

## QUALITY CONTROL STUDIES ON CHANDRAPRABHAVATI

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## ABSTRACT

The present study deals with a comparative quality control studies carried out on two samples of *Chandraprabha vati*. One obtained from market and another in house preparation prepared as per Vaidya Yoga Ratnavali, IMPCOPS, Chennai. In the present work parameters like macroscopic and microscopic standards, along with HPTLC and GC-MS profile were studied and discussed. Besides other parameter such as Hardness, Uniformity of weight, Disintegration time, physicochemical constants, heavy metal content and microbial load were also determined. The data obtained could be useful in deciding the standards for this potential ayurvedic drug and can contribute in the preparation of quality Ayurvedic drugs.

**Keywords:** *Chandraprabha vati*, Analytical specification, Market sample and In-house preparation.

## INTRODUCTION

Ayurveda and Siddha medicines can be explored with the modern scientific tools for better leads in the health care [1]. Since Ayurveda is really the generic term for "traditional medicine" in India, actual practice may be widely divergent. The quality assessment of herbal formulations is of paramount importance in order to justify their acceptability in modern system of medicine [2]. *Chandraprabha vati* is a tablet, used in Ayurvedic treatment to treat cold, cough, diabetes, cancer, diseases of urinary track, rhinitis, bronchitis, asthma and such other respiratory diseases, allergic skin conditions, piles, liver, spleen diseases, anaemia and fistula. It is useful in dental problems like caries, in eye infections and also useful in treating semen defects and gynaecological problems [3].

## MATERIALS AND METHODS

The samples were procured from market and another in house preparation prepared as per Vaidya Yoga Ratnavali, IMPCOPS, Chennai. Both the samples were compared from quality control point of view.

## Physicochemical standardization

Physico-chemical constants like total ash, water soluble ash, acid insoluble ash, water and alcohol soluble extractives, loss on drying at 105°C, heavy metals contents and microbial load were determined as per the WHO guide lines [4].

## Microscopic characterization

For the microscopic examination, a pinch of powdered sample was warmed with chloral hydrate, cooled, washed and mounted in glycerine; A few mg of powder was washed with plain water, treated with iodine and potassium iodide, drop of glycerine was added and mounted; a pinch of sample was taken and stained with phloroglucinol and HCl [5].

## Determination of Microbial Load

The microbial loads were determined as per Ayurvedic pharmacopoeia [6].

## GC-MS analysis

GC-MS analysis of hexane extract was done on a GC clarus 500 Perkin Elmer system interfaced to mass spectrometer (GC-MS) instrument employing standard protocols [7].

## HPTLC analysis

## Sample preparation

*Chandraprabha vati* Market sample (Vati I): About 0.1 gm of chloroform extract of sample was dissolved in 3ml of chloroform. *Chandraprabha vati* In- house Preparation (Vati II): About 50 mg of chloroform extract of in house preparation was dissolved in 3ml of chloroform.

## Instrumentation and chromatographic conditions

HPTLC aluminium plates pre-coated with silica gel 60 F<sub>254</sub> (10 × 10 cm) with 0.2mm thickness (E. Merck, Germany) were used as the stationary phase. The Chloroform extracts of market and in-house preparation of *Chandraprabha vati* were spotted on a 10x10cm Silica gel 60 F<sub>254</sub> precoated plate (E. merck) of thickness 0.2mm and 7mm wide band using automatic TLC applicator Linomat 5. The composition of the mobile phase was Toluene: Ethyl acetate: Diethylamine (70:20:10). The optimized chamber saturation time for the mobile phase was 30 minutes at room temperature. Developed the plate in the solvent system in twin trough chamber to a distance of 8cm. The plate was allowed to dry at room temperature. Then it was scanned densitometrically at 254nm. The images were captured on CAMAG REPROSTAR 3 with win-CATS software [8].

