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Marker-based standardization of Diatrin tablet ensuring safety and efficacy

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Abstract
The growing reliance on herbal formulations as safer alternatives to allopathic medicine necessitates the development of standardized products to ensure consistency, safety, and efficacy. This study focuses on the standardization and comparative evaluation of a marketed polyherbal antidiabetic formulation, Diatrin tablet, and its laboratory-prepared counterpart. The formulation comprises herbal constituents such as *Syzygium cumini*, *Gymnema sylvestre*, *Curcuma longa*, and others known for their antidiabetic and therapeutic properties. Granules were prepared using the wet granulation method, followed by evaluation for physicochemical parameters including bulk density, moisture content,angle of repose, Carr's index, and Hausner's ratio. The final tablets were analyzed through FTIR to assess drug-excipient compatibility, and standard quality control tests were performed, including weight variation, friability, hardness, and disintegration time. Both formulations were subjected to organoleptic, microscopic, and phytochemical evaluations. High-performance thin-layer chromatography (HPTLC) confirmed the presence of quercetin, a flavonoid marker, in both samples with Rf values ~0.9. Quantitative estimation of total flavonoid content using aluminum chloride colorimetric assay revealed 237.85 mg/g and 250.93 mg/g in marketed and laboratory-prepared tablets, respectively. The study demonstrates the suitability and reliability of developing a standardized herbal antidiabetic formulation that meets established pharmacognostic and pharmaceutical standards.

Keywords: Diatrin tablet, standardization, antidiabetic activity, physicochemical analysis and HPTLC

1. Introduction
Herbal Formulations is one of the oldest forms of medicine that people have ever encountered is herbal therapy, which uses whole plants or plant parts to treat a variety of severe diseases or promote overall health. Growing global interest in herbal medicine is largely driven by its widespread acceptance and reputation for offering health benefits with minimal or no side effects [1]. Herbal medication standardization refers to procedure for establishing an array for consistent guidelines and key characteristics to guarantee this items' performance, security, consistency, or purity. This involves developing and adopting technical standards based on systematic research, including experiments and observations. Through this process, specific criteria are identified, which define the attributes of the herbal formulations. As such, standardization plays a crucial role in maintaining quality control in herbal medicine production [2]. The concept of standardization expression encompasses all procedures implemented throughout production process along with quality assurance phases that ensure consistent and reproducible quality in Natural remedies [3]. On the WHO's recommendation, organizations for quality control and assurance procedures are set up that monitor GMP for herbal goods as well as licensing and labelling systems for their manufacture, distribution, and marketing in various nations [4]. Jiva Diatrin tablets have been considered as an appropriate treatment for people who wish to regulate their blood sugar levels because they are made with pure, natural extracts of powerful herbs that have been utilized since ancient Ayurvedic times. By supporting insulin secretion, regulating blood glucose levels, and strengthening the pancreas, the blend of herbal herbs revitalizes the body. The pancreas can be strengthened, urinary issues can be controlled, and metabolism can perform at its best.

2. Materials and Methods
2.1 Materials
Plant materials included powders of *Syzygium cumini*, *Gymnema sylvestre*, *Ficus racemosa*, *Emblica officinalis*, *Aegle marmelos*, *Curcuma longa*, *Momordica charantia*, *Trigonella foenum-graecum*, *Azadirachta indica*, and *Acacia nilotica*. Reagents and solvents used were

ethanol, methanol, aluminium chloride, potassium acetate, HPTLC plates, and standard quercetin.

2.2 Collection of plants and authentication
The plants involved on the label of marketed tablets include the leaves of *Syzygium cumini*, the rhizomes of *Curcuma longa*, bark of *Ficus racemosa*, the fruit of *Emblica officinalis*, and the fruit of *Aegle marmelos*. The leaves of Gymnema sylvestre, *Momordica charantia*, seeds of *Trigonella foenum-graecum*, *Azadirachta indica*, and *Acacia nilotica* were collected from nearby locations and every plant was verified by botanist Prof. Sanjay S. Sathe, Prof. of Botany [5].

2.3 Preparation of granules and compression of Tablet
Acacia and starch act as granulating agents, magnesium stearate acts as a lubricant, and methyl paraben is used as a preservative. To create homogeneous granules by wet granulation method a moist mass was made by passing through sieve number 16. A single punch compression machine was used to punch the prepared granules. Tablets of about 500 mg were produced. The following formula was used to prepare granules [6].

2.4 Evaluation of prepared granules [7]
Moisture level: The drying method was used to calculate the moisture content loss. An instrument called a moisture balance was used to determine it.

Angle of repose
The fixed funnel technique was used to determine prepared granules' angle of repose.

Angle of repose = $\tan^{-1}$ (h / r).

