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# Standardization of a Polyherbal Formulation Vajravalli Chooranam

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**Abstract :** Intention of the study is to standardize Vajravalli Chooranam, a polyherbal components used in the Siddha system of treatment. Powder microscopy, physico-chemical parameters, preliminary phytochemical tests, qualitative inorganic test, TLC photograph documentation, HPTLC finger print profile new release at UV 254nm, UV 366nm before derivatization and in 540nm after derivatization have been carried out. All the information generated are traits to the drug below learn. The set of physicochemical parameters, TLC and HPTLC results could be valuable for first-rate control of the drug.
**Keywords**: *Cissus quadrangularis,* joint less stem, osteo arthritis, calcium,Vajravalli.

## Introduction:

Standardization of any herbal medicines plays a principal function within the herbal drug industry. In this investigation, a drug used in the Siddha system of medication namely Vajravallichooranam (VVC) is undertaken for standardization studies. It is a poly herbal formulation with 5 parts of plant ingredients. *Cissus quadrangularis Linn.* Is an ingredient which is well known amongst South Indian men and women and is consumed as calcium supplement within the type of Thuvaiyal (mixed with baked rice and eaten). Typical healers additionally advise the identical to an ortho sufferer. *Zingiber officinale Rosc*. Is also delivered in many food gadgets like tea, decoction, chutneys (facet dish of many tiffin gadgets), thuvaiyal, sweet, and so forth. For its known therapeutic action of digestion. *Alpiniachinensis Willd.* is aromatic stimulant, bitter, stomachic, carminative, acts as expectorant and the rhizomes are useful in rheumatism (Nadkarni KM, 1996). Syzygiumaromaticum Linn. possesses anti-inflammatory and antioxidant properties; it is a good source of manganese which is responsible for bone strength (Bhowmik D, 2012). *Piper longum Linn.,* is used for rheumatic pain, paralysis, sciatica, for the immune system and bronchial asthma (Manoj P, 2004).

In the Siddha method of medicine, VCC is stated as an effective remedial drug for cervical spondylosis. It's taken orally within the dose of two to four grams twice a day with honey (Chidambarathanu Pillai S, 1992). Any drug can be subjected to scientific trial after the completion of preclinical experiences. The preclinical experiences comprise chemical standardization and toxicity reviews like acute, sub-acute and chronic reports and for the pharmacological undertaking for the therapeutic use claimed. Vajravallichooranam is alleged for

Osteo-arthritis, positive completion of scientific learn will lead to a clinically confirmed anti-osteo-arthritic herbal drug. Consequently standardization of Vajravallichooranam is undertaken in this gain knowledge of.

## Materials and Methods

### Plants materials:

Well matured fresh stem of C. Quadrangularis has been collected in Chennai and different dry components viz., matured rhizome of Z. Officinale& A. Chinensis, ripen fruits of P. Longum and flower bud of S. Aromaticum were procured from the market and authenticated by way of the Pharmacognosist, Siddha central research Institute, Chennai. The list of the ingredients is presented in table. 1

**Table 1**: **List of ingredients of *VVC***

| Sl. No. | Tamil Name | Botanical Name | Part Used | Quantity | % |
|---|---|---|---|---|---|
| 1. | Pirandai | *C. quadrangularis*Linn. | Stalk | 20g | 66.67 |
| 2. | Chitharathai | *A. chinensis*Willd. | Rhizome | 2g | 6.67 |
| 3. | Kirampu | *S. aromaticum* Linn. | Flower bud | 2g | 6.67 |
| 4. | Chukku | *Z. officinale*Rosc. | Dried rhizome | 2g | 6.67 |
| 5. | Tippili | *P. longum*Linn | Fruit | 4g | 13.33 |

### Purification of the raw materials:

Joints of the well grown stalks of C. Quadrangularis were cut at both ends and jointless stalks are collected.Then they are boiled with 2 pints of sour curd mixed with 4 grams of Saindavalavana, until the entire curd evaporates. Then dried the stem in the shade and powdered as fine powder (Chidambarathanu Pillai S, 1992). The rhizome of Z. Officinale were peeled off dermis; the fruits of P. Longum and rhizome of A. Chinensis had been relatively fried on a pan in low flame (nameless, 1992).

