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# THIN LAYER CHROMATOGRAPHY - AS A TOOL FOR STANDARDIZATION OF AYURVEDIC MEDICINE, *TRIKATU CHURNA*

**Sampad Shil[1], Mudiganti Ram Krishna Rao[2*], Prabhu K.[3], Amuthvalli K[4]**

[1]Student, Department of Industrial Biotechnology, Bharath Institute of Higher Education and Research, Selaiyur, Chennai.

[2] Professor, Department of Industrial Biotechnology, Bharath Institute of Higher Education and Research, Selaiyur, Chennai.

[3]Associate Professor, Sree Balaji Medical College and Hospital, Chennai

[4]Research Associate, Prashanth Fertility Centre, Chennai.

**Abstract:**

*Evaluation and standardization of Ayurvedic and other alternative systems of medicine has become highly pertinent to bring them to the main stream of medicinal practice. Trikatu churna is well known polyherbal medicine in Ayurveda consisting of powders of three ingredients, namely, Piper longum, Piper nigrum and Zingiber officinale in equal proportions. Trikatu is also known as "Three Bitters", which is of pungent taste. This combination streamlines the metabolism of the body and is indicated in a wide range of health problems like asthma, fever cold, cough, as purgative, carminative, to reduce obesity, indigestion, high cholesterol, slow metabolism, hypothyroidism, congestion, edema etc. The present study deals with the detection of important phytochemicals such as alkaloid, steroid, terpenoid and flavonoid present in this churna by TLC analysis. The analysis of various constituents of Trikatu separately and also in various combinations was undertaken to understand the influence of one on another. A comparison was also done with the customized sample of Trikatu with seven market samples to find out the variation among them. The activity of these metabolites were in the order of alkaloids, steroids, flavonoids and terpenoids, respectively, as observed by their rf values. When the market samples were compared with the results of CS, it was observed that M1, M2 and M3 were close to that of CS. M4, M5, M6 and M7 were not matching with respect to all the four metabolites. The reasons for such variations are discussed and the need for standardization is emphasized.*

**Key words:** *TLC, Ayurveda, Phytochemical, Alkaloid, Steroids, Terpenoid, Flavonoid*

***Corresponding Author:**

**Dr. Mudiganti Ram Krishna Rao, Ph. D,**

*Professor,*

*Dept of Industrial Biotechnology,*

*Bharath Institute of Higher Education and Research,*

*Bharath University, Chennai- 600073.*

*Phone: +91-9894994567*

*E mail: mrkrao1455@gmail.com*

QR code

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## INTRODUCTION:

In Ayurvedic medical practice Trikatu is known as Heating Formula. Its thermogenic action promotes AGNI or digestive fire which burns the harmful toxins and revitalizing the metabolism. Among the constituents long pepper, Pippali, is well-known for its immunomodulatory and rejuvenating effect on digestive and respiratory system. Sunthi or dry ginger is excellent in rejuvenating the whole body and called as Vishvabhaishjya , which means the medicine of the world. Maricha or black pepper is said to have Pramathi Guna i.e., it forcefully expels out the toxins from the body. Trikatu promotes healthy digestion, improves all gastric functions and increases food absorption and is known to reduce congestion in digestive tract and improve digestion and appetite. Trikatu is attributed with other medicinal functions like improving lung function, reduce excess weight and increases general vitality (Johri *et al*, 1992; Ali *et al*, 2014).

Trikatu, although a simple preparation, requires thorough standardization towards the quality, quantity and adulteration aspects. There are some reports in this direction. Divya *et al*, 2017 have done the physic-chemical and HPLC studies of Trikatu. Vyas *et al*, 2011 have estimated the piperine content in in trikartu by HPTLC studies. High Performance Thin layer Chromatography of Thrikatu churnam was reported by Sunita *et al*, 2011. Chauhan *et al*, 2016 have reported the physicochemical parameters of the ingredients of Trikatu churnam market samples. Some work on the standardization of Trikatu was done by Mahato *et al*, 2016, Gupta *et al*, 2015; Thakur *et al*, 2012. Kumaravelu *et al*, 2017, have developed a novel method of standardization of Trikatu and its constituents by Near Infra-Red Spectroscopy.

Thin Layer Chromatography is a simple, effective and quick approach in analysis of Trikatu. The present study is one step in this direction. The present study has two sections:

1. TLC Analysis of various constituents of Trikatu separately and also in various combinations to understand the influence among them with respect to the presence of the alkaloid, flavonoids, terepenoids and steroids present in the various constituents .

