A STUDY ON HERBAL HAIR OIL FORMULATIONS USING RP-HPTLC METHOD FOR ESTIMATION OF URSOLIC ACID AND DIOSGENIN IN HAIR LOSS AND GREY HAIR ACTIVITY

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Abstract

Hair loss, also called alopecia, is a disorder caused by an interruption in the body's cycle of hair production. Commonly it affects On the scalp. On average, the scalp has 100,000 hairs that cycle through periods of growing, resting, falling out, and regenerating. Grey hair is really hair with reduced melanin, while white hair completely lacks it. Coconut oil softens hair and increases shine. Black seed oil strengthens moisturizes the hair, adds nutrients, and stimulates the scalp. Rosemary oil performed a hair growth promoter, with less scalp itching as a side effect. Different oils can help to grow hair and also add strength and shine. In this present study the different herbs and oils was used to prepared herbal hair oil by using different formulation methods which contain major chemical compounds like ursolic acid diosgenin to improve hair growth and reduce grey hair. After the oil was evaluated by morphological and pharmacological studies. By using HPTLC and microbiological studies we found that two formulations gave good results compare with other f**o**rmulati**o**ns.

Key words: Herbal oil, ursolic acid, diosgenin, hair growth, grey hair, HPTLC etc.

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Introduction:

Many special herbs having the medicinal properties they acts as a biovitalizers for promoting hair growth. Herbal formulations always show a benedictory effects to humans. Proper identification and quality evaluation of crude herbal extracts is a fundamental requirement. Herbal drugs of Poly formulations have multiple numbers of Phyto constituents using HPTLC.(1)

Hair loss:

It can be the result of heredity, hormonal changes, medical conditions or a normal part of aging. Anyone can lose hair on their head, but it's more common in men. Baldness typically refers to excessive hair loss from your scalp. Hereditary hair loss with age is the most common cause of baldness. Hereditary hair loss. Both men and women develop this type of hair loss, which is the most common cause of hair loss worldwide.(2-4) Age, Alopecia areata, Cancer treatment, Childbirth , illness, or other stressors, Hair care, Hairstyle pulls on your scalp, Hormonal imbalance.

Greyingof Hair

Hair greying is a big problem in the metro cities due to exposure of heavy polluted environment.Greying is caused due to the loss of the pigment in the shaft. The colour of the hair mainly depends upon the amount of melanin present in the hair.(5). So, Ayurvedic Polyherbal formulation of hair oil is to impart softness of the hair also help to fill the fissures.

Ursolic acid and its isomer, oleanolic acid, have been used in to nics to enhance hair growth and prevent scalp irritation. (6) Both triterpenoid compounds encourage hair growth by stimulating the peripheral blood flow in the scalp and activating the hair mother cells. Diosgenin in The use of natural products, including steroidal compounds, has been growing not only as therapeutically active agents but also as lead compounds in drug discovery approaches**.** The present study was carried out screen and confirm the presence of Phytoconstituents in each and every ingredient of different hair oil and also in final formulation using HPTLC.(7-9)

High-performance thin-layer chromate graphy (**HPTLC**) High Performance Thin Layer Chromatography (HPTLC) technique is most simple and fastest separation technique based on Planar Chromatography which gives better precision and accuracy with extreme flexibility for various steps. The results showing number of peaks are presented indifferent Tracks. HPTLC spectral analysis of both extracts were done in three different wavelengths- Scan 1:After derivatized with ANS at 560nm and UV800 to 450 nm UV, (10) thin-layer chromatography to automate the different steps, to increase the resolution achieved, and to allow more accurate quantitative measurements. Automation is useful to overcome the uncertainty in droplet size and position when the sample is applied to the TLC plate by hand. One approach to automation has been the use of piezoelectric devices and inkjet printers for applying the sample.[11]

Materials And Methods:

Materials: the herbs such as

Guava (*Psidium guajava* L), *Carica papaya*, Onion (*Allium cepa* L.), methi seeds (Fenugreek), Saragva(Moringa oleifera) , Rosemary oil (Rosmarinus officinalis L), Kalonji (Nigella sativa) and coconut oil. all these herbs were ordered from R.K UNIVERSITY, Rajkot. oils purchased from local market in Ahmadabad. These raw materials were screened for identity, purity and strength. The prepared oils were screened for presence of using HPTLC. Reference standard compound ursolic acid and Diosgenin was purchased from local market.

Herbal Oil Formulation

For the Formulation of herbal hair oil different herbs fresh leaves, seeds and bulb were collected in a container. Coconut milk is used as base oil and boiled along with collected herbs until the oil colour changes to pale yellow and floats on the surface. Now the oil boiled oil is collected into a container by using separating funnel. Eight formulations were prepared by using different concentrations of herbs.

Appearance

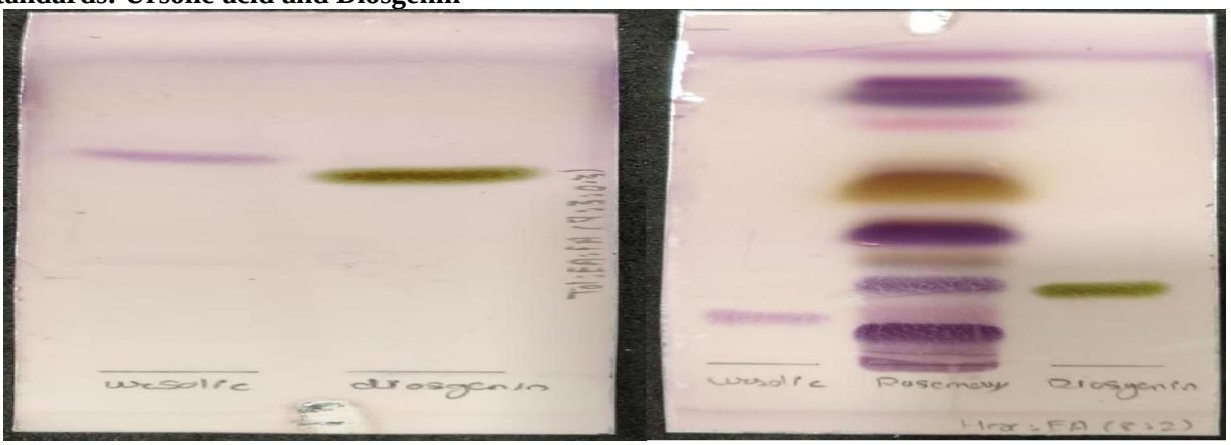
The appearance of the oil was judged by its color and odour.

Sample preparation: Transfer 6 g of oil sample in test tube. Add 2 ml hydrochloric acid and heat the mixture/solution for 2-3 min on water bath, extract above solution with 10 ml methanol. Separate the methanol layer (upper) and evaporate to dryness. Dissolve the 10 mg of residue in 10 ml methanol and use this solution (20 µl) for HPTLC analysis. The standard preparation is about 1mg/ml in methanol and Stationary phase: 20 by 10 TLC plate, Mobile phase: Toluene: Ethyl acetate: Formic acid (7:3:0.3) and Saturation: 20 min, Track length/band width: 8mm and Distance between track: minimum 4 mm Developing distance: 8mm, Derivatization: Anisaldehyde-Sulphuric Acid Reagent (ASR)

Scanning wavelength: Scan 1: 254nm, 365nm, 560nm.

Sample preparation:
6 g.oil was taken and 6 ml methanol was added. Methanol is immiscible with oil hence separated in upper layer. Sonicate for 10 min and centrifuge at 3500 rpm for 10 ml. Collect the supernatant and filter with A grade filter paper TLC spotting: Directly applied above cited Methanolic solution to TLC plates.

Mobile Phase: Toluene: Ethyl acetate: Formic acid (7: 3: 0.3)
Standards: Ursolic acid and Diosgenin



Mobile Phase: n-Hexane: Ethyl acetate (8:2) Standards: Ursolic acid, Rosemary oil, and Diosgenin

Preparation of Standard
The standard diosgenin (100 μg mL^{-1}) was prepared by dissolving 1 mg of diosgenin in 10 mL of chloroform.

HPTLC Separation Condition
The standards and samples were spotted on activated HPTLC plates of silicage l60F254of0.5-mm thickness coating using a CAMAGL inomatV(Switzerland) sample applicator. The plates (10 × 10 cm) and detected by 20% antimony chlorideinchloroform,which was sprayed and dried in a chromatographic oven at 105°C for 10 min. The plate was kept in photo-documentation chamber (CAMAG Reprostar 3), and finally,the plate was fixed inscannerstageandscannedat254 nm (after derivatization – 433 nm). The resolution bands were obtained,and retardation-factor(*R*)was calculated.

