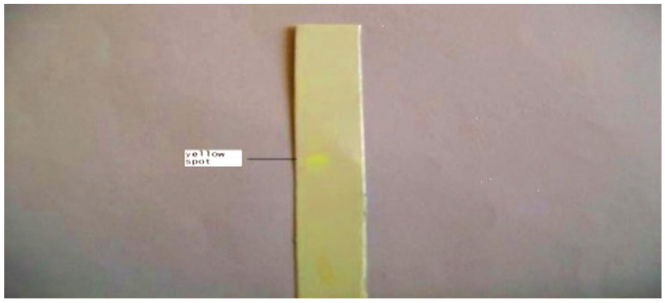


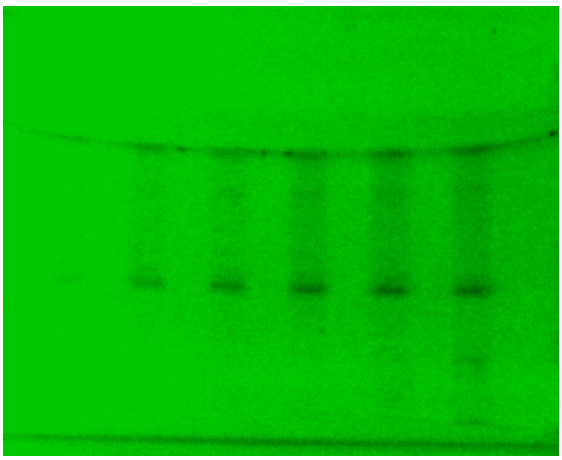
Fig. 6: TLC of isolated PN-1

R_f value was found to be 0.47 (standard 0.5) in toluene: diethyl ether: dioxane (9.4: 3.2: 2.4). Melting point was found to be 125°C which matched with the standard range of 125–126°C. All the data of melting point, TLC, UV, and IR studies of EL-1 while comparing with the literature revealed its confirmation as standard PN-1 and can be considered a marker of PN fruits (Fig. 6).

Quantification of EL-1 and PN-1 in all Formulations by HPTLC

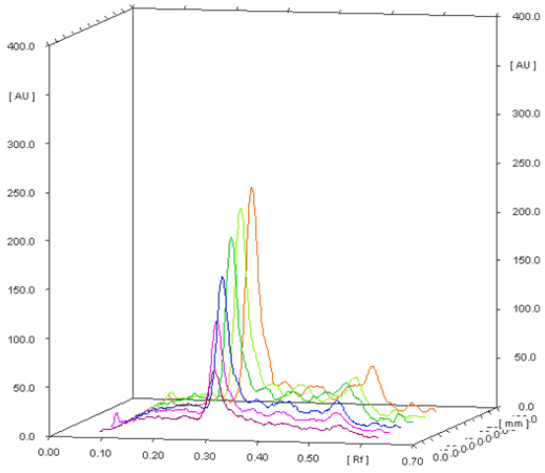
HPTLC chromatograph of calibration of standard EL-1 showed in Fig. 7 and UV spectra 3D at 235 nm for calibration of standard s EL-1 showed in Fig. 8.

HPTLC chromatograph of calibration of standard PN-1 showed in Fig. 9, and UV spectra 3D at 235 nm for calibration of standard PN-1 showed in Fig. 10.



S1 S2 S3 S4 S5 S6

Fig. 7: HPTLC chromatograph of Calibration of standard swertiamarin



Equation: 7.0974x + 547.4

Fig. 8: UV spectra 3D at 235 nm for calibration of standard swertiamarin

Quantification of EL-1

HPTLC chromatograph of EL-1 marker with all formulations and Comparison of standard EL-1 with all the formulations showed in Fig. 11 and 12, respectively.

