

HPTLC FINGERPRINTING OF MAHABHRINGRAJ OIL: A NATURAL HAIR GROWTH PROMOTER

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Abstract

The present study was aimed to develop the high performance thin layer chromatography (HPTLC) fingerprinting of Mahabhringraj Oil and its ingredients. The study was carried out to develop complete profiling of HPTLC and showed the presence of active ingredients which were used to prepare the Oil. Now a days, herbal formulations attracted the people because of their no side effects. The formulations were subjected to chromatographic determinations to determine the presence of active constituents. HPTLC of Mahabhringraj Oil and its ingredients were spotted on silica gel precoated aluminium plates of Merck through Automatic TLC Sampler 4 applicator and using a solvent system Chloroform: n hexane: toluene: Ethyl acetate : GAA(4: 1.3:1.0:0.9:0.1). The R_f values were ranged between 0.02 to 0.82 using the methanol extractions.

Key words: Mahabhringraj Oil, Fingerprinting, Oil, HPTLC, Medicinal herbs, Growth.

Introduction

Mahabhringraja oil contains Bhringraj (*Eclipta alba*) which is the active ingredient and exhibit the antimicrobial properties. *Eclipta alba* (Bhringraja) is also known as the False daisy and is an important Ayurvedic herb which is commonly used in the treatment of hairfall. Some special herbs acts as a biovitalizers for promoting the hair growth. The major chemical constituents of Bhringraja: Ecliptic, Wedelolactone, Des-methyl wedelolactone, Stigmasterol, Heptacosanol, Hentriacontanol, sixteen polyacetylenicthiophenes etc.

Wedelolactone: The main properties of Wedelolactone studied by the various scientists are:

Anti hepatotoxic (NazimUddin *et al.*, 2010)

Anti bacterial (Karthikumar, S *et al.*, 2007)

Trypsin Inhibitor, Antivenom (Vianna- da -silva *et al.*, 2003)

R_f value of Wedelolactone is 0.56 (Shaikh *et al.*, 2012)

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. Hair oils serve as a hair tonic for hairs. Herbs being easily available in mountainous area and is used for the medicinal properties. Identification of herbs and their extracts used in oil is a fundamental requirement. WHO put emphasis on the quality and the authentication of the herbs which are used in the formulations. HPTLC is the technique which creates a revolution in the herbal chemistry. It gives the better resolution and correct documentation of the active constituents. Proper identification and quality evaluation of crude herbal extracts is a fundamental requirement. Herbal drugs of Polyformulations have multiple numbers of Phytoconstituents. The preparation procedure of each polyherbal oil sample was carried out in accordance with pharmaceutical manuscripts of Persian traditional

medicine. (Hamedi *et al.*, 2013). Hair is important for one appearance. It also helps us in the recognition. Many scientists were interested in the biology of hair growth and pigmentation has exposed the hair follicle.

Hair Ageing: Now a days, Youngsters facing the problem of hair ageing. It is due to intrinsic or extrinsic factors. Intrinsic factors includes genetic or epigenetic mechanism, whereas, Extrinsic factors include Ultravoilet radiation, Air Pollution, Smoking, Nutrition & Disturbed lifestyle.

Hair Damage: Hair is sort of fibre. Scalp hair has the longest hair growing phase, It is the subject to more damage hairs at other body sites. Hair fibre is damaged due to excessive hair combing and brushing particularly when wet. The longitudinal fissures between the exposed cortical cells resulting hair fractures (Trichorrhexisnodosa) at these sites.

Graying of Hairs

Hair graying is a big problem in the metro cities due to exposure of heavy polluted environment. Graying 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. Commo *et al.*, (2004) showed that the grey hair undergone a marked reduction in melogenically active melanocyte in the hair follicles. Lack of melanin to enlarged medulla, are directly responsible for the coarseness of white hair. Heavily weathered cuticle and porous scalp is mainly responsible for the damage of the hair. Hair becomes dry when it lost its moisture. Oil is a natural remedy to provide nourishment to the scalp. So, Ayurvedic Polyherbal formulation of hair is to impart softness of the hair also help to fill the fissures.

Materials and Methods

The following ingredients are selected for the preparation of the Oil.

Tila taila, Bhringraj, Manjistha, Padmakasht, Lodha, LalChandan, Kharanti Panchang, Haldi, Daaruhaldi, Nagkesar, Priyangu, Mulethi, Kamal Phool, Anantmool (AFI, 2000).

Preparation of Oil

All the ingredients were collected of good quality. Wash and clean all the ingredients and after that dry them. All the ingredients powered them separately and pass through the 180 μm IS sieve. Transfer all the powdered components in the wet grinder and grind them with the appropriate amount of water to make the good homogenous mixture. Add the Tila Taila and all the ingredients with constant stirring to avoid the lumps by maintaining the temperature between 50-90°. Allow to stand it for overnight. Continue the process of heating for three days and check by rolling between the fingers. (API, 2016).

HPTLC Fingerprinting

High performance thin layer chromatography (HPTLC) is highly advance technique for the identification and separation of different constituents.

Sample preparation

For fingerprinting the 10 ml of oil was taken in 50 ml stoppered conical flask and then add 10 ml of (9 vol Methanol:1 vol Water). Shake it stoppered flask for 15 minutes. Then the stoppered flask were kept in the defridger for overnight. Next day collect the stoppered flask from the defridger. Oil is settled at the bottom. Kept the flask at room temperature and supernatant was collected and centrifuge it and collect the supernatant in vial for spotting.

