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FORMULATION AND STANDARDIZATION OF PATOLADI TABLET – A POLYHERBAL AYURVEDIC DRUG TO COMBAT CHRONIC TONSILLITIS IN CHILDREN

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ABSTRACT

Introduction: Patoladi tablet (PT) is a polyherbal formulation widely used in Ayurvedic clinical practice with multi fold benefits, specifically to combat chronic tonsillitis in children. There is no work on record on formulation and standardization

aspect of compound formulation Patoladi tablet. This paper highlights the preparation, physico-chemical characterization, TLC and HPTLC densitogram profiling of Patoladi tablet which can be applied for authentication of this herbal formulation.

Methods: Eleven herbal drugs namely Patola (Tricosanthus dioica), Shunti (Zingiber officinale), Harithaki (Terminalia chebula), Vibhithaki (Terminalia belerica), Amalaki(Emblica officinalis), Vishala (Cytrullus colocynthis), Brahmi (Bacopa monnieri), Katuki (Picrorrhiza kurroa), Haridra (Curcuma longa), Daruharidra (Berberis aristate), Guduchi (Tinospora cordifolia) were authenticated botanically. The tablets were prepared by combining all these drugs and subjected for detailed physico-chemical and HPTLC analyses.

Results: Set of standardization parameters were derived for the compounded tablet containing eleven herbs by physico-chemical characterisation. The tests proposed would serve as diagnostic parameters for the identity of this polyherbal formulation. HPTLC fingerprint profile which can serve as a fingerprint for the identification of the formulation has been obtained.

Conclusion: The proposed method of making tablet from eleven herbs will aid in yielding concentrated medicament with the same efficacy as per the classically proposed drug dosage at lower dose. Standards for the herbal formulation has been developed for the quality check of the formulation.

Keywords: Patoladi tablet, chronic tonsillitis, physico-chemical characterization, TLC and HPTLC densitogram

1. INTRODUCTION

The process of standardization of herbal medicines is more challenging as they contain more than one active principle and the active compound is frequently unknown **[1]**. Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety, and reproducibility **[2]**. Specific standards are set to carry out the experimentation, which would lead to the development of a set of characteristics exhibited by the particular herbal medicine. Standardization of Ayurvedic formulations is an important step for the establishment of a consistent therapeutic efficacy, a consistent chemical profile, or simply a quality

assurance program for production and manufacturing of herbal drugs **[3-4]**. World Health Organization (WHO) specific guidelines for the assessment of the safety, efficacy and quality of herbal medicines as a prerequisite for global harmonization are of utmost importance **[5]**.

Patoladi Kashaya yoga mentioned in Mukharoga chikitsa prathikarana of Bhaishajya Ratnavali, helps in curing all diseases of oral cavity by its oral administration **[6]**. The classical dosage forms of such yogas would be difficult to be administered to children due to undesirable palatability. Maintaining uniform dosage and storage of the drugs over a period in the classical described form are also difficult. Considering these facts, it was thought to develop tablets from plant parts which would be most appropriate mode of administration. More over the proposed method of making it into tablet will also aid in yielding concentrated medicament with the same efficacy as for the classically proposed drug dosage at lower dose. The drug thus prepared will also be benefited with enhanced shelf life of one year. The development of a composite standardization protocol for Patoladi yoga in tablet form is aimed in the current study.

