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Application of HPTLC in the Standardization of a Homoeopathic Mother Tincture of *Syzygium Jambolanum*

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ABSTRACT

A simple and accurate HPTLC method has been developed for quantification of Syzygium Jambolanum and finger printing of the in-housed mother tincture considered here to be a standard with that of different marketed samples available from manufacturers of homoeopathic medicines in India. This HPTLC method was quantitatively evaluated in terms of stability, repeatability, accuracy and calibration providing the utility in the analysis of the mother tincture.

Key Words: HPTLC, Mother Tincture, *Syzygium Jambolanum*, Fingerprint.

INTRODUCTION

Most of the modern medicines have originated from plant metabolites [1]. There is a growing focus on the importance of medicinal plants in the traditional health care system (viz. Ayurveda, Unani, Homoeopathy, Yoga) in solving health care problems [2-10]. Because of this awareness plant materials and herbal remedies derived from them represent substantial portion of the global market. Most of the developing countries have reviewed the traditional medical practices as an integral part of their culture so much so a large number of herbal remedies coming out to treat so many deadly ailments. But we are still facing problems for the standardization of herbal products and there are no specific prescribed provisions for Herbal Drugs in current Drug Legislation enforced in our country [11,12]. But in view of the growing interest in herbal medicines and photochemical, scientists are trying to develop methods for standardization of herbal drugs used in different formulation [13].

In order to obtain high quality products care should be taken right from proper identification of plants, seasons, area of collection, their isolation and purification process and rationalizing combination in case of polyherbal drugs [14, 15].

Due to lack of availability of suitable experimental and clinical models, the scientific validation and clinical effectiveness of most of the plant products becomes difficult. But in view of the growing interest in herbal medicines and phytochemicals, scientists are trying to develop methods for standardization of the herbal drugs used in different formulations. Though a number of scientific publications are available on various aspects of botanical, pharmacognostic, phytochemical and pharmacological investigations of plant material, no evidence is available by which if one investigates a particular plant material and process in a specific manner. So to develop a standard procedure for herbal product is highly necessary for the generation [16].

To identify and quantify active substances from herbal formulation product it is necessary to develop standard procedure with the use of latest technology. Though official treatise on homoeopathy is available in India it is obvious that various quality control parameters specified in these official books are not sufficient enough to fulfill the requirement as well as different regulations coming out from the homoeopathic medicines [17, 18, 19].

Syzygium Jambolanum is commonly known as *Syzygium cuminii* (Linn) Skeel and belongs to the family of Myrtaceae. It is also known as Black plum (Eugenia jambolana) [20]. A tree found throughout India mostly cultivated for its edible fruits, seed coat, within which tow cotyledons are distinct, loosely adheres with percicarp. Partused to prepare mother tincture are seeds. Seeds are known for their medicinal character to cure diabetes, diarrhea, dysentery and blood pressure. A principle was isolated from black plum seeds which abolished glycosurea, hyperglycemia and reduces polydepsia which occurred in diabetic rats. Lal and Choudhari have reported that black plum seeds extract is capable of lowering blood pressure to the extent of 34.6%. recent studies with ellagic acid by Bhargava et.al. have shown that it markedly lowers blood pressure. It is very likely that characteristics of black plum seeds to lower blood pressure is due to presence of ellagic acid, which is one of the main constituents of the seeds. Lowering of the blood sugar in alloxan diabetic rats was produced by ethanolic extract of seeds.

It contains Glycoside (Jamboline), Tannin, Ellagic Acid and Gallic Acid. It has immediate effect on increasing the blood sugar, glycosuria results. It is chiefly used in homoeopathy as remedy in diabetes mellitus. No other remedy causes in so marked degree the diminution and disappearance of sugar in the urine. It is also used for old ulcer of skin, diabetic ulceration.

Homeopathy is holistic system of therapy which works at reinforcing the body's own natural capacity to heal and achieving a gentle and lasting cure. Mother tinctures (MQ) are defined as the original tincture prepare with the aid of alcohol, directly from the crude drug. They are the precursors of the corresponding potencies of the respective drug and the starting point for the production of most homeopathic medicines [21]. They contain a number of chemical entities. It is not possible to establish the chemical picture of an extract with a single chemical test. Though a series of chemical tests may establish the chemical picture and thus identity of a poor selectivity and time factor involved. The alternative method that is available to establish a chemical picture is chromatographic analysis. The method of chromatographic analysis affords

the advantage if identifying the chemical entities present, which constitutes the chemical plants (herbal) extract and at the same time facilitate to quantity the extract. Chromatography is a technique by which the complex mixtures can be resolved in to individual components. The objective of this work is to make an in-house standard mother tincture and compare it with different marketed samples using its fingerprint characteristics and to further quantity them with specific active principle of the known fraction. This concept of standardization may lead to a solution to the factors which are responsible for variation in the homoeopathic formulations.

