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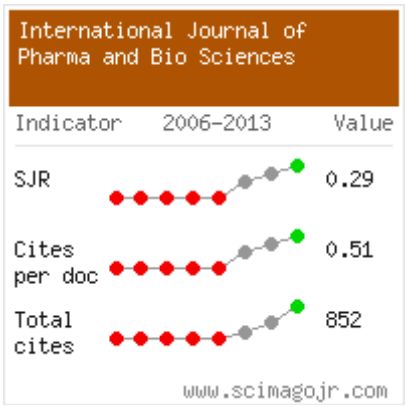

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HPTLC PROFILING OF FLAVONOIDS AND GLYCOSIDES IN THE FLOWERS OF *CROSSANDRA INFUNDIBULIFORMIS* (L.) NEES

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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 antisolar activity. Phytoconstitutents 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.**

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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 Puliyars tribal women[1]. Flower extract of this plant is reported to have wound healing property[2]. Recent research findings report that the methanolic extract of *Crossandra infundibuliformis* flowers showed antimicrobial activity[3], antioxidant activity[4] and significant anti-ulcer activity[5]. 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 evaluated[6]. 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 formulations[7]. Phytochemical screening of various solvent extracts of *C.infundibuliformis* flower revealed the presence of flavonoids, glycosides, alkaloids, saponins, tannins, steroids and terpenoids[8]. HPTLC analysis provides insight about the compounds present in the crude extract. Earlier reports suggest that the HPTLC profiling facilitates effectual separation of phytocompounds from plants by the use of highly polar solvents as mobile phase[9-11]. 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 2011[12] 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 $60F_{254}$ (3 x 10cm) test sample and standard solution 2µl (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 366mn. 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 flavonoids[13] and brown color spot indicates the occurrence of glycosides[14]. 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.