## RESULTS AND DISCUSSION

Table 1: Ingredients of *Chandraprabha vati*

| S. No. | Name         | Botanical Name                      | Anatomical part | Quantity (gm) |
|--------|--------------|-------------------------------------|-----------------|---------------|
| 1.     | Bakuchi      | <i>Psoralea corylifolia</i> Linn.   | seeds           | 3             |
| 2.     | Vaca         | <i>Acorus calamus</i> L.            | Rhizome         | 3             |
| 3.     | Musta        | <i>Cyperus rotundus</i> Linn.       | Rhizome         | 3             |
| 4.     | Kiratatikta  | <i>Swertia chirata</i> Buch.-Ham    | Pl              | 3             |
| 5.     | Devadaru     | <i>Cedrus deodara</i> (Roxb.) Loud. | Heart wood      | 3             |
| 6.     | Haridra      | <i>Curcuma longa</i> Linn.          | Rhizome         | 3             |
| 7.     | Daru haridra | <i>Berberis aristata</i> Dc.        | Stem            | 3             |
| 8.     | Ativisa      | <i>Aconitum heterophyllum</i> Wall. | Root            | 3             |
| 9.     | Pippali mula | <i>Piper longum</i> L.              | Root            | 3             |
| 10.    | Citraka      | <i>Plumbago zeylanica</i> L.        | Root            | 3             |

|     |                      |  |           |    |
|-----|----------------------|--|-----------|----|
| 11. | Trivrt               | <i>Operculina turpethum</i> (Linn.)      | Root      | 12 |
| 12. | Danti                | <i>Bliospermum montanu</i> Muell-Arg.    | Root      | 12 |
| 13. | Patraka              | <i>Cinnamomum tamala</i> Nees & Eberm.   | Leaf      | 12 |
| 14. | Tvak                 | <i>Cinnamomum zeylanicum</i> Blume       | Stem bark | 12 |
| 15. | Suksmaila            | <i>Elettaria cardamomum</i> (Linn.)Maton | Seed      | 12 |
| 16. | Vamsa lochana        | <i>Bambusa bambos</i> Druce              | S.C       | 12 |
| 17. | Dhanyaka             | <i>Coriandrum sativum</i> Linn.          | Fruit     | 3  |
| 18. | Bibhitaka            | <i>Terminalia belerica</i> Roxb.         | Pulp      | 3  |
| 19. | Haritaki             | <i>Terminalia chebula</i> Retz.          | Pulp      | 3  |
| 20. | Amalaki              | <i>Emblica officinalis</i> Gaertn.       | Pulp      | 3  |
| 21. | Cavya                | <i>Piper retrofractum</i> Vahl.          | Stem      | 3  |
| 22. | Vidanga              | <i>Embelia ribes</i> Burm. f.            | Fruit     | 3  |
| 23. | Gajapippali          | <i>Scindapsus officinalis</i> Schoott.   | Fruit     | 3  |
| 24. | Sunthi               | <i>Zingiber officinale</i> Roxb.         | Rhizome   | 3  |
| 25. | Marica               | <i>Piper nigrum</i> Linn.                | Fruit     | 3  |
| 26. | Pippali              | <i>Piper longum</i> L.                   | Fruit     | 3  |
| 27. | Yava ksara           | Extract of <i>Hordeum vulgare</i> Linn./ | Pl        | 3  |
| 28. | Sarji ksara          | Impure sodium carbonate                  | -         | 3  |
| 29. | Saindhava lavana     | Rock salt                                | -         | 3  |
| 30. | Sauvareala lavana    | Black salt                               | -         | 3  |
| 31. | Vida lavana          | Vit salt or Sanchal Salt                 | -         | 3  |
| 32. | Maksika dhatu bhasma | Copper pyrite bhasma                     | -         | 3  |
| 33. | Loha bhasma          | Iron bhasma                              | -         | 6  |
| 34. | Sitajatu             | Asphaltum                                | -         | 24 |
| 35. | Guggulu              | <i>Commiphora wightii</i> (Arn.) Bhand.  | Exudate   | 24 |
| 36. | Sita                 | Sugar                                    | -         | 12 |
| 37. | Madhu                | Honey                                    | -         | QS |

The macroscopic characters of the *Chandraprabha vati* were studied and following features were observed and recorded (Table 2). Starch grains, prismatic crystals, sclereids and Tannin containing cells were present in both market and in In-house prepared samples.