2. Comparison of the presence of the four phytochemicals in customized sample of Trikatu with various market samples.

## MATERIALS AND METHODS

Procedure

Trikatu powder consists of three ingredients, namely, Piper *nigrum (Pn), Piper longum (Pl)* and *Zingiber officinale (Zn)* in equal proportions. The three ingredients were procured from standard Ayurvedic vendors at Chennai. The fruits were washed thoroughly and dried. Equal weights of all the three ingredients were taken, powdered separately and passed through sieve mesh to have uniform sized particles. To prepare the samples for TLC procedure, 10 mg each of the ingredient were and suspended in 1 ml of respective solvent solutions for the phytochemicals namely, Steroids, terpenoids, flavonoids and alkaloids, and kept for 2 hrs to allow the dissolution of the phytochemicals in the solvent. 1 µl of each sample was taken and charged on the TLC plate and spots. The procedure was done and the bands were visualized by putting the plates in Iodine chamber. The plates were photographed and Rf value was calculated for each spot. Two samples were run in each plate. Tests for Steroids, Terpenoids, Flavonoids and Alkaloids were performed for individual components, Customized Sample (CS) and those of market samples (M1-M7).

**Solvent system for phytochemical analysis:**

For Steroid: Hexane: Ethyl acetate (1:1)
For Terpenoid: Hexane: Acetic acid (9:1)
For Flavonoid: Toluene: Acetic acid (9:2)
For Alkaloid: Ethyl Acetate: Methanol: Water (12:35:1.5)
The following sampling system was used for TLC analysis

1. PN, PL and ZN: Individually**.**
2. PN + PL+ Zn - 1:1:1 - Customized Sample (CS)
3. PN+ZN - 1:1; PN+PL -1:1; PL + ZN - 1:1
4. CS+PN - 1:1; CS+PL – 1:1; CS+ZN - 1:1
5. M1, M2, M3, M4, M5, M6 and M7

## RESULTS AND DISCUSSION:

The TLC results of various parameters are indicated as Rf values in Table 1. Table 2 indicates the presence of all the four phytochemicals in Trikatu components, trikatu mixture and in market samples. Figure 1 and Figure 2 indicate the TLC patterns of steroid, Figure 3 and 4 for terpenoids, Figure 5 and 6 for flavonoids and figures 7 and 8 represent those of alkaloids among the various constituents of Trikatu and market samples.

**Table 1: indicates the presence of the four phytochemicals, namely, steroid, terpenoid, flavonoid and alkaloid in various constituents, combinations and in market samples.**

| SAMPLE | STEROID | TERPENOID | FLAVONOID | ALKALOID |
|---|---|---|---|---|
| PN | 0.738 | 0.23 | 0.6, 0.615 | 0.846 |
| PL | 0.646, 0.861 | 0.230,0.692 | 0.6, 0.615 | 0.861 |
| ZN | 0.830, 1 | 0.692 | 0.646 | 0.769 |
| PN+PL+ZN | 0.738 | 0.692, 0.907 | 0.615 | 0.753, 0.846 |
| PN+ZN | 0.569, 0.846 | 0.723 | 0.723, 0.630 | 0.846 |
| PN+PL | 0.846, 0.584 | 0.753 | 0.646, 0.6 | 0.846 |
| PL+ZN | 0.692 | 0.692 | 0.661, 0.538 | 0.8 |
| MPL | 0.692, 0.892 | 0.723 | 0.615, 0.6 | 0.815 |
| MPN | 0.63 | 0.784 | 0.6, 0.538 | 0.538, 0.861 |
| MZN | 0.6307 | 0.692 | 0.6, 0.538 | 0.538, 0.846 |
| MIX | 0.492, 0.830, 0.984 | 0.215,0.769,1 | 0.523 | 0.876 |
| M1 | 0.476, 0.815, 0.969 | 0.215, 0.753, 1 | 0.523 | 0.876 |
| M2 | 0.476, 0.8, 1 | 0.215, 0.738, 1 | 0.476, 0.492 | 0.861 |
| M3 | 0.476, 0.784, 0.984 | 0.230, 0.738 , 1 | 0.476, 0.492 | 0.861 |
| M4 | 0.369, 1 | 0.462, 0.692,1 | 0.5384 | 0.676, 0.753 |
| M5 | 0.338, 1 | 0.462, 0.692,1 | 0.6 | 0.676, 0.753 |
| M6 | 0.985 | 0.784, 0.692, 0.846 | 0.7846 | 0.692 |
| M7 | 0.923 | 0.707, 0.784, 0.846 | 0.8 | 0.7076 |