Calibration Curve For Diosgenin and Urosilic acid:
A stock solution of standard diosgenin (100 μg mL^{-1}) was prepared in chloroform. Different volumes of stock solution,1,2,3,4, 5, and 6 μL, were spotted on TLC plate to obtain the concentration of 100, 200, 300, 400, 500, and 600 ng $spot^{-1}$ of diosgenin, respectively. The data of peak areas were plotted against the corresponding concentration.

UV Analysis:2 g oils samples were dissolved in 10 ml acetone. Used for UV analysis under wavelength at 800 to 450 nm. The blank is acetone and instrument used for Shimadzu 1800, Software: UV probe 2.4.2 in the date of 15-04-2022 with time10:00 am to 2:00 pm.

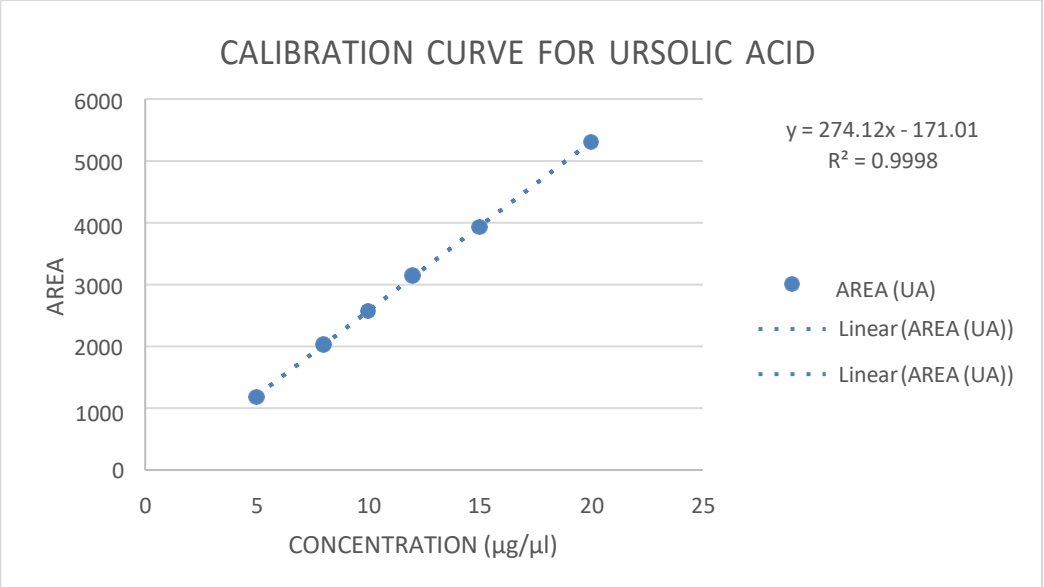
Results and discussion:

Table 1: Different 8 oil samples

s.no	Name of the plant	Botanical name	Weight (gms)	
			F1,F2,F3,F4	F1,F2,F3,F4
1	Guava leave	Psidium guajava.l	10GM	20gm
2	Methi seed	Trigonella foenum-graecum	10GM	20gm
3	Onion bulb	Allium cepa	10GM	20gm
4	Papaya leave	Carica papaya,	10GM	20gm
5	Saragva leave	Moringa oleifera	10 GM	20gm
6	Rosemerry oil	Salvia rosmarinus	E- oil	
7	Kalonji oil	Nigella sativa,	30ml	
8	Coconut oil	Cocos nucifera (L.)	70ml	

Table 2: Calibration curve of ursolic acid and Diosgenin

SR.NO.	CONC. (µg/µl)	AREA (Ursolic acid)
1	5	1180.641
2	8	2030.482
3	10	2568.273
4	12	3150.655
5	15	3929.671
6	20	5302.803
SR.NO.	**CONC. (µg/µl)**	**AREA (DIOSGENIN)**
1	5	1061.382
2	8	1856.825
3	10	2258.206
4	12	2761.657
5	15	3475.039
6	20	4897.317



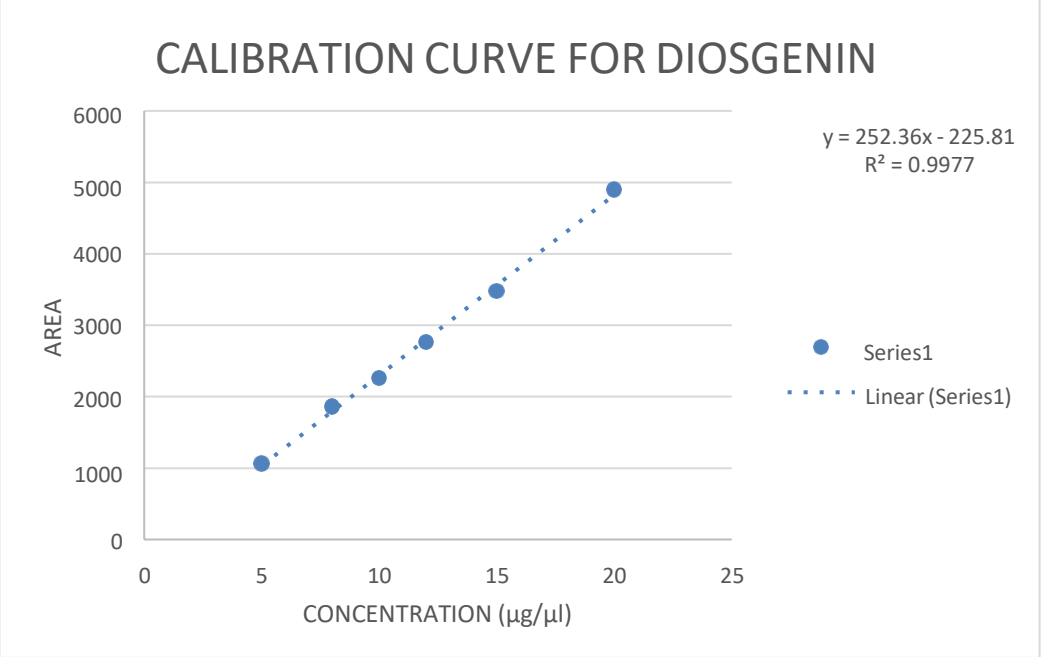
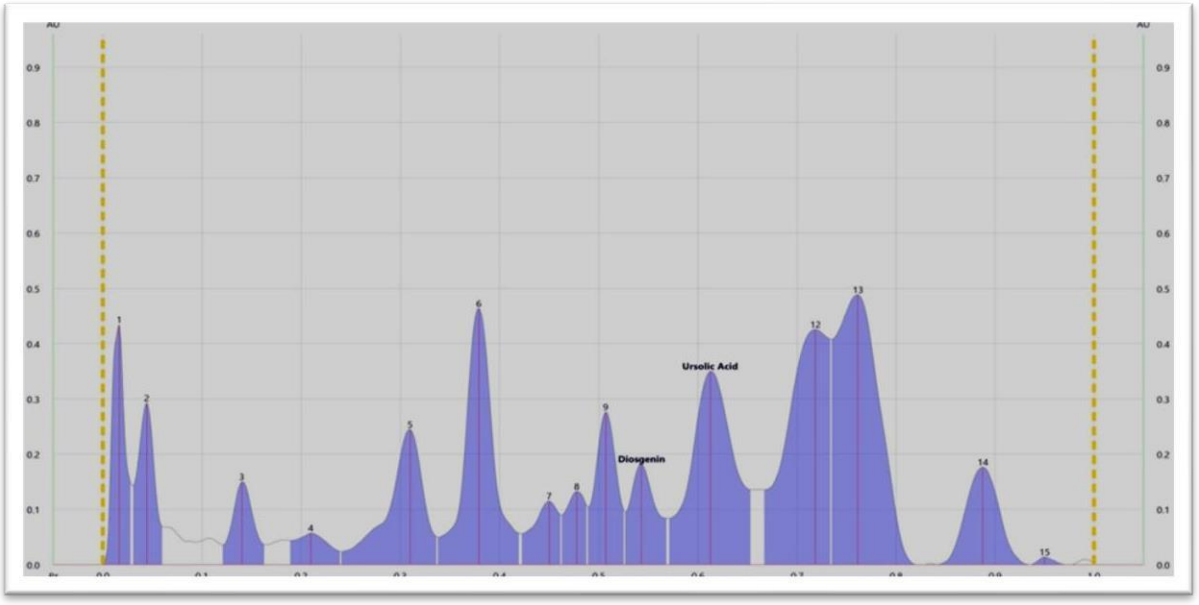
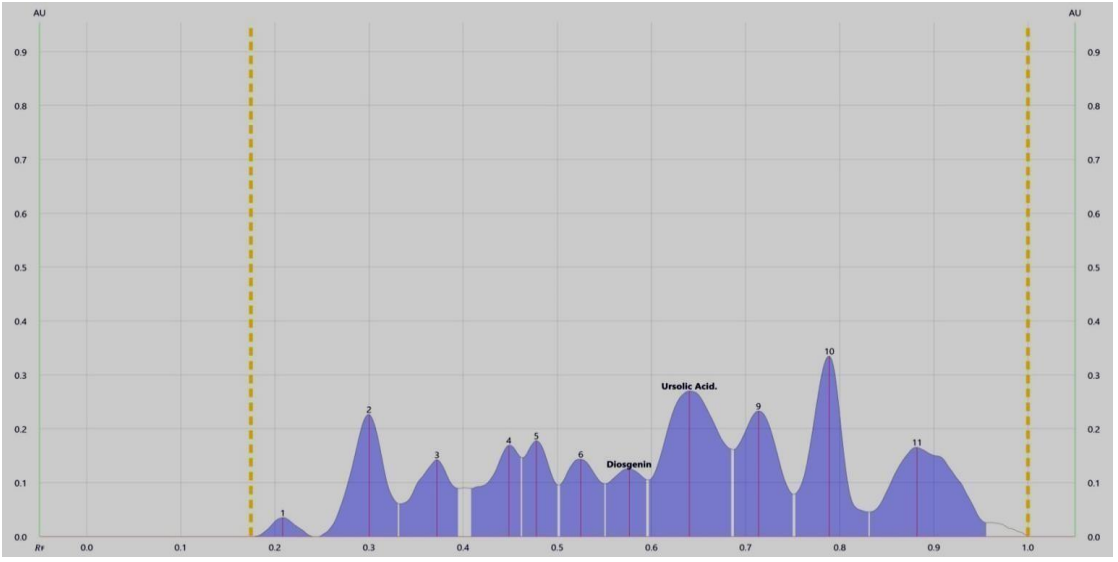


