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Comparative Phytochemical Screening and Thin Layer Chromatographic Analysis of Ethanolic Leaf Extracts of Morphotypes of *Hibiscus Rosa- Sinensis* Linn.

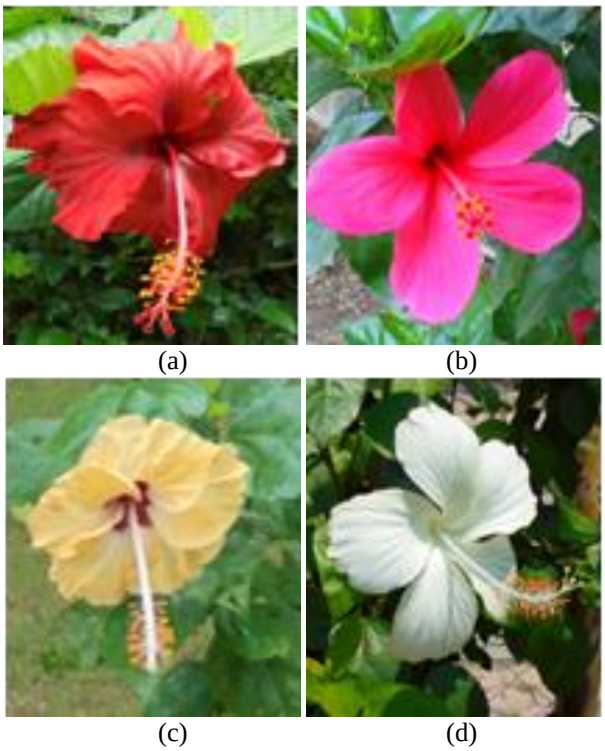
Shilpi Patel and Madhavi Adhav

Abstract
Hibiscus rosa- sinensis Linn. is one of the important medicinal as well as ornamental plant, belongs to family Malvaceae. It is commonly called Gurhal. The extracts (Leaf) are largely used in the treatment of various diseases. The leaf are emollient and slightly aperients (drury, 2010). Young leaves used for wound healing and treatment for dandruff (Trivedi, 2010).
The result of the study revealed the presence of phytochemical constituents such as flavanoids, tannins, glycosides, phenolic compounds, terpenoids, triterpenoids, phytosterols, gum & mucilage.
Alkaloids & saponins were absent in phytochemical screening and TLC analysis. The presence of floavonoids, glycosides, phytosterols, terpenoids phenolic compound, tannins are mainly contributed in the medicinal utility of the plant ; since, these constituents are common in all the four tested morphotypes, any one of them can be used as a substitute for others.

Keywords: *Hibiscus rosa- sinensis* Linn, Malvaceae, phytochemical screening, TLC, Phytoconstituents

1. Introduction
In estimation, the plants were used to cure the diseases and infection during ancient time. Medicinal plants are the foundation of many important drug of the modern world. Plants are now playing an important role in many medicines like allopathic medicine, herbal medicine, homoeopathy and aromatherapy. Many of these local medicinal plants are used as spices and food. The World Health Organization supports the use conventional medicine provided they are established to be effective and safe. Medicinal plants have great significance in health of individuals and communities. The medicinal importance of plants lies in some chemical substances that produce a specific physiological action on the human body.
Hibiscus rosa- sinensis Linn. known as china rose is an important medicinal plant. It belongs to family Malvaceae that comprise around 82 genera and over 1500 species distributed all over the world in warm temperate and tropical region (Lawrence, 1969; Gupta, 1981) [12, 6]. It is an evergreen woody glabrous showy shrub with about 1.5-2.5 m height. Leaves are coarsely toothed above and entire below ovate bright green and 3 nerved base. The flowers are axillary solitary companulate. (Kaushik *et al*., 1999) [8].
Several articles and ancient literature have been shown that the flowers of this plant possess antifertility activity (Pekamwar *et al.*, 2013) [16]. Flower is used for treatment of inflammation (Kritikar and Basu, 1984) [9] and menorrhagia (Nadkarni, 1998) [15]. Flower buds are taken row for relieving stomach pain (Maheshwari, 2000) [13]. The flower of *Hibiscus rosa sinensis* Linn. possess various activity such as anticonvulsants, antidiabetic, antipyretic and antioxidant (Agrawal *et al.*, 2012) [1]. Flower is made into paste and given to treat irregular menstruation (Retnam *et al.*, 2006) [18]. Ethanolic flower extract shows antihyperlipidemic activity (Sikarwar and Patil, 2015) [21]. The juice of the fresh root is given for gonorrhea (Caius, 1986) [3]. In present research work four morphotypes of *Hibiscus rosa -sinensis* Linn. are selected.

