

**VITAMIN D PRECURSOR IN *WRIGHTIA TINCTORIA* AND SCIENCE OF DR. JRK'S 777 OIL****Aruna V\*, Soundharya R, KalpanaT, Amruthavalli GV, Gayathri R**

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**Address for Correspondence:** Aruna V, Department of Research and Development, Dr. JRK's Research and Pharmaceuticals, Chennai**Disclosure statement:** *The authors have no conflicts of interest.***Abstract:**

The present paper establishes the reservoir status of *Wrightia tinctoria* of Vitamin D precursor essentially Sitosterol, Campesterol, Stigmasterol and thereby Dr. JRK's 777 oil formulated with *Wrightia tinctoria* at 50:50w/v on application over skin and exposure to sun induces Vitamin D synthesis in the skin which is essential for treating Psoriasis. Pre-vitaminized with Vitamin D preparation could not offer superior therapeutic value equal to Dr. JRK's 777 oil. Further most of the preparations containing *Wrightia tinctoria* in the market also could not equal the performance of Dr. JRK's 777 oil. Details are presented in the paper.

**Keywords:** Psoriasis treatment, Vitamin D precursor, *Wrightia tinctoria*, Stigmasterol, Campesterol**Introduction:**

Herbal preparations have been in use for ages in the treatment of Psoriasis and with the discovery of *Wrightia tinctoria* for treating Psoriasis by Dr. JR Krishnamoorthy in mid-80's, along with Central council for research in Siddha and Ayurveda, Govt of India, *Wrightia tinctoria* has gained global recognition and attention.<sup>1, 2, 3</sup> Subsequent research studies on *Wrightia tinctoria* however have brought only partial and or inconclusive therapeutic efficacy of *Wrightia tinctoria* based preparations for psoriasis.<sup>4, 5</sup> The rampant commercial exploitation of *Wrightia tinctoria* has taken place in the market but unfortunately no in-depth research studies have been made by most of the commercial beneficiaries of *Wrightia tinctoria* except Dr. JRKs Research & Pharmaceuticals Pvt. Ltd.<sup>6, 7, 8, 9, 10, 11</sup>

One of the proven active constituents of *Wrightia tinctoria* is the precursor of Vitamin D, i.e., Phytosterols, especially beta- sitosterol, Stigmasterol and Campesterol which are abundantly present in *Wrightia tinctoria*.<sup>12</sup> Although *Wrightia tinctoria* is the rich source of the above precursor molecules of Vitamin D but they are either unstable,<sup>13</sup> or may rapidly degrade or not fully extractable during the manufacturing of any such preparations with

*Wrightia tinctoria*. During manufacturing of *Wrightia tinctoria* based preparations, the phytosterols in the plant needs to be catalyzed appropriately to convert them to the next stage for the synthesis of Vitamin D. Otherwise the preparations may not exhibit superior therapeutic efficacy for psoriasis due to the lack or absence of vitamin D.

In the present paper, we have studied various micro and macro aspects that can interfere and or countermand the functioning of Vitamin D synthesis from *Wrightia tinctoria* during manufacturing of *Wrightia tinctoria* based preparations for Psoriasis. Further we have also evaluated the presence of Vitamin D moiety in several commercially available *Wrightia tinctoria* based preparations for Psoriasis that are licensed and marketed in India under Siddha or Ayurveda products.

We have also evaluated the instant effect of Vitamin D on normal skin during sun exposure and the precursor of Vitamin D rich *Wrightia tinctoria* based preparation of the company- Dr. JRK's 777 oil.

We in the present paper wish to syndicate the popular acceptance and rating of Dr. JRK's 777 oil by medical practitioners in India who may have possibly compared the efficacy of Dr. JRK's 777 oil over

other preparations in the market and then rated the superiority of Dr. JRK's 777 oil.

We have adopted stringent quality norms starting from raw material procurement, raw material quality check, raw material therapeutic value check, strict compliance of science during manufacturing, in-process check, finished product check and final approval. Alongside the presence of phytosterols were also tested qualitatively in the finished product. Details are presented in the paper.

### Materials and Methods

#### Preparation of *Wrightia tinctoria* extract

Ethanol extract of *Wrightia tinctoria* leaf was prepared at the ratio of 1:10 solid to solute, weight per volume. Filter the extract after 24 hours and evaporate the filtrate to obtain dry extract.

