



HPTLC PROFILING OF FLAVONOIDS AND GLYCOSIDES IN THE FLOWERS OF *CROSSANDRA INFUNDIBULIFORMIS* (L.) NEES

JENCY SAM^{*1} AND JEYA SUNDARA SHARMILA²

^{1*}Department of Bioinformatics, School of Biotechnology and Health Sciences, Karunya University, Coimbatore, TamilNadu, India.

²Department of Nano Science & Technology, TamilNadu Agriculture University, Coimbatore, TN, India

ABSTRACT

Plants as such are useful, of which the flowering species contributes one tenth part in finding new drug or the lead molecules. *Crossandra infundibuliformis* L. Nees (Acanthaceae) is a tropical evergreen shrub reported to have some medicinal properties as wound healing, antioxidant, antimicrobial, antiulcer and antisolular activity. Phytoconstituents of the flower extract is also screened and display the presence of major phytochemicals. Hence the present work is focused on to identify the flavonoids and glycosides present in the methanolic extract of *C.infundibuliformis* flowers using High Performance Thin Layer Chromatography (HPTLC) method. The HPTLC profile for flavonoids and glycosides identified five flavonoids and four glycosides in the *C.infundibuliformis* flower extract. Flavonoids, glycosides and combination of both have been used in drug research for many years due to their vast pharmacological application. In future, these compounds could be isolated from the flowers part of this plant and used as lead molecule.

KEYWORDS: *Crossandra infundibuliformis*, HPTLC, flavonoids, glycosides, phytochemicals.



*Corresponding author

JENCY SAM

Department of Bioinformatics, School of Biotechnology and Health Sciences,
Karunya University, Coimbatore, TamilNadu, India.

INTRODUCTION

Plants as such are useful, of which the flowering species contributes one tenth part in finding new drug or the lead molecules. *Crossandra infundibuliformis* L Nees (Acanthaceae) is an evergreen shrub widely distributed in regions of India, Malaysia, Srilanka and tropical Africa. The flowers of *C. infundibuliformis* are sometimes referred as 'Tropical flame' or 'Firecracker' has been used as hairdo by the Puliya tribal women¹. Flower extract of this plant is reported to have wound healing property². Recent research findings report that the methanolic extract of *Crossandra infundibuliformis* flowers showed antimicrobial activity³, antioxidant activity⁴ and significant anti-ulcer activity⁵. In China, some flowers have been eaten since ancient times, and some flowers have been used in traditional Chinese medicine. Recently the antioxidant capacities and total phenolic contents of 51 edible and wild flowers have been evaluated⁶. Researchers reported that the fresh aqueous extract of *Crossandra infundibuliformis* flowers exhibited better anti-solar activity than the dry flower extract and have concluded that the flowers of *C. infundibuliformis* can be used in various sunscreen formulations⁷. Phytochemical screening of various solvent extracts of *C. infundibuliformis* flower revealed the presence of flavonoids, glycosides, alkaloids, saponins, tannins, steroids and terpenoids⁸. HPTLC analysis provides insight about the compounds present in the crude extract. Earlier reports suggest that the HPTLC profiling facilitates effectual separation of phytochemicals from plants by the use of highly polar solvents as mobile phase⁹⁻¹¹. Thus the present study is initiated to recognize the flavonoids and glycosides profile of *Crossandra infundibuliformis* flower extract using high performance thin layer chromatography (HPTLC) technique.

MATERIALS AND METHODS

Plant Collection and extract preparation

The flowers of *C. infundibuliformis* were acquired from local market, Salem District, Tamil Nadu, India and authenticated as *Crossandra infundibuliformis* (Acanthaceae family) at the Botanical Survey of India, Coimbatore, India (No: BSI/SC/5/23/2013-14/Tech./705). The flowers were washed, shade-dried and powdered. Flower

powder (30 grams) was extracted with the solvents (150ml each) in the order as petroleum ether, chloroform, ethyl acetate, methanol and aqueous by cold percolation method.