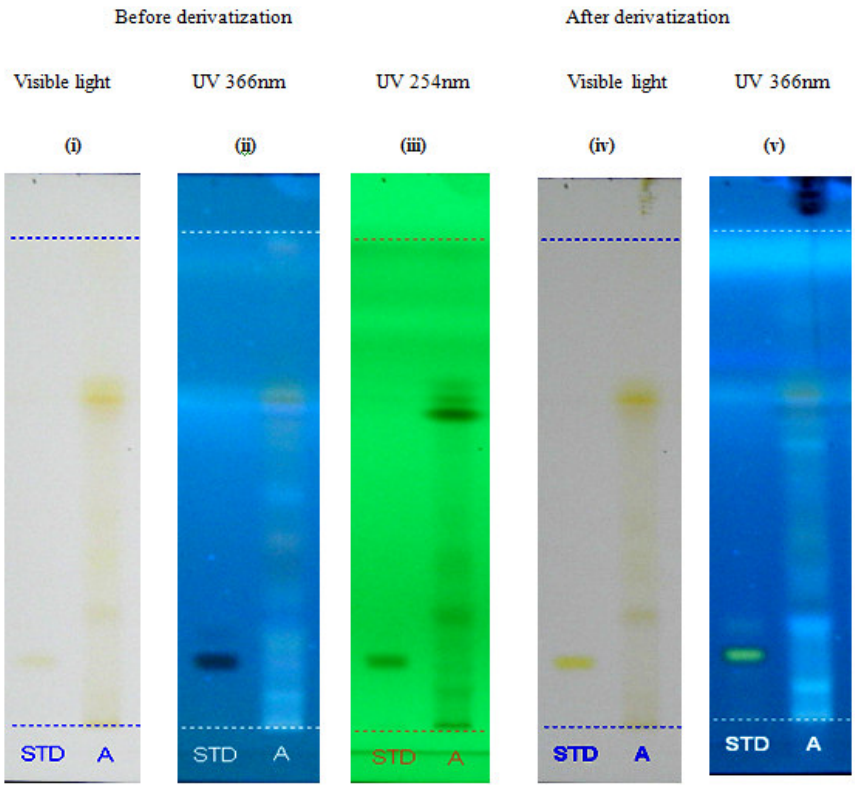


FIGURE 2

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

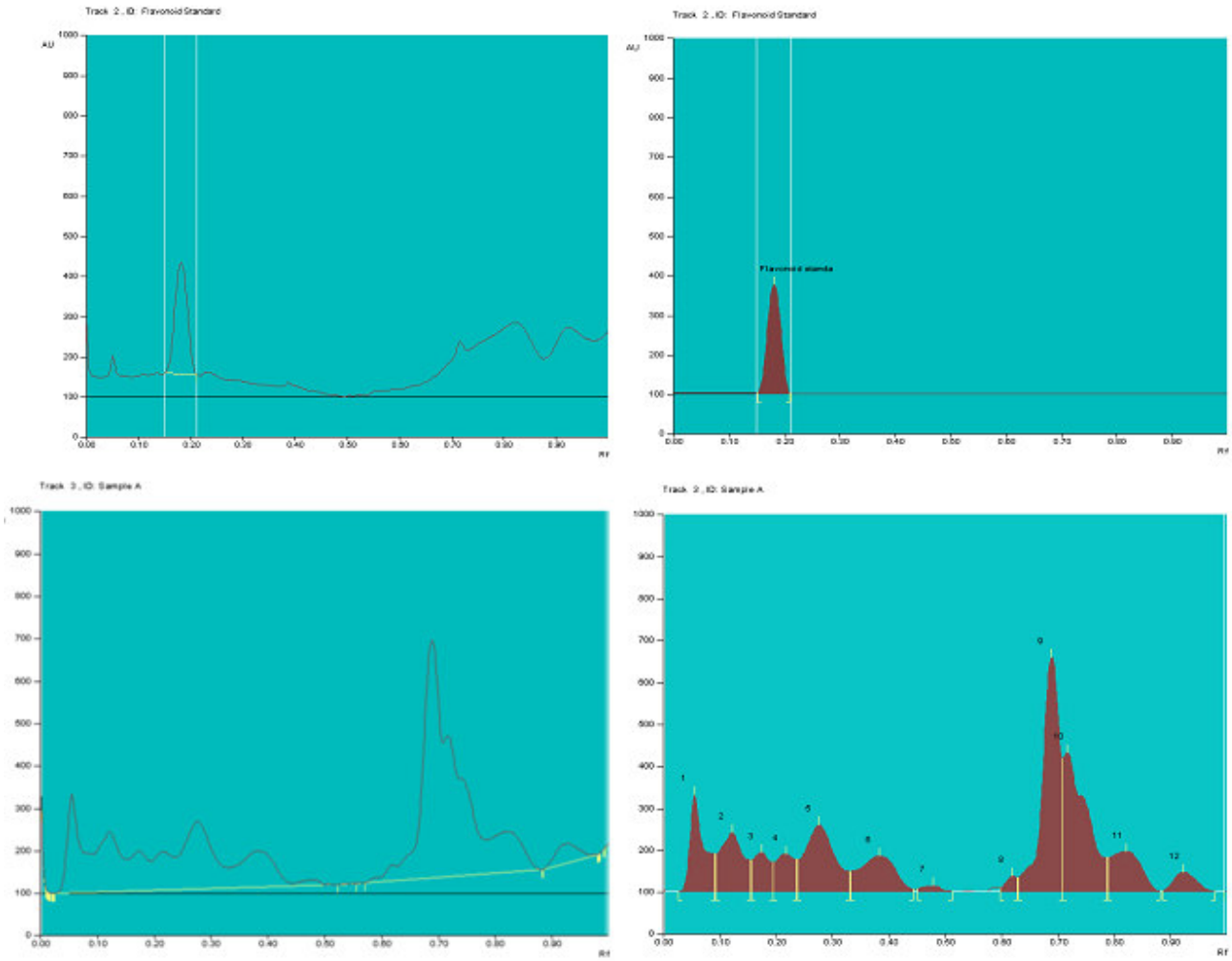


TABLE 2

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.