2. MATERIALS AND METHODS

The eleven herbal drugs **(Table 1)** constituting Patoladi tablet are namely Patola (*Tricosanthus dioica*), Shunti (*Zingiber officinale*), Harithaki (*Terminalia chebula*), Vibhithaki (*Terminalia belerica*), Amalaki (*Emblica officinalis*), Vishala (*Cytrullus colocynthis*), Brahmi (*Bacopa monnieri*), Katuki(*Picrorrhiza kurroa*), Haridra (*Curcuma longa*), Daruharidra (*Berberis aristata*), Guduchi (*Tinospora cordifolia*) were procured from teaching pharmacy of Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan district, Karnataka state, India. The procured drugs were identified and authenticated at Department of Botany, Government Science College, Hassan, Karnataka state, India. The preparation of tablet was carried out at certified pharmacy. The procedures of standardization and HPTLC Photo documentation, Rf values, densitometric scan, 3-D chromatogram were done at SDM Centre for Research in Ayurveda and Allied Sciences, (AYUSH Centre for Excellence and Recognized SIROs by DSIR), Laxminarayana Nagar, P.O., Kuthpady – 574 118, Udupi, Karnataka state, India.

Table 1: showing ingredients of Patoladi tablet

S. No.	Drug	Latin name	Part used
1.	Patola	*Tricosanthus dioica*	Root
2.	Shunti	*Zingiber officinale*	Rhizome
3.	Harithaki	*Terminalia chebula*	Fruit
4.	Vibhithaki	*Terminalia belerica*	Fruit
5.	Amalaki	*Emblica officinalis*	Fruit
6.	Vishala	*Cytrullus colocynthis*	Root
7.	Brahmi	*Bacopa monnieri*	Root
8.	Katuki	*Picrorrhiza kurroa*	Rhizome
9.	Haridra	*Curcuma longa*	Rhizome
10.	Daruharidra	*Berberis aristata*	Stem-bark
11.	Guduchi	*Tinospora cordifolia*	Stem

2.1. Preparation of Patoladi tablet

The raw ingredients of Patoladi tablet was washed and dried **(Figure 1)**. The material (dried roots) was disintegrated **(Figure 2)** and pulverized and made into coarse powder **(Figure 3)** individually and passed through 100# sieve. At the same time coarse powder of drugs taken and 8 times water added, boiled and reduced to 1/4th **(Figure 4)**. The blended mass powder was given three days Bhavana with Patoladi Kashaya and another three bhavanas with lemon juice in a wet grinder **(Figure 5)**. After Bhavana process, it was dried well for four to five days under sunlight **(Figure 6)**. Then dried powder again pulverized to fine consistency **(Figure 7)**. The powder was weighed and compressed into tablets (average weight 500mg) with direct compression method through single rotatory tablet punch machine fitted with suitable dye **(Figure 8)**. In process of tablet compression, color, shape, hardness, uniformity in weight parameters as analyzed.

Figure 1: washed and dried ingredients of Patoladi tablet

Figure 2: disintegrated ingredients of Patoladi tablet

Figure 3: ingredients of Patoladi tablet made into coarse powder

Figure 4: coarse powder of drugs boiled and reduced to 1/4th

Figure 5: bhavana with Patoladi Kashaya and lemon juice

Figure 6: paste kept for drying under sunlight

Figure 7: dried powder again pulverized to fine consistency

Figure 8: prepared Patoladi tablets

2.2. Instrumentation and techniques of drug analysis

Loss on drying at 105°C

10 g of sample was placed in tared evaporating dish. It was dried at 105˚C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.

Total Ash

2 g of sample was incinerated in a tared platinum crucible at temperature not exceeding 450˚C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.

Acid insoluble Ash

To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ashless filter paper (Whatmann 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air-dried drug.

Water soluble ash

Boil the ash for 5 min with 25 ml of water; collect insoluble matter on an ashless filter paper, wash with hot water, and ignite for 15 min at a temperature not exceeding 450˚C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash with reference to the air-dried sample.

Alcohol soluble extractive

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95%). Shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value.

Water soluble extractive

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice. Take the average value.

Uniformity of weight

20 tablets selected randomly and weighed. The average weight was calculated. The individual weight of the tablet was taken. Not more than two of the individual weights deviate from the average weight by more than the percentage shown in the following table and none deviates by more than twice that percentage **(Table 2)**.

