

**Development of spectrophotometric fingerprinting method for *Talisadi Churna***

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**Selective and efficient analytical methods are required not only for quality assurance but also for authentication of herbal formulations. A simple, rapid and validated fingerprint method has developed for estimation of piperine in '*Talisadi churna*', a well known herbal formulation in India. The estimation was carried out in two laboratory batches and three marketed formulation by an ultraviolet spectrophotometric method at 342.8 nm.**

**Key Words:** *Talisadi churna*, Piperine.**INTRODUCTION**

Herbal drugs have been used since ancient times as medicines for treatment of a wide range of diseases. In spite of the great advances in modern medicine in recent decades, crude drugs either of plant, animal or mineral origin still make an important contribution to health care. Ayurveda is a unique holistic system, based on the interaction of body, mind and spirit. In about 400 BC, the first Ayurvedic medical school was founded. Its compendium of writings date c 100 AD and describes 341 plant medicines as well as medicines of animal and mineral origin among them *Visnaga*, *Gotu kola* and others [1].

Churnas are classical Ayurvedic formulations. *Talisadi churna* contains *Piper longum* and *Piper nigrum*, which contain Piperine as the major constituent. Piperine is 1-[5-(1, 3-Benzodioxol-5-yl)-1-oxo-2, 4-pentadienyl]as shown in figure 1. Piperidine is the alkaloid biomarker constituent of *Piper longum* responsible for its pungency and that of black pepper [2].

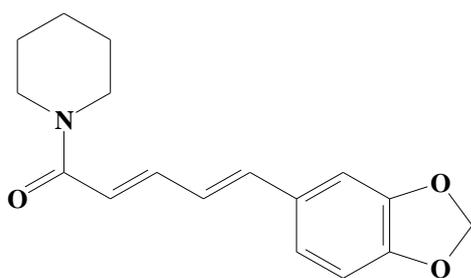


Figure 1: Chemical structure of Piperine

A comprehensive and quantifiable identification method for fingerprint development; is able to reveal chemical information of herbal medicines with spectrogram and other analytical techniques [3]. The identification of herbal medicines from various sources is crucial in order to ensure authenticity, quality, safety and efficacy. Most of the herbal formulations are lacking in their defined quality control parameters. Therefore, they are not well accepted in global market. Hence, WHO has emphasized the need to ensure the quality of medicinal plant products by using modern analytical technique and applying suitable standards [5, 6]. The drug ingredients used in the preparation of *Talisadi churna* as per the Ayurvedic formulary of India are shown in Table 1.

**MATERIALS AND METHODS****Procurement of crude drug**

The crude drugs were procured from the local market and identification was confirmed by macroscopic and microscopic features.

**Preparation of the formulation**

*Talisadi churna*, two laboratory batches (named TLS-I, TLS-II) were prepared in the laboratory according to specifications of the Ayurvedic formulary of India [6]. The available commercially brands TMS-A, TMS-B and TMS-C of *Talisadi churna* were procured from a local Pharmacy.

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**Table 1: Drug ingredients used in the preparation of *Talisadi Churna***

Crude drugs	Botanical name	Parts used	Quantity (g)	Major constituents
Talisa	<i>Abies webbiana</i>	leaf	12	Essential Oil & Alkaloid
Marica	<i>Piper nigrum</i>	fruits	24	Alkaloids (Piperine, Chavicine, Piperidine, Piperetine) & essential oil
Sunthi	<i>Zingiber officinale</i>	roots	36	Cineole zingiberol, sesquiterpene like zingiberene, bisobolene and sesqui phellandrene, gingerosol in the oleo-resin
Pippali	<i>Piper longum</i>	fruits	48	Piperine, Piperlongumine, Piperlonguminine, Essential Oils
Vinsa rocana	<i>Bombusa bambos</i>	Sc.	60	Cyanogenic glucoside-taxiphyllin.
Ela	<i>Elettaria cardamomum</i>	Sd.	6	Essential oil
Tvak	<i>Cinnamomum zeylanicum</i>	Stem bark	6	Essential oil, tannin and mucilage
Sarkara	Sugar	-	384	Sucrose

### Chemicals

All the chemicals and solvents used were of A.R. grade. Standard Piperine (98%) was procured from Lancaster (England).

### Preparation of Piperine standard solution

Ten milligrams of Piperine were dissolved in ethanol to give 100 ml of solution of 100 µg/ml.