Morphotype	I	(*Hibiscus* Red)	- Red flower with 5 petals
Morphotype	II	(*Hibiscus* Pink)	- Pink flower with 5 petals
Morphotype	III	(*Hibiscus* Yellow)	- Yellow flower with 5 petals
Morphotype	IV	(*Hibiscus* White)	- White flower with 5 petals (Kaushik *et al*., 1999) [8]



(a) (b) (c) (d)

Fig 1: Morphotypes of *Hibiscus rosa- sinensis* Linn. (a) *Hibiscus* Red; (b) *Hibiscus* Pink; (c) *Hibiscus* Yellow; (d) *Hibiscus* White.

2. Material and methods

For present investigation the plant material of *Hibiscus rosa sinensis* Linn. (Leaves) were collected from D.A.V.V. campus Indore and Apna garden vishnupuri Indore. The collected plant material was identified with the help of Flora of British India (Hooker J.D., 1875) [7].

To obtain ethanolic extract 100 gms of shade dried plant material was extaract with 500 ml of ethanol (95%) in "Soxhlet Extraction apparatus". Finally the prepared plant was macerated with water for 24 hrs. to obtain aqueous extract. The extract was concentrated by distilling off the solvent (Kokate, 1994; Kokate *et al.*, 1993) [11, 10].

The extract thus obtained was than subjected to TLC screening for identification of various phyto constituents by methods suggested by (Stahl, 1969; Senguttuvan and Paulsamy,2014; T. Indhumathi and S. Mohandass., 2013; Rao *et al*.,2014; Matter Al Maliki 2011;Wagner *et al.*, 1984; Bladt, 2004) [22, 20 23, 17, 14, 25, 2]

3. Result and Discussion

Phytochemical screening

Prelimnary phytochemical screening of ethanolic leaf extracts were performed. The presence of flavonoids, glucosides, phenolic compound, tannins, terpenoids, phytosterols, carbohydrates, proteins, amino acid, gum and mucilages were noted in all the four tested morphotypes. Among the carbohydrates glucose, fructose, galactose was absent while starch was present in all extracts. Alkaloids, saponins, anthocyanin, fixed oil and fats were found absent in all the tested morphotypes. (Table No. 1)

Tannin

Presence of tannin in ethanolic leaf extracts of morphotypes of *Hibiscus rosa sinensis* Linn. were determined by using the solvent system Chloroform: ethyl acetate: ethanol (6:4:4). Leaf extract of all the tested morphotypes gives single spot with orange colour in UV lamp. Leaf extracts of Red, Yellow and White morphotypes shows single spot but extract of pink morphotype gives two spots on TLC plate (Fig No.2 & Table No.2).

Phenolic compound

The TLC studies were performed for detection of phenolic compound using the mobile phase of n -Butanol: acetic acid: water (35: 5: 12) for ehanolic leaf extracts of morphotypes of *Hibiscus rosa sinensis* Linn. Similar separation pattern was observed in all leaf extracts of tested morphotypes (Fig No.3 & Table No.3).

Terpenoids

Terpenoids are also called as Isoprenoids. The suitable mobile phase for terpenoids was determined as the combination of Benzene: ethyle acetate in the ratio of 5:95. In UV lamp single spot was observed in leaf extracts of pink yellow and white morphotype. No separation was observed in leaf extract of red morphotype. In I_2 vapour single spot appears in *Hibiscus* yellow and *Hibiscus* white leaf extracts but extract of *Hibiscus* pink gives two spots (Fig No.4 & Table No.4).