Table 2: Showing average weight of tablet and percentage deviation

Average Weight of tablet	Percentage deviation
80 mg or less	10
More than 80 mg but less than 250 mg	7.5
250mg or more	5

Hardness test

5tablets were taken and tested for hardness. The lower plunger was placed in contact with the tablet. The upper plunger was then forced against a spring by turning a threaded bolt until the tablet fractures. The force of fracture was recorded.

Disintegration time

The tank of the disintegration apparatus was filled with distilled water up to the mark. 750 ml of distilled water in each of the 1000 ml beaker was taken. The timer of the instrument was set for 60 minutes. The temperature of water in beakers to 37°C and that of water in the main tank to 37.5°C was maintained. One tablet was introduced into each tube and, added a disk to each tube. The assembly was suspended in the beaker containing water and the apparatus was operated. The time duration at which the tablet disintegrated was noted.

HPTLC

1gm of sample of Patoladi tablet sample was dissolved in 10.0ml of alcohol kept overnight and filtered. 3, 6 and 9µl of the above extract was applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (9.0: 1.0). The developed plates were visualized in short UV, long UV and then derivatised with Vanillin sulphuric acid reagent subsequently scanned under UV 254nm, 366nm and 620nm (after derivatisation). R_f, colour of the spots, densitometric scan and 3-D chromatograms were recorded.

3. RESULTS

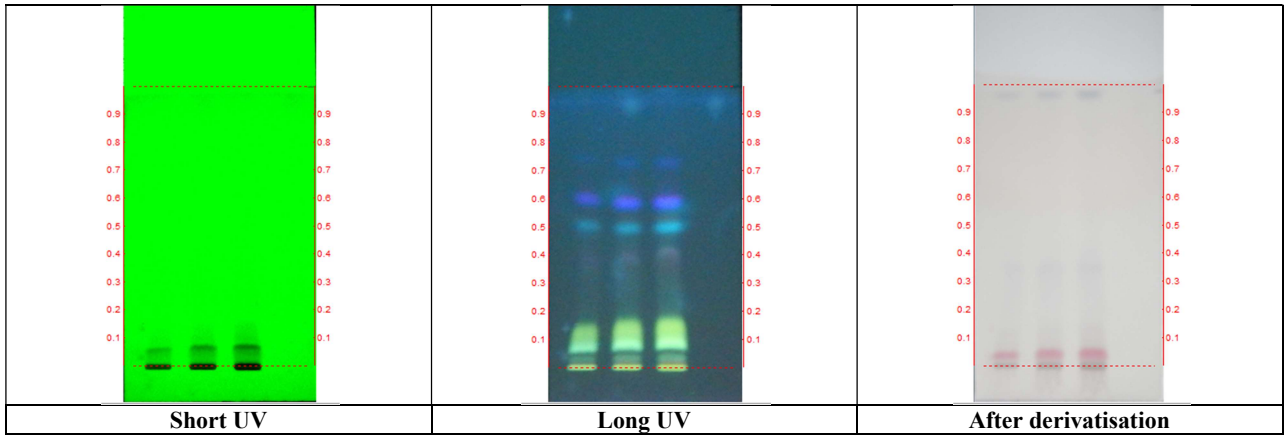
The standardization parameters of Patoladi tablet is detailed in table 3. The results standardization tests for Patoladi tablet is detailed in table 4. The HPTLC Photo documentation of sample of Alcoholic extract of Patoladi tablet is shown in **Figure 9**. The Rf values of sample of Patoladi tablet is detailed in **Table 5**. The densitometric scan of the sample of Patoladi tablet is shown in **Figure 10**.

