

Evaluation of Leaf Extract of *Amorphophallus Paeoniifolius* for Antioxidant potential, TPC, TFC and HPTLC Fingerprinting

Payal Patel^{1*}, Heta Kachhiya²

¹Student, Department of Pharmaceutical Chemistry and Analysis, Indukaka Ipcowala College of Pharmacy, A constituent college of The Charutar Vidya Mandal University, New V. V. Nagar, Anand, Gujarat, India

²Assistant Professor, Department of Pharmaceutical Chemistry and Analysis, Indukaka Ipcowala College of Pharmacy, New V. V. Nagar, Anand, Gujarat, India

***Corresponding Author**

E-Mail ID: hetaben.kachhiya@cvmu.edu.in

ABSTRACT

Amorphophallus paeoniifolius plant belongs to Araceae family, commonly known as Jimmikan, White spot arum, jungli suran, etc. The plant has been investigated and found to have many medicinal properties. Leaves of *Amorphophallus paeoniifolius* are having medicinal values such as anti-diarrhoeal, anti-bacterial properties. Also, recently 13 flavonoids have been isolated from the leaves of *Amorphophallus paeoniifolius*. The present research evaluates the estimation of methanolic extract of leaves of plant for Total Phenolic Content and Total Flavonoid Content. Total phenolic content in methanolic extract of leaf was found to be 21.43 mg/g GAE, along with this total flavonoid content was found to be 11.96 mg/g QE. High performance thin layer chromatography (HPTLC) profile of the methanolic leaf extract was also performed to compare with standard biomarkers such as Resveratrol, Lupeol and β -sitosterol using silica gel G60 F₂₅₄ as stationary phase and Benzene: Ethyl acetate (9:1 v/v) as mobile phase. The quantitative study was carried out using HPTLC which demonstrates that methanolic extract of leaf contains Resveratrol, Lupeol and β -sitosterol with R_f values of 0.03, 0.53 and 0.36 respectively.

Keywords: *Amorphophallus paeoniifolius*, total phenolic content and total flavonoid content, high performance thin layer chromatography (HPTLC), antioxidant potential of *Amorphophallus paeoniifolius* leaf extract

INTRODUCTION [1]

Amorphophallus paeoniifolius (Dennst.) Nicolson also commonly known as Elephant foot yam belongs to family Araceae is an Ayurvedic medicinal plant which is distributed through India.

A strong herbaceous plant with underground hemispherical discouraged dim earthy colored corm; leaves compound, huge, singular, petiole bold, mottled, 60-90cm long, pamphlets 5-1.25cm long of variable width, obovate or oval, intense, unequivocally and many-nerved; male and female inflorescence

touching, fixes missing, member of spadix sub globose or shapeless, equalling or longer than the ripe area, spathe campanulate, pointed, firmly, firmly veined, greenish pink remotely, base inside purple, edges recurved, undulate and crisped, male inflorescence sub turbinate, female 7.5cm or all the more lengthy; natural products obovoid 2-3 cultivated red berries. Parts used is corms.

Properties and uses are astringent, anti-inflammatory, anti-haemorrhoidal, haemostatic, expectorant, carminative, etc. They are valuable in vitiated states of vata

and kapha, elephantiasis, cancers, aggravations, retching, hack, asthma, etc.

MATERIAL AND METHOD[2-4]

Instruments

UV-Visible double beam spectrophotometer with matched quartz cell

Model: UV1900i, Software: Lab-solution, Wavelength range: 200-800, Scan speed: Medium

High performance thin layer chromatography (HPTLC)

Model: Camag Linomat 5 (Semi-automatic applicator), Software: winCATS 1.4.8, Chamber: Twin trough glass chamber (10×10 cm, 20×10 cm), Scanner: Camag TLC Scanner 4, Stationary Phase: pre-coated TLC Silica gel 60 F₂₅₄

Chemicals: Methanol (AR grade), n-Hexane (AR grade), alpha – naphthol solution (LR grade), conc. H₂SO₄ (AR grade), 4% NaOH (LR grade), 1% CuSO₄ (LR grade), 5% Ninhydrin solution (LR grade)

Preparation of Leaves Powder

The leaves of *Amorphophallus paenoniifolius* were collected, washed with tap water. Leaves kept in the shade for a period of 15 days for drying. Afterward, the were crushed into powder using mixer and passed through sieve no. #60 to obtain uniform fine powder which is further stored in air tight container.

