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Chromatographic estimation of maturity based phytochemical profiling of *Ipomoea mauritiana*

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Abstract

Collection of herbs at right maturity is one of such parameter which affect afficacy of medicinal plants. Standard reference markers used in quality control of herbal drugs mostly authenticate identity and not efficacy. In order to derive bioactive markers, knowledge regarding appropriate collection time for each herb is essential. Traditional medical knowledge is bioactivity-oriented and informs about best time of collection for certain medicinal species, as observed in case of *Ipomoea mauritiana Jacq.* (Vidari–Sanskrit). Only *mature* (bigger size) tubers of *Ipomoea mauritiana* are used by Traditional Medical Practitioners (TMP) for preparing galactagogues and immunomodulatory herbal medicines (Rasayan).

Microscopy of transverse sections revealed structural variation between mature and immature tubers and girth of tubers determine the maturity of plant, the difference in phytochemical profiles of mature and immature tubers was observed.

Variation in phytoconstituents of mature and immature tubers was confirmed through proximate analysis, phytochemical screening, qualitative HPLC and HPTLC analysis revealed the variation in phytoconstituents in mature and immature tubers.

Keywords: Microscopy, HPLC, HPTLC, proximate analysis, phytochemical screening.

Introduction

In Ayurveda, an experienced traditional practitioner guides the preparation of herbal formulations, it is not sufficient to just use a raw drug of the correct botanical identity; it is equally important to use a raw drug collected at the right stage of maturity for it to have best potency. This could be one of the reasons for the variability in batch-to-batch preparations of herbal medicines in the market.

Ipomoea mauritiana (*Vidari*-Sanskrit) is used in Ayurvedic formulations as tonic, aphrodisiac, galactogogue, immunomodulator [22]. The tubers are used in various Ayurvedic formulations like Vidaryadikvatha Churna, Vidaryadi Ghrita, Marma Gutika, Manmathabhra Rasa, Pugakhanda (Aparah). Only the mature tubers of the plant are used in traditional preparations and immature ones are discarded. The current study is to investigate the biological and chemical differences between mature and immature tubers. The mature tubers of *Ipomoea mauritiana* was compared with the immature tubers to find out the

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differences in their phytoconstituents and their bioactivities using modern chromatographic tools. The chemical and biological profiles showed that mature tubers possess about twice phytoconstituents than immature tubers which is responsible for biological activity. *Ipomoea mauritiana* is generally called as 'Vidari' consist many phytoconstituents like taraxerol, taraxerol acetate, β-sitosterol, scopoletin and 7-O-β-D-glycopyranosyl scopoletin (Scopolin) which can be isolated from the methanol extract of the root tubers of *Ipomoea mauritiana*[9]. Many anticancer and antimicrobial glycosides were isolated from *Ipomoea* species[32].Numerous reports shows that the herbal products available to consumers are of varying quality. To produce high-quality herbal products, attention must be paid in selection and collection of medicinal plants, phytochemical variation of plant breed, organ specificity, stages of growth, storage etc. Quantitative analysis of sesquiterpene lactones in *Arnica montana* shows that it is of different percentages at different stages of flower maturity. The total content of sesquiterpene lactones increased progressively with maturity of flower (0.512% in buds to 0.943% in withered)[12]. The best product quality with maximum yield is a function of a dynamic relationship between maturity, yield and quality parameters. The above studies reveals the role of maturity in determining the quality of herbal medicines. As per living traditions of Kerala (South India) mature tubers of *Ipomoea mauritiana* are selected for use in formulations as against the immature ones. These drew our attention to study the difference in phytoconstituents of mature and immature tubers. Modern chromatographic techniques like HPLC and HPTLC were used to judge the authenticity of traditional recommendations.

Methods

The mature and immature tubers of *Ipomoea mauritiana* were collected from of Bangalore Karnataka. The tubers were authenticated by qualified plant taxonomists. Voucher specimens were deposited at FRLHT, Bangalore and Raw Drug Collection (K. Ravikumar, No.56052).