Applicator

The sample was applied on silica glass plate with the help of linomat syringe using the Linomat applicator on the glass Merck HPTLC plate silica gel 60 F 254 (20×10). 10 μ l of sample was applied and 15 bands and length is 8.0 mm and the distance between the spots is 11.4 mm.

Solvent System

A number of solvent systems were tried, but a better resolution is not obtained. But the good resolution was obtained in solvent: Chloroform: n-hexane: toluene : Ethyl acetate : GAA. The R_f values were noted using the UV 254, UV 366.

Development of Chromatogram

Firstly the filter paper was put inside the CAMAG Automatic developing chamber 2 and wait for 20 min for the saturation. For developing the chromatogram one stationary was used and other the non polar mobile phase is used. The chromatograms were developed in ADC 2 saturated with solvent Chloroform: n-hexane: toluene: Ethyl acetate: GAA:: 4:1.3:1.0:0.9:0.1 for 20 minutes upto the distance of 80 mm. The development time is 13 min 15 sec and the start temperature is 21°C.

Documentation

After the development of the chromatogram the plate was air dried and put inside the Visualiser for the documentation. The images were taken at the UV 254 and UV 366 through Vision Cats Software (Version 2.4). The colored bands were observed in the images clearly.

Densitometry

CAMAG Scanner 4 was used as a scanner to record the absorbance of the wavelength UV 254 and UV 366 nm. The spectrum speed is 20 nm/s at the data resolution is 1 nm using the slit 6 × 0.45 mm using the Deuterium and Tungsten lamp. The wavelength range is 200 to 450 nm. The different graphs were observed for the each track.

Results

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 in Table-2 and Table-3. HPTLC spectral analysis of both the plant extracts were done in three different wavelengths- under UV at 254 and 366 nm, and white light (Figure 1 and Figure-2). The Isodiametric view of Fingerprinting analysis was shown in (Figure 3). The individual ingredient peaks were evaluated using Scanner 4 presented in (Figure 4)

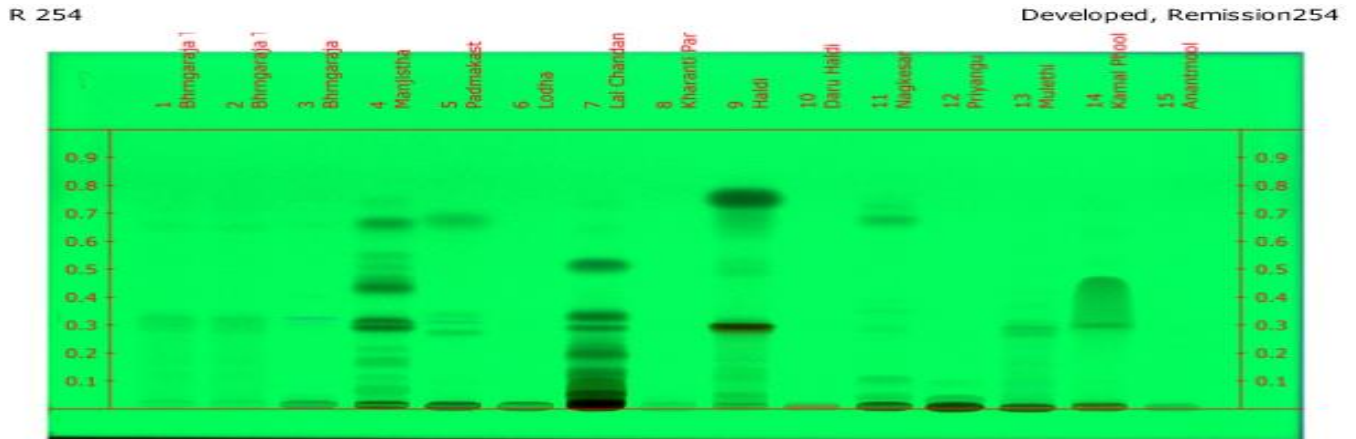


Fig : 1 showed the fingerprinting at UV 254 nm



Fig 2 showed the fingerprinting at UV 366 nm .