EXPERIMENTAL SECTION

Authentic plant (leaves) of *Syzygium Jambolanum* was used to prepare mother tincture. *Ellagic acid* ($C_{14}H_6O_8$, m.p. >350^oC, purity 99 % by HPLC) was purchased from SPIC. The solvents 99.9% absolute ethanol, HPLC water, toluene, ethyl acetate, formic acid were of Analytical Grade Purity (MERCK Ltd.).

Preparation of Standard mother tincture: The dried seeds of plant coarsely powdered, 10 g of this powder was used and the requisite amount of alcohol and water was added as specified in HPI and the standard mother tincture was prepared by the percolation method. This tincture was transferred to suitable glass container and stored for further study [22].

Preparation of Standard *Ellagic acid*: Weigh 5 mg *Ellagic acid* in 5 mL volumetric flask and 5 mL ethanol was added (1µg/µL) in to it. Out of this, 1 mL of the standard solution was taken in another volumetric flask.

2-D Stability: A single spot of 5 µl (starting position 15 mm from left) of the standard mother tincture was applied at the corner, keeping 10 mm distance from the bottom on silica gel $60F_{254}$ aluminium sheet of size 10 × 10 cm. After drying, the plate was developed in Twin Trough Chamber with Toluene: Ethyl Acetate: 100 % Formic Acid (100:60:50) as mobile phase [Fig. 1].

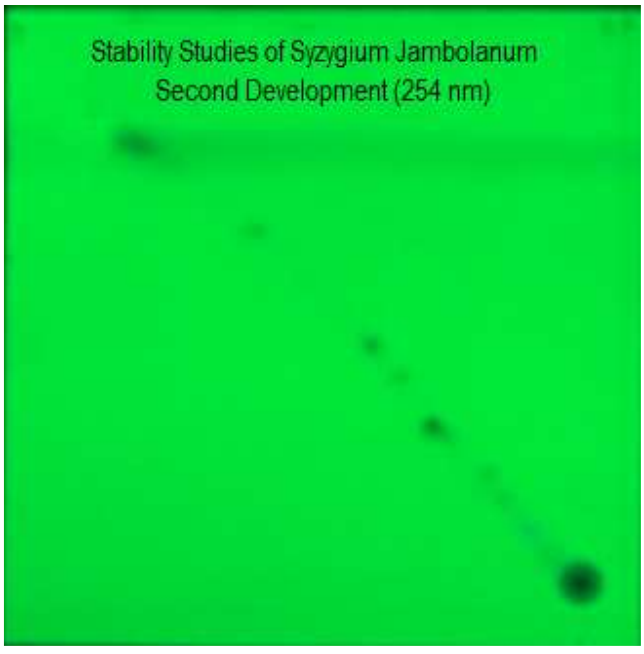

Fig. 1: 2-D Stability o Syzygium Jambolanum

Standardization of standard other tincture: Camag HPTLC system comprising of Linomat 5 as sample applicator TLC Scanner 3 controlled by win CATS software version 1.3.4 was used for quantitative evaluation [23]. Stationary phase used for quantitative evaluation. Stationary phase used was Merck percolated TLC aluminum foil Silica Gel $60F_{254}$ and the mobile phase used was Toluene: Ethyl Acetate: 100 % Formic Acid (100:60:50) v/v. Samples and standard were applied at 8 mm bands with 6 mm distance between the tracks. Tank saturation was given with filter paper for 15 min. Ascending development for a distance of 80 mm in a twin trough chamber was completed in *ca*. 15 min. Volume of standard mother tincture was first optimized at 5 µL for fingerprinting. The λ_{max} of *ellagic acid* was found to be 279 nm after taking the spectra of the standard of *ellagic acid*. Quantitative measurement in the absorbance mode was at 225 nm using a slit dimension of 5.00 × 0.45 mm.

Linearity response: The volume of standard mother tincture was optimized to 5 µL for quantification. It was then simultaneously applied with different concentration of standard *ellagic acid*. The method was found to be linear with a regression of 0.99945 and a standard deviation of 1.82 % and amount of *ellagic acid* was calculated in the mother tincture [Fig. 2].