High Performance Thin Layer Chromatography

To carry out the HPTLC analysis method adopted by Yamunadevi Mariswamy et al 2011¹² is followed. Test solution required for HPTLC analysis was prepared by dissolving 25mg of flower powder in 4ml methanol and centrifuged for 5min at 3000rpm. The HPTLC equipment (CAMAG) with applicator LINOMAT 5 was used wherein in the TLC plate coated with Silica gel 60F₂₅₄ (3 x 10cm) test sample and standard solution 2 μ l (each) was loaded as 5mm band length using Hamilton syringe. In TLC twin trough developing chamber TLC plate loaded with samples was kept and developed up to 90mm with corresponding mobile phases as toluene-acetone-formic acid (4.5: 4.5: 1) for flavonoid profile and chloroform: methanol: water (6.5:2.5:0.4) for glycoside profile. Then the developed plate is kept in hot air for the solvents to evaporate. In REPROSTAR 3, a photo-documentation chamber (PDC) the plate was kept to capture images at different wavelength as UV366nm, UV 254nm and visible light. Spray reagent 1% ethanolic aluminium chloride reagent and Libermann-Burchard reagent was used for flavonoid and glycoside respectively. These reagents were sprayed on to the developed plate. As a final step this plate is kept in scanner and scanned at 366nm. The peak table, peak display and peak densitogram were noted using the scanner software winCATS (v1.3.4).

RESULTS AND DISCUSSION

The result analysis of HPTLC profile for flavonoid and glycoside of *C. infundibuliformis* methanolic extract are given in Table 1 and Table 2 respectively. Yellow color spot is detected in the TLC plate, confirms the presence of flavonoids¹³ and brown color spot indicates the occurrence of glycosides¹⁴. Further, five different flavonoids with Rf value 0.06, 0.22, 0.28, 0.48 and 0.69 were recorded in the chromatogram after derivatization. Among the five flavonoids, the maximum peak area is occupied by Flavonoid 5 (0.69) followed by Flavonoid 3 (Rf 0.28) as 15624.8 and 7685.1

respectively. These Rf values are in line with quercetin (Rf 0.65)¹⁵ and rutin (Rf 0.27)¹². Figure 1 shows the HPTLC chromatogram of flavonoid. Baseline peak and Densitogram peak scanned at 254 nm for the standard and test sample in regard with flavonoid are displayed in Figure 2. The HPTLC chromatogram of glycoside shown in Figure 3 provides four different glycosides with Rf values as 0.06, 0.20, 0.26, and 0.91. Glycoside 3

has the maximum peak area of 14346.3 followed by glycoside 1 with peak area 7821.1. However the Rf value of glycoside 4 is same as that of a standard (anthranol glycoside)¹⁶. Baseline peak and Densitogram peak scanned at 254 nm for the standard and test sample in regard with glycoside are displayed in Figure 4. 3D display for flavonoid and glycoside profiles are depicted in Figure 5 (I and II respectively).

TABLE 1
HPTLC PROFILE FOR FLAVONOIDS OF CROSSANDRA
INFUNDIBULIFORMIS METHANOLIC EXTRACT

Peak no.	Rf value	Height	Area	Assigned substance
1	0.18	302.6	7676.5	Flavonoid Standard
1	0.06	232.0	5315.7	Flavonoid 1
2	0.12	141.2	5394.7	Unknown
3	0.17	93.9	2559.3	Unknown
4	0.22	89.6	2770.1	Flavonoid 2
5	0.28	158.7	7685.1	Flavonoid 3
6	0.38	85.8	4993.1	Unknown
7	0.48	13.7	415.0	Flavonoid 4
8	0.62	37.1	664.0	Unknown
9	0.69	559.8	15624.8	Flavonoid 5
10	0.72	331.8	12661.3	Unknown
11	0.82	96.0	4744.6	Unknown
12	0.92	46.0	1709.3	Unknown

FIGURE 1
HPTLC CHROMATOGRAM OF FLAVANOIDS.