**Table 2: Organoleptic standards of Chandraprabha vati samples**

| S. No. | Parameters  | Market sample | In-house preparation |
|--------|-------------|---------------|----------------------|
| 1.     | Colour      | Puff          | Brown                |
| 2.     | Odour       | Pleasant      | Pleasant             |
| 3.     | Consistency | Solid         | Solid                |

The physico chemical data obtained were presented in the following table

**Table 3: Physicochemical data of Chandraprabha vati Samples**

| S. No. | Parameters                      | Market sample | In-house preparation |
|--------|---------------------------------|---------------|----------------------|
| 1.     | Loss on drying at 105°C (%)     | 6.94          | 12.02                |
| 2.     | Hardness (Kg/cm <sup>2</sup> )  | 2             | 5                    |
| 3.     | Uniformity of weight            | 96-104%       | 94-107%              |
| 4.     | Disintegration time (minutes)   | 20            | more than 20         |
| 5.     | Total ash (%)                   | 11.77         | 19.25                |
| 6.     | Acid-insoluble ash              | 3.88          | 4.02                 |
| 7.     | Alcohol-soluble extractives (%) | 18.43         | 24.73                |
| 8.     | Water-soluble extractives (%)   | 59.86         | 45.40                |

Data of Heavy metal analyses of *chandraprabha vati* were tabulated in table 4.

**Table 4: Heavy metal analysis of chandraprabha vati**

| Sample Code          | Hg (%) | Pb (ppm) | Cd (ppm) |
|----------------------|--------|----------|----------|
| Marketed sample      | 0.33   | 69.31    | BDL      |
| In-house preparation | 0.41   | BDL      | BDL      |

\*BDL – Below Detectable Limits

Microbial load determined for both the samples were presented Table 5.

**Table 5: Total Microbial Load in Chandraprabha vati samples**

| S. No. | Tested Microbial           | Market sample       | In-house preparation | WHO Limits             | Inference     |
|--------|----------------------------|---------------------|----------------------|------------------------|---------------|
| 1.     | Total viable aerobic count | 1.4X10 <sup>6</sup> | 1.8X10 <sup>6</sup>  | <10 <sup>7</sup> CFU/g | Within Limits |
| 2.     | Total fungal count         | 2X10 <sup>2</sup>   | 3X10 <sup>2</sup>    | <10 <sup>4</sup> CFU/g | Within Limits |
| 3.     | Total enterobacteriaceae   | Nil                 | Nil                  | <10 <sup>4</sup> CFU/g | Within Limits |
| 4.     | <i>E.coli</i>              | Nil                 | Nil                  | <10 <sup>3</sup> CFU/g | Within Limits |
| 5.     | <i>Salmonella</i>          | Absent              | Absent               | Absent/10g             | Absent        |

Fig 1 and 2 shows GCMS chromatogram of market sample and In-house prepared sample.