Table 3: spotting or tracking details

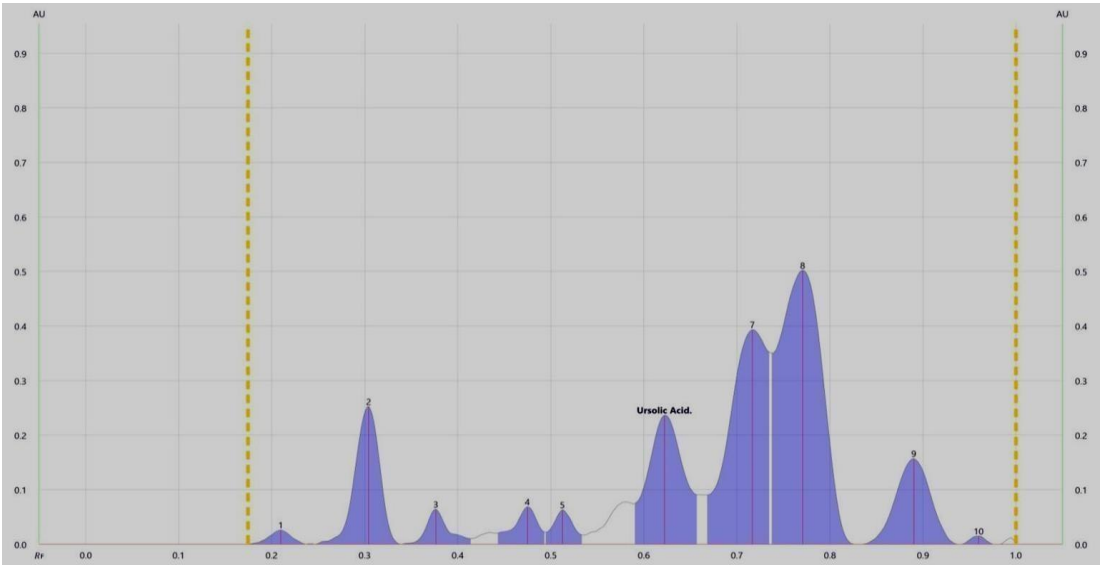
s.no	**Spotting or tracking details**
1	Track 1: F1/10 (5 µL)
2	Track 2: F2/10 (85 µL)
3	Track 3: F3/10 (5 µL)
4	Track 4: F4/10 (5 µL)
5	Track 5: F1/20 (5 µL)
6	Track 6: F2/20 (5 µL)
7	Track 7: F3/20 (5 µL)
8	Track 8: F4/20 (µL)
9	Track 9: Ursolic acid (2µL)
10	Track10: Diosgenin (2µL)
11	Track 11: Rosemerry oil(5 µL)
12	Track 12: Methi MEOH(10µL)
13	Track 13: Guava MEOH(10µL)
14	Track 14: Kalonji oil(2 µL)
15	Track 15: Onion MEOH(5 µL)
16	Track 16: Papaya MEOH(5 µL)
17	Track 17: Saragva MEOH(5 µL)



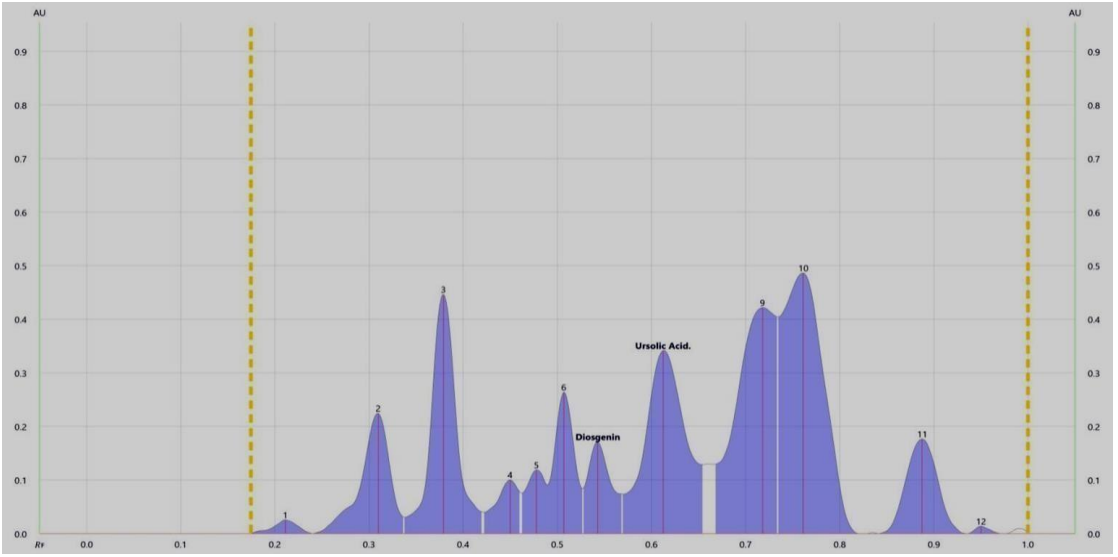
Track 1: F1/10 (5 µL)



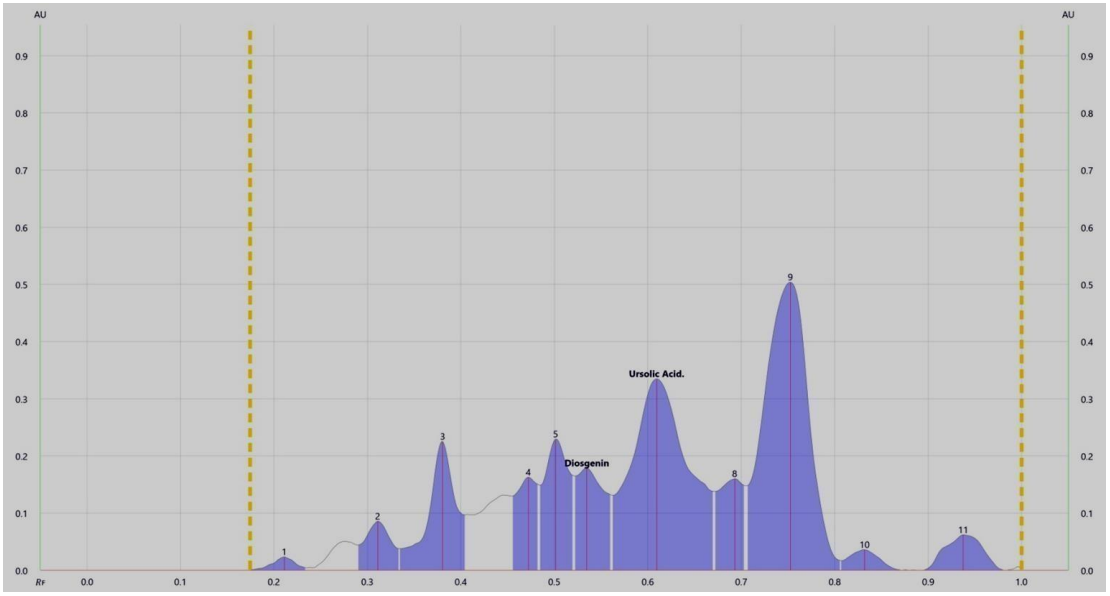
Track 2: F2/10 (10µL)