Bulk density
The cylinder method was used to determine granules' bulk density.

Density $_{(bulk)}$ = Granule's weight / Granule's bulk volume.

Tapped density
Tapped volume was determined by tapping measuring cylinder for 100 times. Tapped density was calculated as;

Density $_{(Tapped)}$ = Weight of granules / Volume $_{(tapped)}$

Carr's index
Carr's Index was calculated based on the values of bulk density and tapped density using the appropriate formula.

Carr's index= (Tapped density - Bulk density) × 100/ Bulk density

Hausner's ratio
To compute Hausner's ratio, divide the tapped density by the bulk density.

Hausner's ratio = Density $_{(Tapped)}$ / Density $_{(Bulk)}$

2.5 Compatibility study of drug and excipients by using FTIR spectroscopy
Compatibility investigation between the excipients used in formulation and herbal medications was conducted by using FT-IR. This pre-formulation study aims to evaluate the potential interactions between drug and the formulation's excipients [8]

2.6 Evaluation of marketed and laboratory prepared tablet [9]**: Weight variation test:** A random sample of twenty tablets was taken and each tablet's weight was determined separately. Mean weight for every tablet determined.

Hardness: A Monsanto hardness tester was used to determine the hardness of both marketed available tablet and those made in laboratories.

Friability
Using a Roche friabilator, the percentage friability of the tablets was determined by first selecting a sample of 20 tablets, recording their initial weights, then placing them inside the friabilator drum, which was rotated at 25 revolutions per minute for 4 minutes. The formula for calculating the percentage friability was as follows:

Percent friability= [W $_{(initial)}$ - W $_{(final)}$] $_{100}$ /W $_{(initial)}$

Disintegration test: Disintegration apparatus was used for this test. Six tablets were selected to evaluate the disintegration time. The beakers were filled with water as the disintegration medium. The water medium was kept at 370^0 C.

2.7 Pharmacognostic evaluation of tablet
Organoleptic evaluation [10]

- **Color:** A visual analysis of the tablet's color was done.
- **Odor:** Just a little of tablet powder was inhaled and the tablet's odor was examined.
- **Taste:** A small amount of the tablet was placed on the tongue, and its flavor was examined.

Physical evaluation
Extractive value [11]**: Alcohol soluble extractive value**
4 grams of tablet powder were put in a conical flask to measure extractive value that was soluble in alcohol, to which 100 ml ethanol was added. For one day, this solution was macerated. The finished solution was filtered after maceration. A porcelain plate containing 25 ml of the sample filtrate was used to evaporate it on a water bath heated at 105 degrees. After cooling in desiccators, the solid residue was weighed. Extractive value of alcohol soluble was computed.

Water soluble extractive value
4 grams of tablet powder and one hundred milliliters of distilled water were added to a conical flask in order to measure the water-soluble extractive value. It was macerate for a full day. 25 milliliters of the filtrate were moved to a porcelain plate after the solution had been filtered. After that, the sample was dried by evaporation in a water bath that was kept at 105°C. Weighing the remaining solid residue allowed us to determine the extraction value.

Ash value [12]
Total ash: For this procedure, 2 grams of tablet powder were transferred into a preheated silica crucible. That sample-containing crucible was kept in a muffle furnace. The muffle furnace was maintained between 400 and 500 degrees Celsius. White ash formed when the sample was burned, demonstrating the absence of organic matter. Following

incineration, cooled via desiccator and weight taken to calculate percentage.

Acid insoluble ash: After being weighed, the ash collected from the silica crucible was shifted to a 100 ml beaker. 25 ml of dil. HCl was used to boil this ash for 5 minutes. In order to remove any acidic residues, insoluble residue was gathered on ash less paper filters, cleaned with warm water, and subsequently burned at a temperature not higher than 450°C. Once chilled in a desiccator, the acid-insoluble ash content was measured.

Water soluble ash

After carefully weighing the total amount of ash from a previously fired sample, it was transferred to a 100 mL beaker. Five minutes were spent heating the mixture in a water bath after adding 25 milliliters of distilled water. Following heating, the solution was filtered, and hot distilled water was used repeatedly to wash the residue until no soluble salts were left in the filtrate. The insoluble ash collected on ashless filter paper was then ignited in a muffle furnace at a constant temperature between 400 and 500°C. Following cooling in a desiccator, the crucible was weighed accurately.

Chemical evaluation

All phytochemical tests are done by following standard procedures for alkaloids, tannins, flavonoids, reducing sugar, proteins, tannins and phenolic compounds etc.

Microscopic evaluation

Microscopic evaluation of tablet powder was done by using SAGLO SGL-11 Digital microscope with SAGLOSOFT Software version 2. The staining reagents used for evaluation are as; Phloroglucinol and Hydrochloric acid, Iodine, Sudan red and Glacial acetic acid etc.