### Preparation of Piperine extract of *Talisadi Churna*

One gram of powdered *Talisadi churna* was refluxed with 60 ml of ethanol for 1 h. The extract was filtered and the marc refluxed with 40 ml of ethanol for 1 h. The combined ethanolic extracts of *Talisadi churna* were evaporated under vacuum until a semisolid residue was obtained. The residue was dissolved in ethanol to yield 100 ml of solution which was filtered through a sintered glass funnel (G-2) using a vacuum filtration assembly. The filtrate was centrifuged at 2000 rpm for 30 minutes, the supernatant thereof collected and volume was made to 100 ml with ethanol [9, 10].

### Preparation of calibration curve for Piperine

Standard solutions of piperine were prepared within the concentration range 2-10 µg/ml in 25 ml volumetric flasks. The absorbances of the piperine solutions were measured at 342.8 nm against ethanol and a calibration curve plotted.

### RESULTS AND DISCUSSION

The developed method was found to be reliable, accurate, precise and sensitive. The method involves measurement of UV absorbance at 342.8 nm for piperine corresponding to the absorption maxima of the herbal formulation *Talisadi churna*. The absorbance characteristics show that piperine obeys Beer Lambert's law within the concentration range 2-10 µg/ml at the λ-max of 342.8 nm. The estimation of piperine content of the *Talisadi churna* and powder of *Piper longum* (Pippali) and *Piper nigrum* (Marica) was carried out separately. The concentration of piperine present in raw material was found to be  $2.12 \pm 0.035$  w/w in *Piper nigrum* and  $0.765 \pm 0.006$  w/w in *Piper longum*. The content of piperine in different batches of *Talisadi churna* was found to be

0.421 ± 0.001 %, 0.385 ± 0.002 % and 0.212 ± 0.004 %, 0.208 ± 0.002 % and 0.223 ± 0.004 % w/w respectively for TLS-I, TLS-II

and TMS- A, TMS-B and TMS-C. The results obtained are summarized in Table 2.

**Table 2: Estimation of piperine content**

S. No.	Name	Piperine Content (% w/w ± SD)	Standard error of mean (SEM)	Confidence Level (95 %)
1.	<i>Piper nigrum</i>	2.120 ± 0.035	0.0155	± 2.128
2.	<i>Piper longum</i>	0.765 ± 0.006	0.0025	± 0.7632
3.	TLS-I	0.421 ± 0.001	0.0005	± 0.42.4
4.	TLS –II	0.385 ± 0.002	0.0007	± 0.3845
5.	TMS-A	0.212 ± 0.004	0.0016	± 0.2135
	TMS-B	0.208 ± 0.002	0.0007	± 0.2086
6.	TMS-C	0.223 ± 0.004	0.0016	± 0.2184

In order to establish the precision and accuracy of the method, recovery studies were performed by adding known amount of piperine into pre-analyzed sample of *Talisadi churna*. The result shows 99.09 %, 98.61 %, 99.17 %, 98.26 % and 98.35 % recovery of piperine.

### CONCLUSION

The developed method was found to be accurate, simple and rapid. It can therefore be applied for routine analysis of piperine in herbal formulation *Talisadi churna*.

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### REFERENCES

- [1] P. Mukherjee, Pharmacological Screening of Herbal Drug, Quality Control of Herbal Drug: An Approach to Evaluation of Botanicals, Eastern Publishers (Business Horizontal Ltd., New Delhi. 2002.
- [2] Indian Herbal Pharmacopoeia, Regional Research Laboratory Jammu, Indian drug Manufacturing Association Mumbai. 1999. Planning, Department of Indian System of Medicine and Homeopathy, Delhi. p 103-119.
- [3] Ayurvedic formulary of India, Part-1, 2nd, edition, Government of India, Ministry of Health and Family
- [4] Yu. Rongmin et al, J. Pharm. Biomed. Anal. 44 (2007) 818–23.
- [5] V. Jain, Swarnlata Saraf, S. Saraf, Asian J. Chem. 19(2) (2007) 1406-1410.
- [6] WHO general guidelines for methodologies on research and evaluation of traditional medicine. (2000): [http://whqlibdoc.who.int/hq/2000/WHO\\_EDM\\_TRM\\_2000.1.pdf](http://whqlibdoc.who.int/hq/2000/WHO_EDM_TRM_2000.1.pdf)