Extraction: Dried material (150g each) of both mature and immature tubers of *Ipomoea mauritiana* was coarsely pulverized to powdered form. Coarse powdered (100g each) of both tubers was extracted using a Soxhlet apparatus with water and methanol at $70\text{-}80^0$c and $45\text{-}55^0$c for 35 complete cycles. The aqueous and alcoholic extracts of both source was dried at $30\text{-}40^0$c using a vacuum evaporator.

Pharmacognostical evaluation: Both mature and immature tubers samples were subjected to morphological and microscopical examination to study the variations among them. Quantitative standards like moisture content, total ash, acid insoluble ash, alcohol soluble and water soluble extractive values for both mature and immature tubers were determined.

Morphology: Both the samples of mature and immature tubers were subjected to macroscopic identification based on color, odor, taste, form, size, milk exudates and fracture.

Fig.1 Photograph of Mature and Immature tubers of *Ipomoea mauritiana*

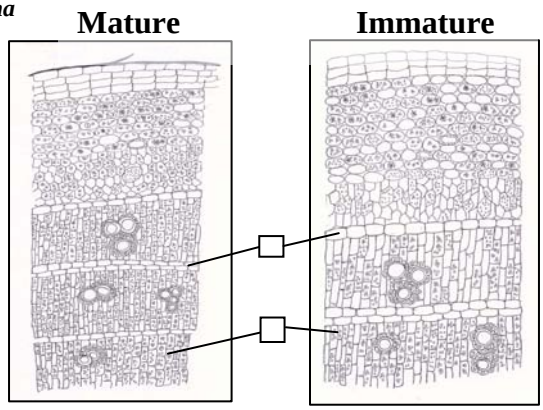

Fig.2 Microscopic sections of Mature and Immature tubers *Ipomoea mauritiana*

Microscopy: The samples of the tubers were soaked in water. Using a sharp blade, transverse sections were taken and observed under the microscope. The

sections were stained with iodine and mounted on glycerin. The intensity of the blue-black color indicates starch content.

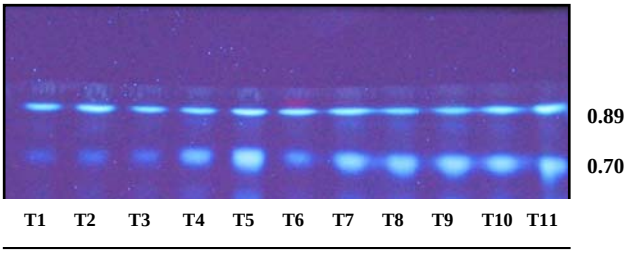

Fig. 3 TLC of samples collected at different stages of growth detection at @366nm

Proximate analysis: The mature and immature tubers were subjected to evaluate their moisture content, total ash, acid insoluble ash, alcohol soluble extractive value and water-soluble extractive value.

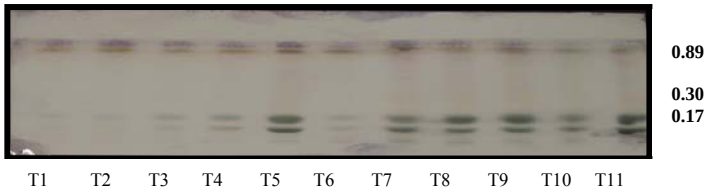

Fig 4. TLC after derivatization with vanillin sulphuric acid reagent
Note:Bands T4, T5, T8, T9 and T11 are samples of same stage.

Moisture Content: Moisture content of the mature and immature tubers was determined using Infrared moisture balance Model-M-3A Deluxe Voltag-230VAC (Advance Research Instrument Co). The percentage of moisture lost was calculated based upon the initial weight of the sample using the formula (P'=P/100-P) where, P'is the percentage of moisture lost by the sample and P is the moisture on the dry basis. To assure complete drying, wait one to three minutes after the weight stops change.