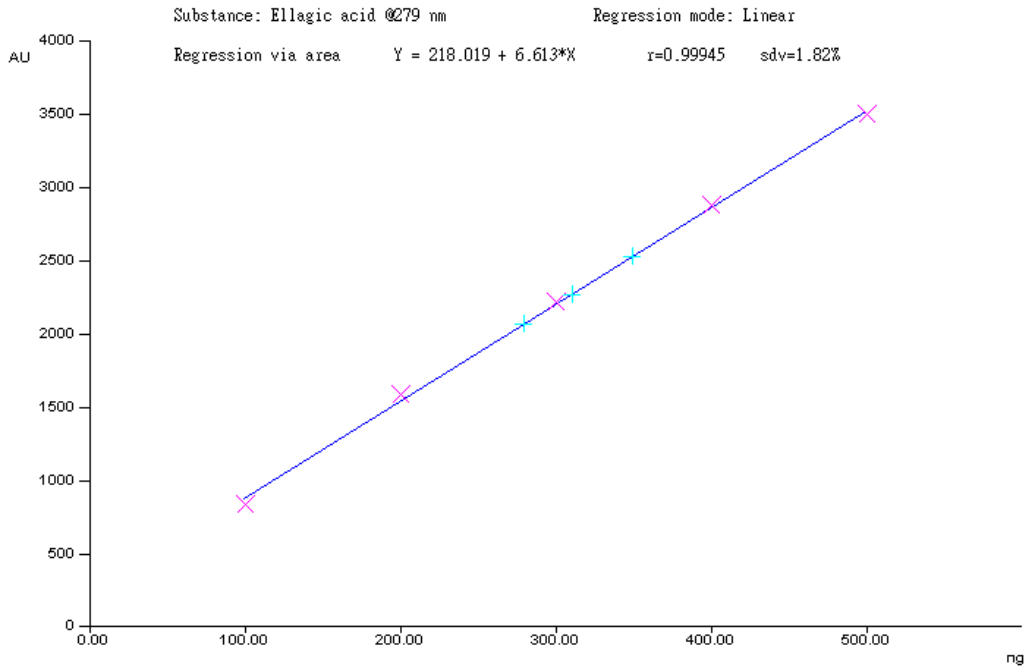

Fig. 2: Calibration curve of *Ellagic acid* (area)

Standardization of the standard mother tincture by fingerprint method: Standardisation of the mother tincture was done by evaluating its fingerprint characteristics, using HPTLC method. Standard mother tincture was chromatographed simultaneously along with four other mother tinctures available in market 5 µL on the same plate for comparison. Multi wavelength (MWL) scanning was done for finding the optimum wavelength. The optimum wavelength was found at 279 nm. The entire plate was further scanned at this wavelength for quantification and spectral

match. Many fractions of standard mother tincture were matched with the help of its characteristic spectra with that of other marketed samples. Individual λ_{max} of each fraction was also found with the help of spectral scanning and then the plate was scanned with this selected wavelength in MWL mode. The pattern of the peaks was compared for the standard mother tincture and marketed samples (Table-1).

It was approved that the response for various concentrations of standard *ellagic acid* was linear in the range of 200 to 600 ng with a coefficient of variation of 0.99916 and standard deviation of 2.32 % [Fig. 3]. *Ellagic acid* was quantified and the amount was calculated in individual mother tinctures. With this method we compared all available mother tinctures and the active principle was also quantified. Thus the method can be said to be standardized.

Quantification of *Ellagic acid* in market samples and standard mother tincture: The amount of *ellagic acid* was calculated in standard mother tincture (A) and market samples (A1 to A4) and the results are tabulated in Table-2.