Triterpenoids

In TLC analysis Ethyl acetate: glacial acetic acid: water: formic acid (100: 11:11:26) used as mobile phase for detection of triterpenoids in ethanolic extracts leaf of morphotypes of *Hibiscus rosa- sinensis* Linn. Leaf extracts of pink, yellow and white morphotype gives two spots on TLC plates while leaf extract of red morphotype gives single spot on TLC plate (Fig No.5 & Table No.5).

Phytosterol

For detection of phytosterol the solvent system P. ether: ethyl acetate (7: 3) were used. Leaf extracts of tested morphotype showed variation in separation pattern on TLC plates. Maximum separation was found in Hibiscus pink leaf extract but no separation was observed in Hibiscus red and Hibiscus white leaf extracts. Four spots were seen in Hibiscus yellow leaf extract (Fig No.6 & Table No. 6).

Glycosides

The suitable mobile phase for detection of glycosides was determined as the combination of Toluene: chloroform in ratio of 9:1.

Leaf extract of pink morphotype shows maximum separation on TLC plate as compare to red, yellow and white morphotype. Leaf extracts of red, yellow and white shows similar separation pattern on TLC plate (Fig No. 7 & Table No. 7).

Alkaloids

In TLC analysis $CHCl_3$: Methanol: Glacial acetic acid [83:17:10] used as mobile phase for detection of alkaloids in ethanolic (leaf) extracts of morphotypes of *Hibiscus rosa sinensis* Linn. All (flower and leaf) extracts of tested morphotypes shows no separation on TLC plates (Fig No. 8 & Table No. 8).

Saponins

Saponins in ethanolic flower and leaf extracts were determine by using the solvent system Chloroform: methanol [30:5]. No separation was found in all leaf extracts of tested morphotypes (Fig No.9 & Table No. 9).

Flavonoids glycosides

The TLC studies were performed for detection of flavonoid using the mobile phase Ethyl acetate: formic acid: glacial acetic acid: water (100: 11: 11:26) for ethanolic leaf extracts of four morphotypes of *Hibiscus rosa- sinnensis* Linn. Leaf extracts of all morphotypes shows the presence of one spot on TLC plate was in I_2 vapour and UV lamp. In I_2 vapour spot appears green colour while in UV lamp spot give greenish brown colour (Fig No.10 & Table No.10).

4. Conclusion

The phytochemical screening and TLC analysis results showed similar in phytochemical constituents in all tested morphotypes. The presence of flavonoids, glycosides, phytosterols, terpenoids phenolic compound, tannins are mainly contributed in the medicinal utility of the plant ; since, these constituents are common in all the four tested morphotypes, any one of them can be used as a substitute for others. All the tested morphotypes differ in their morphology buc similar in phytochemical constituents.

5. Acknowledgement

I wish to express my sincere gratitude to my supervisor Dr. Madhavi Adhav, Assistant Professor, Department of Botany, P.M.B. Gujarati Science College Indore for his valuable guidance & support and I also thank to Principal, P.M.B. Gujarati Science College Indore and Head, Department of Botany, P.M.B. Gujarati Science College Indore for provided full research laboratory facilities throughout my work.

Table 1: Preliminary phytochemical screening of ethanolic extract (Flower and leaf) of morphotypes of *Hibiscus rosa sinensis* Linn.

S No.	Plant constituents Test/Reagents	RL	PL	YL	WL
1.	**Alkaloids**				
	(i) Mayer's reagent	—	—	—	—
	(ii) Wagner's reagent	—	—	—	—
	(iii) Hager's reagent	—	—	—	—
2.	**Carbohydrates**				
	(i) Molisch' s test	+	+	+	+
	(ii) Benedict,s reagent	+	+	+	+
	(iii) Fehling solution	+	+	+	+
3.	**Types of Carbohydrates**				
	(i) Glucose	—	—	—	—
	(ii) Fructose	—	—	—	—
	(iii) Galactose	—	—	—	—
	(iv) Lactose	+	+	+	+
	(v) Starch	+	+	+	+
4.	**Glycosides**				
	(i) Keller kiliani test	+	+	+	+
5.	**Phytosterols**				
	(i) Liebermann's test	+	+	+	+
6.	**Terpenoids**				
	(i) Solkowski test	+	+	+	+
7.	**Fixed oils and Fats**				
	(i)Spot test	—	—	—	—
8.	**Saponins**				
	(i) Foam test	—	—	—	—
9.	**Phenolic Compounds**				
	(i) Ferric chloride solution	+	+	+	+
10.	**Tannins**				
	(i) Lead acetate solution	+	+	+	+
11.	**Protein**				
	(i) Xanthoprotic test	+	+	+	+
	(ii) Biuret test	+	+	+	+
	Amino acid				
	(i) Ninhydrin reagent	+	+	+	+
12	**Flavonoids**				
	(i) Con HCl + Mg ribbon	+	+	+	+
13	**Gums and Mucilages**				
	(i) Alcoholic precipitation	+	+	+	+
	(ii) Molisch's test	+	+	+	+
14	**Anthraquinones**				
	Borntgrager's test	-	-	-	-