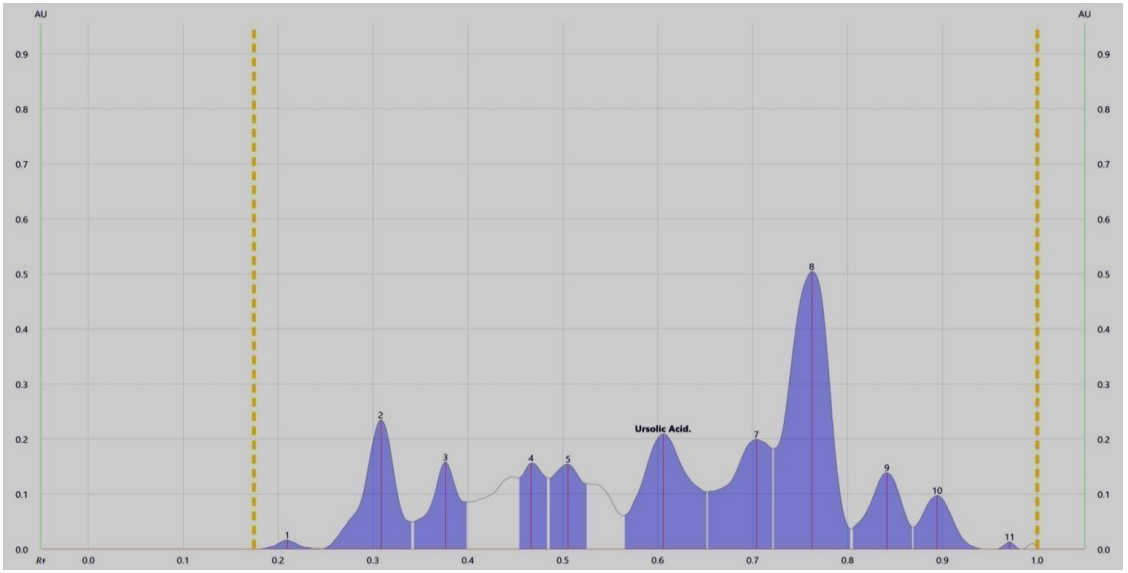
Track 3: F3/10 (10µL)



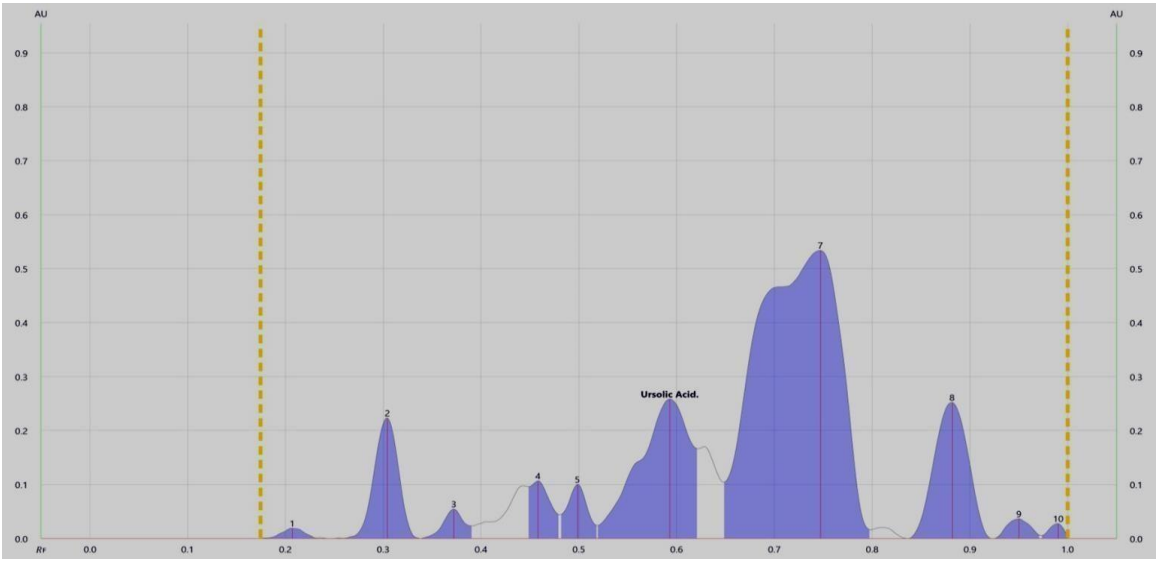
Track 4: F4/10 (10µL)



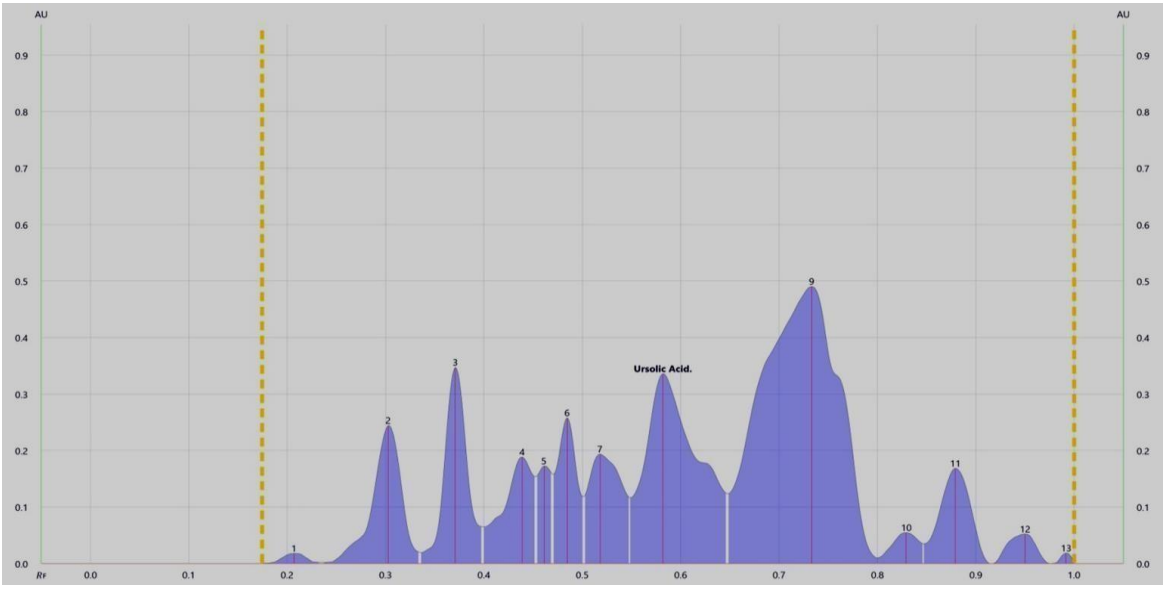
Track 5: F1/20 (10µL)