Total ash value[5]*:* A 3g of the ground air-dried sample was ignited slowly at 450-500^{0}C in the muffle furnance for 3 hours to obtain a carbonized residue. Percentage of ash is calculated using formula. (Ash % = (B-C) x100/A) where, A-Sample weight in g, B-Weight of dish + contents after drying (g), C- Weight of empty dish (g).

Acid insoluble ash: Total ash treated with dilute hydrochloric acid reacts with minerals to form soluble salts and the insoluble ash residue consists mainly of silica, as acid insoluble ash. Acid insoluble ash is calculated as ash % using formula (Acid insoluble ash % = (B-C) x 100/A) where, A-Sample weight in g, B-Weight of dish + contents after drying (g), C-Weight of empty dish (g)

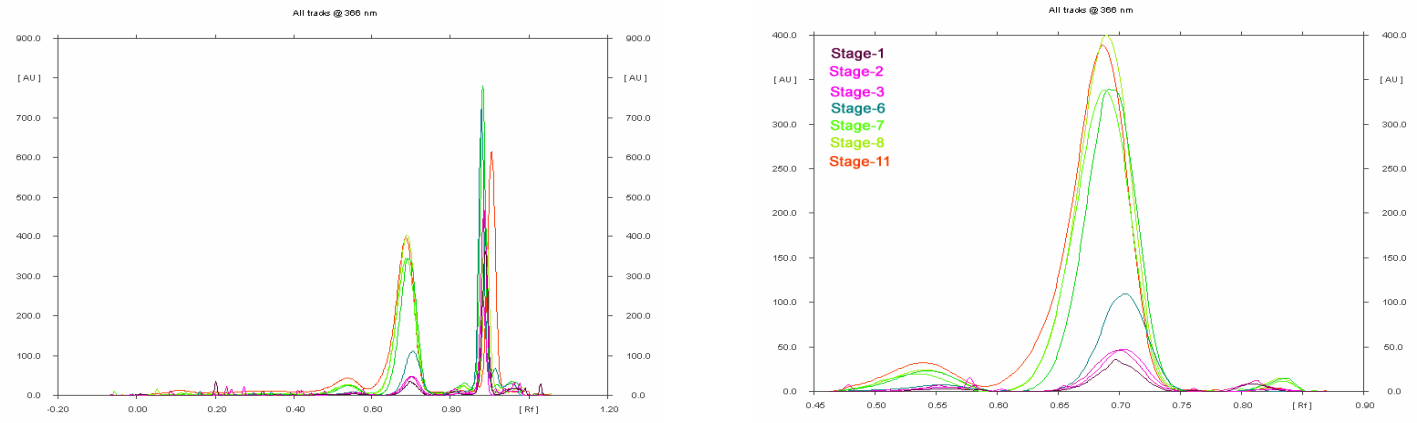

Fig 5. Densitometric scanning of different stages of mature and immature tubers at 366nm before derivatization