2.8 Qualitative estimation of suitable biomarker by HPTLC [11, 12]

AETRON HPTLC system was used for analysis, which consists of SPRAYLIN-VI automatic TLC sample applicator Merck HPTLC plates on aluminum sheets covered with silica gel G60 F254 (0.2 mm thick) were used, along with an ILS micro syringe (100 µl capacity).

Solvent: Ethanol

Stationary phase - Aluminium-backed silica gel G60 F254 and F365 Thin-layer chromatography (TLC) plates measuring 10 cm by 10 cm with thickness of the layer was 0.2 mm (E-Merck, Darmstadt, Germany).

Mobile phase -Toluene, ethyl acetate, glacial acetic acid, ethyl alcohol and methanoic acid

[6: 3: 1(drop): 1: 1(drop)]

2.9 Determination of total flavonoid content [13]

The aluminum chloride technique's fundamental concept

A quercetin stock solution (1 mg/ml) was prepared in methanol. By appropriately diluting the stock solution with methanol, quercetin standard solutions with various concentrations were produced. 2.8 milliliters of distilled water, 0.1 milliliters of 10% aqueous aluminum chloride, 0.1 milliliters of 1 M potassium acetate, and 1.5 milliliters of 95% ethanol were combined with 0.5 milliliters of the standard solution to create a combination. For half an hour, mixture was incubated at room temperature, after which its absorbance was recorded at 445 nm using a UV spectrophotometer. Distilled water was substituted for aluminium chloride to create the blank solution. Aluminium chloride was added as well to a 0.5 ml sample of diatrin tablets. The calibration curve is used to determine the flavonoid concentration.

TFC= C× V/ M

3. Results and Discussion

Collection of plants and authentication

The plants which are used for formulation including *Syzygium cumini*, *Curcuma longa*, *Ficus racemosa*, *Emblica officinalis*, *Aegle marmelos*, *Gymnema sylvestre*, *Momordica charantia*, *Trigonella foenum- graecum*, *Azadirachta indica*, and *Acacia arabica* were collected from local areas of Sangli. Authentication done by botanist Prof. Sanjay S. Sathe, Prof. of Botany. After that, each plant part was chopped into little bits and dried. Using mixer, components of the dried plant were crushed into a smooth powder.

Preparation of tablet

All herbal powders and excipients were mixed together and uniform granules were prepared by using proper formulation method.

Table 1: Formula for tablet preparation

9	Ingredients	Quantity(mg)
1	*Syzygium cumini*	100
2	*Gymnema sylvestre*	100
3	*Ficus racemosa*	50
4	*Emblica officinalis*	50
5	*Aegle marmelos*	50
6	*Curcuma longa*	50
7	*Momordica charantia*	50
8	*Trigonella foenum-graecum*	50
9	*Acacia nilotica*	50
10	Magnesium stearate	5
11	Methyl paraben	5
12	Starch paste in neem decoction	QS

Fig 1: Laboratory prepared Granules.

Fig 2: Laboratory prepared tablets

Evaluation of prepared granules: Granules exhibited a moisture content of 5.72%. The measured bulk density and tapped density were 0.303 g/ml and 0.341 g/ml respectively. Carr's index was calculated to be 12.53%, while Hausner's ratio was 1.122. The angle of repose was determined to be 29.30°, indicating excellent flow characteristics.

Table 2: Evaluation of prepared granules

Sr. No.	Parameters	Mean Observation	±SD
1	Moisture content	5.723%	0.075
2	Bulk density	0.303 g/ml	0.004
3	Tapped density	0.341 g/ml	0.012
4	Carr's index	12.53%	0.045
5	Hausner's ratio	1.122	0.027
6	Angle of repose	29.93^0	0.394

Compatibility study of drug and excipients by using FTIR spectroscopy

The drug's unique peaks in the combinations did not change, with no significant shifts, loss of peaks, or formation of new peaks observed. This indicates the lack of a chemical reaction between the excipients and the drugs (API). The chemical compatibility of drug with the chosen excipients was validated by the lack of any significant spectrum modifications, indicating that it is suitable for additional formulation development.