Preparation of Extract

Soxhlet extraction method was used to prepare methanolic extract of leaf powder. 10 gm of powder was weighed and placed in thimble. Soxhlet extractor was placed onto a flask containing solvent n- Hexane in quantity of 200 ml and process was continued for 3 hours (190 cycle) to remove chlorophyll along with oily and fatty material. Then marc was placed in new thimble and Soxhlet extraction was

performed by using 250 ml of methanol for 8 hours. The methanol extract was transferred in porcelain bowl and allowed to evaporate methanol naturally.

Phytochemical Tests

Test for carbohydrate

Molisch's test: To 2-3 ml of fluid concentrate, add not many drops of alpha-naphthol arrangement, shake and add conc. H₂SO₄ from sides of test tube. Violet ring is framed at the intersection of two fluids.

Test for protein

Biuret test: To 2-3 ml of watery concentrate, add 4% NaOH and scarcely any drops of 1% CuSO₄ arrangement. Violet or pink tone shows up.

Test for amino acids

Ninhydrin test: Heat 2-3 ml of aqueous extract and 3 drops of 5% Ninhydrin solution in the boiling water bath for 10 min. purple or bluish colour appears.

Test for steroid

Salkowski reaction: To 2ml of fluid concentrate, add 2ml chloroform and 2 ml conc. H₂SO₄. Shake well. Chloroform layer seems red and corrosive layer shows greenish yellow fluorescence.

Liebermann- Burchard reaction: Blend 2ml of concentrate with chloroform. Add 1-2ml acidic anhydride and 2 drops conc. H₂SO₄ from the side of test tube. First red, then, at that point, blue lastly green tone shows up

Test for glycosides

Test for Cardiac Glycosides:

Legal's test: To aqueous extract, add 1ml pyridine and 1ml sodium nitroprusside. Pink to red colour appears.

Test for Anthraquinone Glycosides:

Borntrager's test: To 3ml of concentrate, add dil. H₂SO₄. Bubble and channel. To cold filtrate, add equivalent volume

benzene or chloroform. Shake well. Separate the natural dissolvable. Add smelling salts. Ammoniacal layer becomes pink or red.

Modified Borntrager's test: To 5ml of concentrate, add 5ml 5% FeCl₃ and dil. HCl. Heat for 5 min in bubbling water shower. Cool and add benzene or natural dissolvable. Shake well. Separate natural layer, add equivalent volume weaken alkali. Ammoniacal layer shows pinkish red tone.

Test for Saponin Glycosides

Foam test: Shake the drug extract vigorously with water. Persistent stable foam observed.

Test for Coumarin Glycosides

Aqueous extract when made alkaline, shows blue or green fluorescence.

Test for Flavonoids

Shinoda test: to remove, add 5ml 95% ethanol, barely any drops conc. HCl and 0.5g magnesium turnings. Orange, pink, red to purple tone shows up.

Test for Tannins and Phenolic compounds

5% FeCl₃ solution: To 2-3ml aqueous extract, add few drops of 5% FeCl₃ solution. Deep blue- black colour appears.

Lead acetate solution: To 2-3ml aqueous extract, add few drops of lead acetate solution. White ppt. observed.

Dil. KMnO₄: To 2-3ml aqueous extract, add few drops of dil. Potassium permanganate solution. Decolouration appears.

Test for Alkaloids

Dragendorff's test: To 2-3ml aqueous extract, add few drops of Dragendorff's reagent. Orange brown ppt. is formed.

Wagner's test: To 2-3ml aqueous extract, add few drops Wagner's reagent. Reddish brown ppt. is formed.

In Vitro Anti-Oxidant Activity

Total phenolic content (TPC)

Preparation of standard stock solution

Accurately weigh 10mg of gallic acid and dissolve it in 10ml of methanol to make concentration of 1000µg/ml. From the above arrangement 1ml is removed in one more 10ml volumetric cup and make up with methanol (100µg/ml).

Preparation of test solution (extract)

For test solution (extract), take 100 mg of extract and dissolve it in 100 ml with methanol (1000µg/ml).