- Equation: 7.0974x + 547.4, From the equation of calibration and peak area of MF1, MF2, MF3, LF obtained 2417, 2259, 1863, 2706 respectively in chromatogram we can obtain the concentration of EL-1 in MF1, MF2, MF3, LF was found to be 2.6 gm/100 gm, 2.4 gm/100 gm, 1.8 gm/100 gm and 3 gm/100 gm, respectively.

Quantification of PN-1

HPTLC chromatograph of PN-1 marker with all formulations and Comparison of standard PN-1 with all the formulation showed in Fig. 13 and 14, respectively.

- Equation: y = 16.636x + 11211, From the equation of calibration and peak area of MF1, MF2, MF3, LF obtained 18453, 14671, 13116 and 19214 respectively in chromatogram we can obtain the concentration of PN-1 in MF1, MF2, MF3, LF was found to be 0.043 gm/100 gm, 0.020 gm/100 gm, 0.011 gm/100 gm and 0.048 gm/100 gm respectively.

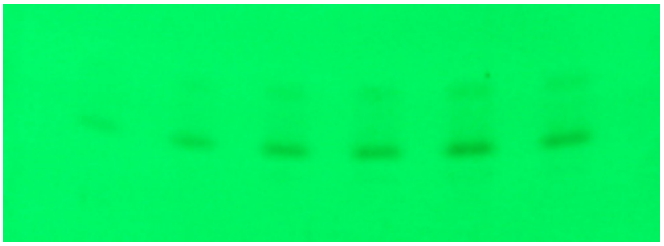
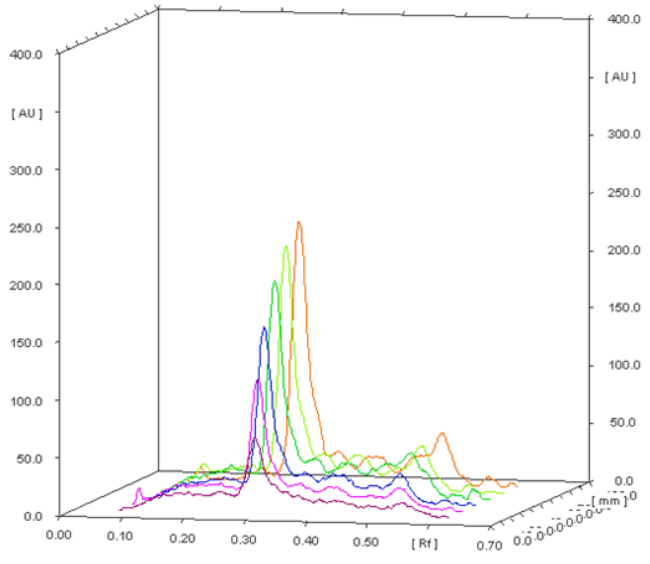


Fig. 9: HPTLC chromatograph of standard piperine



Equation: y = 16.636x + 11211

Fig. 10: 3D UV spectra at 345nm for calibration of standard piperine

Estimation of EL-1 and PN-1in Different Formulations

The percentage of EL-1 marker was highest in LF and lowest in MF3 and in the order as LF>MF1>MF2>MF3. This