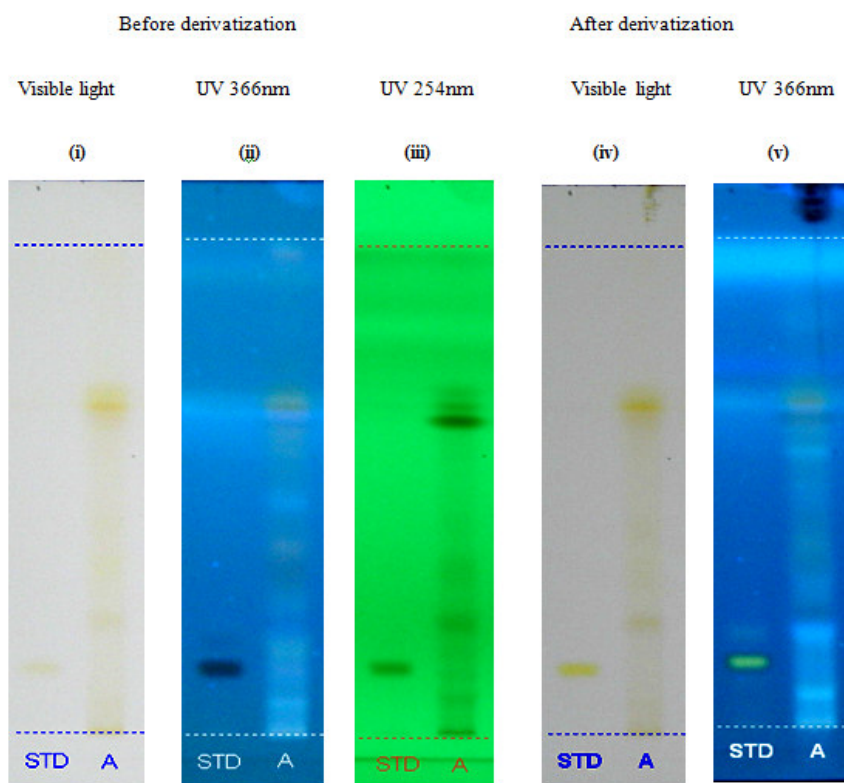


FIGURE 2
BASELINE PEAK (LEFT) AND DENSITOGRAM PEAK (RIGHT) (SCANNED AT 254 NM) OF STANDARD (TOP) AND TEST SAMPLE (BOTTOM) CORRESPONDING TO FLAVONOIDS.