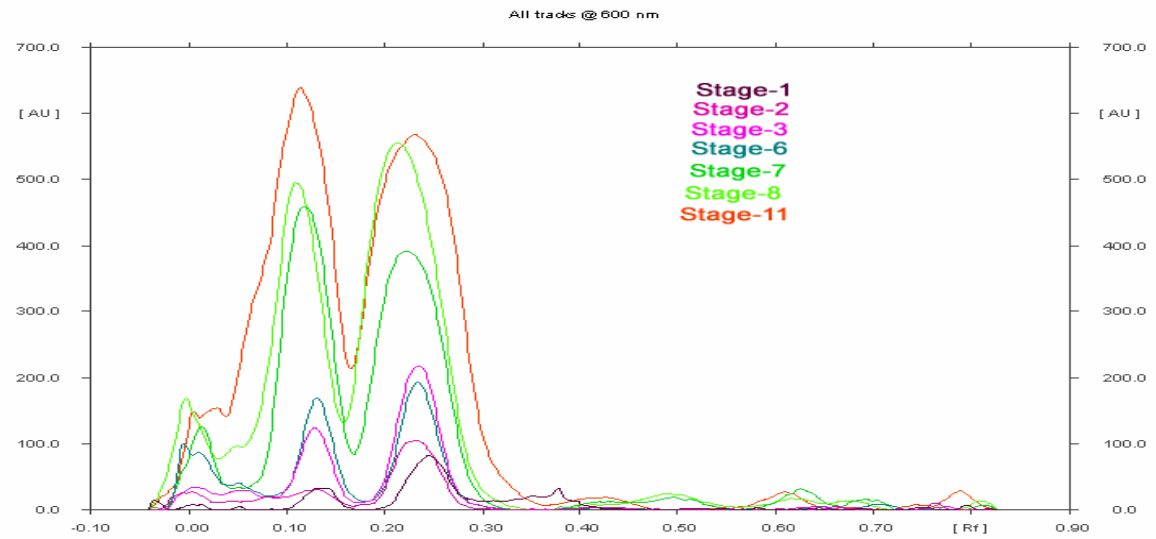

Fig.6 Densitometric scanning of different stages of mature and immature tubers at 600nm after derivatization.

Extractive Values: The determination of water and alcohol soluble extractive value is used as means of evaluating the quality and purity of drugs the constituents of which cannot be readily estimated by other means. Extraction of the drug done by maceration with cold water or by a continuous extraction process in a Soxhlet extractor.

Alcohol soluble extractive value: A 4.0 g of the air dried drug, coarsely powdered and macerated with 100ml of 90% ethanol in a glass stoppered flask for 24 hours, shaking the contents frequently during the first 6 hours and allowing to stand for 18 hours. Thereafter, filter and evaporate 25ml of the filtrate to dryness on a water bath at 105^0C for 1 hour in a hot air oven, remove the dish, cool in a dessicator and weigh. The percentage of ethanol-soluble extractive calculated with reference to the air-dried drug.

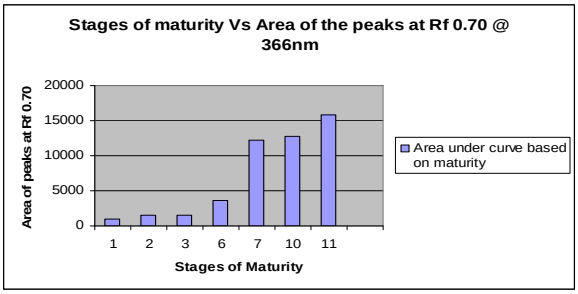

Fig 7. Graphical representation of area of peak at R_f value 0.70 Vs different stages of maturity of tubers of *Ipomoea mauritiana*

Water soluble extractive value: An air dried drug (4g), coarsely powdered, macerated with 100ml of chloroform water in a glass stoppered conical flask for 24 hours, shaking the contents frequently during the first 6 hours and then stand for 18 hours. Thereafter, filter and evaporate 25 ml of the filtrate to dryness on a water bath and 2 ml of alcohol was added., shake and dry the contents on water bath. Dried repeatedly at 105^0C for 1 hour in the hot air oven for 30 minutes,

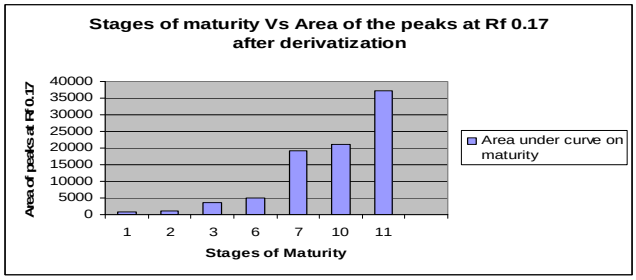

Fig. 8 Graphical representation of area of peak at R_F value 0.17 Vs different stages of maturity of tubers of *Ipomoea mauritiana.*

cool and weighed. The process repeated till the constant. The Percentage of Water soluble/Alcohol soluble extractive value was calculated using formula (B - A X 4 X 100 / W) where, A-Empty weight of the dish (g), B-Weight of dish + residue(g), W-Weight of plant material taken(g).