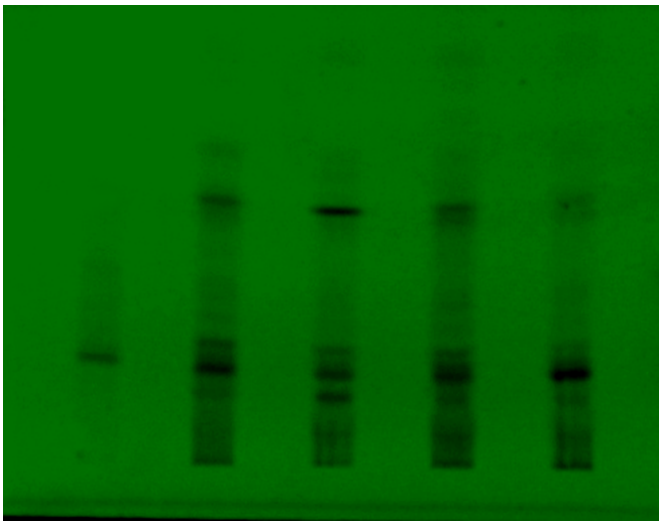


Fig. 11:HPTLC chromatograph of standard swertiamar in with all the formulation

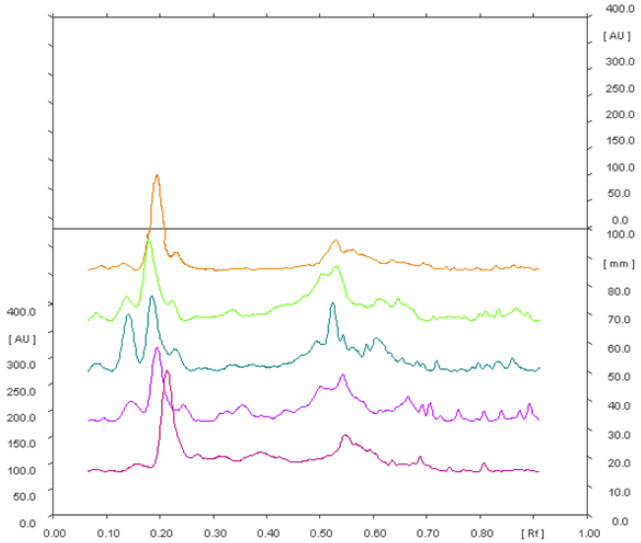


Fig. 12: Comparison of swertiamarin marker with all formulations. Key: violet- standard, purple-MF1, blue-MF2, green- MF3 and orange- LF.

Key: violet- standard, purple-MF1, blue-MF2, green- MF3 and orange- LF.

Table 6: estimation of EL-1 in different formulations.

Formulation	*Peak area*	*Peak area*	*Concentration in ng/µL from calibration curve*	*% of marker compound*
MF 1	2417	2417	263.4	2.6%
MF 2	2259	2259	241.1	2.4%
MF 3	1863	1863	185.3	1.8%
LF	2706	2706	304.1	3%

Table 7: Estimation of PN-1 in different formulations.

Formulation	*Peak area*	*Concentration in ng/µl from calibration curve*	*% of marker compound*
MF 1	18453	87.06	0.043%
MF 2	14671	41.58	0.02%
MF 3	13116	22.9	0.011%
LF	19214	96.2	0.048%