RL, PL, YL, WL, = Leaf extracts of *Hibiscus* Red, *Hibiscus* Pink, *Hibiscus* Yellow, *Hibiscus* White.

Table 2: Comparative TLC observations of Tannin from ethanolic leaf extracts of morphotypes of *Hibiscus rosa- sinensis* Linn.

S no.	**Morphotype**	**Mobile phase - Chloroform : Ethyl Acetate**								
		Eyes			I_2Vapour			UV Lamp		
		Spot No.	Colour	R_f	Spot No.	Colour	R_f	Spot No.	Colour	R_f
1	RL	1	Green	0.78	1	Green	0.78	1	Orange	0.78

2	PL	1 2	Green Yellow	0.78 0.71	1 2	Green Green	0.78 0.74	1	Orange	0.78
3	YL	1	Green	0.78	1	Green	0.78	1	Orange	0.78
4	WL	1	Green	0.78	1	Green	0.78	1	orange	0.78

RL = Red Leaf, PL = Pink leaf, YL = Yellow Leaf, WL = White Leaf

Table 3: Comparative TLC observations of Phenolic compound from ethanolic leaf extracts of morphotypes of *Hibiscus rosa -sinensis* Linn.

S no	Morphotype	Mobile phase -								
		Eyes			I_2Vapour			UV Lamp		
		Spot No.	Colour	R_f	Spot NO.	Colour	R_f	Spot no.	Colour	R_f
1	RL	1	Green	0.77	1	Green	0.77	1	Orange	0.77
2	PL	1	Green	0.77	1	Green	0.77	1	Orange	0.77
3	YL	1	Green	0.77	1	Green	0.77	1	Orange	0.77
4	WL	1	Green	0.77	1	Green	0.77	1	Orange	0.77

RL = Red Leaf, PL = Pink leaf, YL = Yellow Leaf, WL = White Leaf

Table 4: Comparative TLC observations of terpenoid from ethanolic leaf extracts of morphotypes of *Hibiscus rosa -sinensis* Linn.

S no.	Morphotype	Mobile phase -								
		Eyes			I_2Vapour			UV Lamp		
		Spot no.	Colour	R_f	Spot no.	Colour	R_f	Spot no.	Colour	R_f
1	RL	-	-	-	-	-	-	-	-	-
2	PL	1	Green	0.92	1	Green	0.92	1	Orange	0.92
3	YL	1 2	Yellow Green	0.85 0.92	1 2	Yellow Green	0.85 0.92	1	Orange	0.92
4	WL	1	Green	0.92	1	Green	0.92	1	Orange	0.92

RL = Red Leaf, PL = Pink leaf, YL = Yellow Leaf, WL = White Leaf

Table 5: Comparative TLC observations of Triterpenoid from ethanolic leaf extracts of morphotypes of *Hibiscus rosa- sinensis* Linn.

