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Preliminary quantification of Phytochemicals in methanolic extract of *Roylea cinerea* (D. Don) Baillon by using HPTLC technique

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Abstract
Roylea cinerea is a medicinal plant of monotypic genus belongs to family Lamiaceae (Incl. Verbenaceae) family, ranged from Kashmir (India) to Nepal in Western Himalayas. The present study reviews about macroscopical-botanical description for authenticity of selected plant with macroscopical-physical parameters like colour, odour, leaf shape-margins-venation etc. were observed for the identification of *Roylea cinerea.* It was important to authenticate taken plant before its methanolic extraction for preliminary quantification of chemicals constituents. In Phytochemical analysis, constituents are quantified by visualizing TLC plate under UV light at different wavelength *i.e.*, 254 nm, 366 nm and 566 nm to get chromatograph of HPTLC which showed different Rf for different phytochemicals present in methanolic extract of plant.

Keywords: *Roylea cinerea*, phytochemical, HPTLC, chromatograph

Introduction
An evergreen pleasantly aromatic shrub *Roylea cinerea* (D. Don) Baillon belongs to family Lamiaceae (Khare, 2007) [9]. A single rare genus *Roylea* has been distributed through-out the Western Himalayas from Kashmir to Nepal at an altitude of 1200-3700 m where it is used as folk medicine against various disease and disorders (Gaur 1999 [8]; Khare 2007 [9]; Dua *et al*., 2011[7]; Rawat and Vashistha, 2013) [17]. The whole plant shows therapeutic source, so it is nature's gift to Himalayan people and is locally known as 'Titpatti' in upper areas of Himachal Pradesh. Bahuguna Parts of plant such as stem and leaves are characterized with greyish glandular hairs gives velvety appearance. Dark green leaves having lemon odour with ovate shaped, dentate margins along with petiole, reticulate venations are preliminary physical characteristic. Its leaf length varies from 2-4 cm with opposite, ovate, acute, lobed pattern, with scattered hairs on upper surface mainly on veins. White pinkish flowers form axillary clusters (4-12 in numbers) with short peduncle. Whole plant is known for its various traditional therapeutic purposes. In Kumau-Garhwal (Uttrakhand) the aerial parts of plant are widely used as medicines for the treatment of liver disorders like- Jaundice, liver debility. For the same purposes it also being used in other parts of Himalayan region. Plant is found to be used to cure ailments such as fever, malaria, skin diseases, swellings and for diabetes (Majumdar *et al*., 1979) [11]. Its flowers can be snuffed during winters in the high altitudinal areas of Himachal Pradesh (Prakash and Aggarwal, 2010) [13]. The young shoots have insect repellent properties, cattle are exposed to burning smoke during rainy season. Various extraction experiments have been conducted by researchers with respect to its medicinal value. The efficacy of extract taken from *Roylea cinerea* was tested and proved against Plasmodium (cause plasmodial infection) under *in-vitro* conditions (Dua *et al*., 2011) [7]. The crude extract of plant has effective antidiabetic potential (Bahuguna *et al.*, 2015) [1] where compounds like stigmasterol and β-sitosterol are responsible for its antidiabetic potential (specifically for diabetes mellitus) confirmed by Bhatt *et al.*, (2017) [2]. According to Sharma *et al.*, (2017) [18] ethanolic extract of *R. cineria* plant has showed slow progression of diabetes on rat and he also confirmed that plant has antioxidant activities as well. Another methanolic extract of leaves and stem of *Roylea cinerea* showed antioxidant and anticancer potential in -vitro conditions (Bhatia *et al*., 2020) [3]. Moreover, its leaves and shoots have higher pharmacological activities against scabs and other skin infections. Previous phytochemical studies showed the sign of presence of various secondary metabolites like Glycosides, Phenols, Tannin, Steroids, Saponin, Diterpenes, Flavonoids etc. which are effective against tumorous cell, microbes like fugus, protozoa, bacteria etc., against malaria, liver disease etc. and effectively termed as ethnomedicinal plant (Pundir *et al*., 2019) [14].

As this shrub is a known potent herbal medicinal plant, contain various diterpenoids and glycosides constituents. Where, aerial part of *R. cinerea* has resulted in the isolation of diterpenes namely calyenone, epicalyone, calyone and precalyone. Specifically, 'Precalyone' has been reported with anti-tumour activity against P-388 lymphocytic leukemia which was described by Prakash *et al*., (1979) [12].