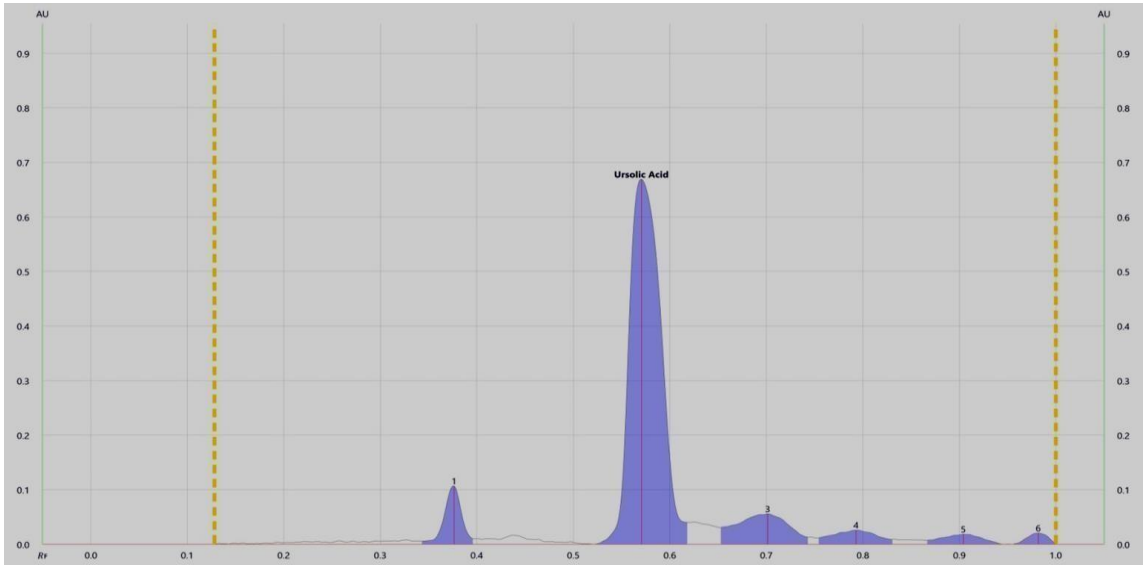
Track 6: F2/20 (10µL)



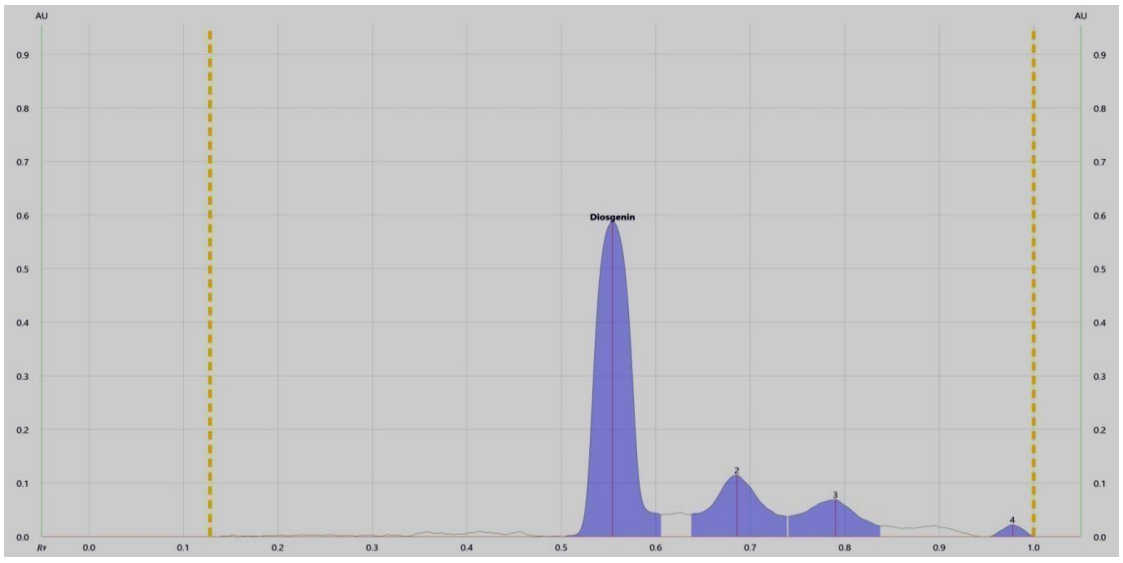
Track 7: F3/20 (10µL)



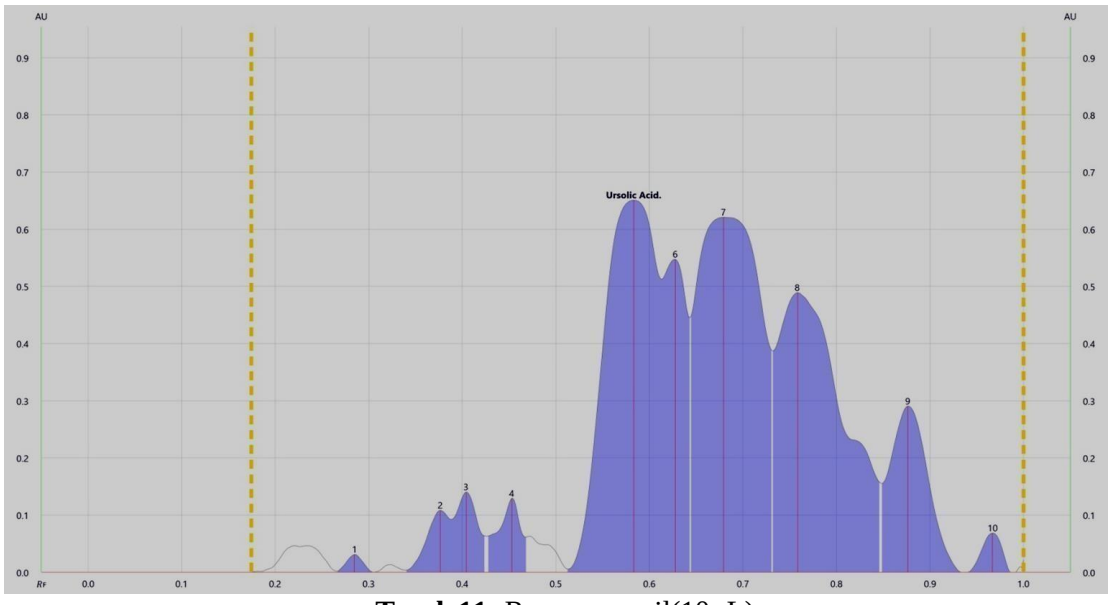
Track 8: F4/20 (10µL)



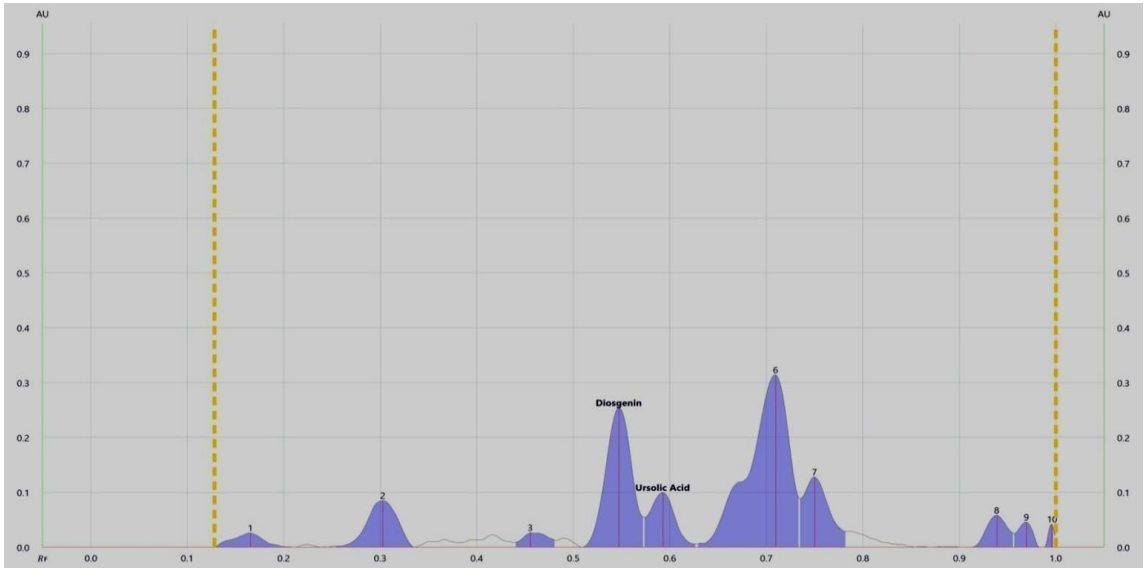
Track 9: Ursolic acid (5 µL)