S no	Morphotype	Mobile phase - Chloroform: Ethyl acetate: Ethanol (6:4:4)								
		Eyes			I_2Vapour			UV Lamp		
		Spot no. on TLC plates	Colour	R_f	Spot no. on TLC plates	Colour	R_f	Spot no. on TLC plates	Colour	R_f
1	RL	1	Light green	0.79	1	Green	0.79	1	Orange	0.79
2	PL	1	Yellowish green	079	1 2	Light Green Green	0.54 0.79	1 2	Orange green Brown	0.54 0.79
3	YL	1	Yellowish green	0.79	1 2	Light green Green	0.54 0.79	1 2	Orange Green brown	0.54 0.79
4	WL	1	Green	0.79	1 2	Light green Green	0.54 0.79	1 2	Orange Green brown	0.54 0.79

RL= Red Leaf, PL = Pink leaf, YL = Yellow Leaf, WL = White Leaf

Table 6: Comparative TLC observations of phytosterol from ethanolic leaf extracts of morphotypes of *Hibiscus rosa sinensis* Linn.

S no.	Morphotype	Mobile phase - P. ether : ethyl acetate(6:4:4)1								
		Eyes			I_2Vapour			UV Lamp		
		Spot no. on TLC plates	Colour	R_f	Spot no. on TLC plates	Colour	R_f	Spot no. on TLC plates	Colour	R_f
1	RL	-	-	-	-	-	-	-	-	-
2	PL	1 2 3 4 5	Yellow Green Green Green Green	0.13 0.19 0.23 0.26 0.46	1 2 3 4 5	Yellow Green Green Green Green	0.13 0.19 0.23 0.26 0.46	1 2 3 4 5	Brown Brown Brown Brown Brown	0.13 0.19 0.23 0.26 0.46
3	YL	1 2 3	Green Green Green	0.19 0.23 0.46	1 2 3	Green Green Green	0.19 0.23 0.46	1 2 3	Brown Brown Brown	0.19 0.23 0.46
4	WL	-	-	-	-	-	-	-	-	-

RL= Red Leaf, PL = Pink leaf, YL = Yellow Leaf, WL = White Leaf

Table 7: Comparative TLC observations of Glycoside from ethanolic leaf extracts of morphotypes of *Hibiscus rosa sinensis* Linn.

S no.	Morphotype	Mobile phase -								
		Eyes			I_2Vapour			UV Lamp		
		Spot no. on TLC plates	Colour	R_f	Spot no. on TLC plates	Colour	R_f	Spot no. on TLC plates	Colour	R_f
1	RL	1 2	Green Green	0.11 0.26	1 2	Green Green	0.11 0.26	1 2 3 4	Green Green Green Green	0.11 0.26 0.46 0.53
2	PL	1 2 3 4 5 6	Green Green Green Yellow Green Green	0.11 0.19 0.23 0.24 0.30 0.34	1 2 3 4 5 6	Green Green Green Yellow Green Green	0.11 0.19 0.23 0.24 0.30 0.34	1 2 3 4 5 6 7	Green Green Green Green Green Green Green	0.07 0.15 0.24 0.26 0.30 0.38 0.53
3	YL	1 2 3	Yellow Green Green	0.23 0.26 0.30	1 2 3	Yellow Green Green	0.23 0.26 0.30	1 2 3 4 5	Green Green Green Green Green	0.07 0.11 0.26 1.19 0.53
4	WL	1 2	Green Green	0.11 0.26	1 2	Green Green	0.11 0.26	1 2 3 4	Green Green Green Green	0.11 0.26 0.46 0.53

RL= Red Leaf, PL = Pink leaf, YL = Yellow Leaf, WL = White Leaf

Table 8 : Comparative TLC observations of alkaloids from ethanolic leaf extracts of morphotypes of *Hibiscus rosa sinensis* Linn.

S no.	Morphotype	Mobile phase - $CHCl_3$: Methanol: Glacial acetic acid [83:17:10]								
		Eyes			I_2Vapour			UV Lamp		
		Spot no.	Colour	R_f	Spot no.	Colour	R_f	Spot no.	Colour	R_f
1	RL	––	––	––	––	––	––	––	––	––
2	PL	––	––	––	––	––	––	––	––	––
3	YL	––	––	––	––	–––	––	–––	––	––
4	WL	––	––	––	––	––	––	––	––	––

RL= Red Leaf, PL = Pink leaf, YL = Yellow Leaf, WL = White Leaf

Table 9: Comparative TLC observations of saponin from ethanolic leaf extracts of morphotypes of *Hibiscus rosa sinensis* Linn.