Estimation of total phenolic content

Total phenolic content was determined with Folin-Ciocalteu reagent. A calibration curve was obtained by using gallic acid as standard. Different concentrations of gallic acid (5-50µg/ml) were prepared by withdrawing 0.05ml, 0.1ml, 0.2ml, 0.3ml and respectively from stock solution in 10ml volumetric flask. Now from each these above prepared sets 1ml of gallic acid was taken in 25ml volumetric flask and mixed with 10ml of distilled water and 1.5ml of Folin-Ciocalteu reagent. After 5 mins of incubation, add 4ml 20% Na₂CO₃. Make up to 25ml with distilled water and keep mixture aside for 30 min. The absorbance was measured at 765nm in a spectrophotometer to determine the total phenolic contents using the formula,

$$C = C_1 \times v/m$$

Where, C= total phenolic content in mg/g in GAE

C₁ = concentration of Gallic acid in mg/ml

V = volume of extract in ml
m = weight of plant extract in g.

Total flavonoid content (TFC)

Preparation of standard stock solution

Accurately weigh 10mg of quercetin and dissolve it in 10ml of methanol to make concentration of 1000µg/ml. From this withdraw 2.5ml and up to 25ml with methanol(100µg/ml). Preparation of test solution(extract): For test solution (extract), take 100mg of extract and dissolve it in 100 ml with methanol (1000µg/ml).

Estimation of total flavonoid content

Total flavonoid content was determined by Aluminium chloride method using

quercetin as a standard. Different concentrations of quercetin (10- 50µg/ml) were prepared by withdrawing 1ml, 2ml, 3ml and respectively from quercetin stock solution 10ml volumetric flask. Now from each of these above prepared sets 1ml of solution was mixed with 4ml distilled water and 0.3ml 5% sodium nitrite. Allow it to stand for 5 mins. Add 0.3ml of aluminium chloride and again incubate for 5 mins. Then add 2ml 1M NaOH and make up to 10ml with distilled water. The absorbance was measured at 510nm to determine the total phenolic contents using the formula,

$$C = C_1 \times v/m$$

Where, C= total phenolic content in mg/g in GAE

C₁ = concentration of Gallic acid in mg/ml

V = volume of extract in ml

m = weight of plant extract in g.

DPPH (1,1- diphenyl 1-2-picryl-hydrazil) free radical scavenging activity

Preparation of 0.1mM DPPH

Weigh accurately 4mg of DPPH and mix with 100ml of methanol to produce 0.1mM concentration. Preparation of standard stock solution: Weigh 10mg ascorbic acid powder in 10ml volumetric flask and make up with methanol to produce 1000µg/ml. Preparation of test solution (extract): Weigh 10mg of extract in 10ml of volumetric flask and make up with methanol to produce 1000µg/ml.

Assay of DPPH scavenging

For both standard solution and test solution concentration set of 10- 100µg/ml were prepared. From standard solution 0.1ml, 0.2ml, 0.4ml and so on were withdrawn to produce 10µg/ml, 20µg/ml, 40µg/ml respectively. Now from each of these above sets of prepared concentration 1ml was taken and mixed with 3ml of methanol and 1ml 0.1mM DPPH solution. Incubate for 45 mins at room temperature, measure absorbance against blank solution at 517nm in UV spectrophotometer.

$$\% \text{ of antioxidant activity} = \frac{[(Ac-As)]}{Ac} \times 100$$

Were, Ac- Control reaction absorbance;

As- Testing specimen absorbance.

HPTLC Screening of Methanolic Leaf Extract

Preparation of standard stock solution

Individually weigh 10 mg of Resveratrol, Lupeol, β -sitosterol in 10ml of volumetric flask and make up with methanol to produce 1000 μ g/ml of each.

Using Linomat 5 applicator, 1 μ l (5000ng/band) and 2 μ l (10000ng/band) of leaf extract was applied by means of Hamilton syringe on 5 $\times$ 10 cm precoated silica plate (G20-F₂₅₄ aluminium sheets). Three bands of 2 μ l (2000ng/ml) each of standard solution of Resveratrol, Lupeol, β -sitosterol was applied on another plate. Mobile phase containing Benzene: ethyl acetate (9:1v/v) was saturated for 15 mins and after that plates were put for chromatographic development up to 8 mm distance on chromatographic plate. HPTLC of leaf extract was scanned under 280 nm for lupeol and resveratrol before derivatization and scanned at 468 nm after

derivatization with anisaldehyde sulphuric acid reagent for estimation of β -sitosterol.