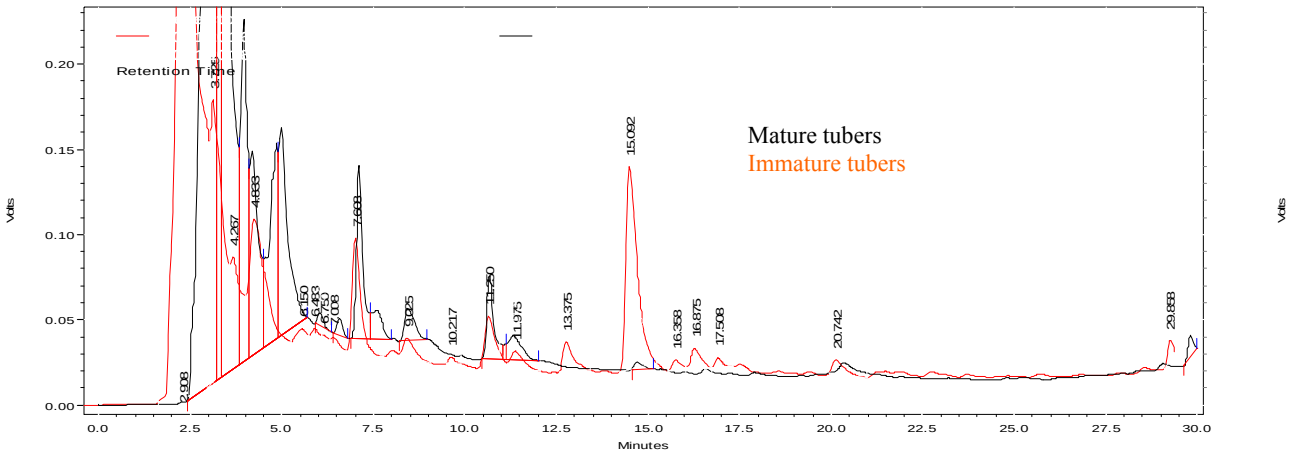

Fig. 9. Overlay HPLC chromatogram of mature and immature tubers of *Ipomoea mauritiana*

Phytochemical screening: Both samples of mature and immature tuber was powdered and subjected to systematic phytochemical screening by successively extracting them in different solvents and testing for the presence of chemical constituents.

High Performance Thin Layer Chromatography (HPTLC): The methanol extract of mature and immature tubers of *Ipomoea mauritiana* (2.5g in10ml) were spotted on precoated silica gel plate $^{60}F_{254}$, with 0.25mm thickness (E.Merck) using micro syringe (100µl, Hamilton) and applicator (Linomat 5). The plate was dried and the chromatogram was developed using Chloroform: Methanol: Formic acid (6:3:1) as a mobile phase, and was dried and scanned using Camag TLC Scanner 3 at 365nm and after derivatization using vanillin-sulphuric acid.

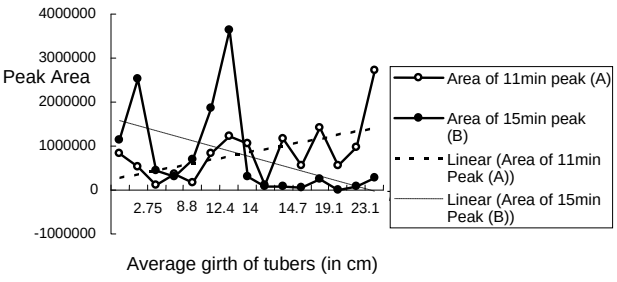

Fig. 10 HPLC peak areas of 11 min and 14.8 min peak against girth of the tubers.