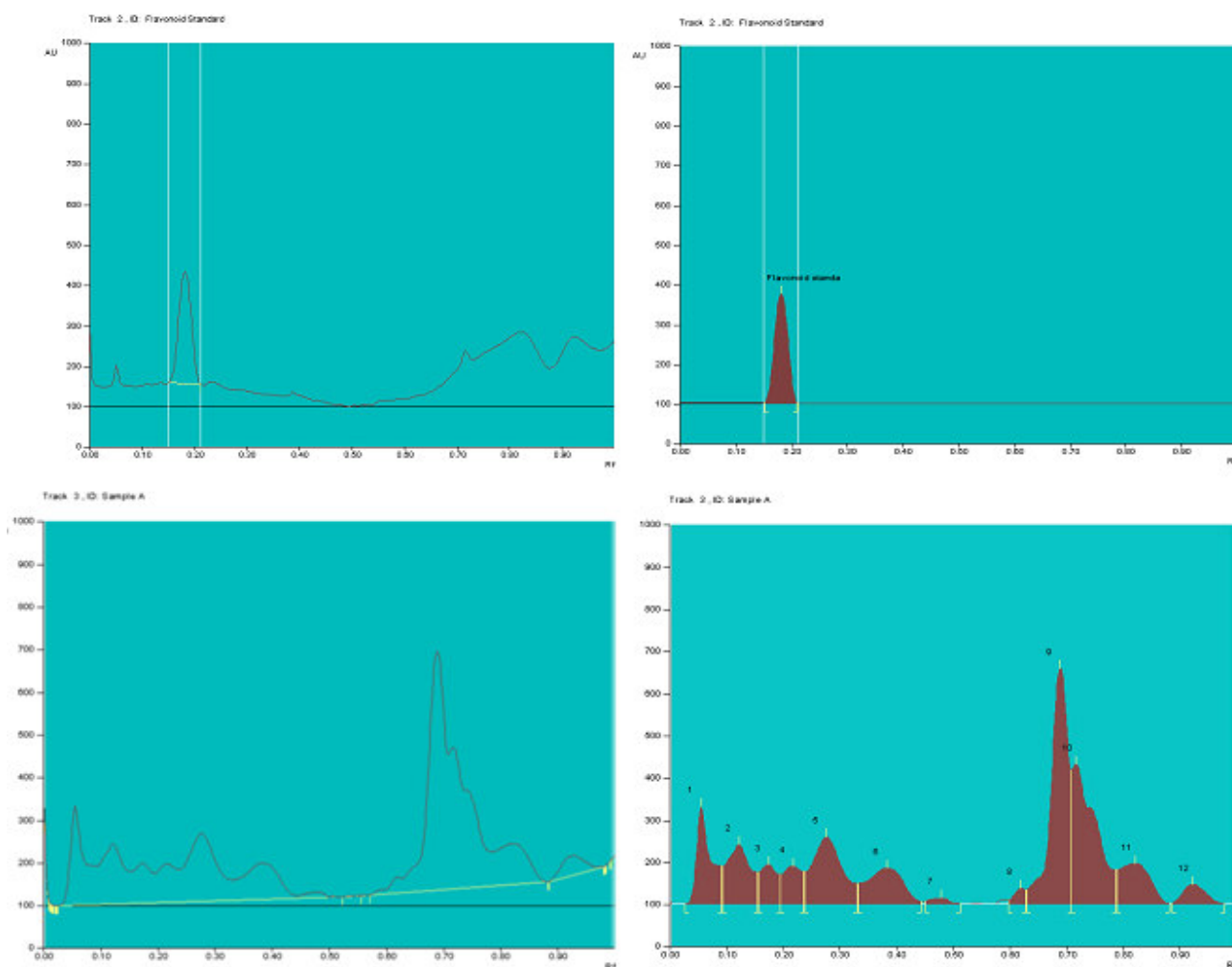


TABLE 2
HPTLC PROFILE FOR GLYCOSIDES OF CROSSANDRA INFUNDIBULIFORMIS METHANOLIC EXTRACT

Peak no.	Rf value	Height	Area	Assigned substance
1	0.06	200.8	7821.1	Glycoside 1
2	0.14	121.4	4864.1	Unknown
3	0.19	107.7	1732.9	Unknown
4	0.20	118.7	1591.6	Glycoside 2
5	0.26	239.4	14346.3	Glycoside 3
6	0.34	147.9	6631.5	Unknown
7	0.41	43.2	703.9	Unknown
8	0.43	37.2	1208.6	Unknown
9	0.59	22.9	418.5	Unknown
10	0.62	19.4	492.4	Unknown
11	0.75	35.4	572.5	Unknown
12	0.78	33.5	458.9	Unknown
13	0.84	11.5	68.0	Unknown
14	0.91	18.5	384.5	Glycoside 4
15	0.98	75.5	854.5	Unknown
1	0.93	277.1	7128.8	Glycoside standard

FIGURE 3
HPTLC CHROMATOGRAM OF GLYCOSIDES.

