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RESEARCH ARTICLE

SAUSSUREA OBVALLATA AND *SAUSSUREA SIMPSONIANA*; A NEW SOURCE OF DEHYDROCOSTUS LACTONE (IMPORTANT ANTICANCER COMPOUND) FROM NORTHWEST HIMALAYA

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Abstract

HPTLC technique for analysis of Dehydrocostus- lactone developed and validated for their etermination of dehydrocostus- lactone, root and flower extracts of Saussurea simpsoniana, Saussurea obvallata. The analytes were extracted with 70% methanol and tested on TLC aluminum plates along with standard. Analysis was executed on pre-coated TLC aluminum plates. Linear ascending development was completed in twin trough glass chamber saturated with mobile phase comprising of toluene: ethyl acetate (9:1 v/v), Spectro- densitometric scanning was performed by TLC scanner IV (CAMAG) in absorbance mode at the wavelength of 530-nm. Present, study revealed that marker compounds are present in higher amount in the roots of *S. obvallata*, (12.19 mg/g), while the concentration was low 5mg/g in the root of *S. simpsoniana* extracts and was not detected in flower sample of *S. simpsoniana.*

Introduction:-

There has been an increasing awareness in the study of medicinal plants as natural products to various parts around the world [1]. The medicinal value of plants relie on bioactive phytochemical that produces definite physiological action in the human body. Based on World Health Organization recommendations, plant origin is important for use in traditional medicine [2]. Now- a- day herbal drug is in demand throughout the world because of their safety and efficacy folk medicine re-evaluated by research for their therauptic use. [3]. Chromatographic and spectral fingerprint analysis plays an essential part in the quality control of complex herbal medicines [4]. Thin layer chromatography (TLC) is the preliminary step to identify the phytochemical constituents in a sample. In our present study phytochemical analysis has been in two species of Saussurea S. simpsoniana (Jogi badsha) collected from the Pir-Panjal ranges of Kashmir Himalaya at an altitude of 5000m Apherwath Gulmarg (J&K) and Saussurea obovallata (Bhramkamal) collected from the valley of flowers from Uttrakhand at an altitude of 4500m. Both the species harbors important medicinal values. Traditionally this herb is used for the treatment of various ailments includes paralysis of limbs, cerebral ischemia, narcotic, expectorant, bronchitis, urinary tract problems, cough [5]. Floral buds and roots of S. obovallata are used as medicines in leucoderma, urinary troubles, bone fractures, wounds, bone pain, cough and cold, reproductive disorder, digestive problems, skin diseases, haematuria, the rhizomes in particular are used as antiseptic and for healing cuts and bruises, treating deaf, headache, paralysis, asthma, cough, old fever, inflammation, and ophthalmic conditions. Its root is used mainly as an antispasmodic in asthma, cough, and treatment of cholera, chronic skin diseases and rheumatism (Kritikar & Basu 1984, Saklani *et al.*2000, Mishra *et al.*2018). The species are also well known to have phytochemical constituents, like

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sesquiterpenoids, flavonoids, phytosterols, and tritrepenoids, which are helpful in chemotaxonomy and also makes the genus an appropriate contender of research interest.

Presently, identification and quantification of dehydrocostuslactone, a natural sesquilactone has been done using HPTLC technique. This compound has caused entire interest in the research because of its anticancer activity in some types of carcinomas (Jinkui wang *et al*.2017) and has previously known in many species such as *Inula helemium* L. and *Saussurea lappa*. Beside this it consists of anti-inflammatory (Kouch, 2001), anti-diabetic, antimicrobial a (Luna *et al*.2007; wedge etal.2000; Fischer et al.1998), antiulcer (Yamhara *et al*.1985) and Carcinogenic property (Gu *et al*.2002, Kawamori *et al*.1995).

Experimental:-

Material Method:-

Samples of Saussurea simpsoniana (Jogi badsha) *S. obovollata* (Bhram kamal) has been collected from Pirpanjal ranges of (J&K), Uttrakhand (valley of flowers) in the month of September 2017. The specimens with the following accession numbers (62268/ 62269) has been submitted to Herbarium, Department of Botany, Punjabi University Patiala (PUN)

Equipment for HPTLC:

Linomate 5 applicator (CAMAG, Switzerland), twin tough chamber (20x10cm), micro syringe (Hamilton-Bonaduz Schweiz CAMAG, Switzerland), precoated silica gel 60F $_{254}$ HPTLC (20×10cm, 0.2mm thick Merck Germany), TLC scanner IV (CAMAG, Switzerland), UV-chamber (CAMAG, Switzerland) and win-CATS software (CAMAG, Switzerland) were used in the study.