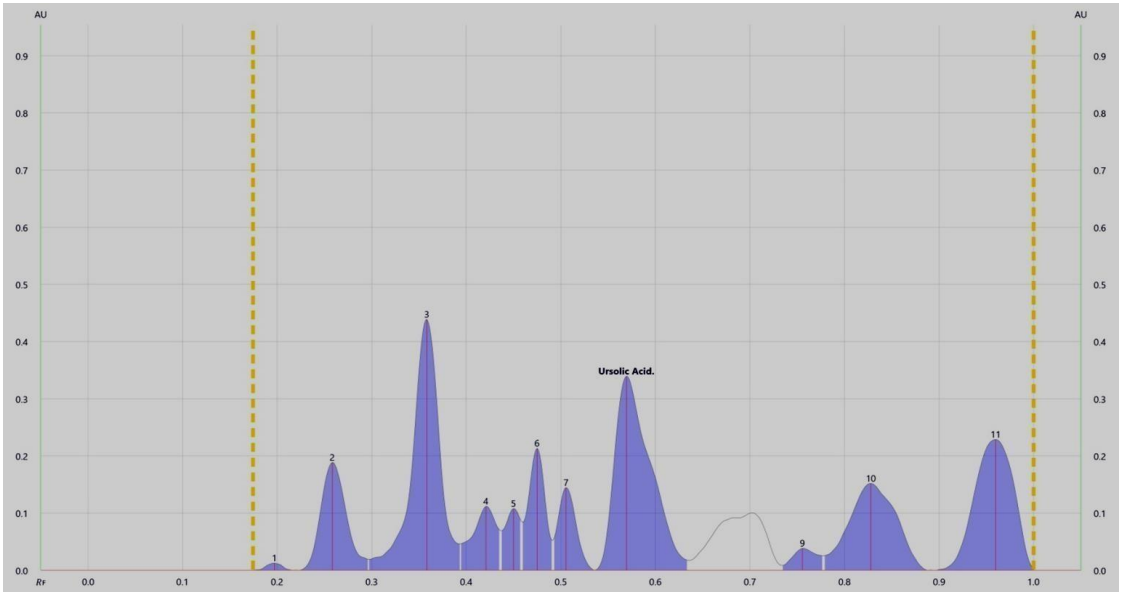
Track10: Diosgenin (5 µL)



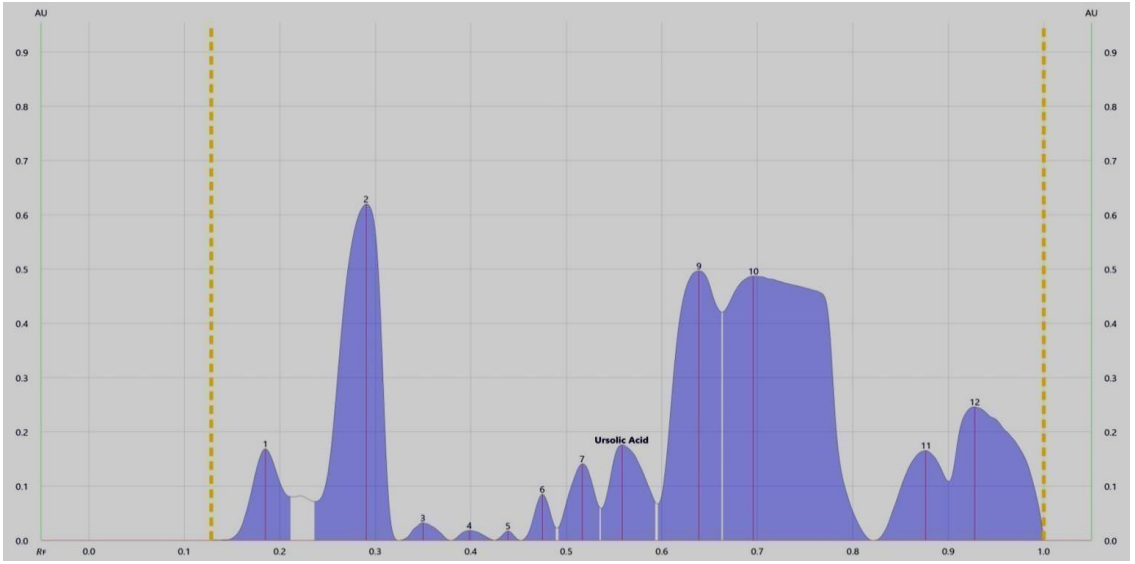
Track 11: Rosemerry oil(10µL)



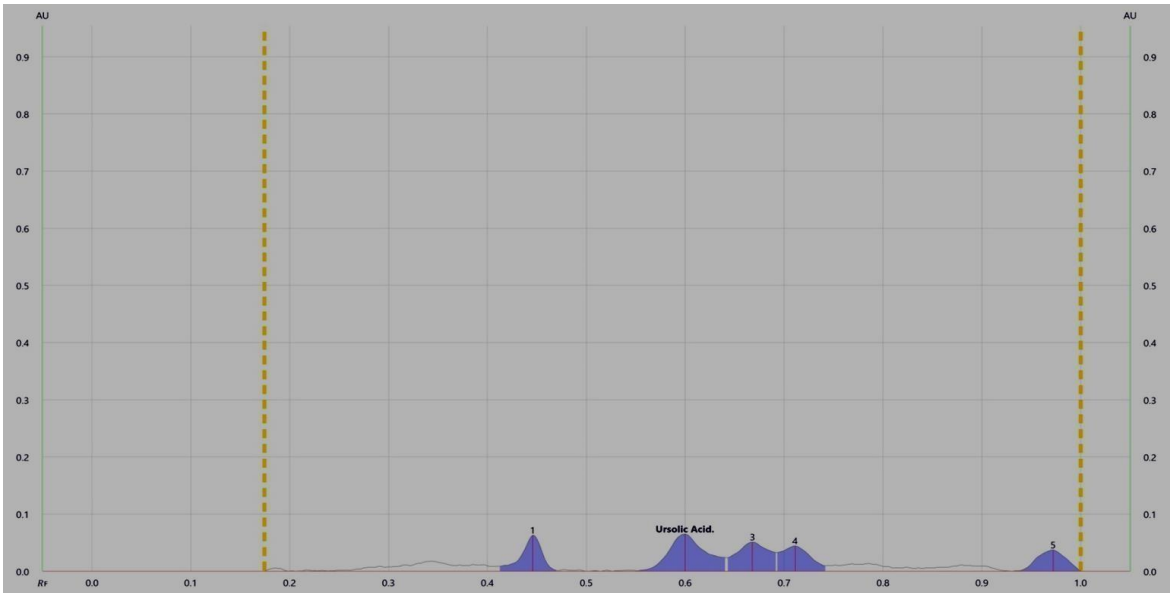
Track 12: Methi MEOH(10µL)



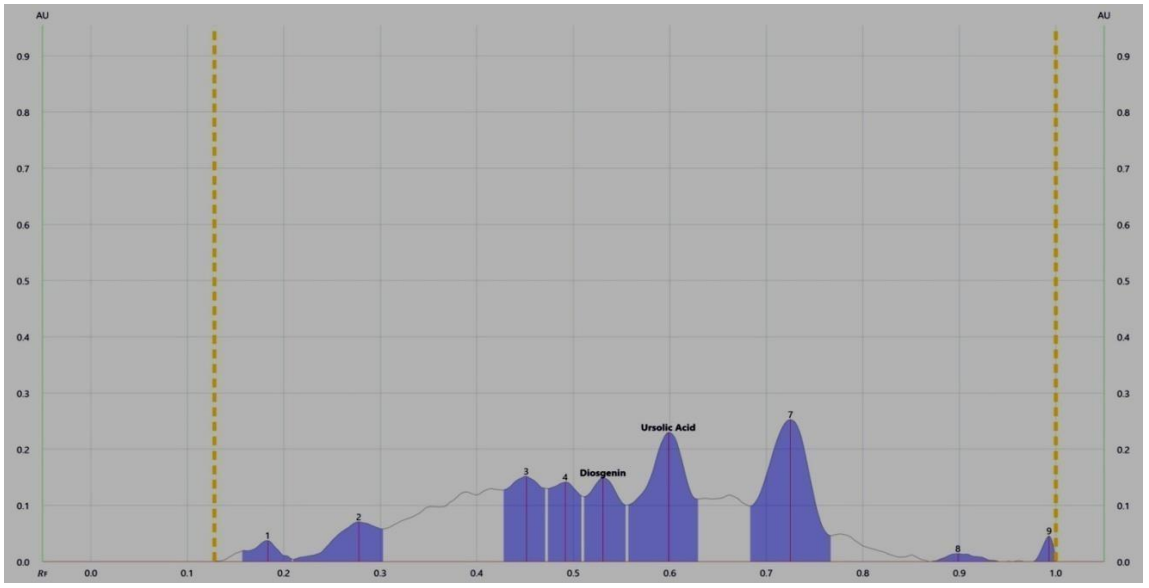
Track 13: Guava MEOH(10µL)