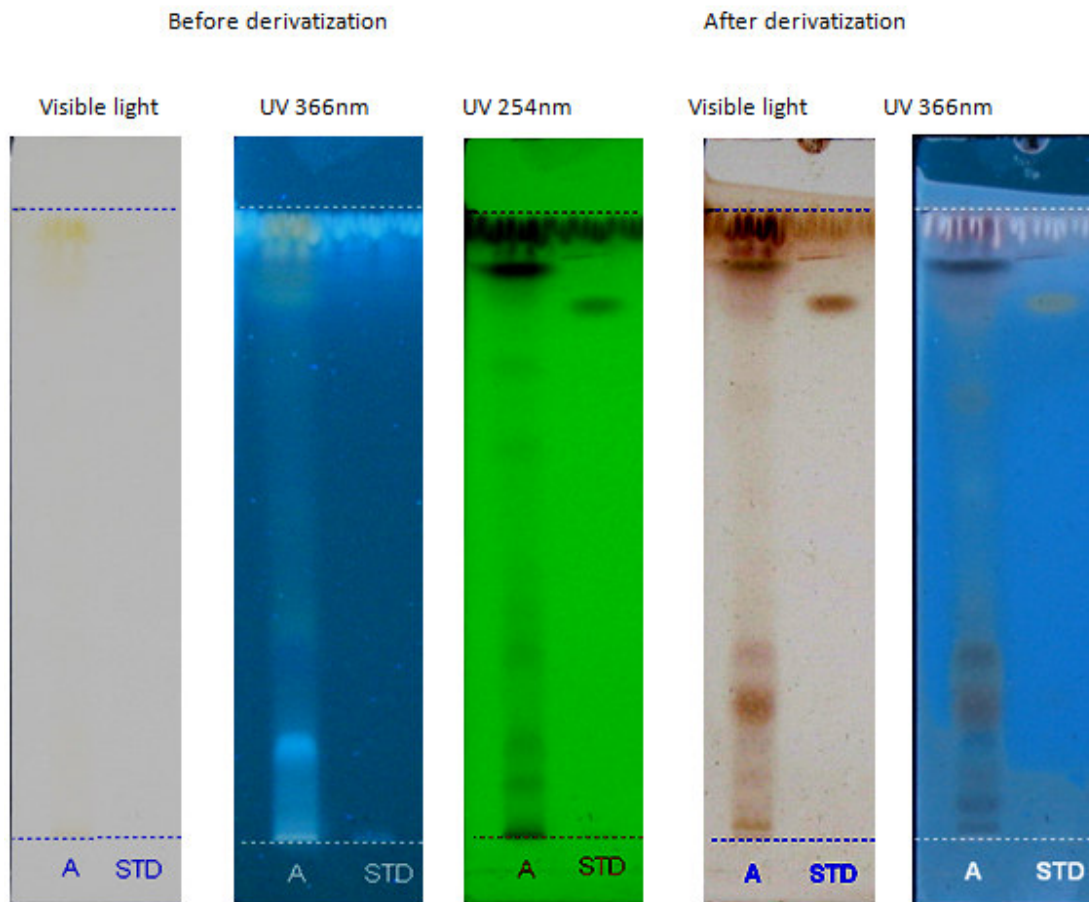