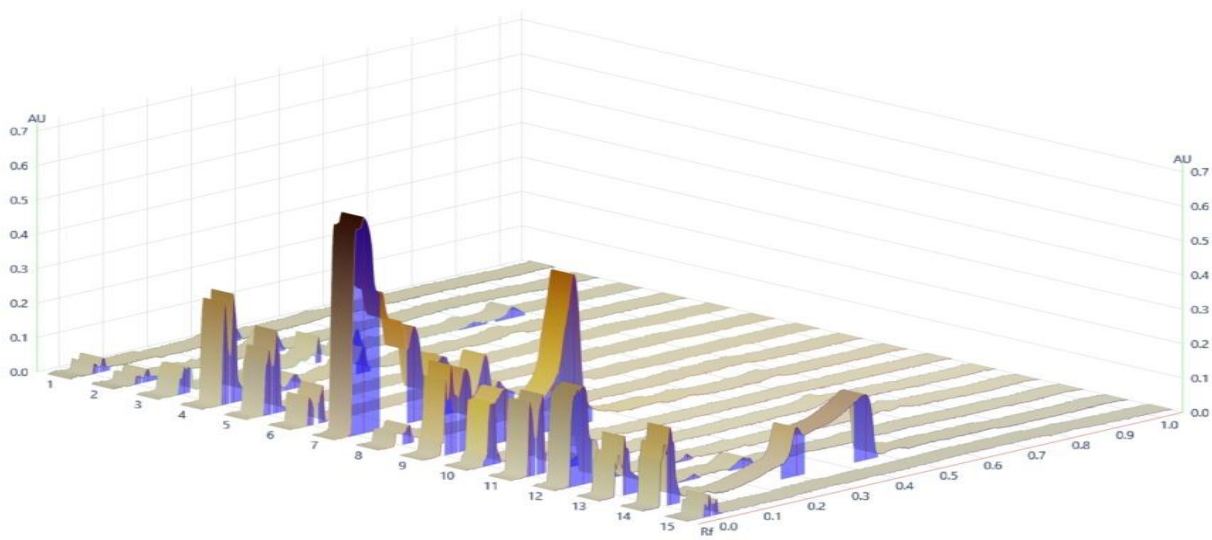
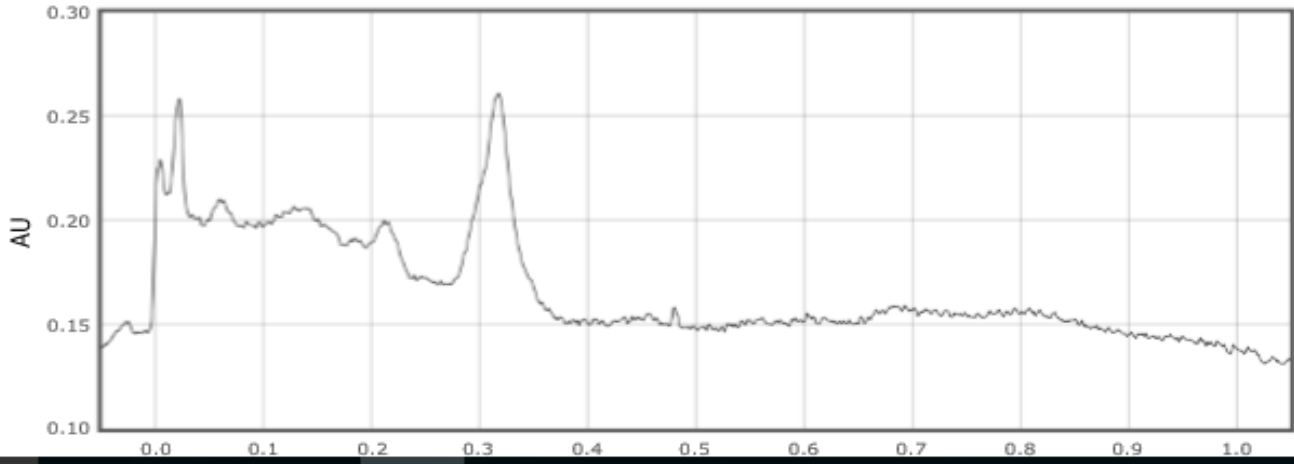


Fig 3: Isodiametric view of all peaks

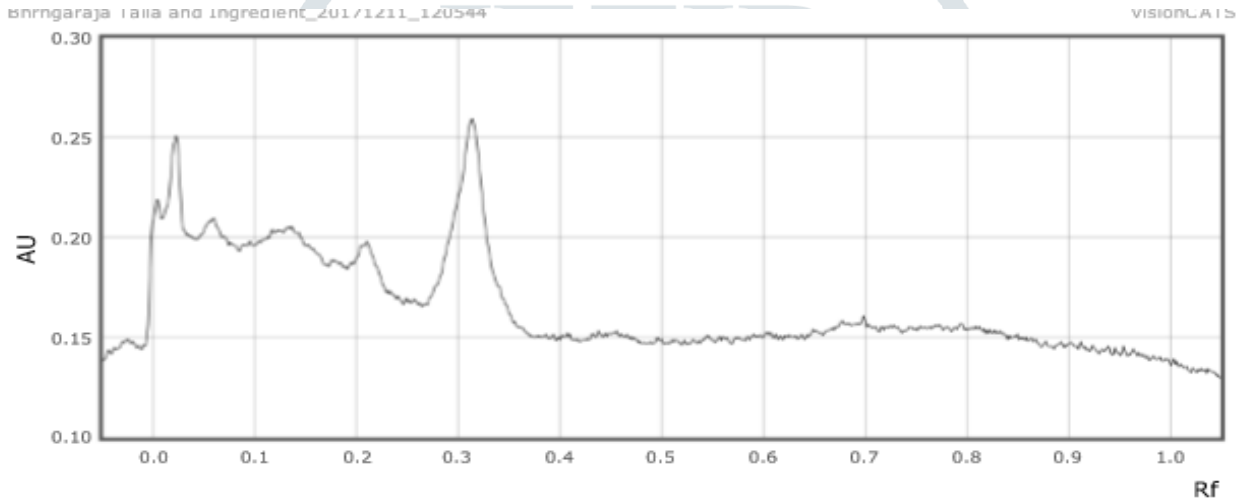
Track 1:

Type Single λ



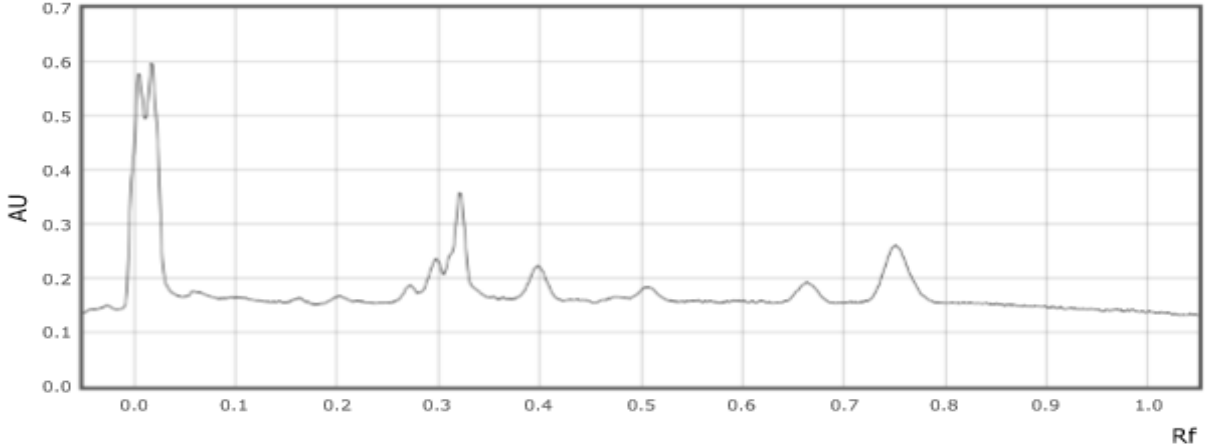
Track 2:

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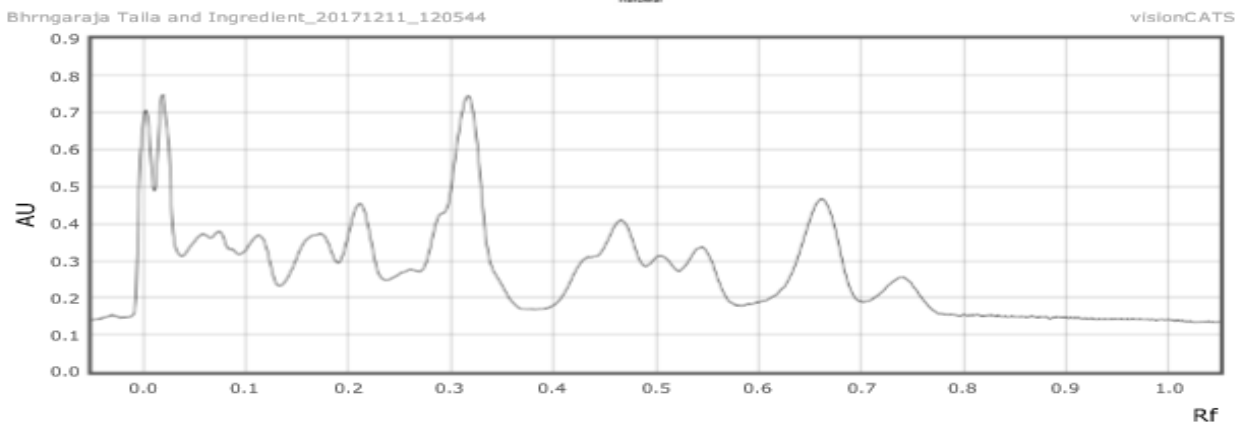
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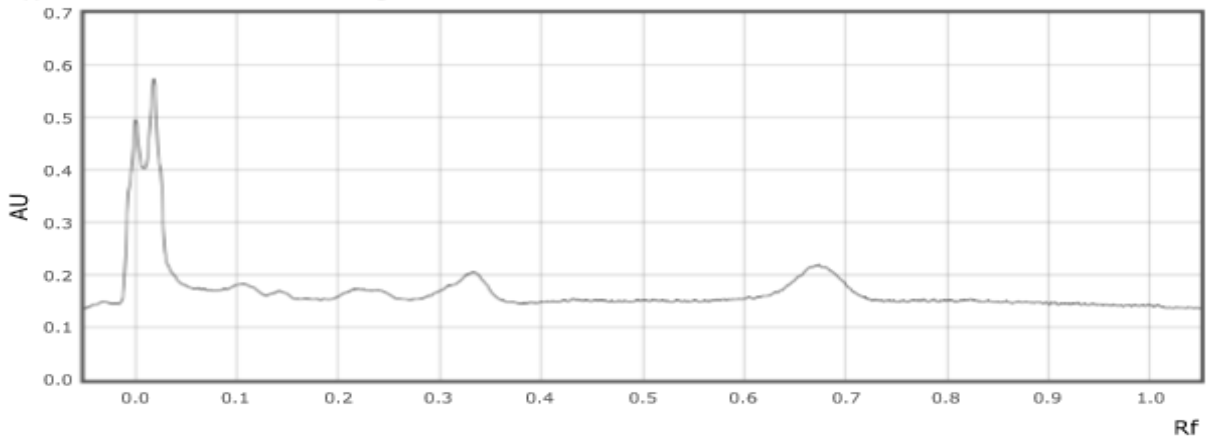
Track 4:

Type Single λ



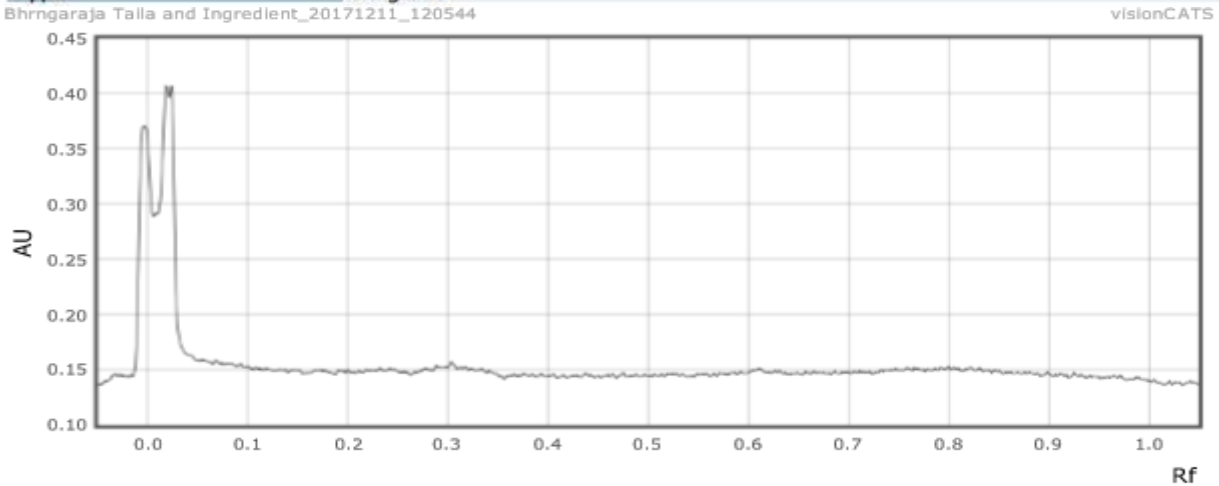
Track 5:

Type Single λ



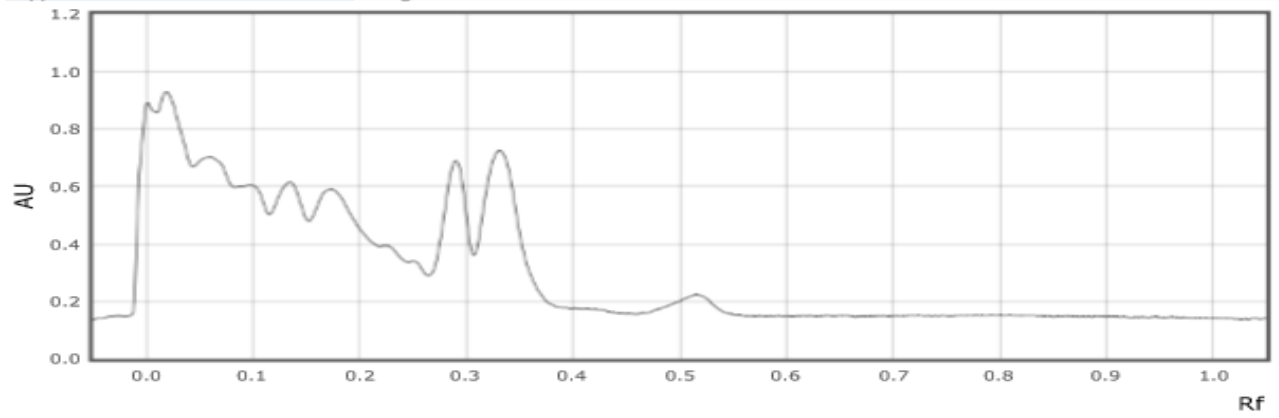
Track 6:

Type Single λ



Track 7:

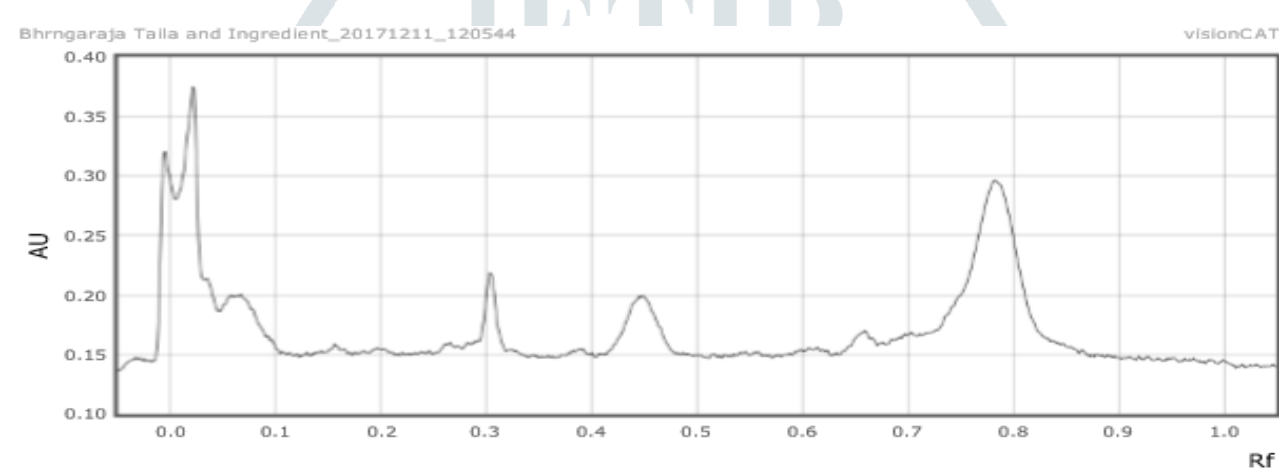
Type Single λ



Track 8:

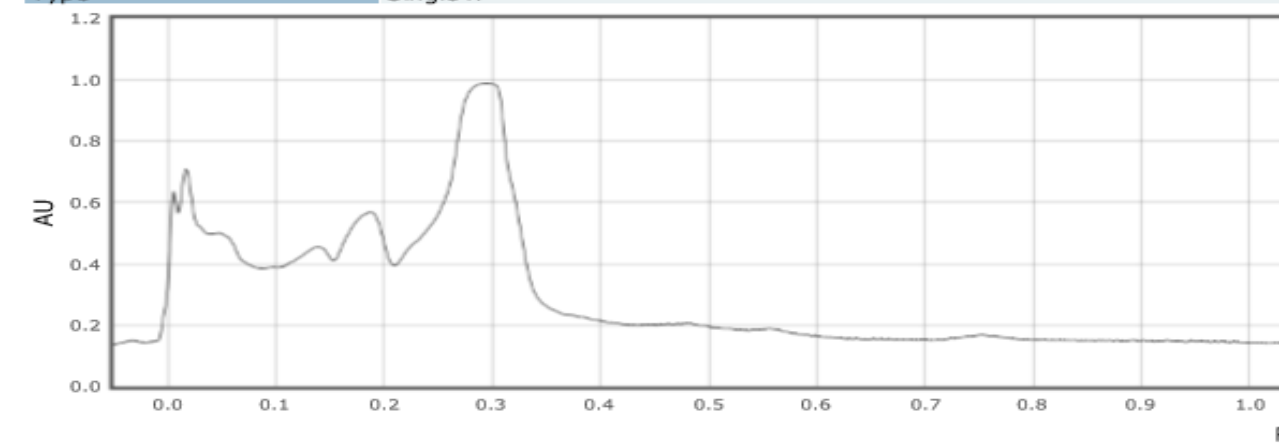
Type Single λ

Bhrrngaraja Talla and Ingredient_20171211_120544 visionCATS



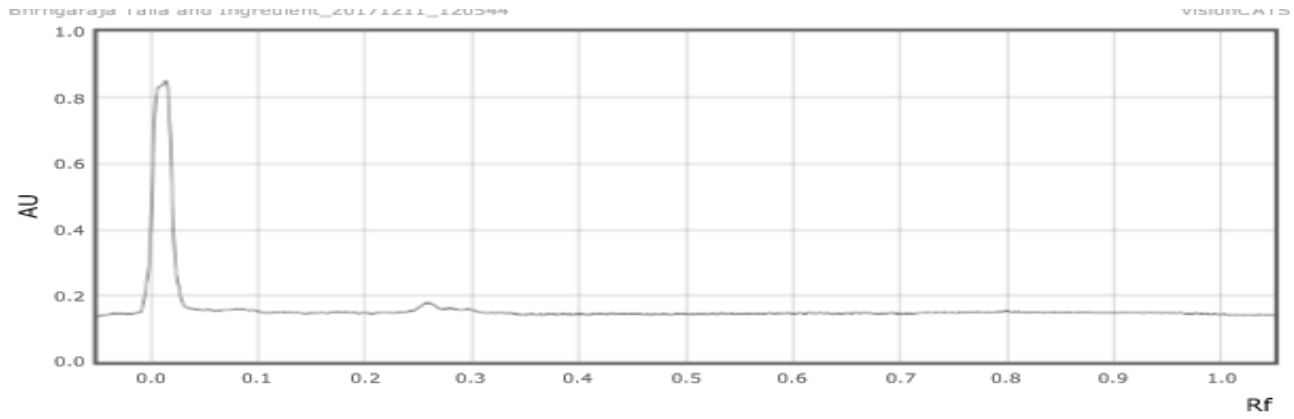
Track 9:

Type Single λ



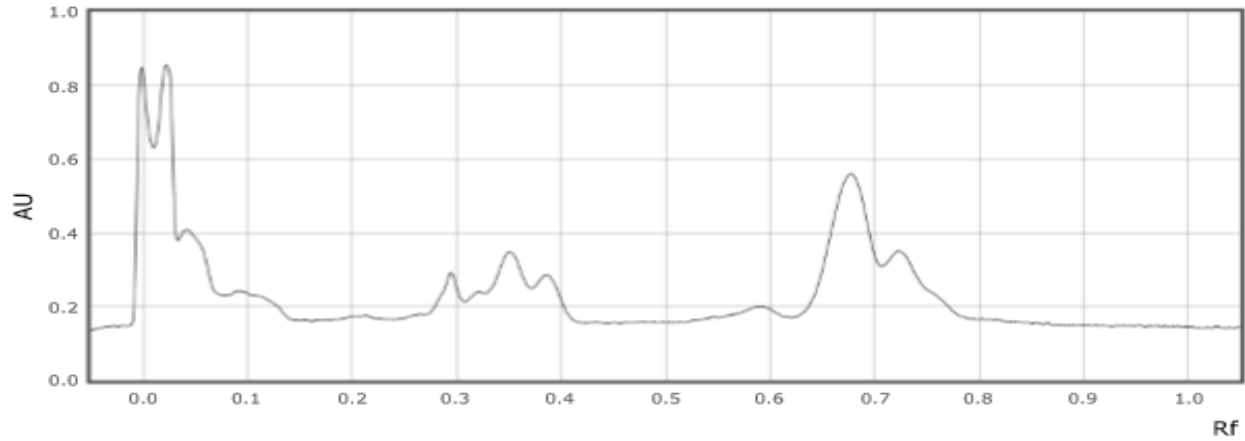
Track 10:

Type Single λ



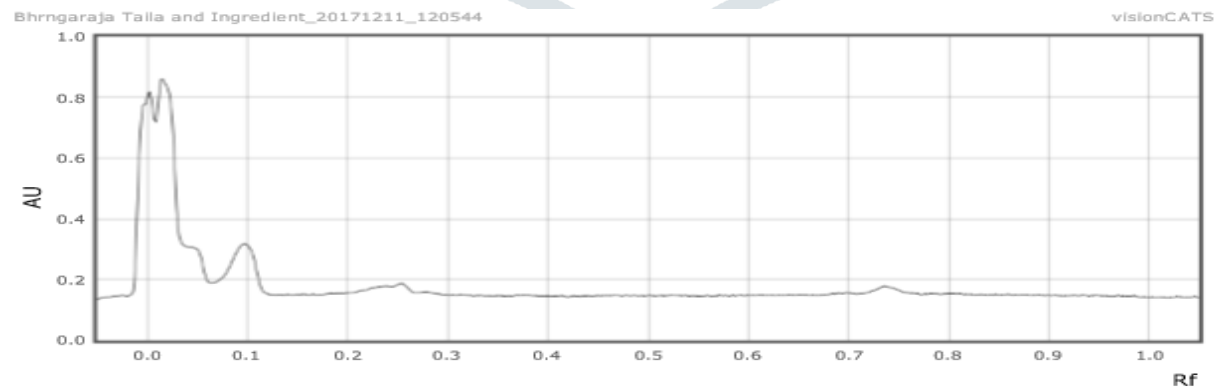
Track 11:

Type Single λ



Track 12:

Type Single λ



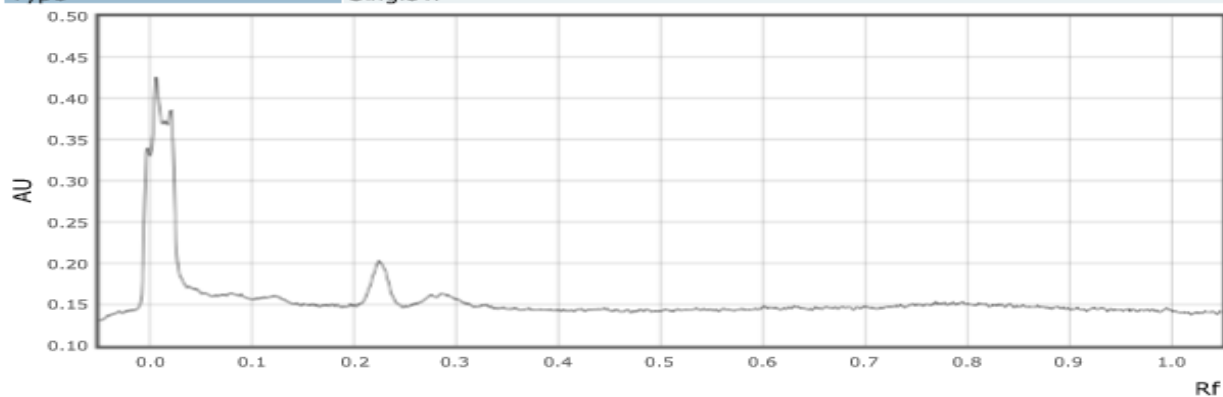
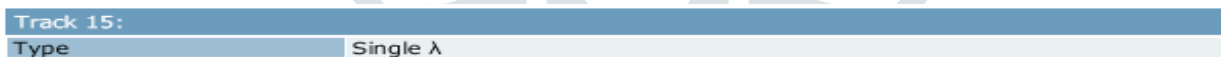
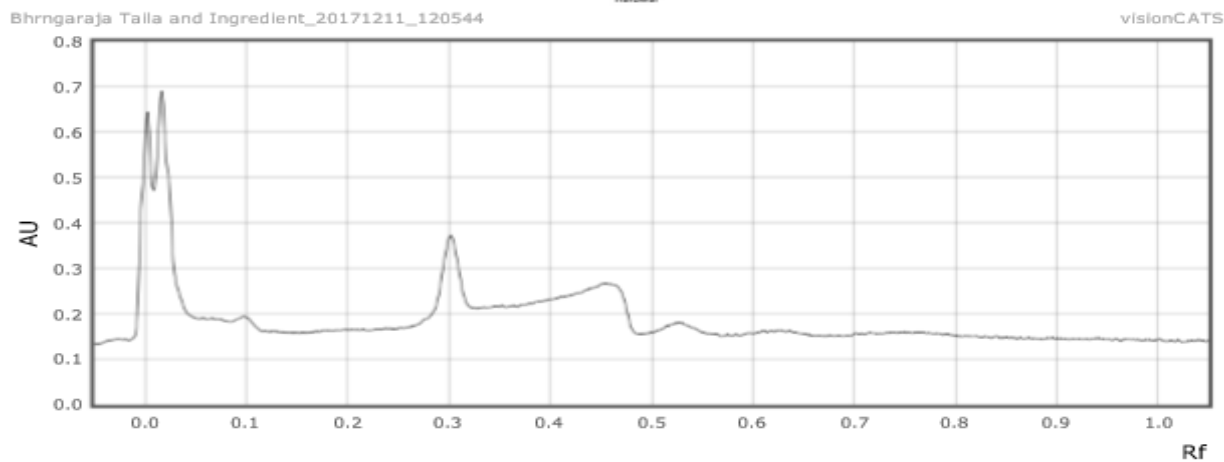
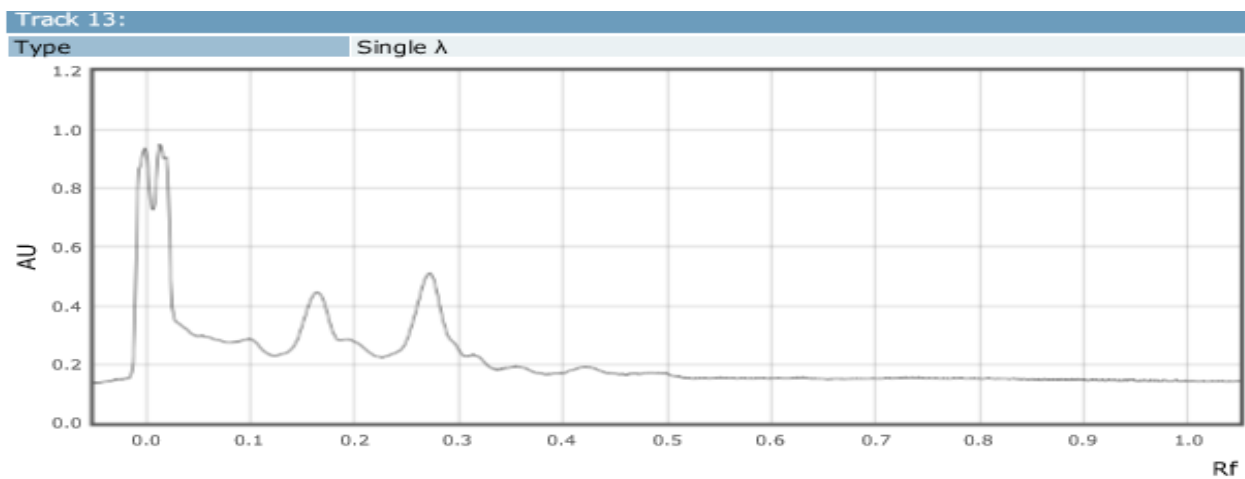