### Preparation of Vajravallichooranam

After purification process, the entire materials were dried, then powdered individually and sieved by white fabric, which is stated as Vasthirakayam in classical Siddha text. The sieved ingredient powders were combined fully to get the Chooranam and stored in a clean and air-tight glass container.

### Powder microscopy

Chooranam, which is passing by way of sieve no. 60 was used for powder microscopy. About 2gm of Chooranam was washed utterly without loss of chooranams, Chloral hydrate, water, phloroglucinol and hydrochloric acid (1:1), Iodine in potassium iodide solution and so on. Were employed as mounting medium. Digital camera lucida used to be used for drawing the salient points of the drug (Wallis TE, 1985).

### Physicochemical/Qualitative analyses

The loss on drying at$105^0$C, total ash content material, water soluble ash content material, alkalinity of the water soluble ash, acid insoluble ash content, water soluble extractives, alcohol soluble extractive, pH and sieve evaluation for particle dimension have been carried as per the approaches described in pharmacopoeial texts (anonymous, 1992 & 1998). Qualitative phytochemical analysis for secondary metabolites had been applied as per the approaches mentioned in general organic books (Harborne JB, 1973; Wagner H and Bladt S, 1996).

### Microbial load

For any internal remedy, the tests for microbial load and designated pathogen are necessary and as a consequence .The tests were carried out as per the WHO ways as advocated through all pharmacopoeias.

**TLC/HPTLC analysis**

**Sample preparation**

4 gram of the drug was soaked in chloroform (100ml) for 18 hours, boiled on a water bath for 10 minutes, filtered, concentrated and made up to 10ml in a standard flask.

**Chemicals and solvents**

Toluene, ethyl acetate, ethyl alcohol, chloroform (Merck), sulphuric acid and vanillin (SDFCL) are of analar grade. For visualization of spots, vanillin-sulphuric acid reagent (1g of vanillin dissolved in ethyl alcohol and sulphuric acid in the ratio 95:5, v/v) was prepared and used as spray reagent.

**TLC plate**

Aluminium sheets precoated with 0.2mm thick silica gel $60F_{254}$ (Merck) as adsorbent was used as TLC plate for the chromatographic analysis.

**Solvent systems**

Many solvent systems with different solvent mixture ratio were attempted in order to achieve better separation of spots. Toluene: Ethyl acetate (6:1.5, *v/v*) was selected as the suitable solvent system.

**Instruments**

For the development of the plate, twin trough chamber (CAMAG) of 10 x 10 sizes was used. Linomat IV TLC applicator, visualizer (CAMAG), TLC scanner 030618 (CAMAG) programmed with WINCATS software were the instruments of TLC photo documentation and HPTLC finger printing.

**Procedure**

Different volumes *viz*., 5, 10 and 15µl of the extract were applied as 8 mm band with 6 mm distance in between and developed up to 8 cm in the above mentioned solvent system. The developed plate was air dried, visualized under UV 254, 366nm for recording the TLC chromatograms; and then scanned in both wavelengths for recording the finger print profiles. The above photo documentation and finger printing was performed at 540 nm after dipping the plate in vanillin-sulphuric acid reagent, followed by heating in an oven till the appearance of color of the spots (Stahl I, 1969; Sethi PD, 1996).

## Results.

**Powder microscopy**

Fragments of tissues showing idoblast containing raphides, fragments of stem epidermal cells in surface view with polyhedral thick walled cells (Pirandai-*Cissus quadrangularis)*; perisperm cells packed with starch grains 3-8µm in diam., and minute crystals of calcium oxalate, oval-elongated spindle shaped stone cells withwide lumen &uniseriate, mulicellular trichomes (Tippili-*Piper longum*); isodiametric idioblasts 40-80µm in diam., containing yellowish-reddish brown oleo-resin, non lignified septate fibres, simple oval-rod shaped starch grains measuring 15-20µm in length, hilum eccentric, lamellae distinct (Chukku-*Zingiber officinale*); pollen grains tetrahedral, spherical, biconvex measuring 15-20µm in diam., fragments of anther walls with characteristic reticulated cells & cluster crystals of calcium oxalate, fragments of parenchyma showing large oval schizolysigenous oil cavities (Ilavangam-Syzygiumaromaticum); starch grains oval-elliptic, 10-20µm in diam., elongated pitted stone cells with a narrow lumen of 50-200µm in length, reddish brown resin cells (Chittarathai-Alpiniaofficinarum).