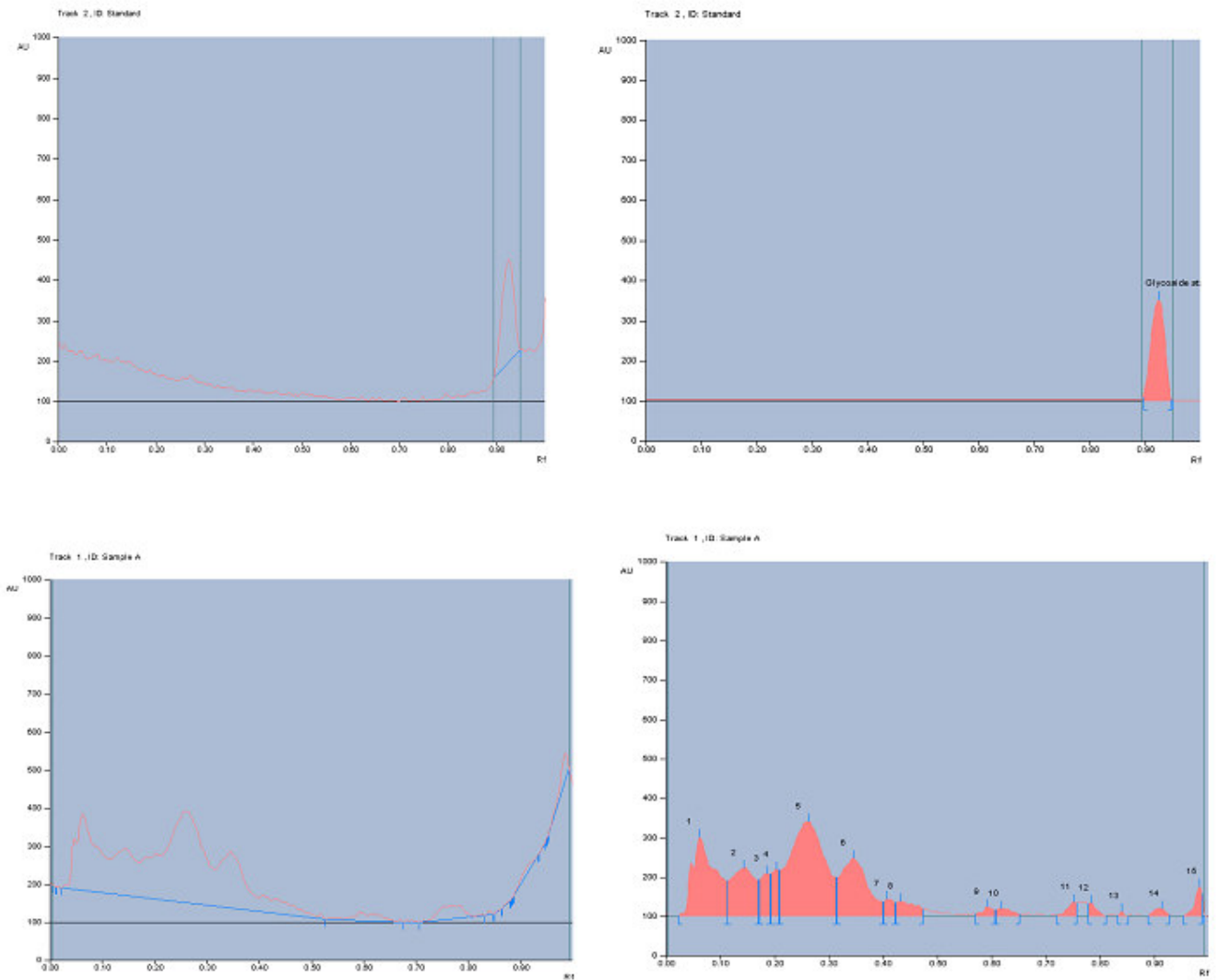