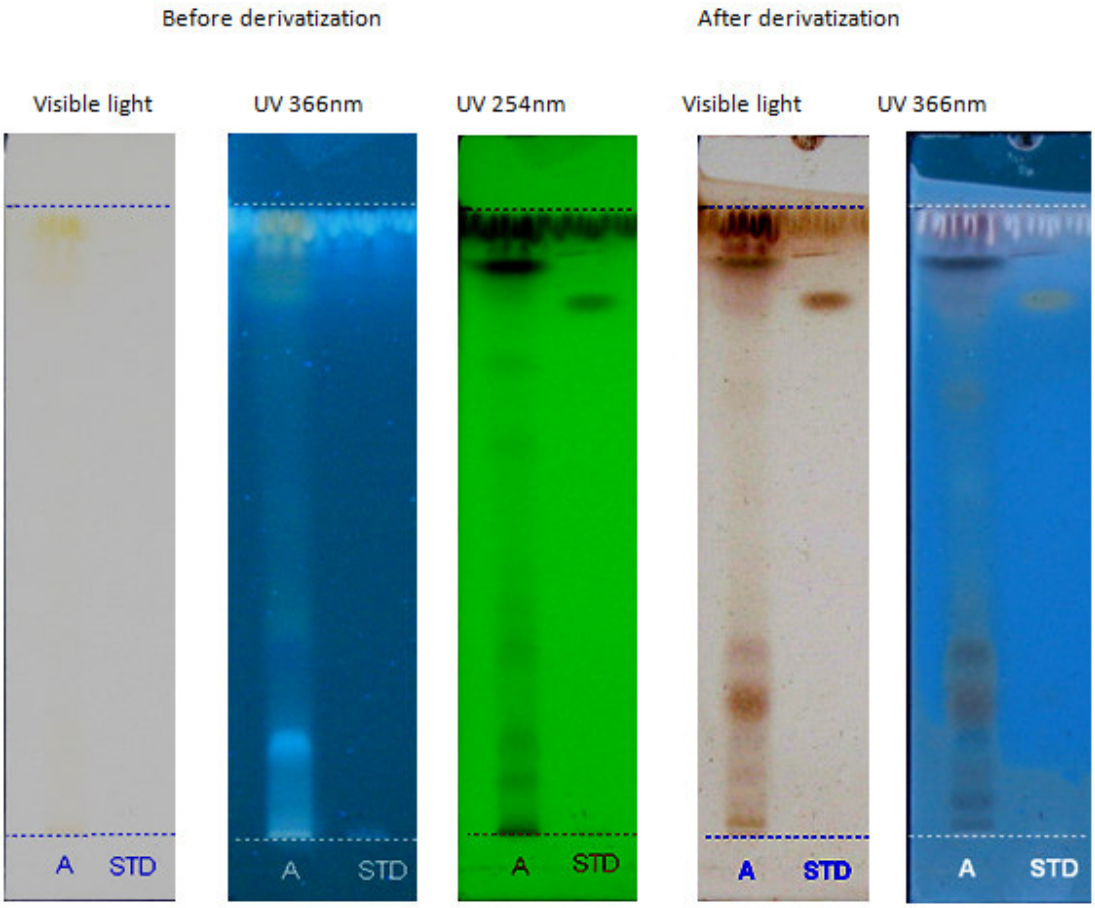
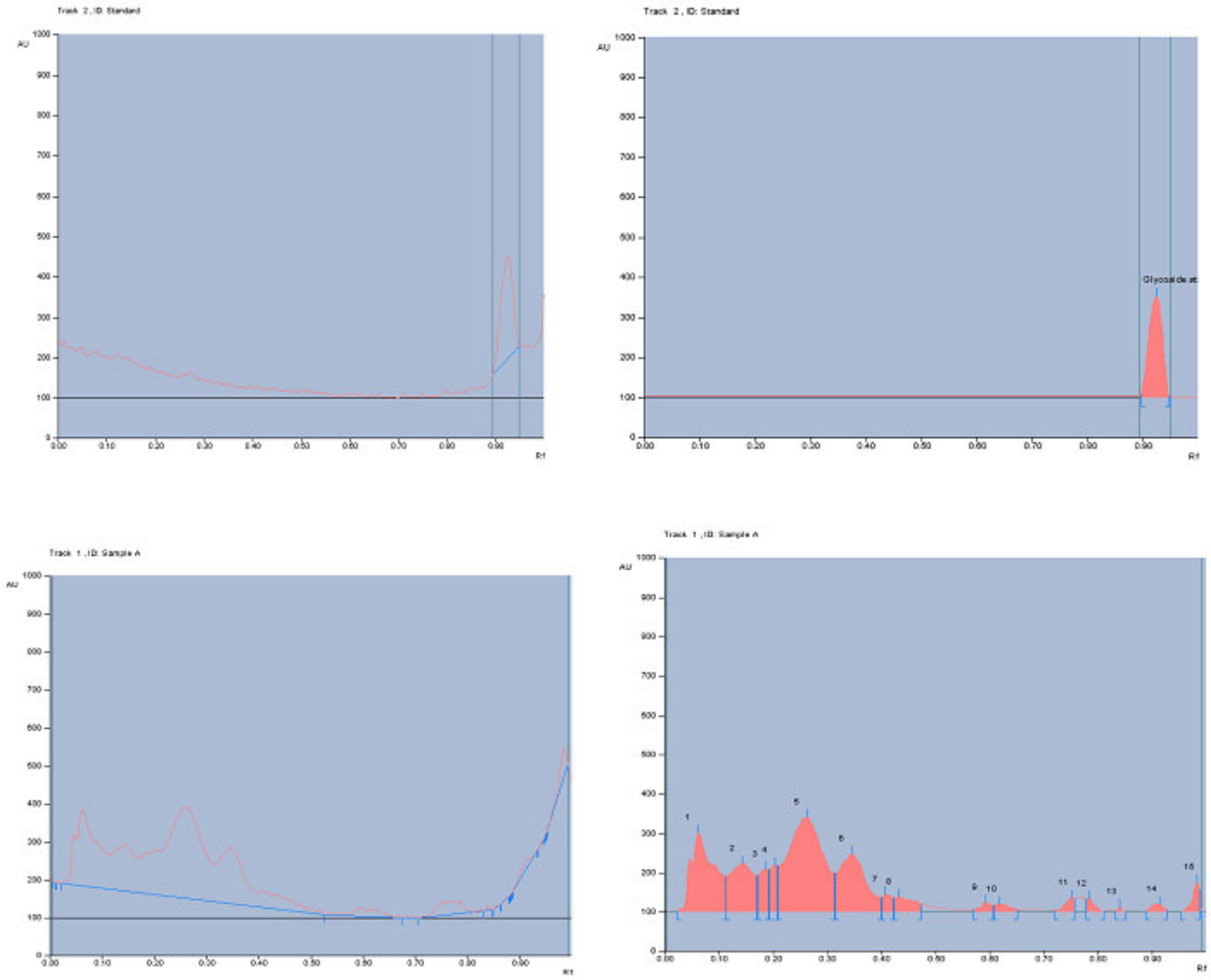