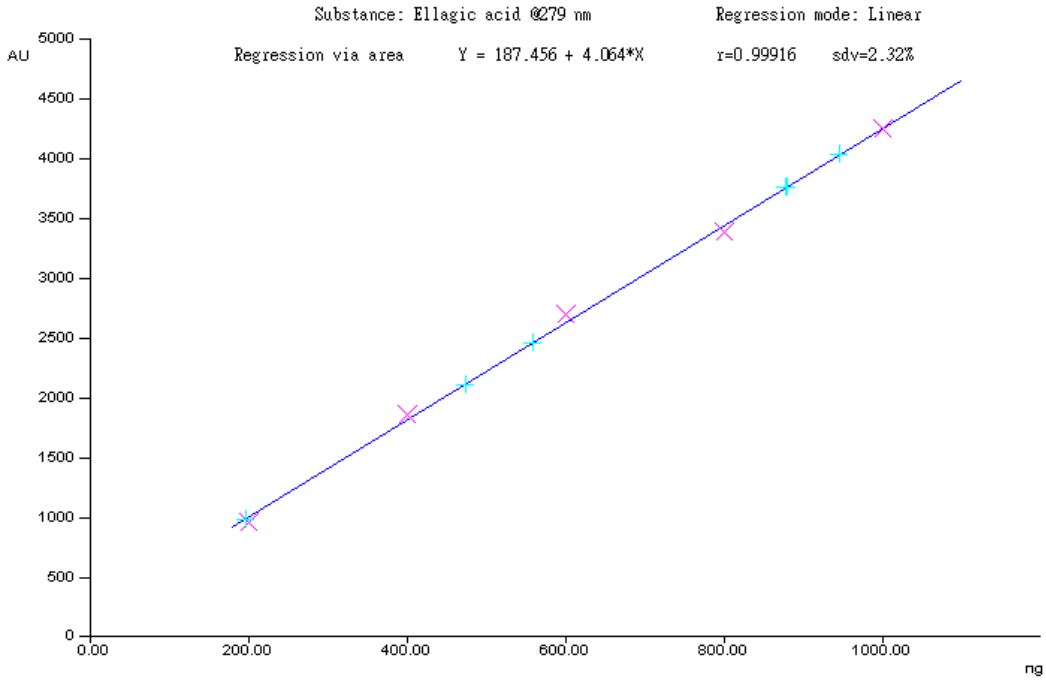

Fig. 3: Calibration curve of Ellagic acid in marketed samples & Std. MQ (area)

RESULTS AND DISCUSSION

The decomposition of the analyte during application or development was confirmed by two-dimensional chromatography. The chromatogram did not show any extra fraction. Repeatability of the method was checked by scanning 15 tracks of 5 µL volume standard mother tincture. The co-efficient of variation (CV) was found to be 0.465 %.

Table 1: Analysis of diffeent *Syzygium jambolanum* mother tincute at scanning wavelength 279 nm

Peak	A			A1			A2			A3			A4		
	R_f	Max. Ht.	Area %	R_f	Max. Ht.	Area %	R_f	Max. Ht.	Area %	R_f	Max. Ht.	Area %	R_f	Max. Ht.	Area %
1	0.09	162.5	34.07	0.07	374.7	57.98	0.10	340.9	95.25	0.08	319.2	53.23	0.07	186.6	61.95
2	0.20	24.8	5.15	0.21	225.8	32.81	0.22	16.0	3.40	0.21	57.5	10.07	0.20	58.6	22.17
3	0.33	41.0	13.29	0.36	34.4	3.95	0.27	14.7	1.36	0.38	29.7	4.66	0.39	30.2	11.02
4	0.48	15.0	2.25	0.46	19.4	1.21	-	-	-	0.70	125.9	30.58	0.77	18.8	4.87
5	0.72	141.0	45.24	0.54	38.1	3.35	-	-	-	-	-	-	-	-	-

Table-2: Amount of in *Syzygium jambolanum* mother tinctures

Sample	Wt. of *Ellagic acid* (mg) in 100 mL sample
A	18.31 mg
A1	15.36 mg
A2	8.8 mg
A3	16.58 mg
A4	11.72 mg

Accuracy: The percentage recovery of *ellagic acid* values was calculated using the above method. The average recovery values obtained were 97.00 to 101.75 %, which confirms that the method is validation.

CONCLUSION

The HPTLC fingerprinting characteristic of *Syzygium Jambolanum* mother tinctures obtained from manufacturer (A1 to A4) and the in-house standard mother tincture (A) had been scanned at 279 nm wavelengths. From the results obtained after densitometric scanning, it was observed that the standard mother tinctures (A) of *Syzygium Jambolanum* shows 4 peaks. The marketed samples A1 shows 2 peaks, A2 shows 3 peaks, A3 shows 3 peaks and A4 shows 4 peaks.

Value of the four marketed tinctures (A1 to A4) was found to show minimum 3 different peaks with R_f values similar to standard mother tinctures (A) and they are similar within themselves. So from this study, it was confirmed that *Syzygium Jambolanum* tincture contain different components with R_f values (0.07-0.10, 0.20-0.22, 0.33-0.39, 0.46-0.48, 0.54, 0.70-0.77). These components must be considered to determine quality of any further sample of the same. The spectral analysis indicates that spectra with particular R_f values of various components (0.08, 0.21, 0.36, 0.46, 0.54, 0.77) have similar pattern within themselves. It may be concluded that samples procured from the market that are showing lesser peaks may not be up to the standard level.

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