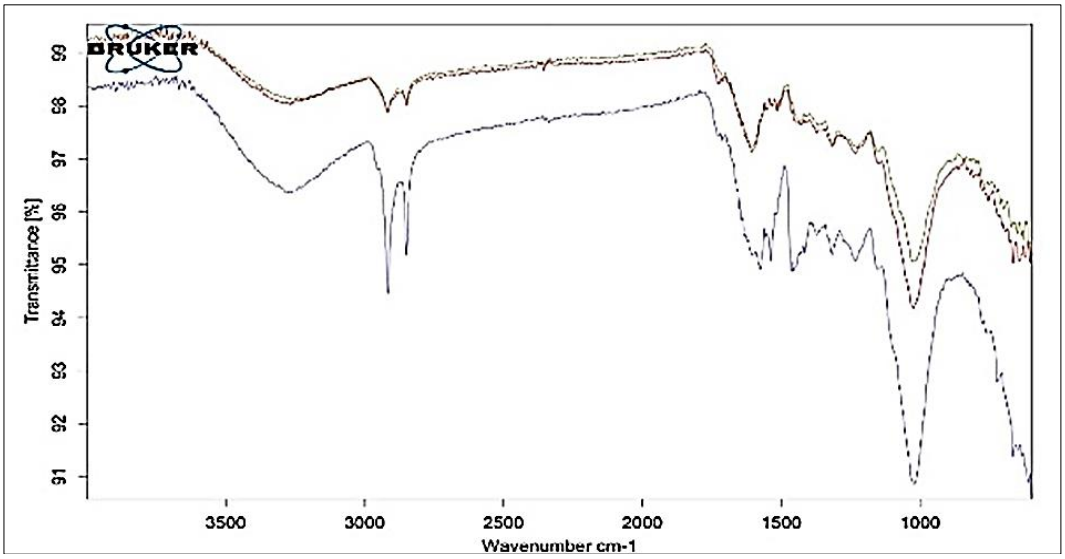

Fig 3: FTIR of Physical mixture of plant drug, Excipient Mixture and Physical mixture of herbal plants with excipients

Evaluation of marketed and laboratory prepared tablet

All tablets comply with the acceptable weight variation limit i.e. ±5%. Both marketed formulation and the laboratory-prepared tablets successfully met the criteria for the weight variation test. The friability of marketed tablet and laboratory prepared tablet was found to be 0.286% and 0.299% respectively. It was found within the limits i.e. NMT 1%. Hardness of marketed tablet was found to be 1.5 kg/cm2 and for laboratory formulation it was 1.5 kg.cm2. It shows no difference in hardness of marketed and laboratory prepared tablet. The disintegration times for the marketed and laboratory-prepared tablets were recorded as 22.45 minutes and 22.11 minutes, respectively. Both formulations complied with the disintegration test requirements.

Table 3: Evaluation of marketed and laboratory prepared tablet

Sample	Weight variation (Mean weight)	Hardness (kg/cm^2)	Friability (%)	Disintegration time (Minutes)
Marketed tablet	522.8 ± 0.7637	1.5 ± 0.00	0.286 ± 0.001	22.45 ± 0.050
Laboratory prepared tablet	492 ± 0.5000	1.5 ± 0.00	0.299 ± 0.001	22.11 ± 0.103

Pharmacognostic evaluation of tablet

Organoleptic evaluation: The organoleptic evaluation of the marketed tablet and laboratory prepared tablet revealed that both the tablets were yellowish green in colour along with a strong aromatic odour and the taste was slightly bitter.

Table 4: Organoleptic evaluation of tablet

Sample	Colour	Odour	Taste
Marketed tablet	Yellowish green	Aromatic	Slightly bitter
Laboratory prepared tablet	Yellowish green	Aromatic	Slightly bitter

Fig 4: Marketed Diatrin tablet

Fig 5: Laboratory prepared tablet

Physical evaluation

The alcohol-soluble extractive value of marketed Diatrin tablet was determined to be 10.3%. For laboratory prepared tablet by using excipients magnesium stearate, methyl paraben and starch this extracting value was found to be 11.5%. It was discovered that the marketed diatrin tablet had a water-soluble extractive value of 22.35%. The water-soluble extractive value for the tablet made in lab was 23.1%. The total ash content of the marketed tablet was measured at 11.35% w/w, while the laboratory-prepared tablet showed a total ash content of 10.63% w/w. The acid-insoluble ash content was determined to be 3.5% w/w for the marketed tablet and 2.9% w/w for the laboratory-prepared tablet. The water-soluble ash content was measured at 1.85% w/w for the marketed tablet and 2.45% w/w for the laboratory-prepared tablet.

Table 5: Extractive values of marketed and laboratory prepared tablets

Sample	Alacohol soluble extractive value		Water soluble extractive value	
	% w/w alcohol soluble extractive value	±SD	% w/w water soluble extractive value	±SD
Marketed tablet	10.3	0.0005	22.35	0.003
Laboratory prepared tablet	11.5	0.0006	23.1	0.005

Table 6: Ash values of marketed tablet and laboratory prepared tablets.