For this study, methanolic solvent was used for extraction as it is first choice by various phyto-chemical analyst or researchers to observe therapeutic potentials of medicinal plants. Methanolic extract of medicinal plants such *Artemisia spp., Acorus calamus, Salvia Rosmarinus, Ocimum spp.* have been used as preliminary to find plant potential as antimicrobial, antifungal, antioxidant, antidiabetic respectively. (Ramezani *et al.*, 2004 [15]; Dhiman and Dutt, 2018 [6]; Chang *et al.*, 1977 [5]; Bihari *et al.*, 2011 [4]). And for quantification, HPTLC technique method has been standardized for *Roylea cinerea*.

HPTLC Technique

HPTLC is an advance type of thin layer chromatography (TLC) which conducts efficient separation with the help of favorable coating material. Where, chromatography is defined as physical process of effective separation of components in concentration-zone/phase different from those in which they are originally present, irrespective of the nature of the force or forces causing the substances to move from one phase to another (William and Weil, 1952) [19].

With the HPTLC technique, various merits over traditional TLC, like this reduced the analysis time, reduce the amount of mobile face used, less amount of sample needed, enhance the data acquisitions and interpretation etc. The HPTLC differ from TLC in term of resolution of mixture (separation of two components of a mixture in a chromatogram). The resolution of mixture increased number of times as compared to TLC; therefore, it is termed as HPTLC works with principle of separation may result due to adsorption or partition or by both phenomena. Chemical analysts are preferring HPLC to identify and quantify the phytochemical for fast result (Marston, 2007) [10], but many of them using HPTLC and considered it as excellent (Reich and Schibli, 2007) [16] for preliminary quantification before HPLC technique.

Material and Methods

Plant material collection

Samples (arial part of plants) for study were collected from wild *Roylea cinerea* at Solan (H.P.) during the month of March, 2018. Denizens of Solan call this plant locally as 'Karu'. The plant material was identified and confirmed with different macroscopic leaf characters (Colour, Odour, Shape, Petiole, Apex, Margin, Venation, size) in the Department of Botany SILB Institute, Solan.

Different observed macroscopic characters of the leaves of *Roylea cinerea* are given in Table 1.

Table 1: Observed macroscopical characters of *Roylea cinerea*

S. No.	Characters	Observations
1	Colour	Dark green but lighter at adaxial side
2	Odour	Lemon like
3	Shape	Ovate
4	Petiole	Present
5	Apex	Acute
6	Margin	Dentate
7	Venation	Reticulate
8	Size	Length: 2-8cm, Width: 1-8cm

Preparation of Extract (Arial part)

The collected plant material was first cleaned against dust and dried under shade for one week. After removing extra moisture from the sample, it is ground to powdery form mechanically by using mortar and pestle. Then sample is stored in air tight packet for further extraction. The powdered sample of plant initially extracted with methanol using Soxhlet-apparatus. The process was repeated four times for complete extraction. Subsequently, filtration was done by using Whatmann filter paper and solvent was forced to evaporate by using vacuum rotary evaporator to get dry crude extract.

Preliminary photochemical analysis

Selection of chromatographic plate and its washing

The TLC plate of dimension 10 cm X 6 cm was selected having support material (Aluminum) and sorbent (Silica gel) over it. After selection pre-washing of plate with using Methanol: Water (9:1) was done to remove Iron impurities which is commonly found along with sorbent (Silica gel).

Sample preparation

For sample preparation 5 mg of plant extract was dissolved in 20 ml of methanol in a conical flask. Then flask was heated for 5 minutes at 20℃ for proper mixing with covering its neck with aluminum foil. Then extract was filtered in beaker after proper dilution. Solvent system of TEF (5:4:1 Toluene: Ethyl acetate: Formic acid) was prepared separately in a jar. Simultaneously, a detector reagent; Anisaldehyde-Sulfuric reagent was prepared by dissolving 5 ml Anisaldehyde, 10 ml acetic acid, 80 ml methanol and 5 ml concentrated H_2SO_4 together in a separate beaker.

Application of sample

Syringe was washed first using methanol and then sample of 20.2 µl was taken with care to avoid bubbles in it otherwise it will affect the sample volume. TLC plate and syringe were placed in its position on sample injector. After pressing enter key on applicator, machine belt started to perform its work (samples were loaded automatically) and sample from syringe will be laid on TLC plate placed on applicator. Repeat the process again, as 2 replicates R1 and R2 are taken for spotting and profiling on same plate. Then TLC plate was displaced without touching on sample spots and was allowed for air drying.

Mobile phase and detector reagent

On the other side, Twin trough chamber was left earlier before 30 minutes for saturation which contained solvent mixture i.e., Toluene: Ethyl acetate: Formic acid (5:4:1) of 10 ml. Air dried TLC plate was marked at 8cm from its bottom and was placed in the twin trough chamber. This chamber was

placed stationary so that mobile phase moves up without any disturbance.