S no.	Morphotype	Mobile phase - Chloroform: methanol [30:5].								
		Eyes			I_2Vapour			UV Lamp		
		Spot no.	Colour	R_f	Spot no.	Colour	R_f	Spot no.	Colour	R_f
1	RL	––	––	––	––	––	––	––	––	––
2	PL	––	––	––	––	––	––	––	––	––
3	YL	––	––	––	––	–––	––	–––	––	––
4	WL	––	––	––	––	––	––	––	––	––

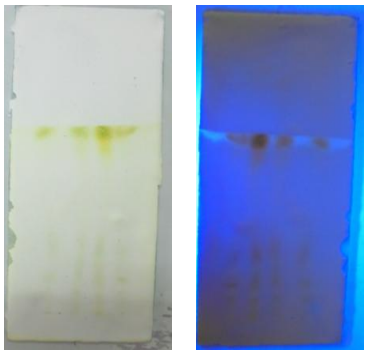
RL= Red Leaf, PL = Pink leaf, YL = Yellow Leaf, WL = White Leaf

Table 10: Comparative TLC observations of Flavonoid glycoside from ethanolic leaf extract of morphotypes of *Hibiscus rosa sinensis* Linn

S no.	Morphotype	Mobile phase - **Formic acid: Glacial acetic acid : H_2O (100:11:11:26)**								
		Eyes			I_2Vapour			UV Lamp		
		Spot no. on TLC plates	Colour	R_f	Spot no. on TLC plates	Colour	R_f	Spot no. on TLC plates	Colour	R_f
1	RF	1	Yellowish green	0.72	1	Yellowish green	0.72	1 2	Green Yellow	0.36 0.72
2	PF	1	Yellowish green	0.77	1	Dark green	0.77	1 2 3	Green Yellow Green	0.31 0.59 0.77
3	YF	1	Yellowish green	0.81	1	Dark green	0.81	1	Green	0.81
4	WF	1	Yellowish green	0.81	1	Dark green	0.81	1	Green	0.81

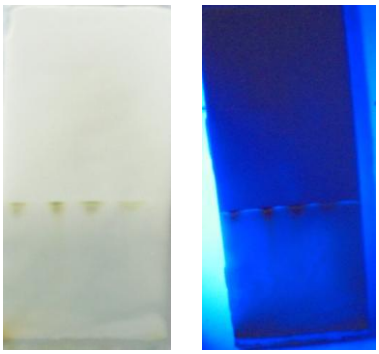
S no.	Morphotype	Mobile phase - Formic acid: Glacial acetic acid : H_2O (100:11:11:26)								
		Eyes			I_2Vapour			UV Lamp		
		Spot no. on TLC plates	Colour	R_f	Spot no. on TLC plates	Colour	R_f	Spot no. on TLC plates	Colour	R_f
1	RL	1	Green	0.86	1	Green	0.86	1	Greenish brown	0.86
2	PL	1	Green	0.86	1	Green	0.86	1	Greenish brown	0.86
3	YL	1	Green	0.86	1	Green	0.86	1	Greenish brown	0.86
4	WL	1	Green	0.86	1	Green	0.86	1	Greenish brown	0.86

RL = Red Leaf, PL= Pink leaf, YL = Yellow Leaf, WL = White Leaf



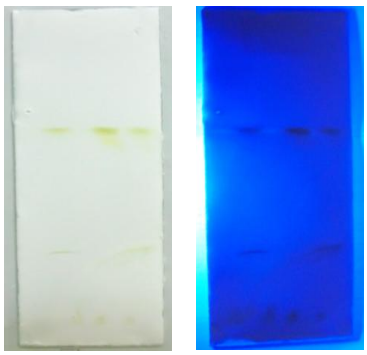
I_2 Vapour UV lamp

Fig 2: Comparative TLC observations of Tannin from ethanolic flower and leaf extract of morphotypes of *Hibiscus rosa sinensis* Linn.