#### Evaluation of key marker compounds in *Wrightia tinctoria*- Thin Layer Chromatography

TLC was performed as per standard procedure. The mobile phase used was Petroleum ether:ethylacetate (8:1) ratio. The RF value was calculated using standard formula.

#### Confirmation of Sterols from TLC plate- UV Spectrophotometer

The sterol band was scraped of carefully from TLC plate and then the compound was re-dissolved in the solvent; the silica residue was separated and then tested by Spectrophotometer. A prominent peak value was obtained between 200 to 400nm showing the presence of Sterols.

### Dermatological effect of sterol and Vitamin D

5 healthy volunteers were used for the present study. Vitamin D 2µg/ml and the sterols extracted from *Wrightia tinctoria* 4µg/ml were prepared separately in coconut oil. The above samples were applied separately in the volar forearm region of the volunteers keeping the concentration as 2mg/cm<sup>2</sup>. After 5 minutes, the samples applied sites were exposed to sun for 5 minutes and then the dermal response were ascertained visually.

### Result

#### Evaluation of key marker compounds in *Wrightia tinctoria*

Ethanol extract of *Wrightia tinctoria* leaf was prepared at the ratio of 1:10 solid to solute, weight per volume. 5 samples of *Wrightia tinctoria* leaf sourced from different parts of India were tested for the above.

The *Wrightia tinctoria* leaf used by company showed the rich presence of Indigotin, Indirubin and sterol (Plate 1 to 3). Indigotin showed blue colored band whereas Indirubin appeared in pink colour. Unstained TLC doesn't show the presence of sterol, however on staining with anisaldehyde sulphuric acid, showed the presence of sterols in purple color band.

The *Wrightia tinctoria* sourced from different sources (never used by the company) did not show the presence of the marker compounds such as Indigotin and sterols significantly. Plate (4 to 6)