Then detector chamber and detector reagent (Anisaldehyde-Sulfuric reagent) were used to detect the constituents which are not visible with day-light. From the detector chamber TLC plate was placed in the oven at 105℃ for 5 minutes to darken the spots on TLC plate.

Scanning

TLC scanner was used to visualize plate under UV at different wavelength i.e., 254 nm, 366 nm and 566 nm. Size of plate (dimensions) and wavelength were set on the monitor screen. And finally, by spot recording or automatization peaks of different constituents were formed on the screen.

Result and Observation

The chromatograph with Rf value of different peaks at different wavelength of 566 nm, 366 nm and 254 nm are shown in Table 2. At UV light with wavelength of 566 nm, maximum numbers of peaks *i.e.,* 14 (R2) and 13 (R1) were obtained. For both replicate samples, peaks were broadened and merged with each other. At wavelength of 566 nm, minimum numbers of peaks *i.e.,* 8 (R1) and 8 (R2) were obtained for both replicates. And for wavelength of 254 nm about 9 peaks for R1and 8 peaks for R2 were noticed with clear narrow peak area shown in chromatographs.

Rf value ranged from 0.13 to 1.03 for replicate (R1) and from 0.12 to 1.03 for replicate (R1) with number of peaks about 13-14 were noticed at wavelength of 566 nm. Other values of Rf ranged from 0.13 to 0.88 for R1 and from 0.14 to 0.87 for R2 with 8 peaks for both sample replicates were obtained at wavelength of 366 nm. Rf values of 0.14 to 0.94 and 0.14 to 0.85 with 8 and 9 peaks were noticed for both R1 and R2 sample replicates respectively on the chromatographs at wavelength of 254nm.

Table 2: HPTLC analysis of methanolic extract of *Roylea cinerea* (D. Don) Baillon

No. of Peaks	Inferences (Rf values at different wavelengths)					
	UV (566 nm)		UV (366 nm)		UV (254 nm)	
	R1	R2	R1	R2	R1	R2
1	0.13	0.12	0.13	0.14	0.14	0.14
2	0.17	0.16	0.32	0.32	0.32	0.31
3	0.25	0.25	0.43	0.43	0.43	0.43
4	0.33	0.32	0.54	0.53	0.54	0.53
5	0.37	0.37	0.59	0.59	0.60	0.60
6	0.42	0.43	0.69	0.68	0.68	0.68
7	0.52	0.51	0.76	0.76	0.75	0.75
8	0.54	0.61	0.88	0.87	0.85	0.85
9	0.61	0.66			0.94	
10	0.66	0.74				
11	0.75	0.85				
12	0.85	0.98				
13	0.97	1.03				
14	1.03					