Table 2. Indiactes the presence of all the four phytochemicals studied in Trikatu, individual components and in market samples

| SL.NO. | Phytochemicals in trikatu, individuals, market samples | Presence(+)/ absent(-) |
|---|---|---|
| 1 | Steroid | + |
| 2 | Terpenoid | + |
| 3 | Flavonoid | + |
| 4 | Alkaloid | + |

**A. Steroid profile pattern analysis**


Figure 1. TLC Profiles of STEROID present in various study samples

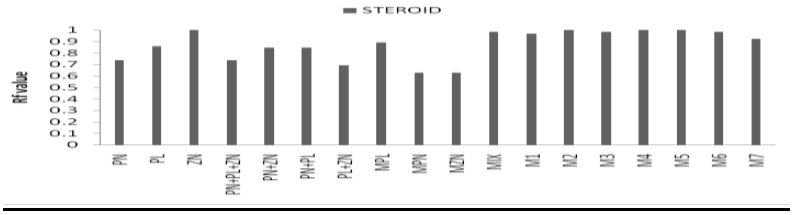


Figure 2. Indicates the steroid Rf values of various study samples.

From Figure I and 2 it is clear that among the three ingredients, rf value for ZN was maximum (0.830 and 1.0) followed by PL (0.646 and 0.861) and PN (0.738). The rf value of CS was interestingly matching exactly with that of PN (0.738). Thus it seemed that although ZN and PL showed more rf values separately, their presence in the CS was similar to the rf value of PN. To understand this phenomenon better, CS was mixed with each ingredient separately at 1:1 ratio. The rf values of CS+PL, CS+PN and CS+ZN were 0.892, 0.630 and 0.630. Here also it is observed that the expression of ZN was masked by PL and PN although in the case of CS+ZN, ZN was double the quantity. Further, when PL+ZN and PN+ZN were observed, the expression of ZN was more in case of PN+ZN (0.846) as compared to PL+ZN (0.692)

The above results indicate that three types of steroids present in the three ingredients could be different and when they come into the solution behave differently.

Further, TLC result of Customized Sample was compared with seven market samples to understand the steroid patterns in each. It was observed that the rf values of steroids present in CS and those in various market samples were not uniform. The rf value of M3 and M6 were matching with that of CS (0.984/0.985). The other market samples showed rf values which were not matching with CS (M1-0.969; M2-1.0; M4-1.0; M5-1.0; M7-0.923). From these results it could be surmised that quality of M1, M2, M4, M5 and M7 were away from that of CS, which could be due to improper proportions of the ingredients, sample collection process, seasonal or geographical variation, changes while storage, transport etc.

**B. Terpenoid profile pattern analysis**

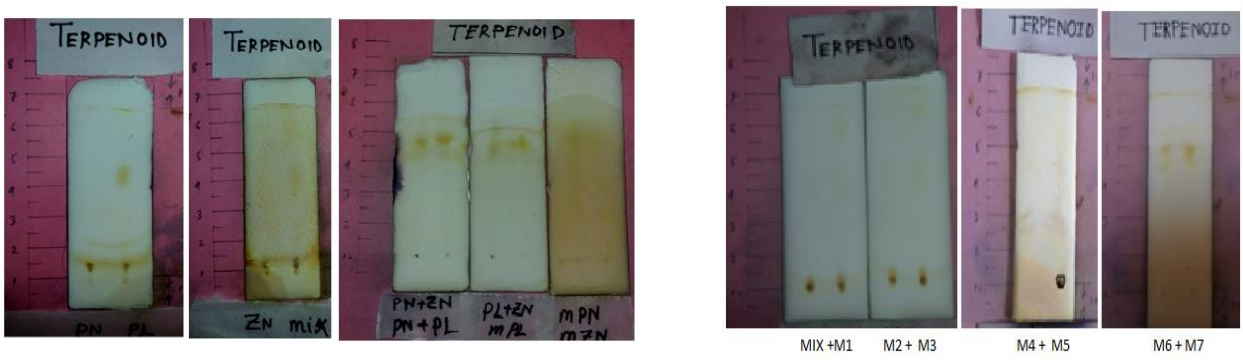


Figure 3. TLC Profiles of TERPENOID present in various study samples

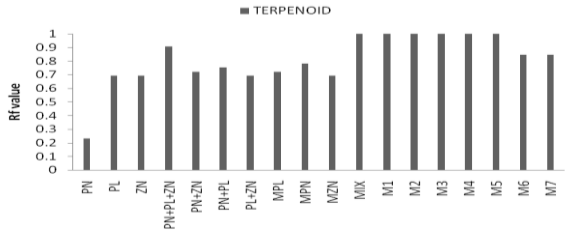


Figure 4. Indicates the steroid Rf values of various study samples.