**Physico-chemical/Microbial load/TLC photo documentation/ HPTLC finger printing**

The results of analytical tests are presented in table 2.

**Table 2: Analytical Results of *VVC***

| S. No | Parameter | Mean (n=2) |
|---|---|---|
| 1. | Loss on drying at 105°C, % | 3.43 |
| 2. | Total ash, % | 11.25 |
| 3. | Water soluble ash, % | 3.45 |
| 4. | Alkalinity (ml of 0.1N HCl/g) | 2.10 |
| 5. | Acid insoluble ash, % | 0.20 |
| 6. | Water soluble extractive, % | 14.35 |
| 7. | Alcohol soluble extractive, % | 10.30 |
| 8. | pH | 6.85 |

The qualitative phytochemical test results and the qualitative inorganic analysis results are enlisted in table 3.

**Table 3: Qualitative Test Results of *VVC***

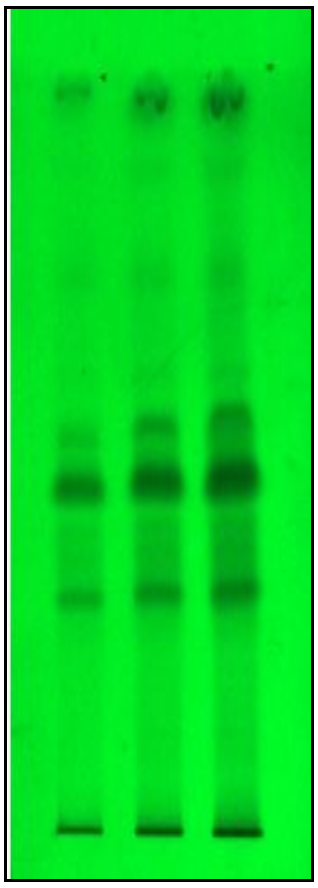
| S. No. | Qualitative Tests | Inference |
|---|---|---|
| 1. | Alkaloids | + ve |
| 2. | Quinones | - ve |
| 3. | Coumarin | + ve |
| 4. | Flavonoid | + ve |
| 5. | Glycoside | + ve |
| 6. | Proteins | + ve |
| 7. | Saponin | - ve |
| 8. | Steroid | + ve |
| 9. | Tannins | + ve |
| 10. | Triterpene | + ve |
| 11. | Volatile oil | + ve |
| 12. | Sodium | + ve |
| 13. | Potassium | + ve |
| 14. | Calcium | + ve |
| 15. | Magnesium | + ve |
| 16. | Lead | - ve |
| 17. | Cadmium | - ve |
| 18. | Arsenic | - ve |
| 19. | Mercury | - ve |

The results of microbial load and specific pathogens are presented in table 4.

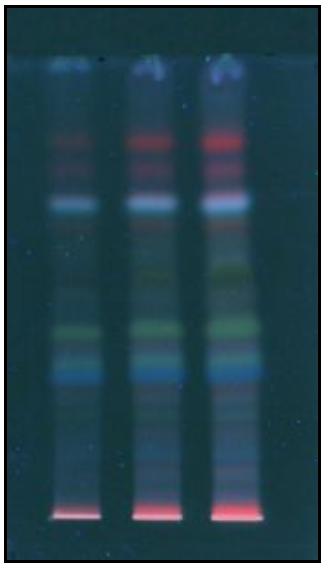
**Table 4:Microbial load and pathogens**

| S. No | Parameter | Value | WHO Limit(CFU/g) |
|---|---|---|---|
| 1. | E. coli | Absent | 10 |
| 2. | Salmonella spp. | Absent | None |
| 3. | Pseudomonoas aeruginosa | Absent | Absent |
| 4. | Staphylococcus aureus | Absent | Absent |
| 5. | Enterobacteriacea | 700 | $10^3$ |
| 6. | Total Bacterial count | 3,80,000 | $10^5$ |
| 7. | Total Fungal count | 2100 | $10^3$ |

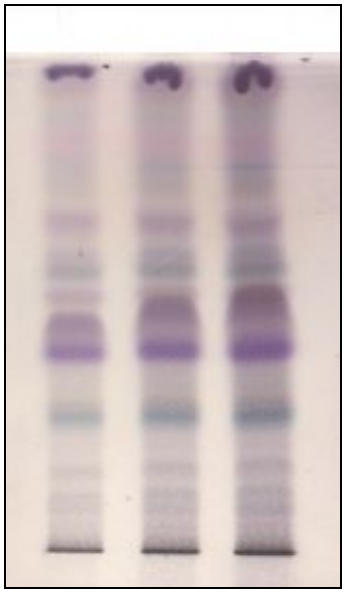
Fig. 1A-C show the TLC photo documentations in different conditions and the table 5 shows the $R_f$ values & colour of spots detected under UV254 nm, 366 nm and after derivatization with vanillin-sulphuric acid reagent.