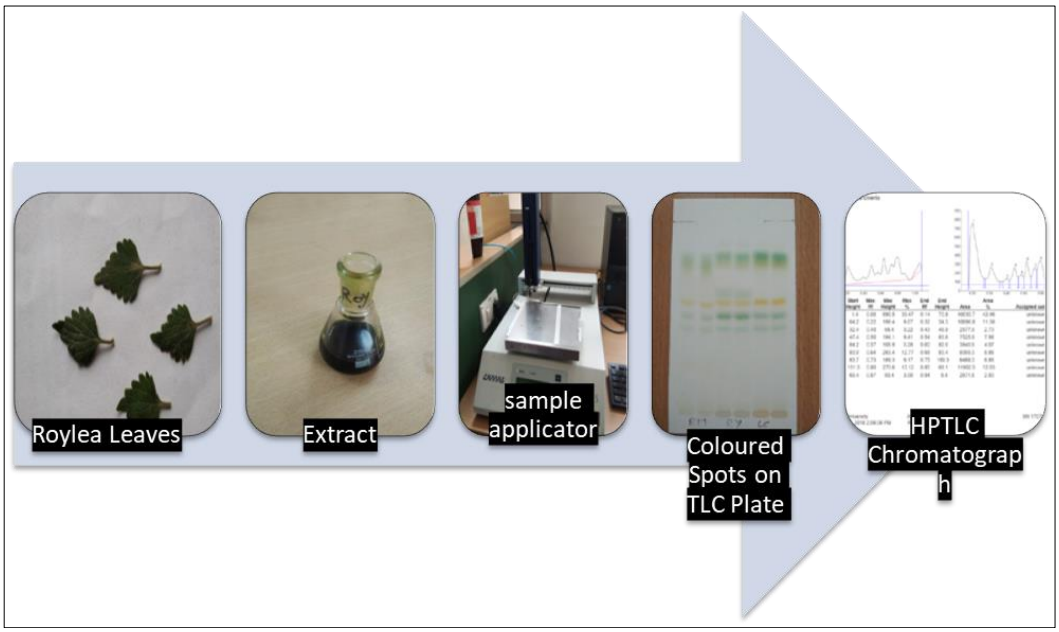
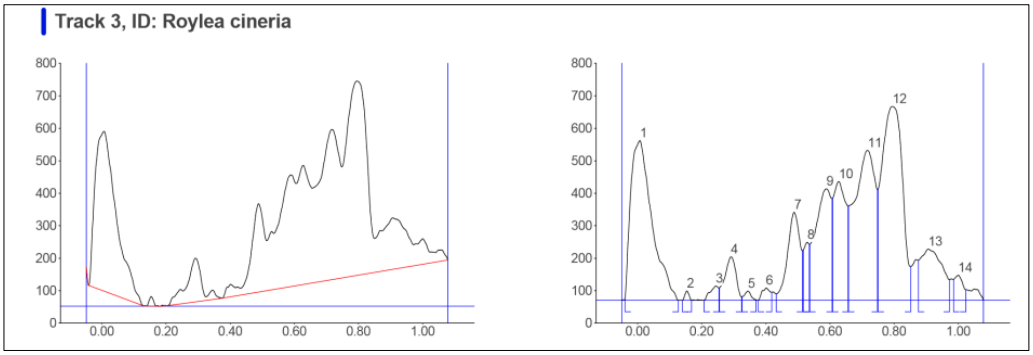
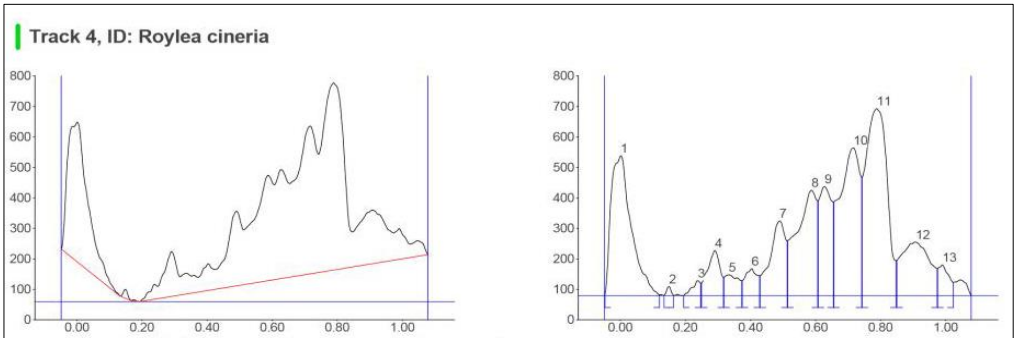


Fig 1: Pictorial representation of Methodology up to Chromatograph

Chromatograph displayed at wavelength 566 nm (UV)
(Track 3 represents Replicate-1 and Track 4 represents Replicate-2)