RESULTS AND DISCUSSION

Preparation of Leaves Powder

Collected leaves powder of *Amorphophallus paeoniifolius* were weighed after sieving process and final weight of leaves powder was found to be 32.3 gm and it was green in colour.

Preparation of Methanolic Leaf Extract

Soxhlet apparatus was used to achieve methanolic leaf extract of *Amorphophallus paeoniifolius* from 10 gm of leaves powder, extract obtained 1.93 (n=3) $\pm$ 0.052. Colour of leaf extract obtained was dark green and texture was sticky.

Result of Phytochemical Tests

Phytochemical analysis on methanolic leaf extract showed presence of carbohydrates, steroids, anthraquinone glycosides, coumarin, flavonoids, alkaloids, tannins and phenolic compound.

Table 1: Phytochemical test on methanolic leaf extract.

Sr No.	Phytoconstituents	Chemical Tests	Observation	Remark
1.	Carbohydrate	Molisch's test		Present
2.	Protein	Biuret test		Absent

3.	Amino acids	Ninhydrin test		Absent
4.	Steroids	Salkowski test		Present
5.	Steroids	Liebermann-Burchard test		Present
6.	Cardiac glycoside	Legal's test		Negative
8.	Anthraquinone Glycosides	Borntrager's test		Present

9.	Anthraquinone Glycosides	Modified Borntrager's test		Present
10.	Saponin Glycosides	Foam test		Absent
11.	Coumarin Glycosides	Fluorescence test		Present
12.	Flavonoids	Shinoda test		Present
13.	Tannins and Phenolic compounds	5% FeCl ₃		Present
14.	Tannins and Phenolic compounds	Lead acetate solution		Present

15.	Tannins and Phenolic compounds	Dil. KMnO_4			Present
16.	Alkaloids	Dragendorff's test			Present
17.	Alkaloids	Wagner's test			Present

In vitro Antioxidant Activity

Total phenolic content estimation

Total phenolic content present in mg/g GAE in methanolic leaf extract was found to be 21.43 mg/g.

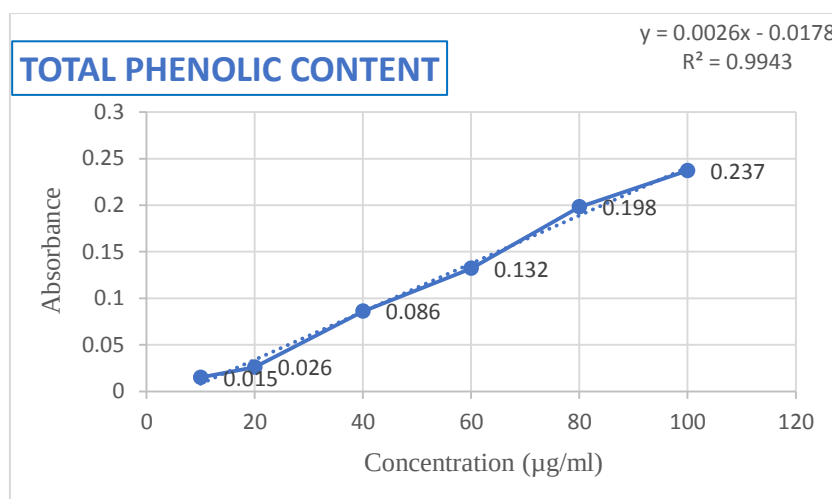


Fig. 1: Calibration curve of Gallic acid (10-100 $\mu\text{g/ml}$).

Total flavonoid content estimation

Total Flavonoid content present in mg/g QE methanolic leaf extract was found to be 11.96 mg/g.