High Performance Liquid Chromatography (HPLC): Powdered tubers of *Ipomoea maurtiana* was extracted by refluxation with water. Extraction of 2.5g of both mature and immature tubers were powdered and refluxed using water as solvent for 45min. The extracts were filtered and concentrated using Rotary evaporator. The sample (2.5 gm of aqueous extract in 10ml water) was subjected to HPLC and the profile for both mature and immature tubers were same. An HPLC instrument of Shimadzu made with injection volume of 20µl at flow rate of 1.0ml/minute run for 30minutes. The HPLC system consisting of LC-10AT$_{VP}$ pump, a rheodyne injector, SPDA$_{VP}$ UV-Visible detector and CLASS-VP6 software was used for the analysis. Merck C-18 (250 x 4.6mm) column with stationary phase of 5µ particle size was used and equilibrated with the initial solvent ratio for an hour. The mobile phase, consisting a gradient system of methanol and water where Pump A: Water and Pump B: Methanol. Concentration of methanol increased from 10-50% within 0.01-20min and raised to 100% at 30.00min. The detection was done at 254nm.

Results and Discussion

Morphology: The morphological variation of mature and immature tubers are given in Table-1 and the picture of tubers are shown as Figure-1

Microscopy: The microscopic variation of immature and mature tubers is expressed through transverse section of samples through Figure-2. The major difference observed through microscopy was the presence of large number of growth rings in mature while only one ring was found in immature tuber. The starch content was found to be more in the mature as compared to immature tubers.

Table 1. Data showing morphological features in both mature and immature source of *Ipomoea mauritiana*

Characterstic	Mature tuber	Immature tube
Colour	Outer surface brownish with white inner p	Outer surface pale brown with pale white inner porti
Odour	Agreeable	Agreeable
Taste	Mucilagenous, Slightly	Mucilagenous, Slightly swee
Form	Obovoid to subcylindr	Cylindrical having tapering r
Size (leng	9.0-12.2mm	4.0-12.5mm
Size (Girt	23-23.1mm	6.0-10.1mm
Latex	Milky white	Milky white

Proximate analysis: The data of proximate analysis of mature and immature tuber is expressed in Table-2. The moisture content of mature tuber was found to be 15% whereas it is 10% in case of immature tubers. The total ash, acid insoluble ash, water soluble and alcohol soluble extractive values of mature tubers are slightly higher compared to immature tubers.

Phytochemical screening: Both mature and immature tubers showed the presence of alkaloids, carbohydrate, glycosides, saponins, phytosterols, resin, flavnoides, and proteins and no such variation observed in presence of chemical constituents determined using Qualitative tests. Result of chemical tests is expressed in Table-3. Starch content was found to be more in mature (5.8%) as compared to immature (2.1%) whereas, Protein and Saponin content was found to be more in immature (6.6% and 7.28%) as compared to mature (4.4% and 1.65%) tubers. Comparative data of protein, saponins and starch content of mature and immature tubers is expressed in Table-4.

Table 2: Data showing values of moisture content, total ash , acid insoluble ash in both mature & immature tubers of *Ipomoea mauritiana.*

Sample Identity	Moisture Content (%)	Total Ash (%)	Acid insolul ash (%)	Alcohol soluble extractive (%)	Water soluble extractive (%)
Mature	15	17.35	1.4	4.08	11.85
Immature	10	16.9	1.2	3.12	12.25

Table 3: Qualitative chemical tests of the extracts of mature& immature tubers of *Ipomoea mauritiana*