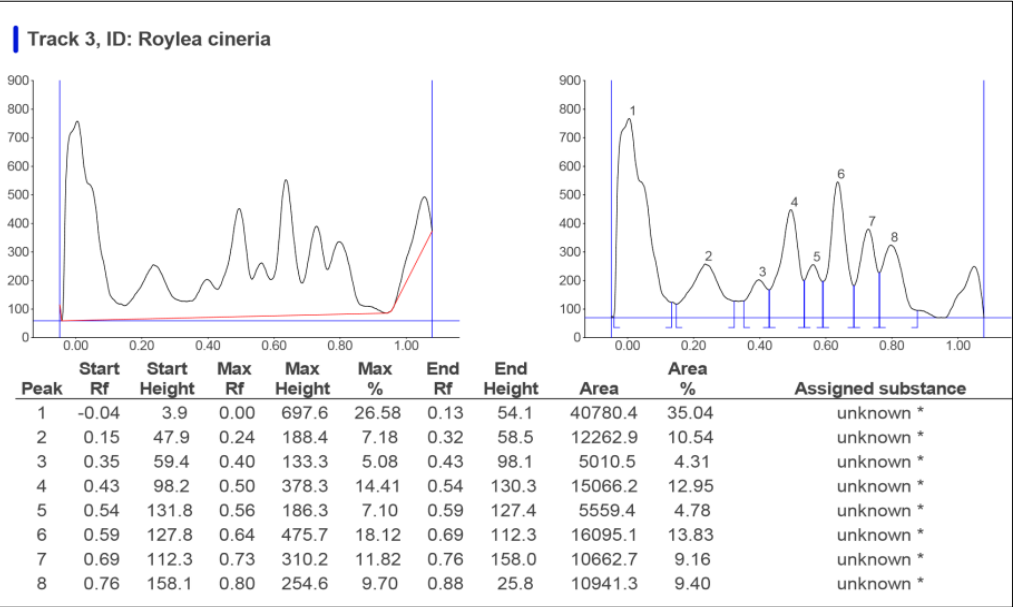


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	7.8	0.01	492.1	15.27	0.13	0.5	23452.6	19.62	unknown *
2	0.14	0.2	0.15	29.3	0.91	0.17	0.9	269.0	0.23	unknown *
3	0.21	1.1	0.25	44.6	1.38	0.25	38.7	843.7	0.71	unknown *
4	0.26	39.3	0.29	134.2	4.16	0.33	11.2	3393.8	2.84	unknown *
5	0.33	11.6	0.35	28.5	0.89	0.37	1.9	433.1	0.36	unknown *
6	0.38	0.0	0.40	38.1	1.18	0.42	25.3	698.2	0.58	unknown *
7	0.43	20.5	0.49	271.5	8.43	0.52	153.8	7689.3	6.43	unknown *
8	0.52	155.3	0.53	179.2	5.56	0.54	172.7	2215.5	1.85	unknown *
9	0.54	173.0	0.59	343.8	10.67	0.61	314.2	12145.3	10.16	unknown *
10	0.61	315.2	0.63	365.9	11.35	0.66	291.2	10268.4	8.59	unknown *
11	0.66	291.6	0.72	462.0	14.34	0.75	341.5	21367.6	17.88	unknown *
12	0.75	343.1	0.80	597.5	18.54	0.85	104.3	27529.9	23.03	unknown *
13	0.88	124.2	0.91	158.2	4.91	0.97	64.9	7686.5	6.43	unknown *
14	0.99	65.2	1.00	78.0	2.42	1.03	33.4	1539.5	1.29	unknown *