**[Toluene: Ethyl acetate (6:1.5, v/v)]**
**Fig. 1A: TLC profile of chloroform extract of *VVC* under UV 254 nm in the solvent system**



**[Toluene: Ethyl acetate (6:1.5, v/v)]**
**Fig. 1B: TLC profile of chloroform extract of *VVC* UV 366 nm in the solvent system**



**[Toluene: Ethyl acetate (6:1.5, v/v)]**
**Fig. 1B: TLC profile of chloroform extract of *VVC* After derivatization in the solvent system**

**Table 5: $R_f$ and colour of spots of TLC of chloroform extract of *VVC***

| S. No | Under UV 254 nm | Under UV 366 nm | | After Dipping in Vanillin-Sulphuric acid | |
|---|---|---|---|---|---|
| | $R_f$ value | $R_f$ value | Colour of the spot | $R_f$ value | Colour of the spot |
| 1 | 0.33* | 0.02 | Pink | 0.08 | Purple |
| 2 | 0.47* | 0.11 | Blue | 0.12 | Purple |
| 3 | 0.55* | 0.14 | Blue | 0.18 | Purple |
| 4 | 0.61* | 0.24 | Blue | 0.27 | Blue |
| 5 | 0.75* | 0.26 | Blue | 0.40 | Purple |
| 6 | 0.88* | 0.32 | Blue | 0.49 | Purple |
| 7 | - | 0.35 | Bluish grey | 0.52 | Purple |
| 8 | - | 0.41 | Bluish grey | 0.57 | Blue |
| 9 | - | 0.53 | Green | 0.59 | Blue |
| 10 | - | 0.64 | Pink | 0.66 | Purple |
| 11 | - | 0.67 | Blue | 0.73 | Grey |
| 12 | - | 0.73 | Pink | 0.77 | Blue |
| 13 | - | 0.80 | Pink | 0.82 | Pink |
| 14 | - | - | - | 0.88 | Grey |
| *Green colour | | | | | |

Figs. 2,4 & 6 represent the HPTLC finger print profiles under UV& Visible conditions. The tables 6-8 show the $R_f$ value and their relative percentage peak areas. Figs. 3,5,7 represent the 3D chromatograms of all three tracks before and after derivatization.