Fig 4: Densitometry graphs of all the peaks

DISCUSSION

Mostly, the entire chromatographic profiles are used to identify or to evaluate the quality of the herbs. This research paper gives the complete individual profiling of all ingredients. Similarly, Nicoletti *et al.*, (2013) report the application of HPTLC fingerprint method on analysis of different neem products. HPTLC helps in differentiating the adulterant of all species which is to be used. Herbal oil was prepared for nurturing the hairs. Usually, the preparation procedure of each polyherbal oil sample was carried out in accordance with pharmaceutical manuscripts of Persian traditional medicine. (Hamedi *et al.*, 2013). Ayurvedic oil is purely herbal which have no side effects i.e why the medicinal plants is commonly used in hair oil all over India for healthy black and long hair (Roy RK, *et al.*, 2008). In Present times, mostly youngsters are also suffering from the hair loss due to many reasons (Week in studies, Carrer pressure, Job, stress) etc. In Ayurveda, hair diseases are described and treated under three types as *Khalita* (loss of hairs), *Palita* and *I_drabidda/I_dralupta* (white patches of hair fall on scalp as alopecia areata, totalis universalis (Bouldin *et al.*, 1999). *Glychriza glabra* serve as a vital ingredient in medicinal oils used for the treatment of rheumatism, hemorrhagic diseases, epilepsy and paralysis as described by Kaur *et al.*, 2013. Roughness of the hair is most common problem due to the exposure in the polluted environment. Pollution in the atmosphere increases day by day and Our hairs get directly in contact with polluted environment. So, there is the urgent need for the nourishment of the scalp which reduce the harmful effects of the environment. The herbal hair formulation is herbal product which not only nourishing the scalp but also relax the nerves of the brain which are sometimes under stress. This HPTLC fingerprinting showed the identification and gives the complete profiling of the herbs which are present in the Oil. Number of hair nourishing creams (which gives the shining, herbal oils containing extracts of mehndi, amla, Bhringraj have been prepared, characterized and compared with marketed products for dyeing as well as growth enhancing activity (Parwal *et al.*, 2011). Classical formulations, Bhringamalakaditaila prepared with the fresh juice of *Eclipta alba* and *Embilica officinalis* and Nilibhringaadi taila prepared with fresh juice of Nilika leaf, which is the natural herb and use of *Eclipta alba* and *Embilica officinalis* have been used to dye hair and promote hair growth since many years. Many diseases are responsible for causing hairfall for example, typhoid, Malaria, Jaundice etc. The use of Chemotherapeutic agents are also causing hairfall (Bertolino *et al.*, 1993). Mahabhringraj Oil is prepared by complete Ayurvedic formulation. Oil of various characteristics could be produced through simple fractionation process i.e just by collecting the oil at various time intervals or mass intervals (Hassan *et al.*, 2000). Hair loss is distressing condition that is associated with a multitude of natural and also due to lack of proper nutritional conditions. The females are facing the thinning of the Long hairs. Hairs are the beauty of the womens. This female pattern hairloss, is frequently referred to as androenetic alopecia, however the role of androgens in this type of hair loss remains uncertain (Birch *et al.*, 2002). Whereas the male pattern hair loss may be associated with the hypeandrogenism, but the majority of female hair loss pattern have a normal androgen levels. Fertilin level is assessed by Clinicians the to rule out iron deficiency. If the women is having fertilin level less than 70 mg/ml then iron supplementation is recommended to maintain the adequate level of fertilin. (Trost, LB. *et al.*, 2006). Another main problems associated with hair such as pigmentation problems such as fading dandruff and falling of hairs (Adhirajan *et al.*, 2001). Mahabhringraj constitutes the herbs like Manjistha, Lodha, Yasti, Bhringraj, Lal Chandan, Daaruhaldi which gives marvelous results after using this. Mahabhringraj hair Oil not only promotes hair growth they also provide necessary moisture to scalp rendering in beautiful hair (Kapoor, 1990). Mahabhringraj oil is considered as a nourishing agent ad also a good moisturizer and also brings shine to hair (Monfalouti *et al.*, 2010). So, the HPTLC profiling of herbs describes the active constituents of herbs which play an important role in the oil and is very beneficial for one's hair growth and is a solution of many hair problems.

Conclusion

HPTLC is a modern technique which gives you the quick results in less time and is a cost effective. The automatic and semi automatic applicator is used for the rectangular bands which gives the good separation. The quality is evaluated using this technique simultaneously, it gives the correct identification of the herbal constituents. In herbal formulations, fingerprint analysis is mostly considered for correct identification and also for the estimation of marker compounds. Present paper deals with the HPTLC profiling showed the presence of herbs which are used in the formulation.

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