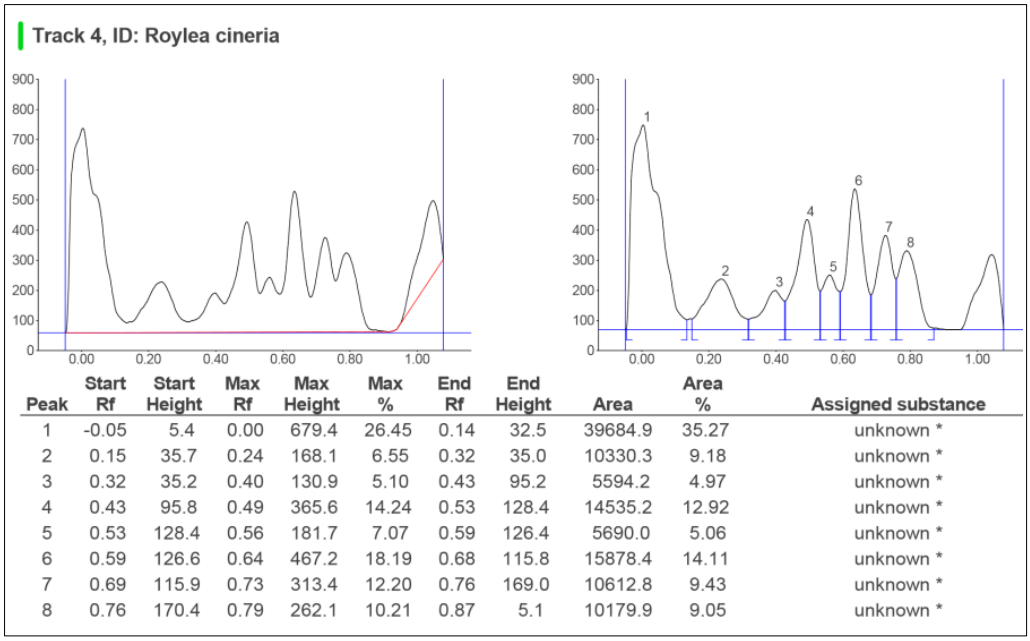


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.05	4.5	0.00	458.7	14.50	0.12	2.1	20507.1	16.07	unknown *
2	0.14	0.7	0.15	29.7	0.94	0.16	0.4	280.5	0.22	unknown *
3	0.20	0.3	0.24	49.0	1.55	0.25	40.9	881.8	0.69	unknown *
4	0.25	41.0	0.29	147.7	4.67	0.32	60.2	4140.5	3.24	unknown *
5	0.32	60.2	0.33	67.9	2.15	0.37	48.8	2103.2	1.65	unknown *
6	0.38	49.4	0.40	87.8	2.78	0.43	65.5	2418.0	1.89	unknown *
7	0.43	65.6	0.49	245.0	7.75	0.51	179.9	8404.8	6.58	unknown *
8	0.52	180.7	0.59	345.6	10.93	0.61	311.5	15051.3	11.79	unknown *
9	0.61	312.2	0.63	357.2	11.29	0.66	307.2	9982.6	7.82	unknown *
10	0.66	307.8	0.72	484.6	15.32	0.74	387.8	21478.0	16.83	unknown *
11	0.75	388.2	0.79	613.4	19.39	0.85	115.0	29003.4	22.72	unknown *
12	0.85	115.7	0.91	175.5	5.55	0.98	89.3	10990.9	8.61	unknown *
13	0.98	89.7	0.99	101.0	3.19	1.03	43.8	2395.6	1.88	unknown *

Chromatograph displayed at wavelength 366 nm (UV)
(R1 -Track 3 and R2 – Track 4)

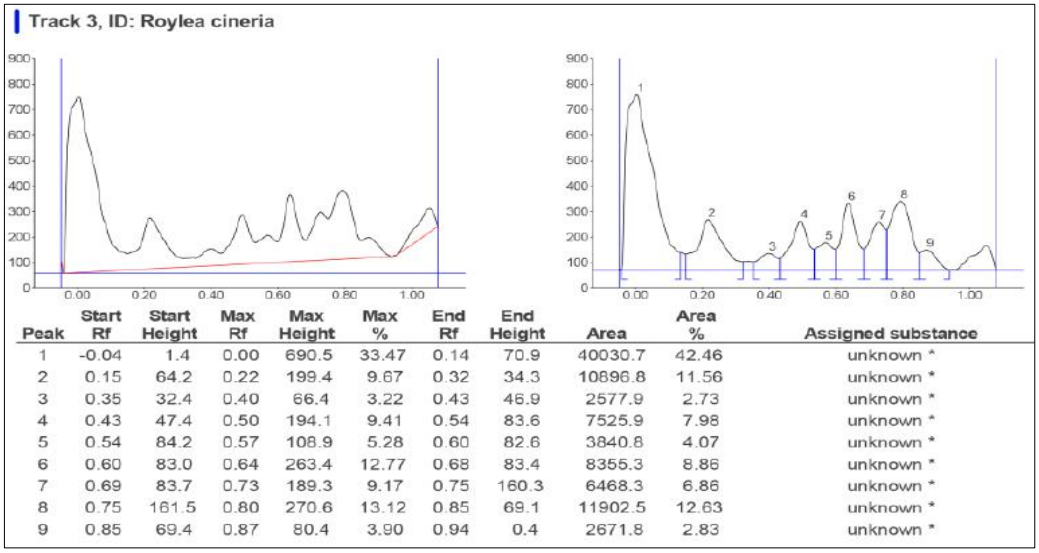


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	3.9	0.00	697.6	26.58	0.13	54.1	40780.4	35.04	unknown *
2	0.15	47.9	0.24	188.4	7.18	0.32	58.5	12262.9	10.54	unknown *
3	0.35	59.4	0.40	133.3	5.08	0.43	98.1	5010.5	4.31	unknown *
4	0.43	98.2	0.50	378.3	14.41	0.54	130.3	15066.2	12.95	unknown *
5	0.54	131.8	0.56	186.3	7.10	0.59	127.4	5559.4	4.78	unknown *
6	0.59	127.8	0.64	475.7	18.12	0.69	112.3	16095.1	13.83	unknown *
7	0.69	112.3	0.73	310.2	11.82	0.76	158.0	10662.7	9.16	unknown *
8	0.76	158.1	0.80	254.6	9.70	0.88	25.8	10941.3	9.40	unknown *