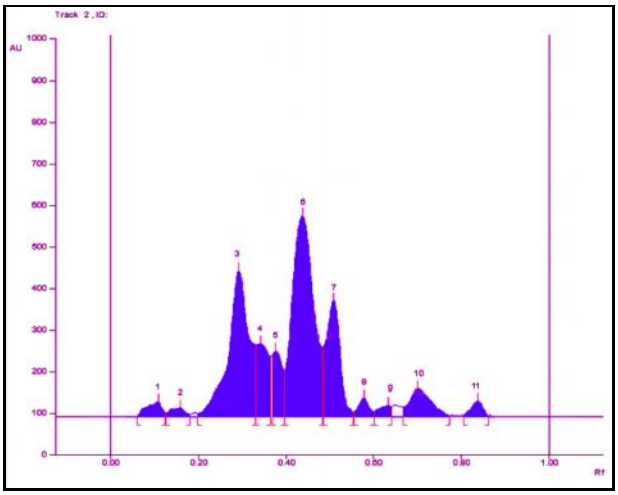


**Fig. 2: HPTLC finger print profile of chloroform extract of *VVC* at UV 254nm**

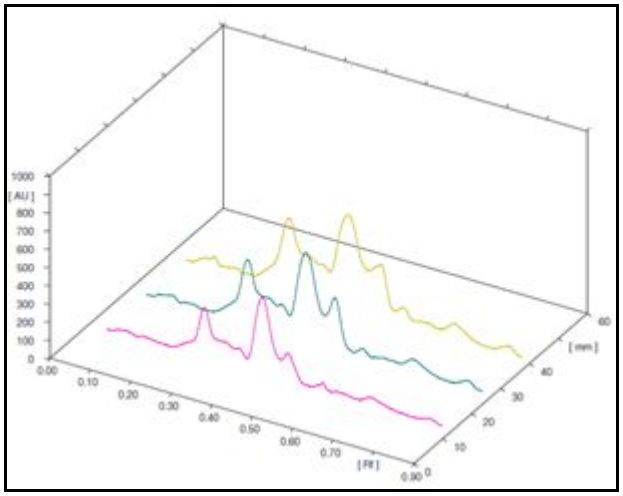


**Fig. 3: HPTLC 3D chromatogram of all tracks of chloroform extract of *VVC* at 254nm**

**Table 6: $R_f$ value and % area of peaks of chloroform extract of *VVC* at 254 nm**

| Peak | Start Rf | Start Helght | Max Rf | Max Helght | Max % | End Rf | End Helght | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.06 | 0.7 | 0.11 | 36.5 | 2.17 | 0.13 | 9.2 | 1160.4 | 1.91 |
| 2 | 0.13 | 9.2 | 0.16 | 22.9 | 1.36 | 0.18 | 6.2 | 679.4 | 1.12 |
| 3 | 0.20 | 7.4 | 0.29 | 350.7 | 20.82 | 0.33 | 172.3 | 15290.4 | 25.11 |
| 4 | 0.33 | 172.5 | 0.34 | 175.4 | 10.41 | 0.37 | 146.5 | 4627.1 | 7.60 |
| 5 | 0.37 | 146.1 | 0.38 | 157.0 | 9.32 | 0.40 | 110.7 | 3395.3 | 5.57 |
| 6 | 0.40 | 111.8 | 0.44 | 482.1 | 28.63 | 0.48 | 166.9 | 21836.7 | 35.85 |
| 7 | 0.49 | 167.3 | 0.51 | 280.5 | 16.66 | 0.55 | 10.1 | 8190.6 | 13.45 |
| 8 | 0.56 | 10.6 | 0.58 | 46.2 | 2.74 | 0.60 | 10.9 | 1010.8 | 1.66 |
| 9 | 0.60 | 11.3 | 0.63 | 28.3 | 1.68 | 0.64 | 24.3 | 729.3 | 1.20 |
| 10 | 0.67 | 22.9 | 0.70 | 66.5 | 3.95 | 0.77 | 2.2 | 3024.0 | 4.97 |
| 11 | 0.81 | 4.1 | 0.84 | 38.1 | 2.26 | 0.86 | 2.0 | 960.1 | 1.58 |