Sample	Total ash		Acid insoluble ash		Water soluble ash	
	% w/w Total ash	±SD	% w/w Acid insoluble ash	±SD	% w/w Water soluble ash	±SD
Marketed tablet	11.35	0.002	3.5	0.002	1.85	0.001
Laboratory prepared tablet	10.63	0.0015	2.9	0.001	2.45	0.001

Chemical investigation (Phytochemical evaluation)

The presence of carbohydrates, reducing sugars, flavonoids, alkaloids, tannins, and phenolic compounds was confirmed in both the marketed tablet formulation and the laboratory prepared tablet.

Table 7: Phytochemical evaluation of tablets (+ stands for Positive test, - stands for Negative test)

Sr. No	Chemical test	Marketed tablet	Laboratory prepared tablet
1	**Test for carbohydrates**		
	Molisch's test	+	+
2	**Test for reducing sugar**		
	Fehling test	+	+
	Benedict test	+	+
4	**Test for flavonoids**		
	Shinoda test	+	+
	Alkaline reagent test	+	+
	Zinc and HCl test	+	+
	Lead acetate test	+	+
4	**Test for proteins**		
	Biuret test	-	-
	Million's test	-	-
	Xanthoprotein test	-	-
5	**Test for alkaloids**		
	Dragendroff's test	+	+
	Hager's test	+	-
	Wagner's test	+	+
	Tannic acid test	-	-
6	**Test for tannins and phenolic compounds**		
	Ferric chloride solution test	+	+
	Lead acetate test	+	+
	Dil. Iodine solution test	-	+
	Dil. HNO3 solution test	-	+
	Bromine water test	-	+
7	**Test for Saponins**		
	Foam test	-	-

Microscopic evaluation

During observation of microscopic images; trichomes, vessels, stone cells, lignified cells, starch grains, oil glands and calcium oxalate crystals was found to be present in marketed as well as laboratory prepared tablet powdr.