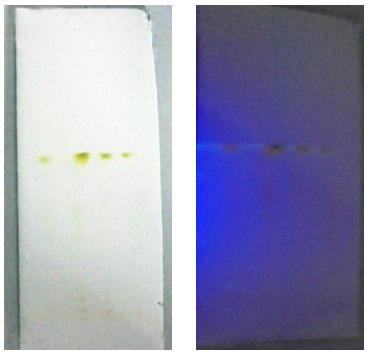
I_2 Vapour UV lamp

Fig 3: Comparative TLC observations of Penolic compounds from ethanolic flower and leaf extract of morphotypes of *Hibiscus rosa sinensis* Linn.



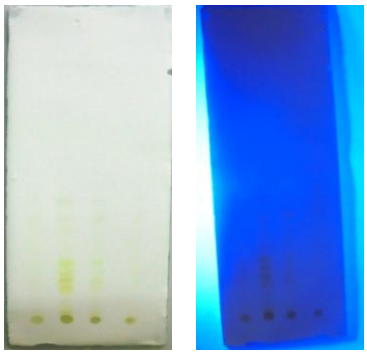
I_2 Vapour UV lamp

Fig 4: Comparative TLC observations of terpenoied from ethanolic flower and leaf extract of morphotypes of *Hibiscus rosa sinensis* Linn.



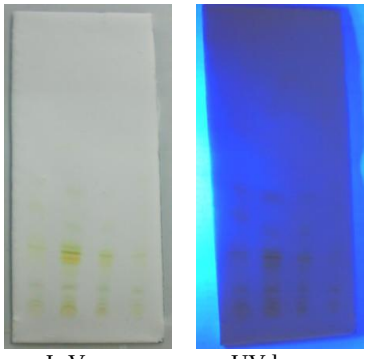
I_2 Vapour UV lamp

Fig 5: Comparative TLC observations of triterpenoid from ethanolic flower and leaf extract of morphotypes of *Hibiscus rosa sinensis* Linn.



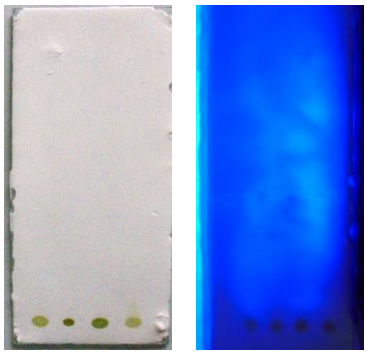
I_2 Vapour UV lamp

Fig 6: Comparative TLC observations of phytosterols from ethanolic leaf extract of morphotypes of *Hibiscus rosa sinensis* Linn.



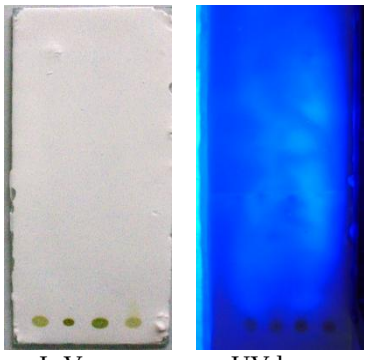
I_2 Vapour UV lamp

Fig 7: Comparative TLC observations of glycosides from ethanolic leaf extract of morphotypes of *Hibiscus rosa sinensis* Linn.



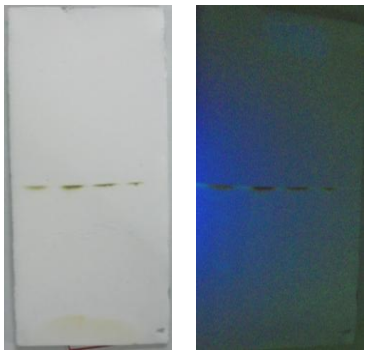
I_2 Vapour UV lamp

Fig 8: Comparative TLC observations of alkaloid from ethanolic leaf extract of morphotypes of *Hibiscus rosa sinensis* Linn.



I_2 Vapour UV lam

Fig 9: Comparative TLC observations of saponin from ethanolic leaf extract of morphotypes of *Hibiscus rosa sinensis* Linn.



I_2 Vapour UV lamp

Fig 10: Comparative TLC observations of Flavonoid glycoside from ethanolic flower and leaf extract of morphotypes of *Hibiscus rosa sinensis* Linn.

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