FIGURE 4

BASELINE PEAK (LEFT) AND DENSITOGRAM PEAK (RIGHT) (SCANNED AT 254 NM) OF STANDARD (TOP) AND TEST SAMPLE (BOTTOM) CORRESPONDING TO GLYCOSIDES.



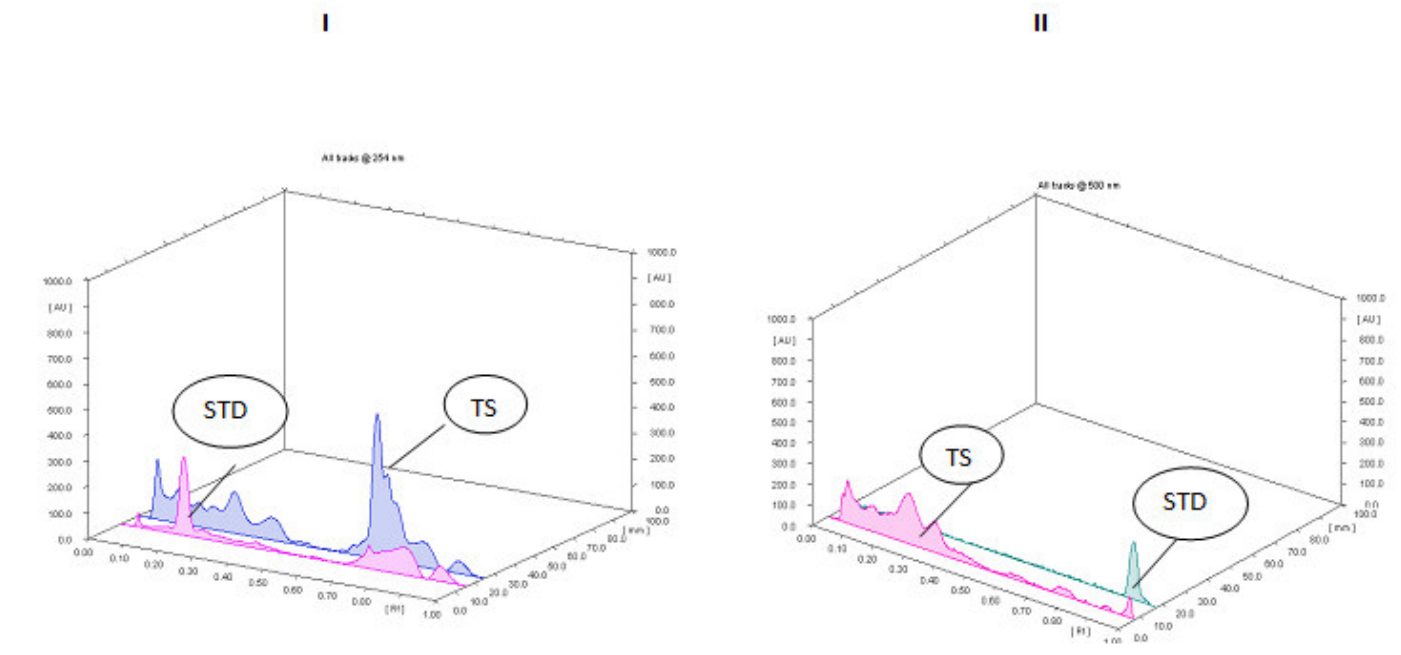


FIGURE 5
THREE DIMENSIONAL VIEW OF STANDARD (STD) AND TEST SAMPLE (TS) OF FLAVONOIDS (I) AND GLYCOSIDES (II) HPTLC PROFILE.

CONCLUSION

High Performance Thin Layer Chromatography (HPTLC) is a more proficient and faster method that uses solvents of high polarity requisite for good separation of phytochemicals. Electronic image of chromatogram and densitogram from HPTLC analysis displays the presence of standard compounds in the test sample. In the present work, HPTLC analysis of *C.infundibuliformis* flower extract displayed five flavonoids in the flavonoid profile and four glycosides in the glycoside profile. Flavonoids such as rutin¹² and quercetin¹⁵ are found to be present in the *C.infundibuliformis* flower extract. In Unani medicine, to treat diabetes mellitus, pomegranate flowers were used¹⁷. Over a period of years flavonoids are considered as the major class of polyphenols, because of the

pharmacological application associated with it. Most important flavonoids function as defense anti-oxidants¹⁸. Flower petals emit brilliant fluorescence because of the presence of flavonoids. Frequently anthocyanin pigment is present in the flowers and yellow pigmentation represents the presence of flavonoid glycosides¹⁹. Drugs such as vancomycin, *Bleomycin* are glycosides and many other glycodrugs available in the category of antibiotics because of the reason that the glycosidic moiety contributes primarily for the activity²⁰. Among the 4 different glycosides detected in the flower extract, R_f value of glycoside 4 is same as that of standard, anthranol glycoside. This HPTLC analysis confirms the presence of flavonoids and glycosides in the methanolic extract of *C.infundibuliformis* flowers.

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