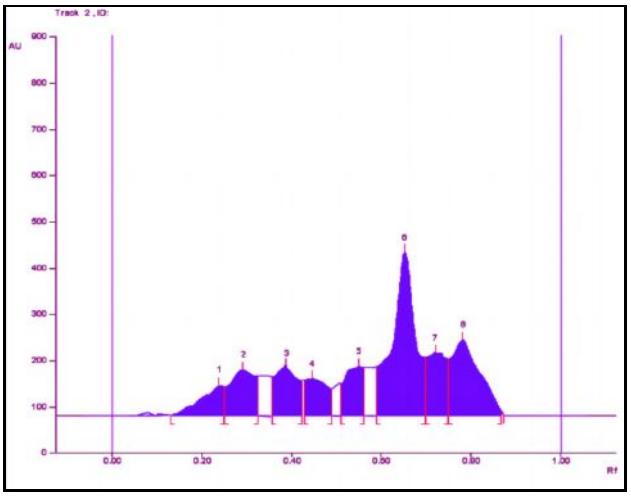


**Fig. 4: HPTLC finger print profile of chloroform extract of *VVC* at UV 366nm**

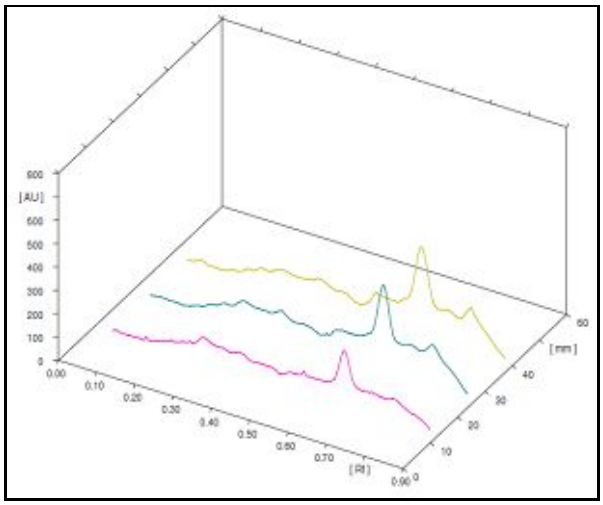


**Fig. 5: HPTLC 3D chromatogram of all tracks of chloroform extract of *VVC* at UV 366nm**

In the HPTLC finger print profiling at UV 366 nm, eight peaks were observed in which the peaks at Rf 0.65 (31.79%), 0.78 (18.40%), 0.72 (10.27%), 0.29 (9.81%) and 0.39 (9.29%) were the major peaks and the area of other peaks at Rf were 0.24 (5.90%), 0.45 (6.85%) and 0.55 (7.69%).

In the HPTLC finger print profiling at 540nm, twelve peaks were observed in which the peaks at Rf 0.47 (26.80%), 0.39 (26.37%), 0.26 (14.19%) and 0.55 (10.26 %) were the major peaks and area of other peaks were found to be ranging from 0.99 % to 7.89%.

**Table 8: $R_f$ value and % area of peaks of chloroform extract of *VVC* at 540 nm**

| Peak | Start Rf | Start Helght | Max Rf | Max Helght | Max % | End Rf | End Helght | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.06 | 0.9 | 0.07 | 32.0 | 2.57 | 0.09 | 24.8 | 559.6 | 0.99 |
| 2 | 0.09 | 24.9 | 0.10 | 49.1 | 3.95 | 0.12 | 0.2 | 873.3 | 1.55 |
| 3 | 0.13 | 0.3 | 0.16 | 57.6 | 4.63 | 0.18 | 0.2 | 1199.4 | 2.13 |
| 4 | 0.18 | 0.2 | 0.20 | 12.6 | 1.01 | 0.20 | 10.9 | 162.5 | 0.29 |
| 5 | 0.21 | 11.0 | 0.26 | 166.2 | 13.34 | 0.31 | 64.3 | 7986.9 | 14.19 |
| 6 | 0.33 | 57.3 | 0.39 | 289.6 | 23.25 | 0.43 | 180.1 | 14838.3 | 26.37 |
| 7 | 0.43 | 180.3 | 0.47 | 242.6 | 19.47 | 0.53 | 75.8 | 15079.6 | 26.80 |
| 8 | 0.53 | 76.5 | 0.55 | 129.2 | 10.37 | 0.61 | 49.0 | 5773.9 | 10.26 |
| 9 | 0.61 | 49.2 | 0.64 | 111.3 | 8.98 | 0.69 | 14.2 | 4422.9 | 7.86 |
| 10 | 0.69 | 13.9 | 0.72 | 42.4 | 3.41 | 0.74 | 37.1 | 1357.2 | 2.41 |
| 11 | 0.74 | 37.3 | 0.75 | 49.3 | 3.96 | 0.76 | 47.8 | 777.0 | 1.38 |
| 12 | 0.76 | 48.1 | 0.80 | 63.1 | 5.06 | 0.86 | 1.8 | 3241.4 | 5.76 |