Chemical Constituents	Tests	Petroleum ether extrac	Chloroform extract	Methanol extract	Ethanol extract	Water extract
Alkaloids	Mayers test	-	-	+	+	-
	Dragendroff' test	-	-	-	-	-
	Wagners test	-	-	-	-	-
	Hagers test	-	-	-	+	
Carbohydrates	Molisch's test	-	-	-	-	+
	Benedicts test	-	-	-	-	+
	Fehling's test	-	-	-	-	+
Glycosides	Modified borntragers	-	-	-	+	+
	Legal test	-	-	-	-	-
Saponins	Foam test	-	-	-	-	+
	Froth test	-	-	-	-	+
Phytosterols	Salkowski tes	-	+	-	-	-
	Libermann Burchard	-	-	–	+	+
	Tschugajew te					
Fats and oil	Stain test	-	-	-	-	-
Resins	Acetone water	-	-	-	+	-
Flavanoids	Gelatin test	-	-	+	+	+
	Lead acetate	-	-	-	-	+
	Shinoda test	-	-	+	+	+
Proteins	Ninhydrin test	-	-	-	-	+
	Biuret test	-	-	-	-	+

High Performance Thin Layer Chromatography

The methanol extract of different stages of mature and immature tubers of *Ipomoea mauritiana* (2.5g in 10ml) were spotted on precoated silica gel plate $^{60}F_{254}$, with layer thickness 0.25mm (E.Merck) using micro syringe (100µl, Hamilton). Mobile phase: (Chloroform: Methanol: Formic acid::6:3:1). The general TLC fingerprinting is similar for both mature and immature tubers. The specific bands of TLC was found to be intense in immature compared to mature tubers.and fingerprints are shown in Fig-3,4. Densitometric scanning at 366nm shows a decrease in

the intensity of the spot with Rf 0.70 from immature tubers to mature tubers.(Fig-5) Scanning after derivatization at 600nm shows a decrease in intensity of the spots at Rf values 0.17 and 0.30 from mature tubers to immature tubers (Fig-6). Representation of area under curves of mature and immature tubers at Rf-0.70 and 0.17 before and after derivatization is given in Fig-7,8. and Table-5.

Table. 4 Data showing percent estimation of Starch, Proteins, and Saponins.

Maturity	Starch content	Protein content	Saponins content
Mature	5.8	4.4	1.65
Immature	2.1	6.6	7.28

Table. 5. Data showing Area of the peak at Rf 0.17 after derivatization

Stages	Track No	Details of samples	Area under peak	
			Rf 0.17 after derivaization	Rf 0.70 before derivaization
1	T1	Immature tubers	890.4	1006.8
2	T2		1183.5	1507.9
3	T3		3681.8	1546.3
4	T4		37176.1	15876.8
5	T5		37176.1	15876.8
6	T6		4953.7	3593.6
7	T7		19048.1	12227.2
8	T8		37176.1	15876.8
9	T9		37176.1	15876.8
10	T10		21192.5	12769.3
11	T11	Mature tubers	37176.1	15876.8

High Performance Liquid Chromatography: The aqueous extracts of different samples of mature and immature tubers of *I. mauritiana* were subjected to HPLC analysis. Samples collected from different stages of maturity were used for analysis. The HPLC profile of mature and immature tubers remains same but the aqueous extracts of mature and immature tubers consistently exhibited a quantitative difference in the phytoconstituents particularly those eluting at 11 minute and 15 minute retention time (RT) in the HPLC chromatograms. It is interesting to note that the peak at 15 minute decreases with increasing girth quite significantly while at 11 minute peak increased. The ratio of 10 peaks at 11 minute (A) to 15 minute (B) RT increased with size (girth) of the tubers. Coupled HPLC chromatogram of stagewise tubers at different retention time is expressed in Fig-9 and graphical representation is expressed in Fig-10.

Conclusion

The current study was done to investigate the concentration of chemical constituents between mature and immature tubers. The mature tuber of *Ipomoea mauritiana* is compared with the immature tuber to find out the differences in their phytoconstituents using modern tools like HPTLC and HPLC. The aqueous extracts of both mature and immature tubers of *Ipomoea mauritiana* were subjected to HPLC and HPTLC analysis, the results reflect the quantitative phytochemical difference among mature and immature tubers. The mature tubers contain better concentration of phytoconstituents than the immature source, which enables us to conclude the authenticity of traditational recommendation.

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