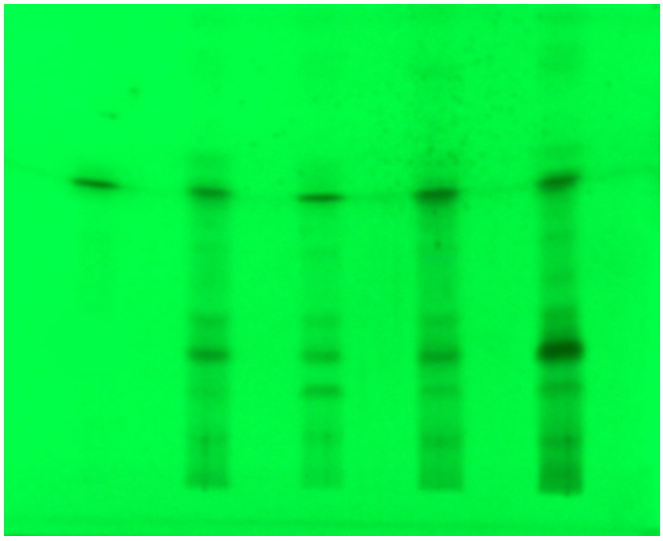


Fig. 13: HPTLC chromatograph of piperine marker with all the formulations

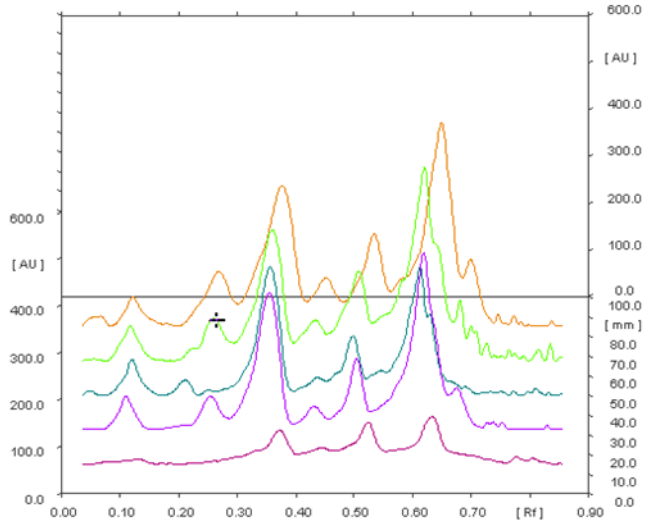


Fig 14: Comparison of standard piperine with all formulation
Key: violet- standard, purple-MF1, blue-MF2, green- MF3and orange- LF.

Key: violet- standard, purple-MF1, blue-MF2, green- MF3and orange- LF.

variation may be due to probable geographical differences of raw materials in Table 6.

The percentage of PN-1 marker was highest in LF and lowest in MF3 and in the order as LF>MF1>MF2>MF3. This variation may be due to probable geographical differences of raw materials in Table 7.

Discussion and Conclusion

Successive solvent extraction of all the four formulations and the chemical tests proved the presence of alkaloids, flavonoids, carbohydrates, tannins, phenols, and phytosterols.

Comparing the data from the melting point testing, TLC, UV, and IR studies of EL-1 and PN-1 with standard EL-1 and PN-1 through literature survey; EL-1 and PN-1 can be assumed to be the marker of EL and PL. Further, the calculation of EL-1's and PN-1's concentration was done with the help of the calibration equation and the peak area derived from the chromatogram. As a result, the concentration of EL-1 in MF1, MF2, MF3, LF was found as 2.6 gm/100 gm, 2.4 gm/100 gm, 1.8 gm/100 gm and 3 gm/100 gm respectively, and the concentration of PN-1 in MF1, MF2, MF3, LF was found to be 0.043 gm/100 gm, 0.020 gm/100 gm, 0.011 gm/100 gm and 0.048 gm/100 gm respectively.

The study showed that active principles EL-1 and PN-1 potential anti-diabetic activity, substantiating that the standardized bioactive phytocompound is more efficacious compared to MFs from the results. The phytocomponent formulation developed can be regarded as a promising natural and safe remedy for preventing diabetic complications. Further studies are required to establish a long-term safety profile, pharmacological screening, and assessment of the effect on vital and metabolic organs for the formulation for better results and ensure anti-diabetic activity.

Thus following this study, it can be concluded that the concentration of bioactive phytocompounds in LF is more than MFs. So LF is more efficacious to than of MFs.

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Abbreviations

%w/v	:	Percentage weight/Volume
%w/w	:	Percentage weight/weight
mg	:	Milligram
kg/cm^2	:	Kilogram/Centimeter
gm	:	Gram
DM	:	Diabetes mellitus
SSE	:	Successive Solvent Extraction
LF	:	Laboratory Formulation
MF1	:	Marketed Formulation 1
MF2	:	Marketed Formulation 2

MF3 : Marketed Formulation 3
EL-1 : Swertiamarin
PN-1 : Piperine
TLC : Thin Layer Chromatography
UV : Ultra Violet Spectroscopy
FTIR : Fourier Transfer Infrared Spectroscopy
HPTLC : High Performance Thin Layer Chromatography
EL : *Enicostemma littorale*
AH : *Aconitum heterophyllum*
PK : *Picrorhiza kurroa*
PL : *Piper longum*
M.P : Melting Point

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