A) In Phloroglucinol and HCl

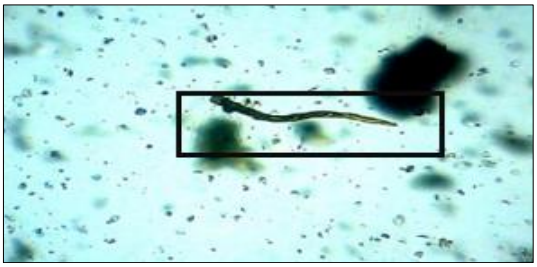

Fig 6: Trichome in marketed tablet

Fig 7: Trichome in laboratory prepared tablet

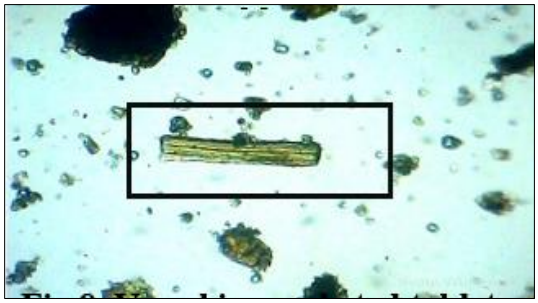

Fig 8: Vessel in marketed tablet

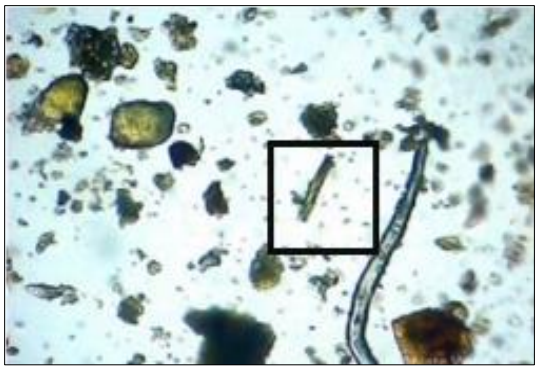

Fig 9: Vessel in laboratory prepared tablet

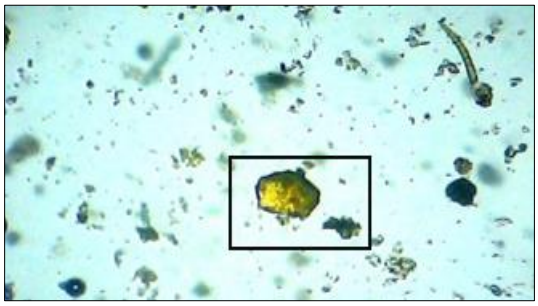

Fig 10: Stone cell in marketed tablet

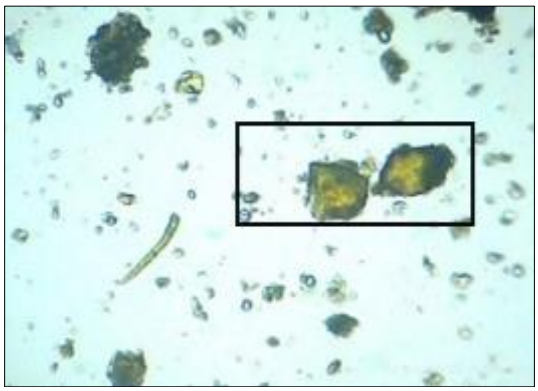

Fig 11: Stone cell in laboratory prepared tablet

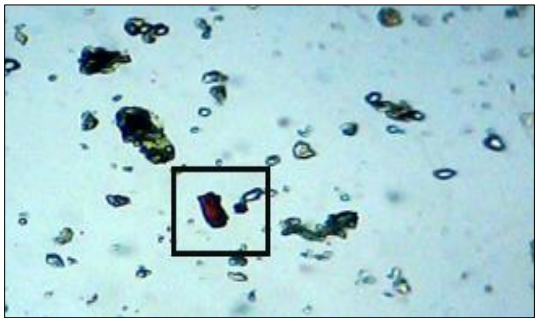

Fig 12: Lignified cell in marketed tablet

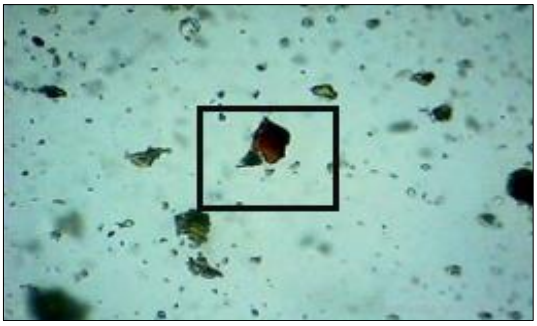

Fig 13: Lignified cell in laboratory prepared tablet

B) In Iodine

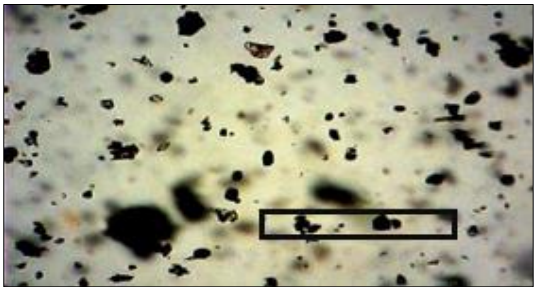

Fig 14: Starch grains in marketed tablet

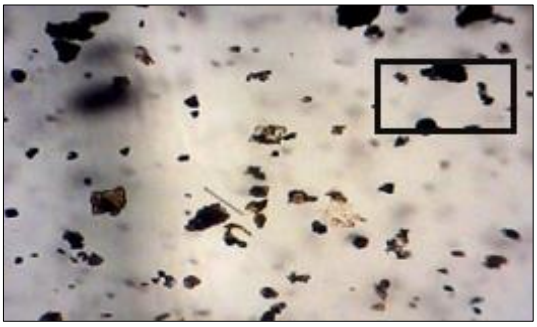

Fig 15: Starch grains in laboratory prepared tablet

C) In Sudan Red

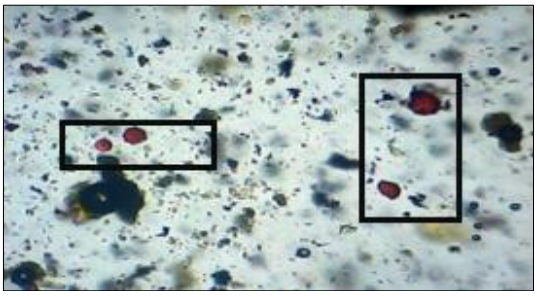

Fig 16: Oil glands in marketed tablet

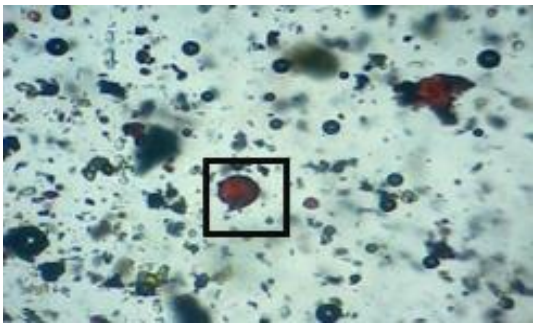

Fig 17: Oil glands in laboratory prepared tablet

D) In Glacial acetic acid

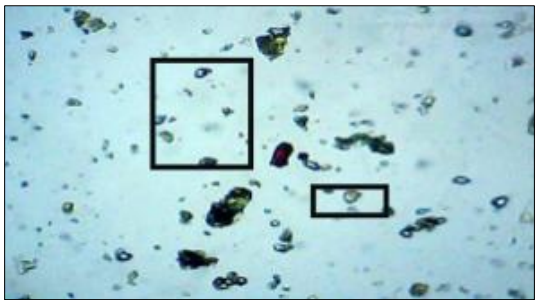

Fig 18: Calcium oxalate crystals in marketed tablet

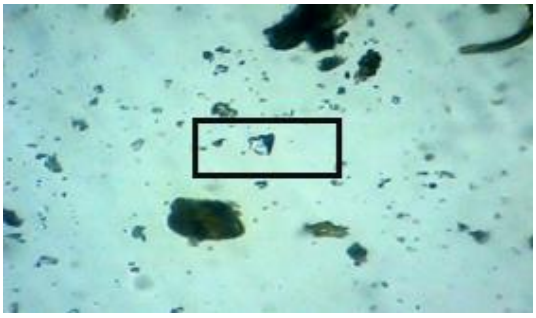

Fig 19: Calcium oxalate crystals in laboratory prepared tablet.

Qualitative estimation of suitable biomarker by HPTLC [14-16]