Table 3: Standardization parameters of Patoladi tablet

Parameters	Results n = 3 %w/w
Loss on drying	6.30±0.00
Total ash	10.68±0.00
Acid insoluble ash	1.91±0.01
Water soluble ash	4.21±0.00
Alcohol soluble extractive	7.05±0.00
Water soluble extractive	22.54±0.01

Table 4: Results of standardization tests for Patoladi tablet

Parameters	% deviation allowed	Results n=3 %w/w
Tablet average wt		0.524
Tablet average wt (Average wt ± SEM)		0.524 ± 0.00
Variation in weight (%)	5.0%	None of the tablets are outside 0.4975 - 0.5499 so tablet passes the test
Hardness test (kg/cm^2)		2.0
Disintegration time (min: sec)		11:34



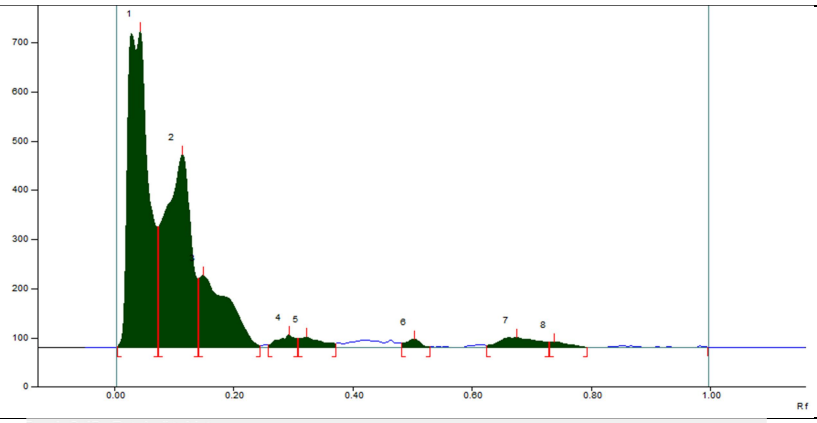
Short UV	Long UV	After derivatisation

Figure 9: HPTLC Photo documentation of sample of Alcoholic extract of Patoladi tablet Track 1: Alcoholic extract of Patoladi tablet- 3µl
Track 2: Alcoholic extract of Patoladi tablet- 6µl
Track 3: Alcoholic extract of Patoladi tablet- 9µl
Solvent system: Toluene: Ethyl acetate (9.0: 1.0)

Table 5: Rf values of sample of Patoladi tablet

Short UV	Long UV	After derivatisation
-	0.05 (F. green)	0.04 (Pink)
0.07 (Green)	0.07 (F. green)	-
-	0.13 (F. green)	-
-	-	0.35 (Purple)
-	0.40 (F. red)	-
-	0.50 (F. aqua blue)	-
-	0.59 (F. purple)	-
-	0.74 (F. blue)	-
-	-	-