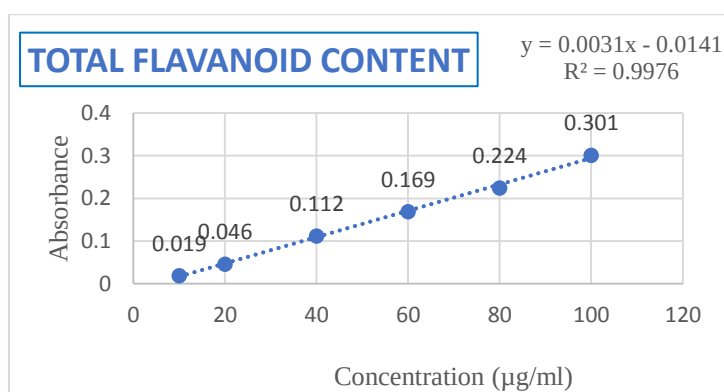


Fig. 2: Calibration curve of Quercetin (10-100µg/ml).

DDPH (1,1- diphenyl 1-2-picryl- hydrazil) scavenging assay

DPPH free radical scavenging activity showed that maximum % inhibition for standard Ascorbic acid was found to be 85.71% and for methanolic leaf extract maximum % inhibition was 88.36%.

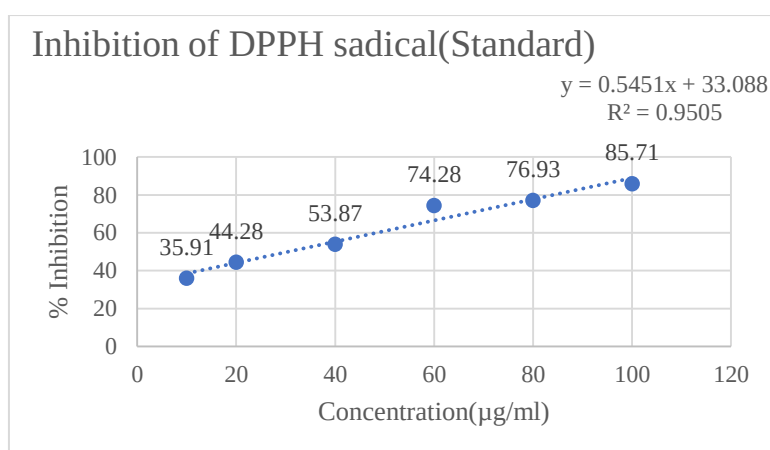


Fig. 3: Inhibition of DPPH free radical by ascorbic acid.

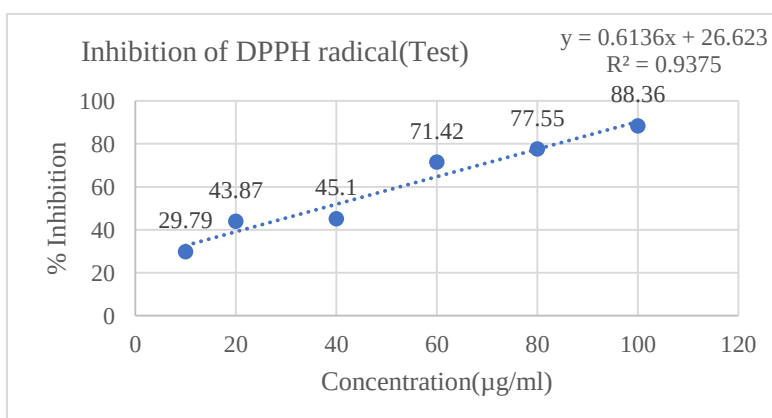


Fig. 4: Inhibition of DPPH radical by methanolic leaf extract.

HPTLC Screening of Methanolic Leaf Extract

HPTLC data showed presence of bands of Resveratrol, Lupeol and β -sitosterol were observed with the R_f value of 0.03, 0.53

and 0.36 respectively. In methanolic leaf extract also, bands at same R_f values were obtained which confirms the presence of selected three biomarkers in it.

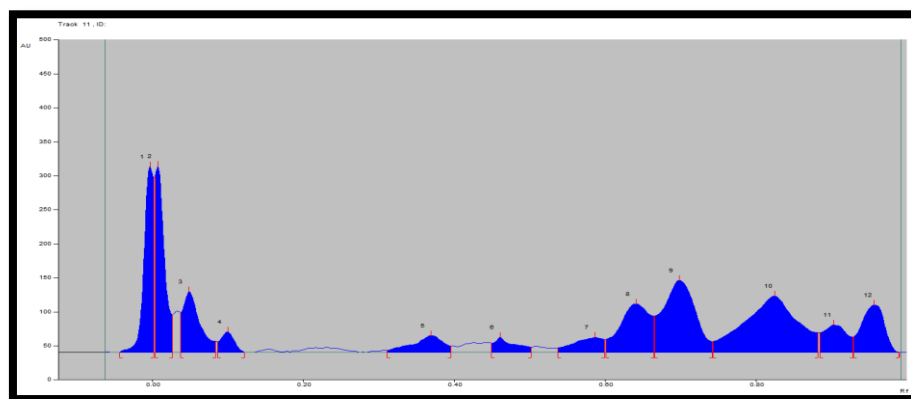


Fig. 5: Densitogram of methanolic extract of leaf.