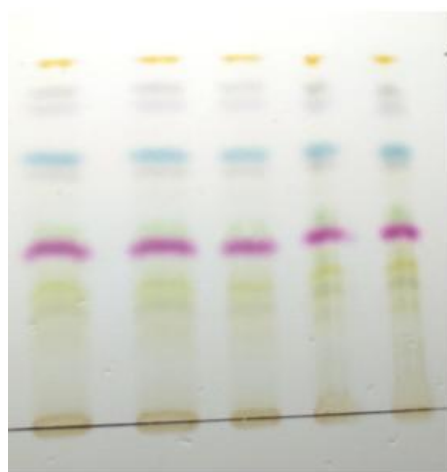


Plate-1 Visible light

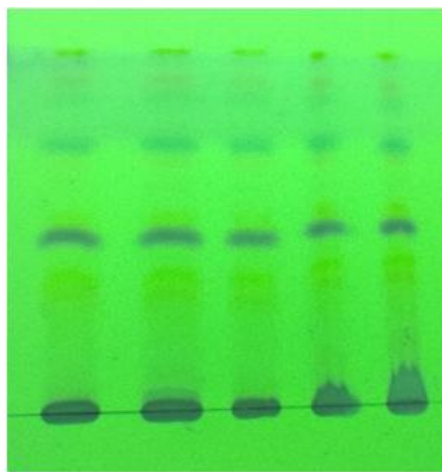


Plate- 2 – UV Visible

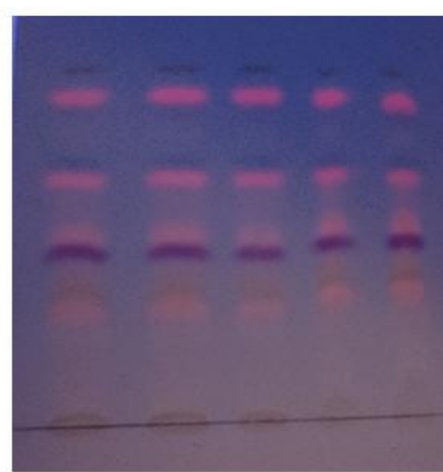
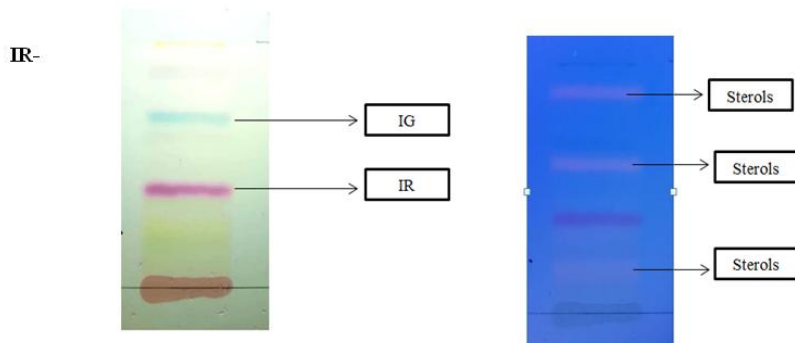
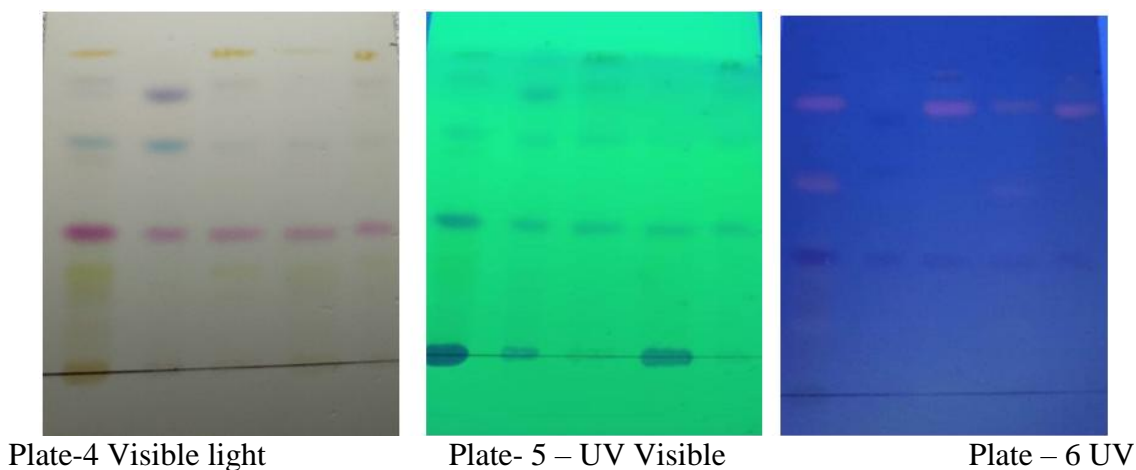


Plate – 3 UV

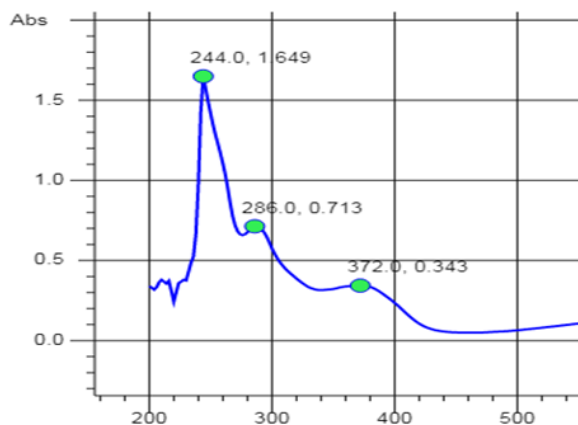


**Indirubin, IG- Indigotin, Sterols- shows fluorescence under UV light**

**Confirmation of Sterols from TLC plate**

The sterol band was scraped of carefully from TLC plate and then the compound was re-dissolved in the solvent; the silica residue was separated and then tested by Spectrophotometer. A prominent peak value

was obtained between 200 to 400nm showing the presence of beta-Sitosterol, campesterol and stigmasterol suggesting that the *Wrightia tinctoria* used by the company contains the sterols the precursor of Vitamin D. Graph-1



**Dermatological effect of sterol and Vitamin D**

The sterol applied site showed appreciable melanogenesis and erythema formation than the Vitamin D suggesting the role of plant sterol in the

biogenesis of Vitamin D in skin during sun exposure, whereas, the Vitamin D may have relatively poor absorption through skin and hence the response seen was meagre.