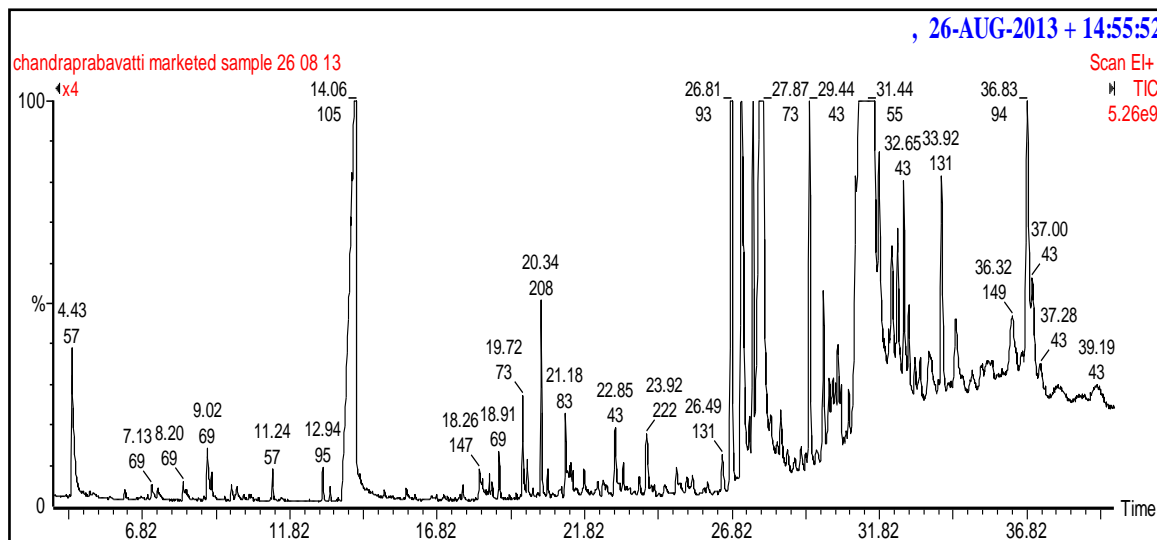


Fig. 1: GC-MS chromatogram of market sample of *Chandraprbha-vati*

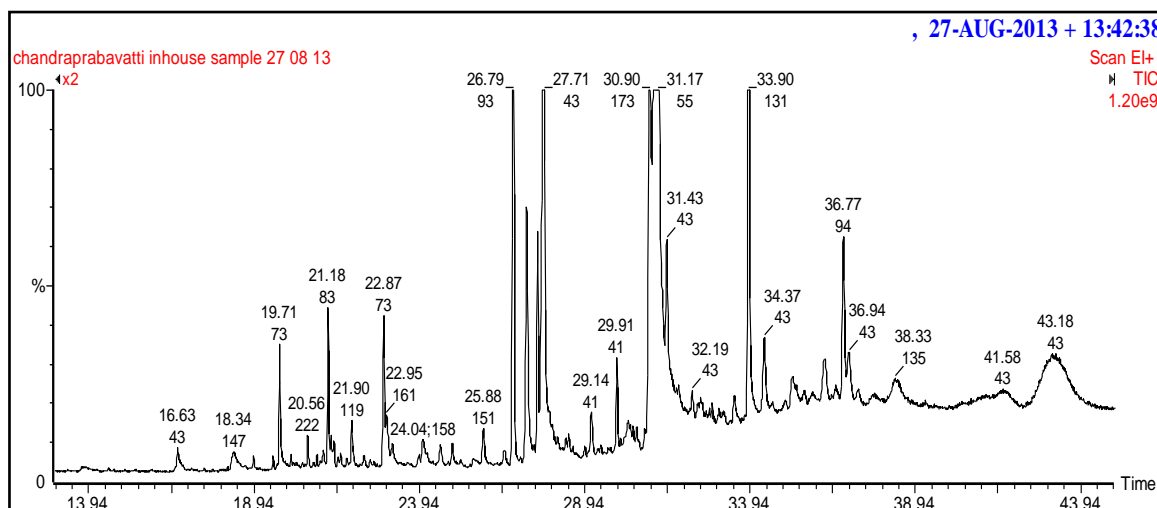


Fig.2 GC-MS chromatogram of In-house prepared sample *Chandraprbha-vati*

The data of the results obtained on chemical constituents identified were presented in Table 6.

Table 6: GC MS Data of *Chandraprabha vati*

| S. No. | Peak Name  | Retention time | % peak area of marked sample | % Peak area of In house preparation |
|--------|--|----------------|------------------------------|-------------------------------------|
| 1.     | n-Decanoic acid; C10H20O2  | 16.63          | 0.0268                       | 0.8237                              |
| 2.     | 1,6,10-dodecatrien-3-ol,3,7,11-trimethyl-  | 19.51          | 0.0157                       | 0.1322                              |
| 3.     | Apiol; C12H14O4  | 20.56          | 0.1504                       | 0.4389                              |
| 4.     | Ar-tumerone; C15H20O   | 21.18          | 0.4183                       | 1.5044                              |
| 5.     | Ethanone, 1-[2-(5-hydroxy-1,1-dimethylhexyl)-3-methyl-2-cyclopropen-1-yl]-; C14H24O2 | 22.44          | 0.1396                       | 0.1051                              |
| 6.     | Tetradecanoic acid; C14H28O2   | 22.87          | 0.6112                       | 1.4875                              |
| 7.     | 2,2,7,9-Tetramethyl-3-oxatricyclo [6.3.1.0(4,9)]dodecane; C15H26O                    | 23.92          | 0.6832                       | 0.888                               |
| 8.     | Thunbergene; C20H32  | 26.81          | 7.3491                       | 11.1396                             |
| 9.     | Aromadendrene; C15H24  | 27.14          | 5.8545                       | 5.3799                              |
| 10.    | á-Elemene; C15H24  | 27.53          | 3.2170                       | 2.1398                              |
| 11.    | Phenol, 2-methyl-5-(1,2,2-trimethylcyclopentyl)-, (S)- C15H22O                       | 33.92          | 2.0341                       | 8.5817                              |
| 12.    | 9,12,15-Octadecatienoic acid, ethyl ester, (Z,Z,Z)-; C20H34O2                        | 36.83          | 3.7676                       | 2.9325                              |