For Standard Quercetin Rf value was found to be **0.9**. Rf value of Quercetin present in marketed tablet and laboratory prepared tablet was found to be **0.92** and **0.91** respectively. The HPTLC analysis of Marketed Diatrin tablet and laboratory prepared tablet successfully revealed the presence of Quercetin under 365 nm. This flavonoid profile also provides a basis for further quantitative analysis and correlation with potential biomarkers of antidiabetic activity.

Fig 20: HPTLC plate under visible light

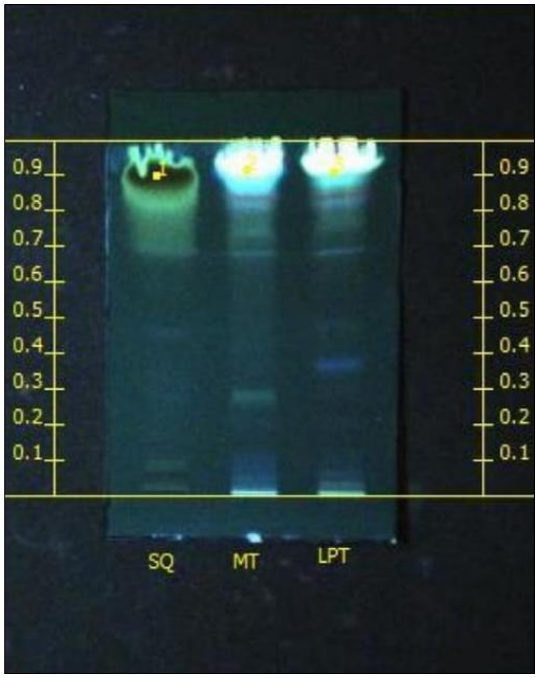

Fig 21: HPTLC plate scan under 365 nm

Determination of total flavonoid content

The standard quercetin solution's λ max was found to be 445 nm. Five standard solutions of quercetin at different concentrations were made and their absorbance was measured at 445 nm using UV spectrophotometer in order to create calibration curve. From the experimental work, the calibration curve of quercetin was obtained with equation: $y = 0.0007x + 0.0537$ $R^2 = 0.997$. TFC was assessed in both commercially available Diatrin tablet and the laboratory-formulated version using the aluminium chloride colorimetric technique, employing quercetin as the reference compound. The analysis revealed that the marketed tablet contained 237.85 mg/g of flavonoids, whereas the laboratory-prepared tablet showed a slightly higher amount of 250.93 mg/g. These findings align with expectations for polyherbal products known to contain flavonoid-rich components.