Volunteer	Sterols extracted from <i>Wrightia tinctoria</i>		Vitamin D	
	Melanin	Erythema	Melanin	Erythema
1	Strong	Strong	Weak	Poor
2	Strong	Moderate	Strong	Moderate
3	Strong	Moderate	Moderate	Weak
4	Moderate	Strong	Weak	Weak
5	Strong	Strong	Poor	Weak

Strong = Visible change; Moderate = Change requires hand lens to observe; Weak = requires hand lens + illuminated light to observe

#### Possible factors that may adversely interfere in the functioning of sterols in *Wrightia tinctoria*

In rancid oil, the phytosterol of *Wrightia tinctoria* was found to be unstable and it disappeared in 14 days as we could not establish its presence of the same either by TLC or by spectrophotometer.

#### Role of temperature

Heating of *Wrightia tinctoria* leaves in oil is found to cause denaturation of sterols as the heat treated leaf did not yield the extraction sterol.

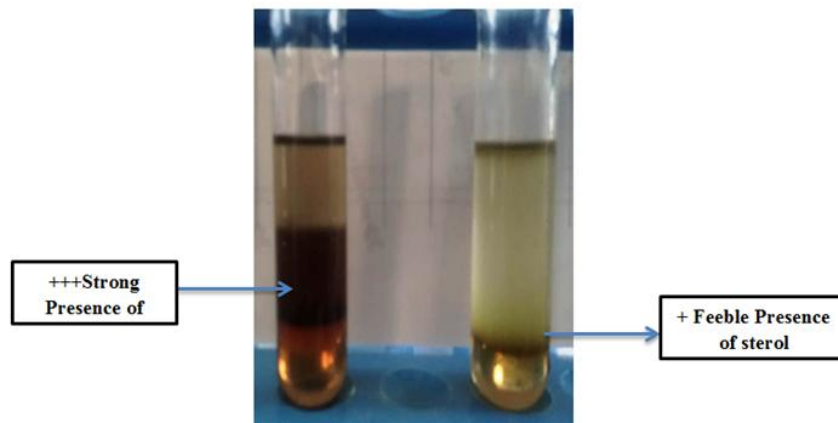
#### Pharmacognostic analysis of sterol in the leaf

Salkowski reaction was performed by dissolving the leaf extract in chloroform and then a drop of sulfuric acid was added and observed for sterol ring that would appear in reddish colour in interphase.

Wrightia tinctoria used by JRK	Salkowski reactions- Presence of sterols
Sample-1	+++
Sample-2	+++
Sample-3	+++
Sample- 4	+++
Sample-5	+++
Wrightia tinctoria procured from different sources	
Market sample- 1	+
Market sample- 2	+
Market sample- 3	+
Market sample- 4	+
Market sample- 5	+

+ = Very feeble presence; +++ = Very strong presence

Salkowski reactions



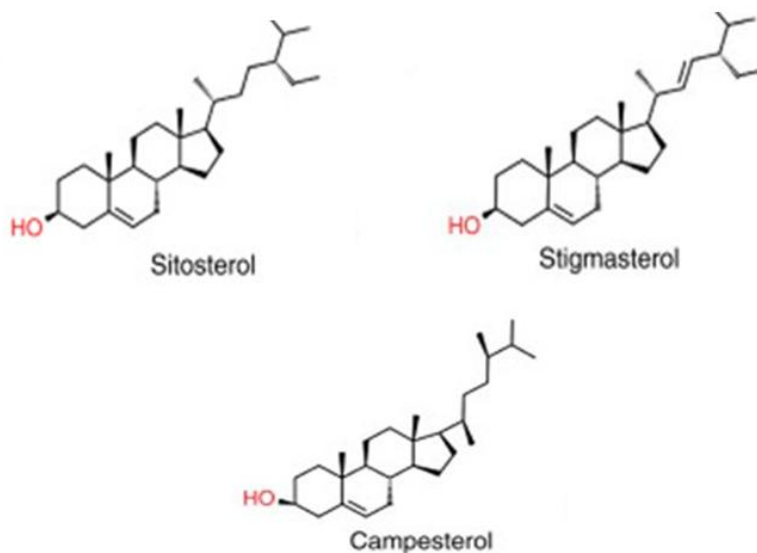
Attempt to detect sterols in 5 batches of Dr. JRK's 777 oil vis-à-vis market products

Five *Wrightia tinctoria* based oil preparations available in the market were procured and then tested for phytosterols. Similarly 5 different batches of Dr. JRK's 777 oil was also used. In brief methanolic fraction was separated and then the solvent was removed. The extract thus obtained was dissolved in chloroform and then a few drops of conc. sulfuric acid was added and observed for the presence of red coloured ring at the interphase indicative of sterols.