FIGURE 4

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



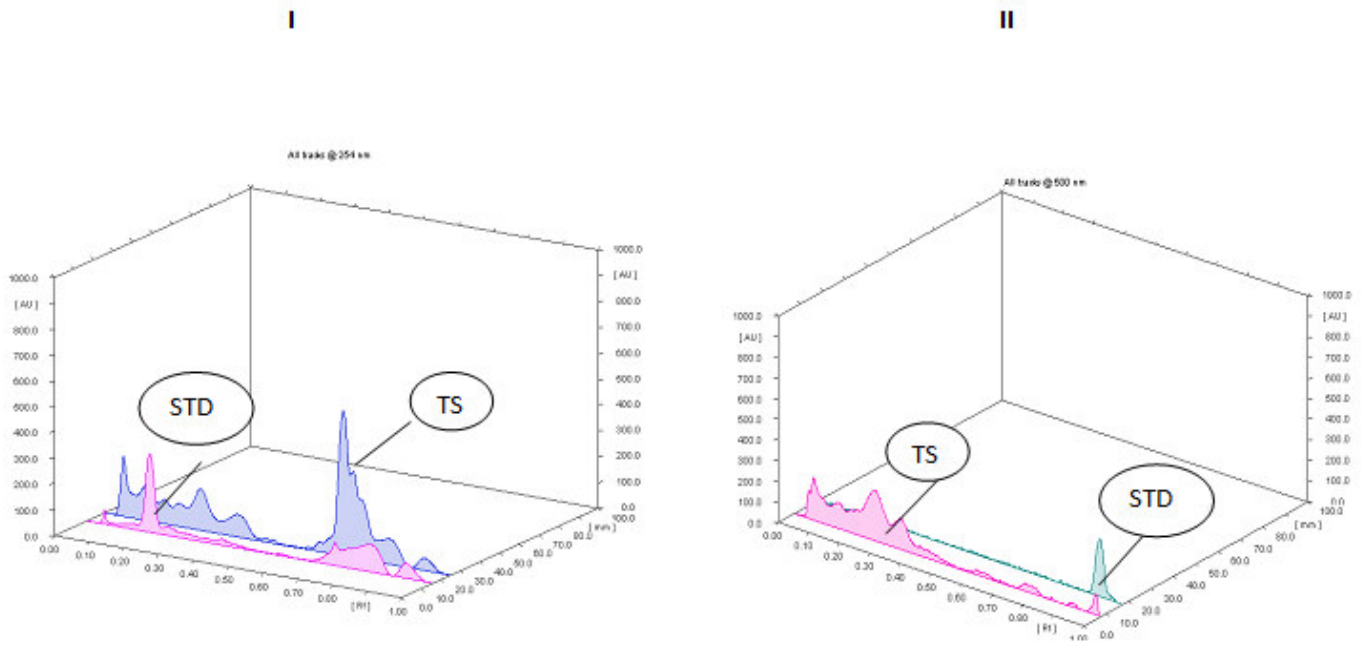


FIGURE 5
THREE DIMENSIONAL VIEW OF STANDARD (STD) AND TEST SAMPLE (TS) OF FLAVONOIDS (I) AND GLYCOSIDES (II) HPTLC PROFILE.

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 phytocompounds. 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 rutin[12] and quercitin[15] are found to be present in the *C.infundibuliformis* flower extract. In Unani medicine, to treat diabetes mellitus, pomegranate flowers were used[17]**.** 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-oxidants[18]. 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 glycosides[19]. 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 activity[20]. Among the 4 different glycosides detected in the flower extract, Rf 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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