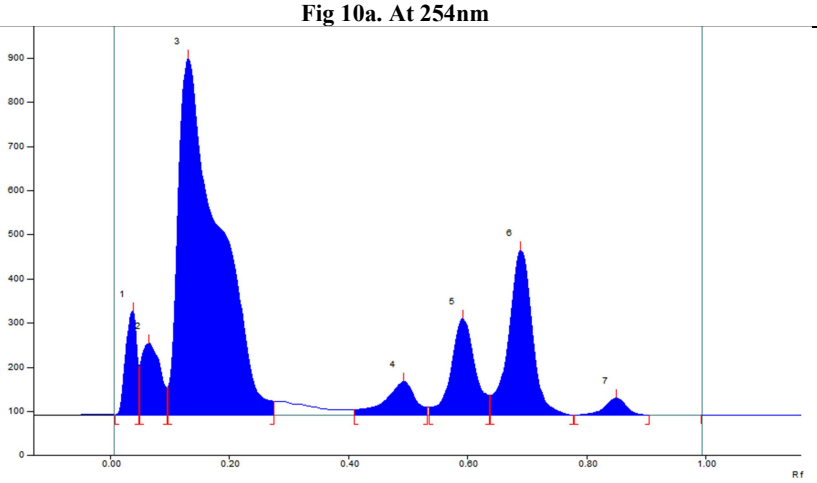
***F- fluorescent**



Track 3, ID: Patoladi tablet

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	2.2 AU	0.04 Rf	642.8 AU	50.31 %	0.07 Rf	45.1 AU	15747.1 AU	44.29 %
2	0.07 Rf	245.7 AU	0.11 Rf	391.8 AU	30.67 %	0.14 Rf	40.1 AU	11912.8 AU	33.51 %
3	0.14 Rf	140.7 AU	0.15 Rf	146.5 AU	11.47 %	0.25 Rf	3.6 AU	5301.7 AU	14.91 %
4	0.26 Rf	4.9 AU	0.29 Rf	26.0 AU	2.04 %	0.31 Rf	17.8 AU	530.0 AU	1.49 %
5	0.31 Rf	17.8 AU	0.32 Rf	21.6 AU	1.69 %	0.37 Rf	8.2 AU	568.9 AU	1.60 %
6	0.48 Rf	8.6 AU	0.50 Rf	17.2 AU	1.34 %	0.53 Rf	1.7 AU	300.4 AU	0.84 %
7	0.63 Rf	4.3 AU	0.68 Rf	20.2 AU	1.58 %	0.73 Rf	10.2 AU	928.3 AU	2.61 %
8	0.73 Rf	10.2 AU	0.74 Rf	11.7 AU	0.91 %	0.79 Rf	0.0 AU	262.1 AU	0.74 %

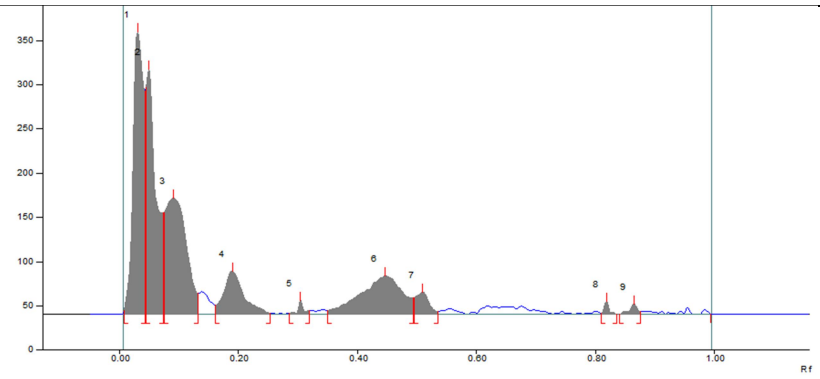
Fig 10a. At 254nm



Track 3, ID: Patoladi tablet

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.5 AU	0.04 Rf	235.3 AU	12.29 %	0.05 Rf	09.9 AU	3102.2 AU	4.66 %
2	0.05 Rf	110.7 AU	0.06 Rf	163.9 AU	8.57 %	0.10 Rf	62.0 AU	3681.3 AU	5.53 %
3	0.10 Rf	63.2 AU	0.13 Rf	808.0 AU	42.23 %	0.28 Rf	31.6 AU	39212.9 AU	58.92 %
4	0.41 Rf	12.6 AU	0.49 Rf	76.2 AU	3.98 %	0.53 Rf	17.9 AU	2760.1 AU	4.15 %
5	0.54 Rf	17.9 AU	0.59 Rf	218.4 AU	11.41 %	0.64 Rf	45.1 AU	6239.1 AU	9.37 %
6	0.64 Rf	45.2 AU	0.69 Rf	373.3 AU	19.51 %	0.78 Rf	0.0 AU	10548.8 AU	15.85 %
7	0.78 Rf	0.1 AU	0.85 Rf	38.4 AU	2.01 %	0.91 Rf	0.2 AU	1009.7 AU	1.52 %