From Figures 3 and 4 it indicates that the presence of terpenoid was more is pl as compared to PN. The mixture rf value for terpenoid was exactly matching to that of PL.

To verify this phenomenon, TLC was carried out in different combinations for terpenoid like: 1. Mixture of any two individual components at a time (1:1) . 2. Mixing of three individual components (1:1:1) and addition of one individual component, that is (1:1:2=pn:pl:zn), (1:2:1=pn:pl:zn)/ (2:1:1=pn:pl:zn).

The rf value of PN+ZN was 0.723, which was dramatically much ahead of the rf values of individual components (Pl-0.230 and ZN-0). Similarly, PL+PN mixture also showed marked higher rf value (0.753) as compared to that of the components (PN-0.230 and PL-0.692). When additional quantity of ZN was mixed with CS, the rf value was (0.692) thus indicating that there is no expression of ZN. Thus it seems the terepnoid present in PL has an overwhelming effect on other two ingredients.

The TLC profile for Terpenoid of customized sample (CS) was compared with seven market samples namely, M1, M2, M3, M4, M5, M6 and M7. It was observed that there was similarity among CS, M1, M2, M3, M4, M5 whereas it was different for M6 and M7.

**C. Flavonoid profile pattern analysis**

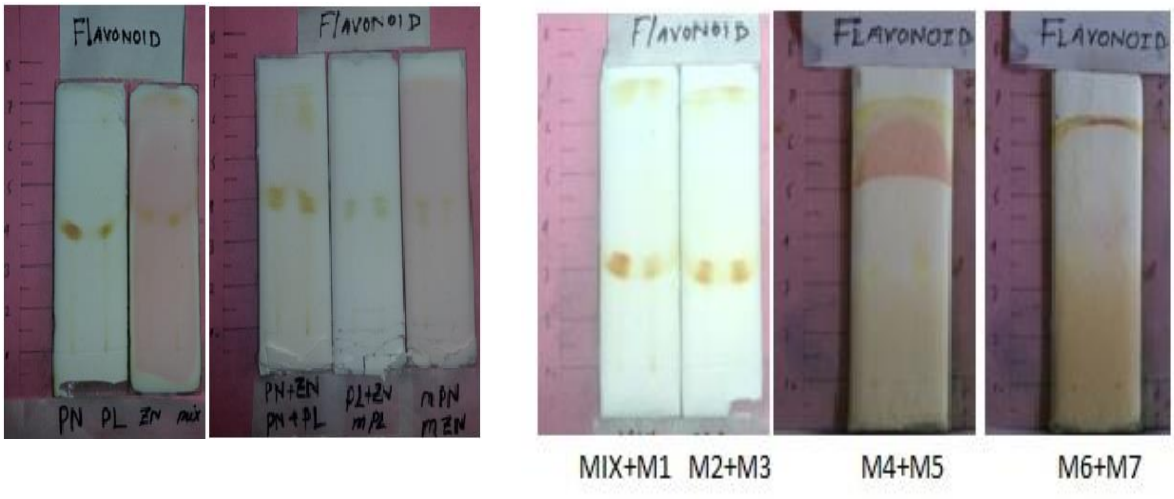


Figure 5. TLC Profiles of Flavonoid present in various study samples

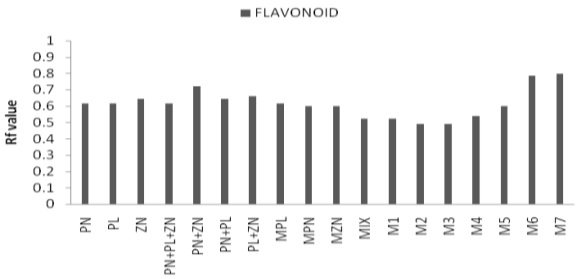


Figure 6. Indicates the Flavonoid rf values of various study samples.

From Figures 5 and 6 it was observed that the presence of flavonoid was almost similar in all three components as indicated by the rf values (PN, PL, ZN, 0.6, 0.615, 0.646, respectively). When PN+ZN and PL+ZN combinations were observed it was found that ZN seemed to be dominant with rf values 0.723 and 0.661.

The rf value of CS was comparable with those of PN and PL, although that of ZN was (0.646). Even when the quantity of ZN was doubled in the mixture the rf value did not increase and stayed at (0.60). This lack of expression of ZN could be due to the masking effect of PN and PL.