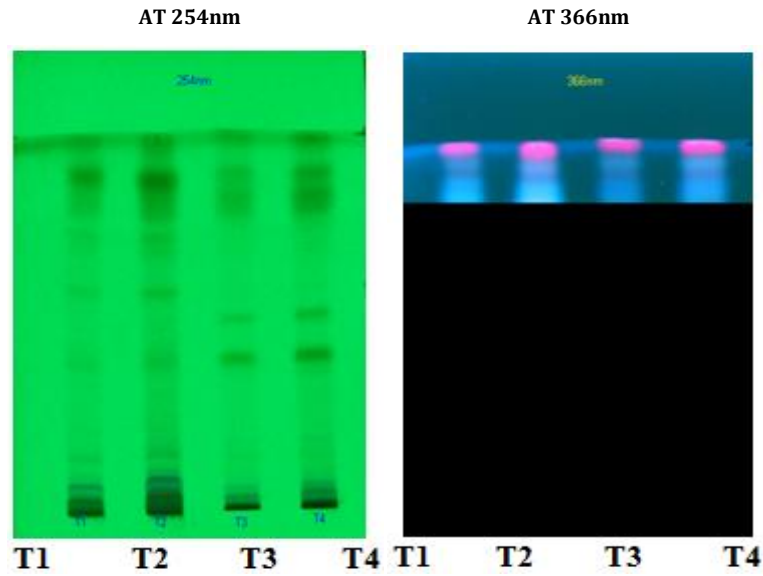
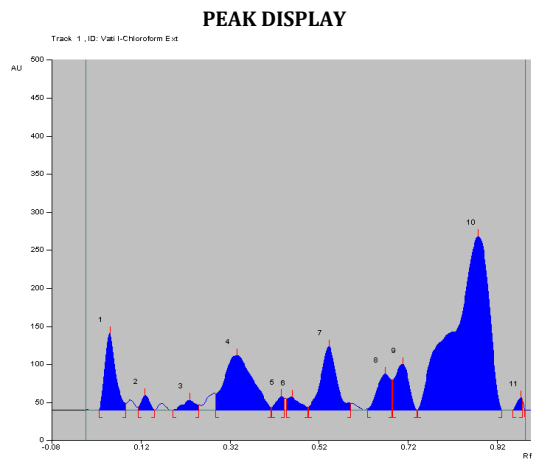


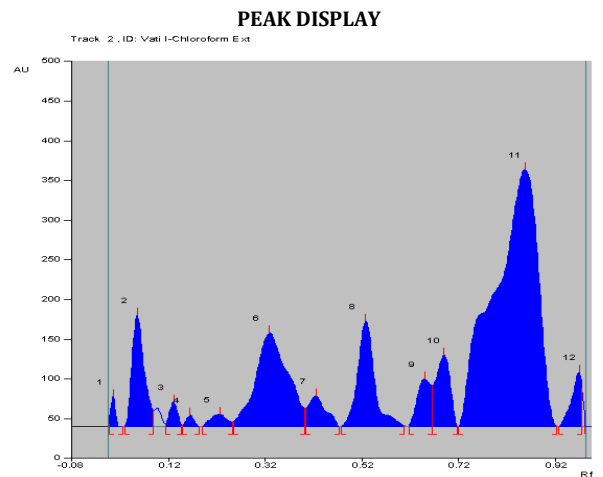
Fig. 3 HPTLC fingerprinting profiles of *Chandraprabha vati* Samples