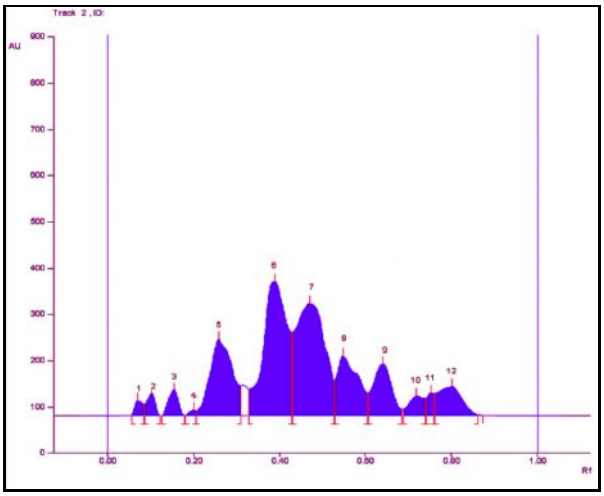


**Fig. 6: HPTLC finger print profile of chloroform extract of *VVC* at 540nm**

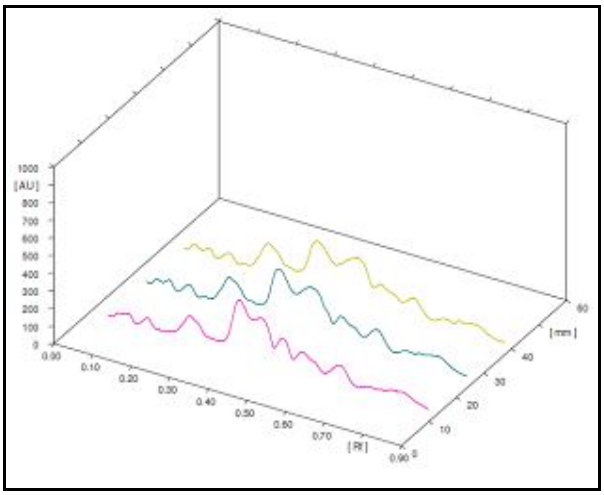


**Fig. 7: HPTLC 3D chromatogram of all tracks of chloroform extract of *VVC* at 540nm**

The HPTLC 3D chromatograms at UV254/366 nm and at 540 nm showed the proportionate increase in the peak areas of all tracks.

## Discussion

The powder microscopic characters showed the presence of all the ingredients. The loss on drying at $105^0$C was calculated as 3.43%, which makes the drug less susceptible to microbial contamination. The total ash of 11.25% indicates the inorganic content present in the drug among which 3.45% are water-soluble and 0.2% is acid insoluble in nature. The extractive value in water is higher than that of alcohol, which may be due to the water-soluble inorganic content present in the drug. The pH value of 6.85 represents that the drug is slightly acidic in nature. In the preliminary phytochemical test, quinone and saponin are found to be absent and

other such as alkaloid, coumarin, flavonoid, glycoside, protein, steroid, tannins, triterpene and volatile oil are present. Similarly in the qualitative inorganic analysis, the cations such as sodium, potassium, calcium, magnesium are found to be present and heavy metals viz., lead, cadmium, arsenic and mercury were found to be absent. Even though the pH of the drug was near to neutral, the test for microbial load and specific pathogens were found to be within the acceptable limits of WHO.

The TLC photo documentation of chloroform extract at UV 254 nm showed six visible spots at Rf values 0.33, 0.47 (major), 0.57, 0.61, 0.75 and 0.88 (all green); at UV 366 nm showed thirteen spots at Rf value 0.02 (pink), 0.11 (blue), 0.14 (blue), 0.24 (blue), 0.26 (blue), 0.32 (blue), 0.35 (bluish grey), 0.41 (bluish grey), 0.53 (green), 0.64 (pink), 0.67 (blue), 0.73 (pink), 0.80 (pink); at 540 nm after derivatization with vanillin-sulphuric acid showed fourteen spots at R¬f values 0.08 (purple), 0.12 (purple), 0.18 (purple), 0.27 (blue), 0.40 (purple), 0.49 (purple),0.52 (purple), 0.59 (Blue), 0.66 (purple), 0.73 (grey), 0.66 (purple), 0.73 (grey), 0.77 (blue), 0.82 (pink) and 0.88 (grey). The spot at $R_f$ is commonly seen in both shorter wavelength and in white light. Similarly the spot at $R_f$ value 0.73 is present in longer wavelength and in white light. All other spots are different and or different nature. Further, merging of spots was observed in the 10µl and 15µl tracks after derivatization and 5µl is sufficient to make better separation

In the HPTLC finger-print profiling at UV 254 nm, elevan peaks were observed in which the peaks at $R_f$ 0.44 (35.85%), 0.29 (25.11%), 0.51 (13.45%) and 0.34 (7.6%) were the major peaks and area of other peaks varied from1.20 % to 5.57 %.

## Conclusion

The generated data from powder microscopy, physico-chemical, preliminary phytochemical, TLC photo documentation and HPTLC finger printing will serve as an reference tool for analyzing and controlling the quality of the drug. Also, it could be considered as pharmacopoeial standard of Vajravalli chooranam.

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