All the 5 batches of Dr. JRK's 777 oil showed rich presence of sterols whereas none of the market samples of *Wrightia tinctoria* based preparations showed the presence of sterols.

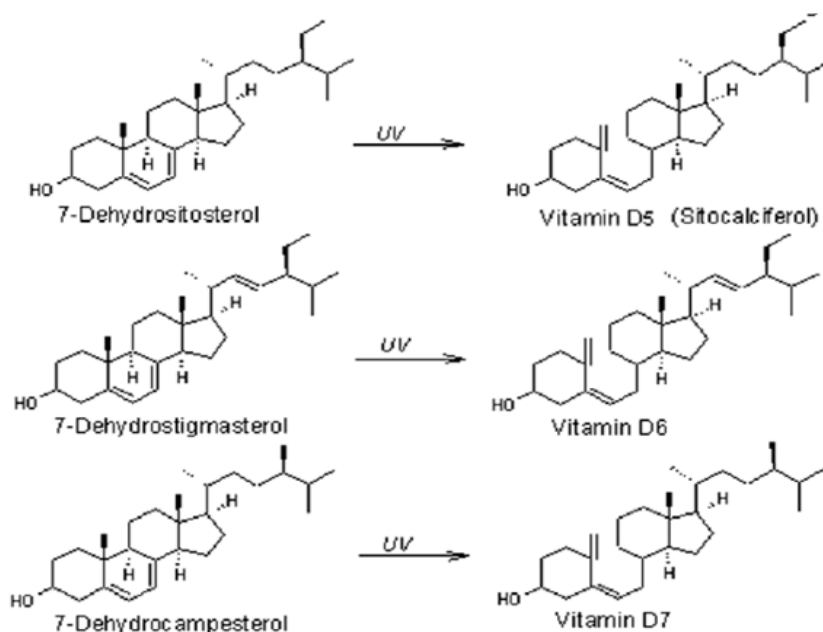
### Discussion

*Wrightia tinctoria* contains the following sterols and the structural chemistry is given below,



In the present study we have established that the sterol moieties are responsible for the treatment success in Psoriasis when *Wrightia tinctoria* based oil preparation is used.

**The sterols are precursor of Vitamin D and hence their dermal absorption and UV exposure may lead to the formation of Vitamin D which in turn offer therapeutic benefit to Psoriasis.<sup>13</sup>**



We have evaluated the direct implication of Vitamin D on skin vis-à-vis the sterols and found that sterols caused greater dermal change than Vitamin D possibly due to poor absorption of Vitamin D. Whereas the phytosterols absorb into the skin and then react with UV and thus participating dermal synthesis of Vitamin D.

The precursor of Vitamin D in *Wrightia tinctoria* appears to be quite unstable and can be altered greatly by several factors such as acid value of the base oil used for preparing *Wrightia tinctoria* based preparations for Psoriasis and the heat treatment employed in the manufacturing process of the same.

If the phytosterols in the plant is allowed to react in UV or heat, the participation of phytosterols in the dermal Vitamin D synthesis is unlikely or can be abysmally low and hence such preparations may not show superior therapeutic effect. Therefore the base oil used for the preparation of *Wrightia tinctoria* preparations should have very low acid value, should not be subjected to heat or sun exposure. Besides that the sterol content in the plant also may vary greatly due to several factors. Therefore sterol content estimation is essential prior to acceptance of the raw *Wrightia tinctoria* herb.

Dr. JRK's 777 oil is the most preferred drug of choice for Psoriasis by medical practitioners in India. We believe that the therapeutic value of Dr. JRK's 777 oil may be the reason why medical practitioners rate the product great.

We have estimated the presence sterols in five market samples of *Wrightia tinctoria* based preparations and surprisingly found that none of the samples showed any significance presence of sterols in them.

The process stringency during manufacturing of Dr. JRK's 777 oil along with the use of therapeutic value approved *Wrightia tinctoria* leaves by the company is the reason for the superior therapeutic effect of Dr. JRK's 777 oil over other market samples. Further our study also has shown that sterol moieties in *Wrightia tinctoria* may hold the secret for its effect in Psoriasis.

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