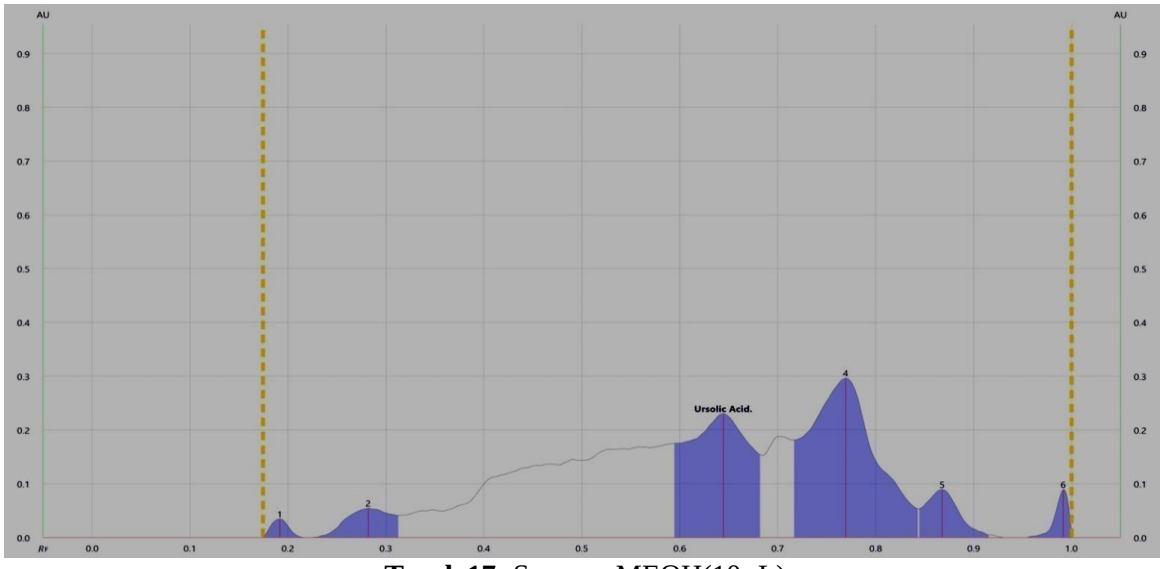
Track 14: Kalonji oil(10µL)



Track 15: Onion MEOH(10µL)



Track 16: Papaya MEOH(10µL)



Track 17: Saragva MEOH(10µL)