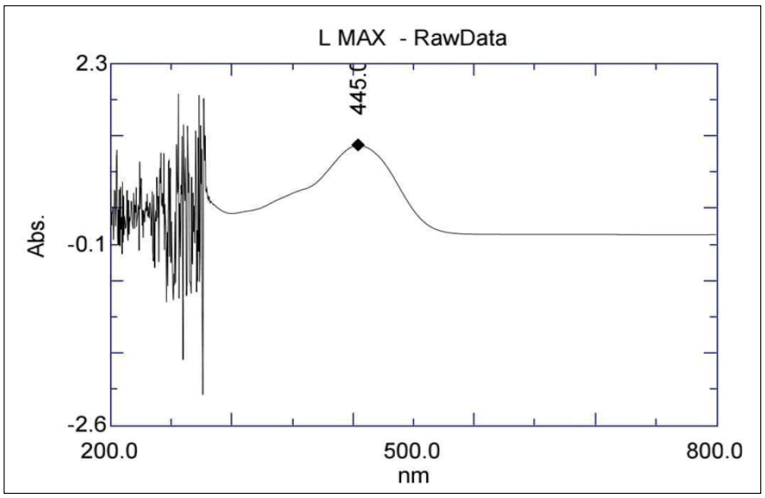

Fig 22: λ Max of Quercetin

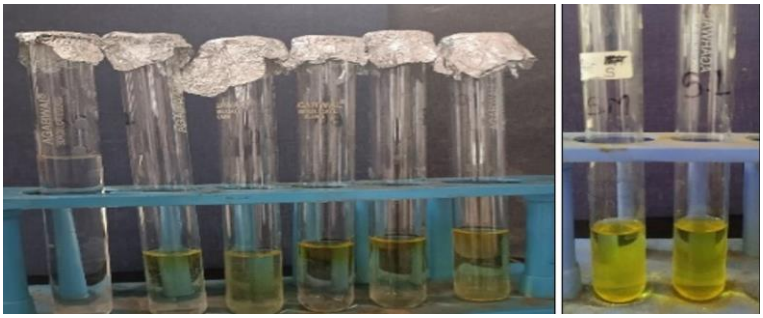

Fig 23: Dilutions of standard quercetin, Marketed tablet and laboratory prepared tablet

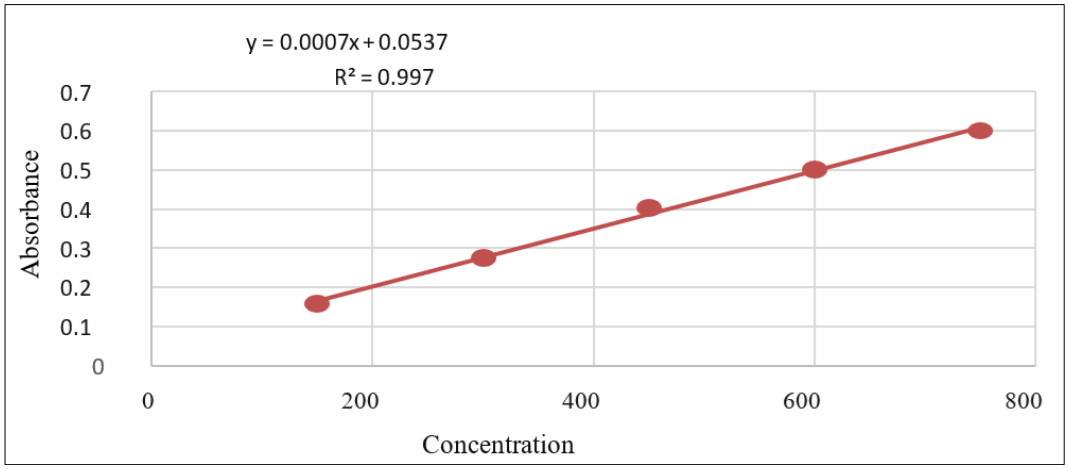

Fig 24: Calibration curve of Quercetin

Table 8: Absorbance of standard Quercetin dilutions

Concentration (µg/ml)	Volume of Stock (1000µg/ml)	Absorbance			Mean	SD	%RSD
150	1500µL	0.158	0.159	0.157	0.158	0.0010	0.6329
300	3000µL	0.274	0.275	0.276	0.274	0.0015	0.5474
450	4500µL	0.402	0.400	0.402	0.402	0.0005	0.1243
600	6000µL	0.501	0.503	0.502	0.501	0.0012	0.2395
750	7500µL	0.598	0.600	0.600	0.600	0.0034	0.5666

Table 9: Total Flavonoid Content (mg quercetin equivalent/g)

Sr. No.	Sample Name	Absorbance	Concentration of flavonoid in sample (µg/ml)	Total Flavonoid Content (mg quercetin equivalent/g)
1	Marketed tablet	0.372	454.714	237.85
2	Laboratory prepared tablet	0.405	501.857	250.93

4. Conclusion

The standardization study between the marketed tablet formulation and the laboratory-prepared replica was successfully conducted to assess standardization parameters. Physical evaluation (weight variation, hardness, friability, disintegration) demonstrated that both formulations fall within pharmacopeial limits, indicating acceptable tablet integrity and consistency. Chemical and microscopic analyses confirmed that the lab-prepared tablets exhibit comparable quality to the marketed product, with no major differences in internal structure or composition. Qualitative HPTLC profiling showed a similar phytochemical fingerprint of Quercetin, suggesting that the active constituents were effectively preserved and uniformly extracted in both formulations. Moreover, the TFC showed consistency between both samples, suggesting comparable levels of key bioactive compounds. From the findings, it can be inferred that laboratory-prepared tablet is both pharmaceutically and phytochemically comparable to the marketed formulation.

5. References

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