Fig 10b. At 366nm



Track 3, ID: Patoladi tablet

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	4.5 AU	0.03 Rf	319.6 AU	36.02 %	0.04 Rf	52.4 AU	4046.7 AU	27.19 %
2	0.05 Rf	255.9 AU	0.05 Rf	276.9 AU	31.21 %	0.07 Rf	14.7 AU	3429.7 AU	23.04 %
3	0.08 Rf	115.1 AU	0.09 Rf	131.3 AU	14.79 %	0.13 Rf	23.4 AU	3360.9 AU	22.58 %
4	0.16 Rf	9.9 AU	0.19 Rf	48.8 AU	5.49 %	0.25 Rf	0.7 AU	1172.4 AU	7.88 %
5	0.29 Rf	1.2 AU	0.30 Rf	15.7 AU	1.77 %	0.32 Rf	4.1 AU	108.8 AU	0.73 %
6	0.35 Rf	4.2 AU	0.45 Rf	43.4 AU	4.89 %	0.50 Rf	18.2 AU	2174.5 AU	14.61 %
7	0.50 Rf	18.5 AU	0.51 Rf	25.2 AU	2.84 %	0.54 Rf	3.3 AU	398.0 AU	2.67 %
8	0.81 Rf	1.2 AU	0.82 Rf	14.7 AU	1.66 %	0.84 Rf	0.1 AU	77.7 AU	0.52 %
9	0.84 Rf	0.0 AU	0.87 Rf	11.8 AU	1.33 %	0.88 Rf	2.6 AU	114.7 AU	0.77 %

Fig 10c. At 620nm

Figure 10: Densitometric scan of the sample of Patoladi tablet

4. DISCUSSION

Polyherbal formulation standardization is a challenge in Ayurveda, this is one such attempt in quantitatively and qualitatively standardizing Patoladi tablet formulation. The moisture content in any formulation should be optimum as presence of moisture can harbor microbial growth the moisture content was 6.30%w/w, Total ash content which includes physiological and non-physiological ash amounts to 10.68%w/w, Acid insoluble ash accounts for 1.91%w/w which directs towards presence of earthy silicacious matters present, water soluble ash 4.21%w/w is physiological in nature. Water soluble extractive value refers to polar constituents dissolved in water 22.54% and ethanol soluble extractive values is indicative of mid polar and polar constituents of the formulation getting dissolved in ethanol was 7.05%w/w is suggestive of the fact that there are more polar constituents present than mid polar and non-polar constituents.

The average tablet weight was found to be 0.524, since the deviation allowed for tablets weighing more than 250mg is 5%, the tablet fall between lower and upper limit of deviation allowed. Tablet hardness determined using Monsanto tablet hardness tester was 2.0kg/cm^2 which means tablet is hard enough to bear the impact during shipping and wear and tear at the same time it holds its integrity as a dosage form.

Disintegration time for the sample of Patoladi tablet is 11min 34sec. HPTLC densitometric scan at 254nm showed 8 peak among which peaks at Rf 0.04 (44.29%), 0.11 (33.51%), 0.15 (14.91%) were major ones, at 366nm showed 7 peaks major ones being Rf 0.13 (58.92%) and 0.69 (15.85%), at 620nm there were 9 peaks present among which 0.03(27.19%), 0.05(23.04%), 0.09(22.58%), 0.45(14.61%) were major peaks.

5. CONCLUSION

Standardization is needed to establish quality control parameters for each traditional drug before it is released for use without the fear of toxicity and contamination. The unique R_f values, densitometric scan and densitogram obtained at different wavelengths pre- and post-derivatisation can be used as fingerprint to identify the polyherbal formulation, Patoladi tablet and can be used as reference while setting the pharmacopoeial standards to ensure the quality. Hence, efforts have been made to provide scientific data on formulation and standardization of Patoladi tablet. The novel concentrated compound formulation Patoladi tablet thus developed yield better acceptability in terms of palatability, increased shelf life and as compared to prescribed forms in a lesser dose.

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7. DATA AVAILABILITY: The data pertaining to the manuscript will be made available to the public after query.

8. CONFLICTS OF INTEREST: None declared.

9. FUNDING INFORMATION: Self-funded.

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