It was interesting to observe that the rf values of CS and that of M1, M2 and M3. But those of M4, M5, M6 and M7 were 0.5384, 0.60, 0.7846 & 0.8, respectively.

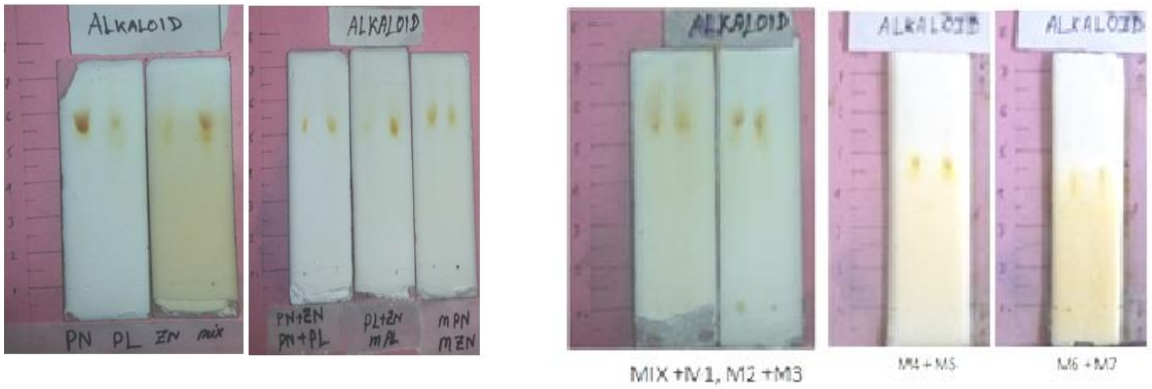


Figure 7. TLC Profiles of Alkaloid present in various study samples

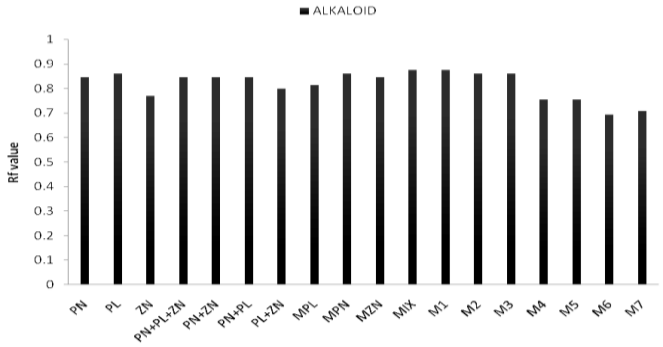


Figure 8. Indicates the alkaloid Rf values of various study samples.

From Figures 7 and 8 the alkaloids TLC profile, the rf values of PL, PN, ZN were 0.861, 0.846 and 0.769. The rf values of PN+PL, PN+ZN and PL+ZN were 0.846, 0.846 and 0.800 respectively. The rf value CS was 0.753 and 0.846 when each ingredient was doubled along with CS not much significant change was observed, thus indicating the three ingredients contribute alkaloids equally.

The comparison of Rf values of CS with other market samples indicated that M1, M2 and M3 were comparable with that of CS (0.876, 0.876, 0.861 and 0.861, respectively. The rf values of M4, M5, M6 and M7 were away from the CS i.e. 0.753, 0.753 and 0.692, 0.707, respectively.

From the above results the following conclusions could be drawn.

The above study was conducted as per the schedule mentioned in the earlier part of materials and methods, was to thoroughly understand the behavior of the components vis a vis mixtures.

All the four important phytochemicals, namely steroids, terpenoids, flavonoids and alkaloids were present in components and in Trikatu sample. The activity of these metabolites were in the order of alkaloids, steroids, flavonoids and terpenoids, respectively, as observed by their rf values.

When the market samples were compared with the results of CS, it was observed that M1, M2 and M3 were close to that of CS. M4, M5, M6 and M7 were not matching with respect to all the four metabolites. This mismatch of these market samples could be uneven proportion of components, sample collection process, seasonal or geographical variation, changes while storage, transport etc.

## CONCLUSION:

The purpose of this study was to compare the various market samples with customized sample to find if any variation existed. And the results are evident. It is high time to standardize the protocols for procurement of the right raw material, process controls, phytochemical content analysis, hygiene and other safety mechanisms in place at manufacturing site, storage, packaging and transport, fixing expiry dates etc. It is suggested that the present approach could be suggested to all the manufactures so that the system of Ayurveda and sidhha could come to the rescue of the populations.

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**COMPETING INTERESTS**

This is to inform that no conflict of interest exist among the authors.

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