Reference compounds and chemicals:-

The chemicals and solvents were of analytical grade from E. Merck, India. The HPTLC plates Si 60F_{254} (20 cm × 10 cm) of 0.20 mm layer thickness were purchased from E. Merck (Germany). The marker compound Dihydrocostuslactone was purchased from Sigma-aldrich (New Delhi India).

Preparation of samples for HPTLC:

The air dried 5gms of Root and flower powder of two species of *Saussurea* was accurately weighed and 70% methanol was used for extraction with the help of soxhlet apparatus (JSGW1195) under reduced pressure. Then the extract were lyophilized and powered extracts were re-dissolved in to make the mg/m concentration and 5µl of samples was loaded on the TLC plate with the help of automatic sample spotter for the quantification of marker compounds from plant extracts

HPTLC analysis of Plant extract:-

The concentration of 2,4,6,8,10,12 µl of standard solutions, (1mg/ml) along with 5µl of suitably diluted sample solution were applied to (20×10cm) silica gel plate 60F_{254} TLC plates (Merck, Germany).The solution was applied as 4mm wide band,6mm apart and 10 mm from the edge of the plate, by means of Camag Linomate-5 applicator. The TLC plates were developed with toluene and ethyl acetate 9:1 ratio in the twin trough chamber previously equilibrated (30 min) with the mobile phase. The development distance was 60 mm at 25 ± 2ºC and 40% relative humidity, after development, the plates were removed from the chamber and air dried for about 5-10 min and was sprayed with freshly prepared ansi-aldehyede sulphuric acid (derivatizing reagent) and further dried at 105-110ºC for 5-10 min in oven. After development, the components of all the marker compounds were visualized in the form of purple pink bands at Rf. 0.05. The plates were scanned densitometrically at wave length 530nm using CAMAG TLC scanner-4 in absorbance mode using tungsten lamp. Peak area was recorded and the amount of Dehydrocostus lactones present in samples were calculated from the calibration plot obtained by plotting peak area against the amount of standards by using Win-Cats software programmer.

Validation of the Method: The linearity of the standards was obtained by applying the different concentrations of standard solution ranging from 2-12µl on TLC plate. The calibration curves were obtained by plotting peak area versus concentrations. The LOD (limit of detection) and LOQ (Limit of quantification) were determined using the given equations.

$$\text{LOD} = \frac{3.3 \times \text{Standard Deviation of the y-intercept.}}{\text{Slope of the calibration}}$$

$$LOQ = 10\times \frac{\text{Standard Deviation of the y-intercept}}{\text{Slope of the calibration curve}}$$

The linearity range was calculated with the linearity regression of the species at Rf.0.05 the LOD 2.8 and LOQ 4.4.which indicates the adequate sensitivity of the method. The identity of the band and the marker compound was confirmed with Rf. value and by UV absorption spectrum.

Result and Discussion:-

During our present research, the root and flower samples of S obovalata, S. simpsoniana were evaluated for the presence of the said marker compounds. The maximmum amount of dehydrocostus lactones were found in the roots of Saussurea obovalata (12.19 mg/g), and then about (9.8mg/g) were found in the flower sample of same spp. In another species S. simpsoniana 5mg/g of marker compound has been found in the root but this compound was not detected in flower sample of S. simpsoniana. Our finding is first report of this compound in these novel species of Saussurea and the compound has been first time reported in the species, the result obtained we concluded that the roots of S. simpsoniana and S. obovallata are the best source of this novel compound and can be used for the formation of herbal drug. And the method is the best method for estimation of dehydrocostus lactones. The developed HPTLC method for the estimation of the referred standard, i.e. dehydrocostus lactones was validated using the parameters according to the ICH guidelines (Table 1). The solvent system we opt was the best for the resolution of the said compound. The TLC procedure was optimized with a view to quantify the herbal extractions. Different mobile phases were tried but the best resolution was found (Touleane: Ethyl acetate 9:1). This mobile phase enabled satisfactory separation of the compound with best resolution of the mixture investigated