**TLC DETAILS**

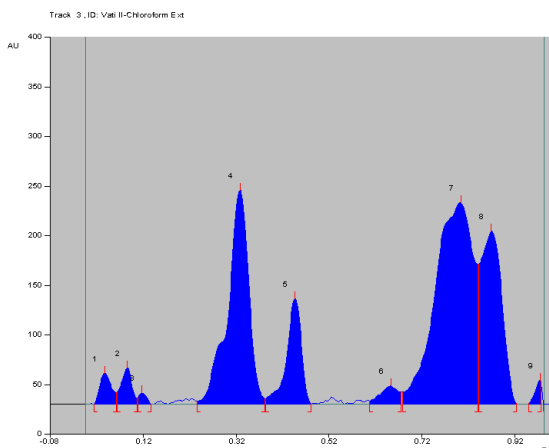
- Track 1-5µl of Sample (chloroform Extract)
- Track 2-10µl of Sample (chloroform Extract)
- Track 3-10µl of Sample (chloroform Extract)
- Track 4-10µl of Sample (chloroform Extract)



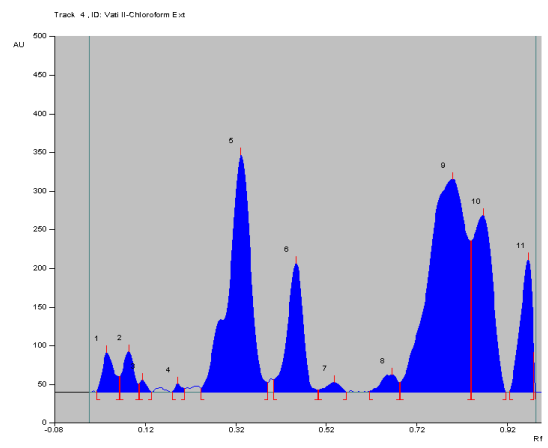
(5µl of Sample chloroform Extract)  
**PEAK DISPLAY**



(10µl of Sample chloroform (Extract))  
**PEAK DISPLAY**



(5µl of Sample chloroform Extract)  
**PEAK DISPLAY**



(10µl of Sample chloroform Extract)  
**PEAK DISPLAY**

Physico-chemical parameters were determined for both the samples of *Chandraprabha-vati*. In-house preparation had more moisture content, hardness, ash and extractive values depicting purity and strength of the preparation (Table 3).

The heavy metal contents were analyzed for both the samples and they were below detectable limits (Table 4). The microbial load determined that the presence of microorganism were within limits as per WHO. Preparations were free from *E. Coli* and *Salmonella spp.* (Table 5).

Through GC-MS analysis, forty three fragmented compounds were presented in in-house sample whereas thirty two compounds were present in In-house prepared sample which were presented in Table 6 chromatograms were given in Figure 1 & 2. GC-MS data revealed that there were 12 types of similar fragmented chemical constituents in both the samples (Table 5). Out of these 12 compounds, Thunbergene a terpene was found to be present in higher concentration. Phenolic compounds were also found to be high in In-house prepared sample (8.5817%). In-house prepared sample shows high area percentage than marketed sample for most of the compounds.

HPTLC finger print profile of the *Chandraprabha-vati* formulations were done for alkaloids which indicated the uniformity in the preparation of in-house and marketed sample formulation. HPTLC finger print profile of chloroform extract of both samples, after scanning at 254 nm showed ten major spots with  $R_f$  values at 0.14, 0.17, 0.23, 0.33, 0.43, 0.53, 0.65, 0.69, 0.86, 0.97. All the bands were blue in colour and 0.97 with pink in colour. In-house prepared sample shows nine major spots at  $R_f$  values 0.12, 0.20, 0.34, 0.46, 0.54, 0.67, 0.80, 0.87, 0.97. The colour of the bands was same as for market sample which shows similarity of ingredients present in the samples. Peak display for the marketed and In-house prepared

sample of *Chandraprabha vati* at different concentration was given in the Fig.3.

## CONCLUSION

To conclude the quality control parameters such as organoleptic standards, physicochemical parameters, microbial load limits, heavy metal contamination along with GCMS and HPTLC profiles determined in the present work can contribute in deciding the quality, purity and strength of *Chandraprabha vati*, a known Ayurvedic formulation which is often prescribed for urinary tract infections, liver and kidney disorders.

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