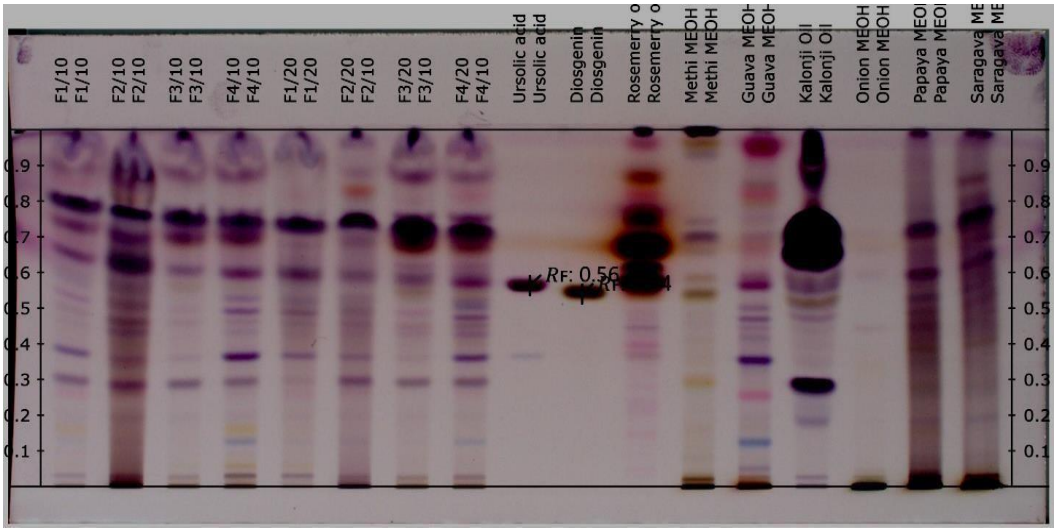


Fig 18: white-Derivatized remission vis

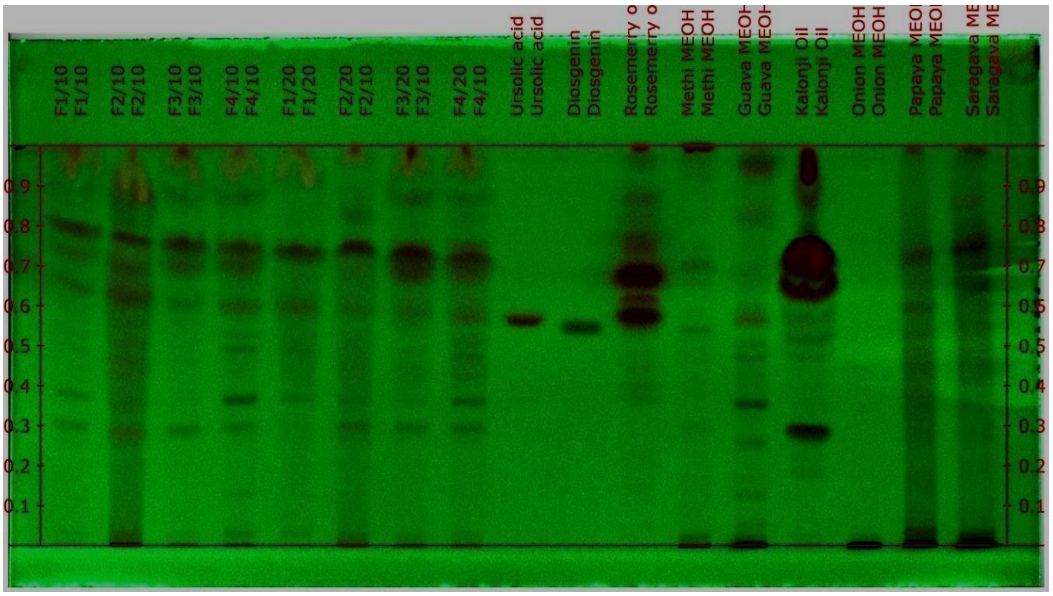


Fig 19: Derivatized 1a remission 254

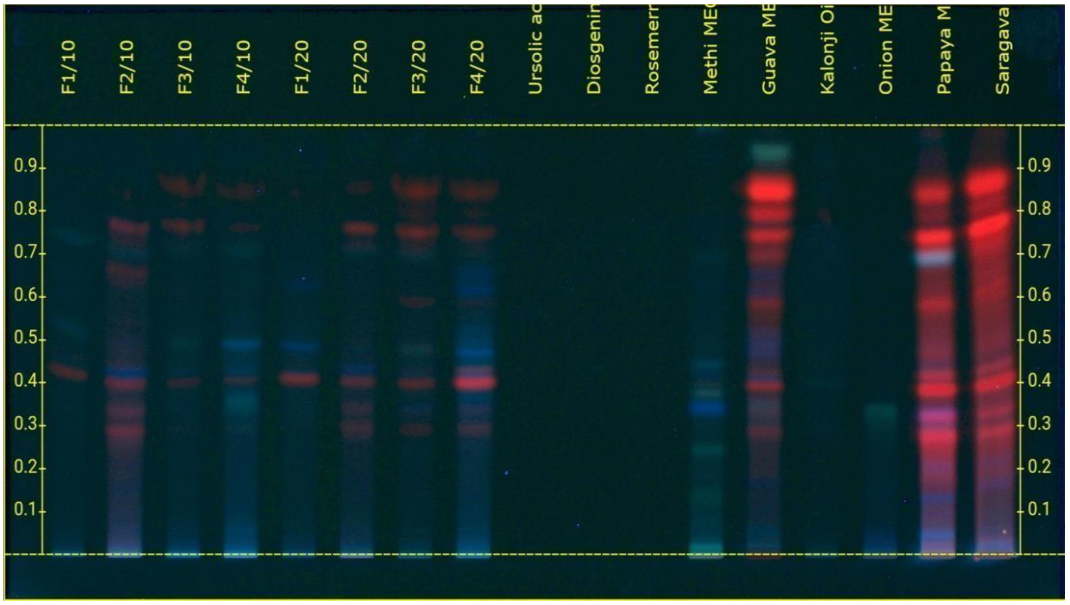


Fig 20: Derivatized 1a remission 366

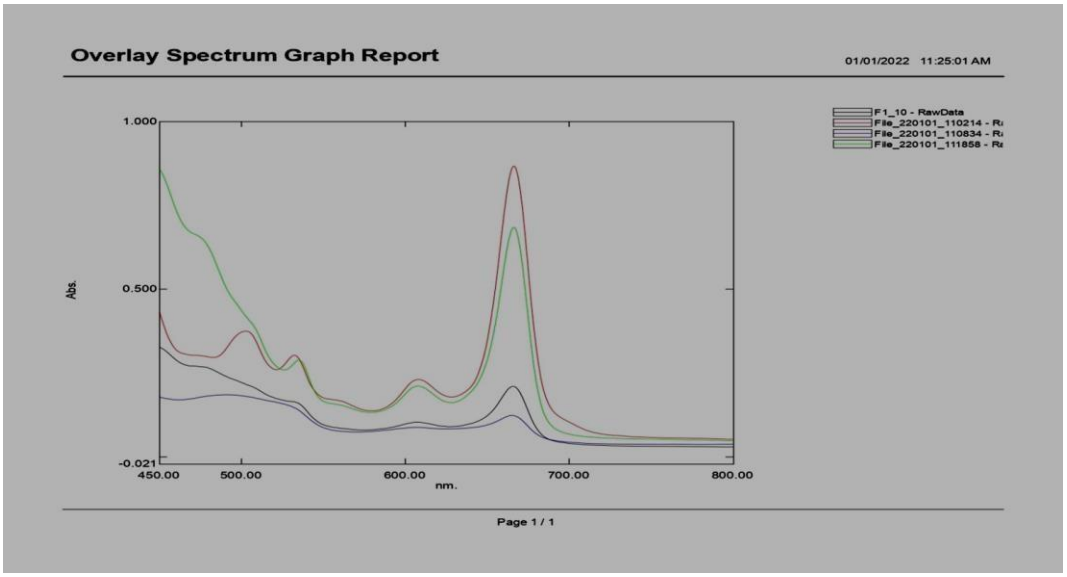


Fig 21: 10% overlay spectrum graph report

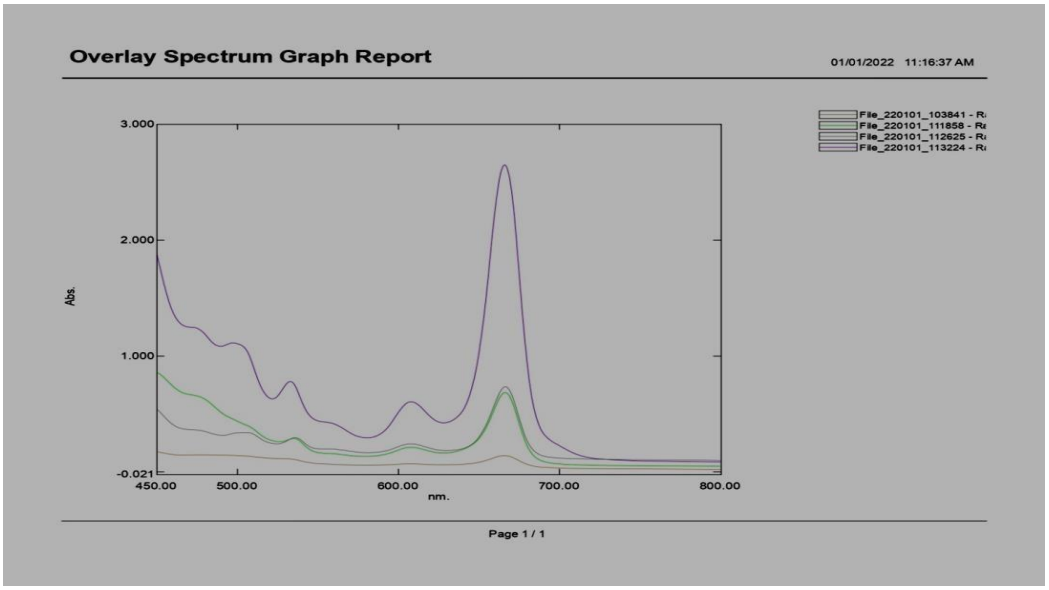


Fig 22: 20% overlay spectrum graph report

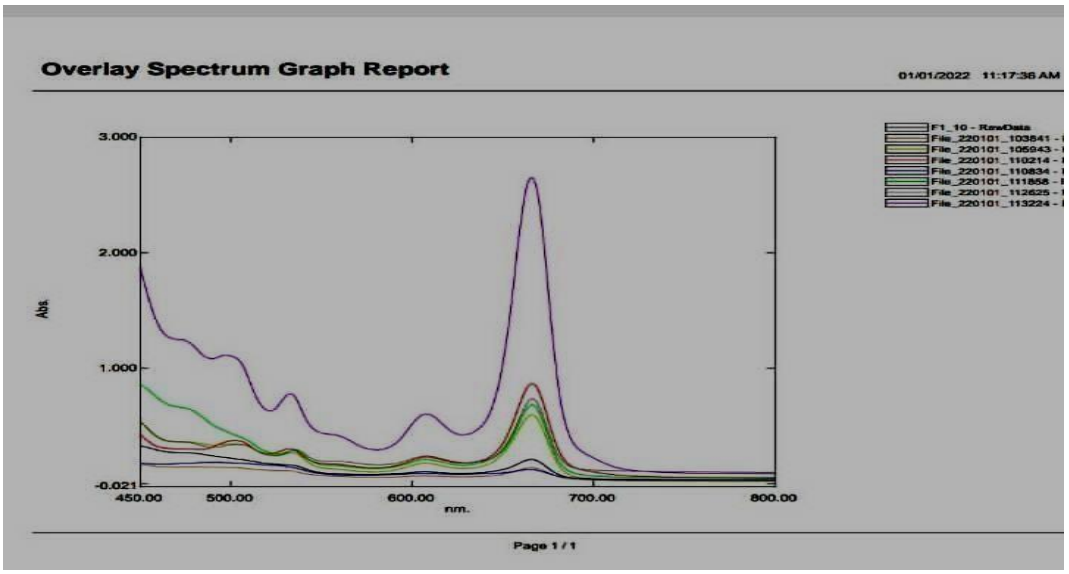


Fig 23: overlay spectrum graph report

Discussion:

According to literature survey, Ursolic acid treatment improves the health of skin and hair. Ursolic acid and its derivatives form oil-resistant barriers on the skin and hair, just as they form the waxy coating of fruits. The major chemical compounds like ursolic acid diosgenin to improve hair growth and reduce grey hair after the oil was evaluated by morphological and pharmacological studies, HPTLC and microbiological studies we found that two formulations gave good results compare with market compounds using HPTLC Analytical methods.[(15)]The present study is an effort to formulate hair growth promoting activity of different formulations. oils have been used on the scalp with the belief that, hair oils in the long run prevents hair loss, brings shine, volume to the hair and prevents graying of hair. Hair oiling involves combing the hair, which is followed by oiling from the roots to the tips of the hair often with a hair braid once a week before shampooing. This belief has been exploited by the cosmeceutical industry, with numerous personal hair care products available over the counter. Ingredient soft he formulation are reported to have activity on hairfall, dand ruffand prematuregreying ofhair.[16] The present study was carried out screen and confirm the presence of phytochemical constituents of different herbal oils and formulations .and also in final formulations using HPTLC.[(17-21)]

The formulation F4 shows good results when compared to other formulations. it shows less side effect on hair growth and less effective in loss of hair.

Conclusion:

This HPTLC is simple and accurate for quantitative monitoring of diosgenin and ursolic acid content in *different* extracts and its formulations. This may be useful for the estimation and characterization of diosgenin and ursolic acid from other species. It also serves as a quality control parameter for its formulations and may help to draw the pharmacopoeial standards for *different extracts.* It encourages nerve growth and improves blood circulation. The blood circulation is so important to hair growth is that without a proper blood supply, the follicles do not get the nutrients they need to grow the hair, and they can die off. ”which research has shown can play a major role in accelerating aging processes, like hair graying. By actively fighting against those free radicals, rosemary oil may also be able to slow down the graying process. Considering the overall results of present study could be concluded that the prepared herbal formulation is less effective than that off or mulations in the of management Grey hair.

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