Thin-layer chromatography (TLC) is used for the separation and identification of natural compounds in different medicinal Herbs (Polish Pharmacopoeia 2002, European Pharmacopoeia. 2007). High-performance TLC (HPTLC) is powerful instrumental analysis technique, and optimized quantitative HPTLC using densitometric, evaluation can produce results similar to those obtained with high-performance liquid chromatography (HPLC) (Wagner, 2001). HPTLC is turning into a routine analytical technique because of its advantages of low operating cost, high sample throughput and requirement for minimum sample clean-up. More ever main advantage of HPTLC is that a number of samples can be run together using a small amount of mobile phase, not at all like HPLC, thus lowering analysis time and cost (Nile, 2009).

For the fingerprint analysis, the plates were derivatized with freshly prepared anisaldehyde–sulfuric acid and were heated at 110°C for 5 min. The spots were identified with peak resolution at a retention factor (Rf.) of 0.5. These plates were scanned for densitometry measurement, spectra recording. The purity of the bands was confirmed by the overlaid absorption spectral of marker compound and sample track. From the results obtained, it is concluded that the method we opt is easy feasible method the best method for the quantification of said marker compound for the spp. of Saussurea. No reports have been published yet on the quantative estimation of dehydrocostus lactone in the *S. simpsoniana / S. obovallata* by HPTLC method.

Conclusion:-

HPTLC densitometry is a rapid, reproducible, accurate, and selective alternative to HPLC for the separation of the dehydrocostus lactones, further, the two species is enriched with the desired constituents can be used for preparations of polyherbal formulations. The advantage of HPTLC is that it results from less amounts of samples and requires less amount of solvent. Besides, this simultaneous quantification of number of samples can be done in lesser time period.

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Conflict of Interests:

The authors declare that there is no conflict of interests regarding the publication of this paper

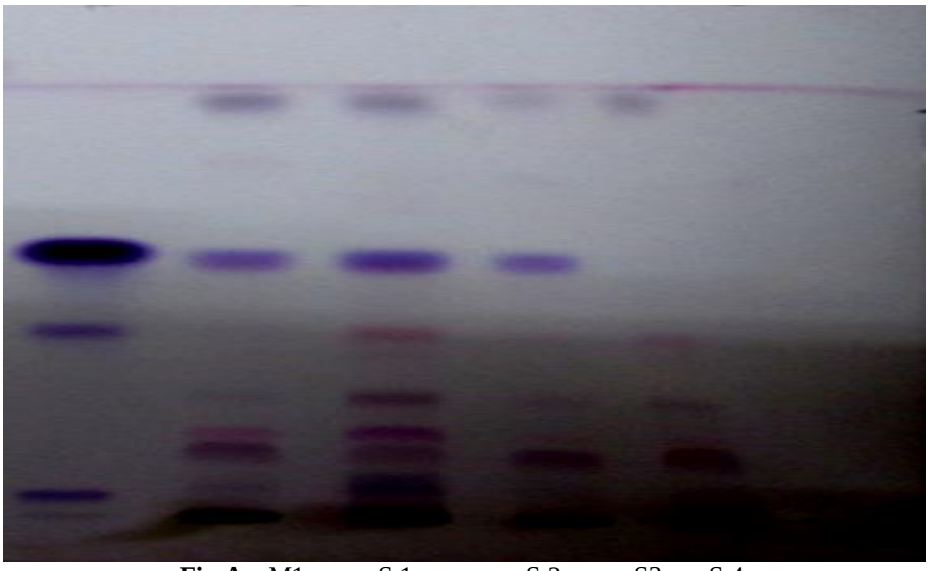
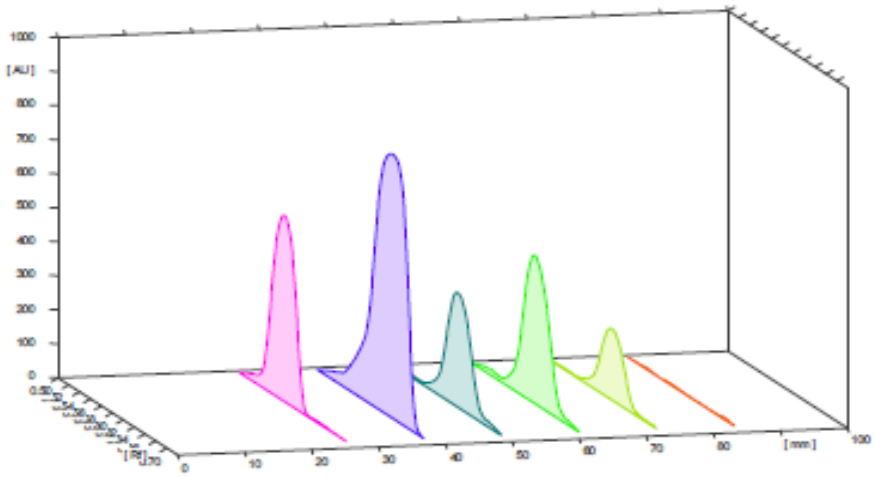