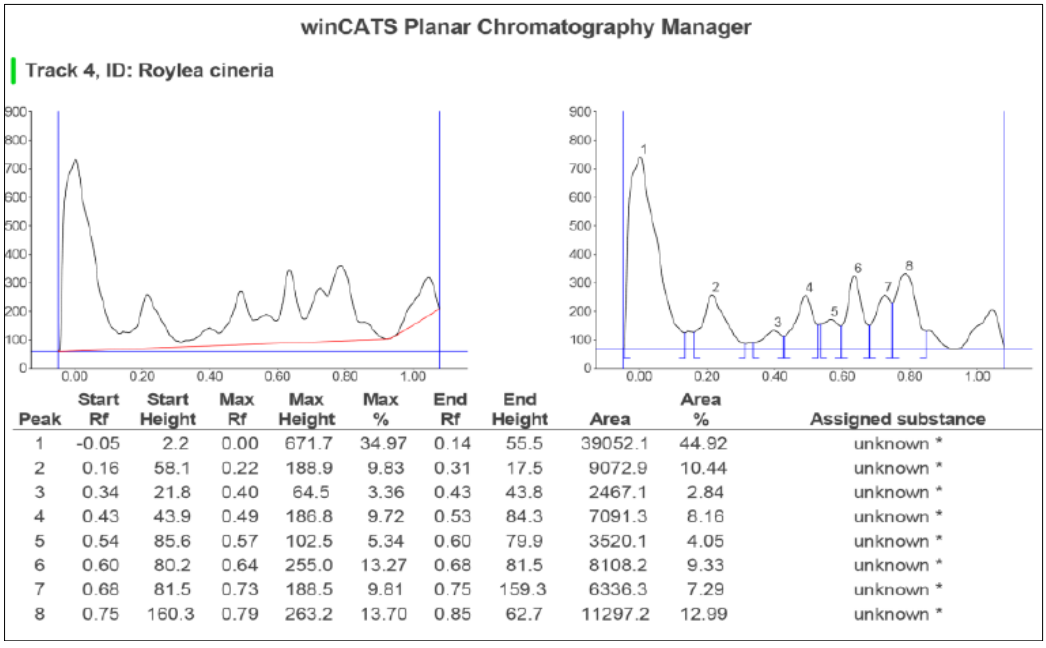


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.05	5.4	0.00	679.4	26.45	0.14	32.5	39684.9	35.27	unknown *
2	0.15	35.7	0.24	168.1	6.55	0.32	35.0	10330.3	9.18	unknown *
3	0.32	35.2	0.40	130.9	5.10	0.43	95.2	5594.2	4.97	unknown *
4	0.43	95.8	0.49	365.6	14.24	0.53	128.4	14535.2	12.92	unknown *
5	0.53	128.4	0.56	181.7	7.07	0.59	126.4	5690.0	5.06	unknown *
6	0.59	126.6	0.64	467.2	18.19	0.68	115.8	15878.4	14.11	unknown *
7	0.69	115.9	0.73	313.4	12.20	0.76	169.0	10612.8	9.43	unknown *
8	0.76	170.4	0.79	262.1	10.21	0.87	5.1	10179.9	9.05	unknown *

Chromatograph displayed at wavelength 254 nm (UV)
(R1 -Track 3 and R2 – Track 4)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	1.4	0.00	690.5	33.47	0.14	70.9	40030.7	42.46	unknown *
2	0.15	64.2	0.22	199.4	9.67	0.32	34.3	10896.8	11.56	unknown *
3	0.35	32.4	0.40	66.4	3.22	0.43	46.9	2577.9	2.73	unknown *
4	0.43	47.4	0.50	194.1	9.41	0.54	83.6	7525.9	7.98	unknown *
5	0.54	84.2	0.57	108.9	5.28	0.60	82.6	3840.8	4.07	unknown *
6	0.60	83.0	0.64	263.4	12.77	0.68	83.4	8355.3	8.86	unknown *
7	0.69	83.7	0.73	189.3	9.17	0.75	160.3	6468.3	6.86	unknown *
8	0.75	161.5	0.80	270.6	13.12	0.85	69.1	11902.5	12.63	unknown *
9	0.85	69.4	0.87	80.4	3.90	0.94	0.4	2671.8	2.83	unknown *



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.05	2.2	0.00	671.7	34.97	0.14	55.5	39052.1	44.92	unknown *
2	0.16	58.1	0.22	188.9	9.83	0.31	17.5	9072.9	10.44	unknown *
3	0.34	21.8	0.40	64.5	3.36	0.43	43.8	2467.1	2.84	unknown *
4	0.43	43.9	0.49	186.8	9.72	0.53	84.3	7091.3	8.16	unknown *
5	0.54	85.6	0.57	102.5	5.34	0.60	79.9	3520.1	4.05	unknown *
6	0.60	80.2	0.64	255.0	13.27	0.68	81.5	8108.2	9.33	unknown *
7	0.68	81.5	0.73	188.5	9.81	0.75	159.3	6336.3	7.29	unknown *
8	0.75	160.3	0.79	263.2	13.70	0.85	62.7	11297.2	12.99	unknown *

Conclusion

The main aim of this work is to develop and validate a chromatographic method for analysis of various constituents present in the methanolic extract *Roylea cinerea* using the technique of HPTLC. About 8 (R2) and 9 (R3) clear peaks were noticed at wavelength of 254 nm, another 8 peaks for both replicates at 366nm, and maximum of about 13 (R2) and 14 (R1) peaks were obtained at 566 nm wavelength. Different peaks with different wavelength represent different unknown phyto-constituents. On the basis of present preliminary HPTLC experiment with crude plant extract, we are hoping for subsequent further studies which required for reconfirmation of phytoconstituents present in leaves of *Roylea cinerea* by using High Performance liquid chromatography to study its therapeutical potential and pharmacological activities.