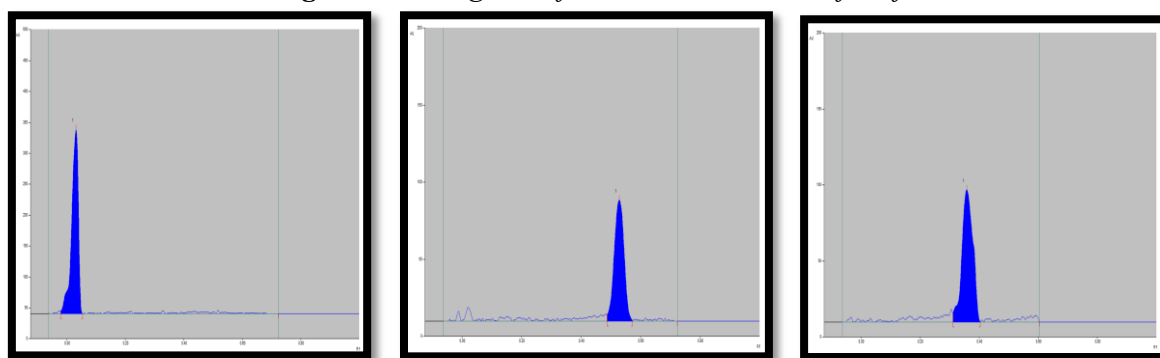


Fig. 6: Densitogram of Resveratrol (0.03), Lupeol (0.53), β -sitosterol (0.36).

CONCLUSION

Leaves of *Amorphophallus paeoniifolius* possess various pharmacological effects such as anti-bacterial and anti-diarrhoeal, etc. Powder of leaves was used to prepare methanolic extract of leaves using Soxhlet apparatus, extractive value obtained was 1.93 gm which was sticky in texture. This study finding's reveals that presence of numerous phytoconstituents like carbohydrate, steroids, anthraquinone glycosides, cardiac glycosides, coumarin glycosides, flavonoids, alkaloids, tannins and phenolic compounds. Total phenolic content in methanolic extract of leaf was found to be 21.43 mg/g GAE, along with this total flavonoid content was found to be 11.96 mg/g QE. In vitro antioxidant

potential of methanolic leaf extract was carried out by DPPH free radical scavenging activity and maximum % inhibition for standard Ascorbic acid was found to be 85.71% and for methanolic leaf extract maximum % inhibition was 88.36%. The qualitative study was carried out using HPTLC which demonstrates that methanolic extract of leaf contains Resveratrol, Lupeol and β -sitosterol with R_f values of 0.03, 0.53 and 0.36 respectively.

REFERENCES

- Warrier, P. K. (1993). *Indian medicinal plants: a compendium of 500 species* (Vol. 5). Orient Blackswan.

2. Khandelwal, K.R. & Sethi, V.K. *Practical Pharmacognosy*, 26th Edn. Nirali Prakashan, 25.1- 25.6.
3. Siddiqui, N., Rauf, A., Latif, A., & Mahmood, Z. (2017). Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (*Nepeta bracteata* Benth). *Journal of Taibah university medical sciences*, 12(4), 360-363.
4. Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., ElSohly, M. A., & Khan, I. A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. *Evidence-based complementary and alternative medicine*, 2014.

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Availability of Data and Material

The data presented in this research work is based on experimental work conducted by the author in the pharmaceutical chemistry and analysis department of Pharmacy, Indukaka Ipcowala College of Pharmacy, India.

Compliance with Ethical Standards Conflict of Interest

The authors have no conflict of interest.

Code Availability Software

Lab solution software on SHIMADZU company made UV1900i
WinCATs software on CAMAG company made HPTLC system.