Fig.A:- M1 S 1 S 2 S3 S 4



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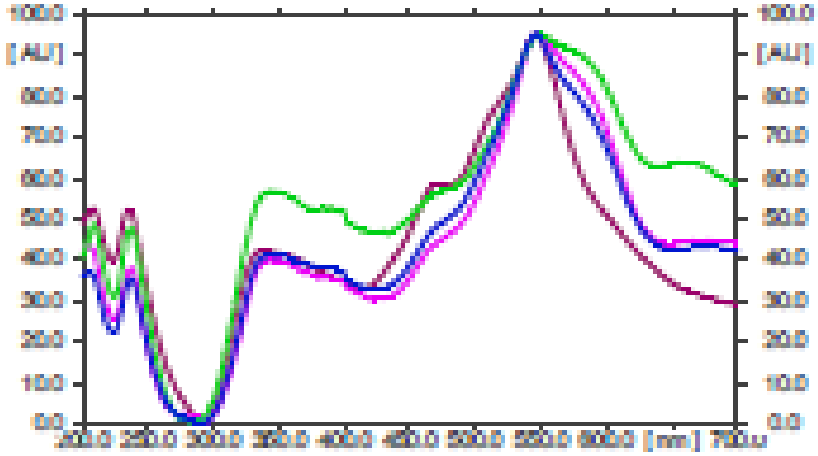

Figure1:- (a) Detection and quantification of dehydrocostuslactone from root and flower at 530nm. Track details: (M1): Marker compound, (S1-S2), Root and flower of S. obovallata ,(S3-S4) Root and flower of *S. obovallata* (b) 3-D chromatogram of sample with dehydrocostuslactone (c) UV absorption spectra of the marker compound in the sample track along with standard dehydrocostuslactone.

Table 1:- Validation data of HPTLC method.

No	Parameters	Dehydrocostus lactone
1	Linearity range ,ng/band	2-12ng/
2	Regression equation	Y=3428x
3	Correlation coefficient	0.994
4	Spacifity	Specific
5	Amount of standard in root sample *S. simpsoniana*	9.8 mg/g
6.	Amount of standard in flower *S.simpsoniana*	12.19 mg/g
7.	Amount of standard in root *S. obvallata*	5mg/g
8.	Amount of standard in flower *S.obvallata*	ND
9.	Rf.	0.5
10	LOD	2.8
11	LOQ	8.4

Table 2:- Optimized chromatographic method for HPTLC.

1.	Stationary Phase	Pre- coated silica gel $60F_{254}$
2.	Mobile phase	Toulene: ethyl acetate (9:1)
3.	Derivatizing reagent	Ansialdehyde sulphuric acid
4	Chamber saturation time	25-30 min
5	Run distance	60mm
6	Temperature	Room temperature
7	Plate development technique	Ascending

Table 3:- Interday and intraday precision of HPTLC.

Marker compound	**Concentration** (µg)	**Intra-day precision Mean area**	**%RSD**	**Intr-day precision Mean area**	**% RSD**
Dehydrocostus lactone	2	1505.6	1.2	1641.1	2.1
	4	1788.7	0.43	3212	1.1
	6	3187.7	0.52	46515	0.5
	8	4716	0.31	6145.1	0.62
	10	6108.1	0.62	7121.0	0.68

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