References

1. Bahuguna RP, Jangwan JS, Singh R, Bhatt UP. In vitro cytotoxic, *in-vitro* and *in-vivo* antidiabetic activity of *Roylea cinerea*. Science Reviews and Chemical Communications 2015;5(2):69-76.
2. Bhatt UP, Sati SC, Bahuguna RP, Semwal RB, Semwal DK. Two antidiabetic constituents from *Roylea cinerea* (D.Don) Baill. Natural Product Research 2017;32:1281-1286.
3. Bhatia A, Buttar HS, Arora R, Singh B, Singh A, Kaur S *et al*. Antiproliferative Effects of *Roylea cinerea* (D. Don) Baillon Leaves in Immortalized L6 Rat Skeletal Muscle Cell Line: Role of Reactive Oxygen Species Mediated Pathway. Frontiers in Pharmacology 2020;11:322.
4. Bihari CG, Manaswini B, Keshari PS, Kumar TS. Phytochemical investigation & evaluation for antidiabetic activity of leafy extracts of various *Ocimum* (Tulsi) species by alloxan induced diabetic model. J Pharm Res 2011;4:28-9.
5. Chang SS, Ostric-Matijasevic BI, Hsieh OA, Huang CL. Natural antioxidants from rosemary and sage. Journal of Food Science 1977;42(4):1102-6.
6. Dhiman B, Dutt B. Evaluation of *Acorus calamus* L. rhizome extract as a bio preservative against wood fungal decay. Journal of Applied and Natural Science 2018;10(1):37-40.
7. Dua VK, Verma G, Agarwal DD, Kaiser M, Brun R. Antiprotozoal activities of traditional medicinal plants from the Garhwal region of North West Himalaya, India. Journal of Ethnopharmacology 2011;136(1):123-128.
8. Gaur RD, Flora of District Garhwal North West Himalya, Transmedia Pub. Srinagar Garhwal, Uttranchal, India 1999,475.
9. Khare CP. Indian Medicinal Plants – An Illustrative Dictionary (Heidelberg, Germany: Springer Publishers). 2007,559.
10. Marston A. Role of advances in chromatographic techniques in phytochemistry. Phytochemistry 2007;68(22-24):2786-2798.
11. Majumdar PL, Maity RN, Panda SK, Mal D, Raju MS, Wenkert E. Journal of Organic Chemistry 1979;44:2811-2812.
12. Prakash O, Bhakuni DS, Kapil RS, Rao GS, Ravindranath B. Diterpenoids of *Roylea calycina* (Roxb.) Briq. Journal of the Chemical Society, Perkin Transactions 1 1979,1305-8.
13. Parkash V, Aggrawal A. Traditional use of ethnomedicinal plants of lower foothills of Himachal Pradesh-1. Indian journal of Traditional knowledge 2010;9:519.
14. Pundir S, Mahindroo N. *Roylea cinerea* (D. Don) Baillon: Ethnomedicinal uses, phytochemistry and pharmacology: A review. Journal of Ethnopharmacology 2019;232:193-200.
15. Ramezani M, Fazli-Bazzaz BS, Saghafi-Khadem F, Dabaghian A. Antimicrobial activity of four Artemisia species of Iran. Fitoterapia 2004;75(2):201-3.
16. Reich E, Schibli A. High-performance Thin-layer Chromatography for the Analysis of Medicinal Plants. Thieme Medical Publishers, Inc., New York 2007.
17. Rawat R, Vashistha DP. *Roylea cinerea* (D. Don) Baillon: a traditional curative of diabetes, its cultivation prospects in Srinagar valley of Uttarakhand. International Journal of Advances in Pharmacy, Biology and Chemistry 2013;2:372–375.
18. Sharma R, Yadav D, Asif M, Jayasri MA, Agnihotri VK, Ravikumar PC. Antidiabetic and antioxidant activities of *Roylea cinerea* extract. Indian Journal of Experimental Biology 2017;55:611-621.
19. Williams TI, Weil H. Definition of Chromatography. Nature 1952;170(4325):503-503.