



## Research Article

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### APPLICATION OF CLASSICAL AND INSTRUMENTAL METHODS FOR EVALUATION OF POLYHERBAL CAPSULE FORMULATION

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**ABSTRACT**

Contudol capsule, a combination of four herbal extracts namely *Boswellia serrata*, *Curcuma longa*, *Moringa oleifera* and *Zingiber officinale* was chosen for the study. Two lots of Contudol capsule were analyzed qualitatively and quantitatively by applying various classical and instrumental methods. Along with general test parameters of capsules like average weight, filled average weight, uniformity of weight, disintegration time, blends of both the capsule lots were tested for loss on drying, total ash content, acid insoluble ash, extractive values, pH by appropriate standardized classical and instrumental methods. Active components like Boswellic acid content was found to be around 129.15 and 131.33 mg per capsule by titration method in respective lots, whereas, spectrophotometric analysis for Curcumin and titrimetric analysis of Tannin content showed 20.806 mg, 20.005 mg and 2.063 mg, 1.883 mg per capsule respectively in corresponding lots. Presence of gingerol was well illustrated by modern sophisticated HPTLC technique, where, in Diethyl ether : Hexane (7:3) system, after spraying with VSR, resembling blue colored spots were observed at  $R_f$  about 0.20, 0.40 and 0.70 in reference standard, extract powder and blend of the capsule. Heavy metals like Arsenic, Mercury, Lead and Cadmium were found to be at the ppm level within the limits set by AYUSH, by Inductively Coupled Plasma spectrometry. Microbiological testing was carried out as per the USP/ BP guidelines and findings were complying with the pharmacopoeial specified values. In this way, by considering these entire test parameters one can very well establish and maintain the quality of polyherbal formulations.

**Keywords:** Qualitative, Quantitative, Polyherbal formulation, Classical, Instrumental**INTRODUCTION**

In the present age of extreme competition, everybody is exposed to a continuous struggle for his very existence. These days the Ayurvedic drugs are increasingly coming from the industry rather than the Ayurvedic physician compounding them impromptu. Commercially, Ayurvedic drugs can make a dent in international market, which are looking towards alternative medicine for the cure of ailments to which even modern system has no answer. These ailments include metabolic lifestyle-induced problems of heart, diabetes and cancer, dementia, age related disorders, immunological disorders and gynecological problems. Evaluation of Indian traditional medicine is possible through the proper exploitation and exploration of wide biodiversity and great ancient treatises of traditional medicine with the light of modern tools and techniques.<sup>1</sup> Various herbs are identified, proved for their effective medicinal activity and successfully used to cure different types of diseases. These medicinal herbs are used in various forms like crude, coarse powder, extracts; spray dried powders, tinctures and so on, in different formulations. Nowadays due to our modern, exhaustive and stressful lifestyle, bone and musculo-skeleton related disorders are increasing. Osteoarthritis and other rheumatic disorders are very common. It affects not only the aging population but also the younger age groups due to physiological and psychological stresses of modern living. A few anti-inflammatory agents are useful but have the risk of side effects such as gastric ulcers and bleeding. That is the reason people are turning to gentle herbal preparations. Different phytochemicals can be qualitatively estimated by applying various standard classical methods.<sup>2</sup> However; we are also focusing on quantitative estimation of active ingredients along with

qualitative parameters. Contudol, a combination of four herbs – *Boswellia serrata*, *Curcuma longa*, *Moringa oleifera* and *Zingiber officinale* – is a natural herbal anti-arthritic. Composition of the Contudol capsule is; Each capsule contains

- *Boswellia serrata* (Shallaki) extract (Standardized for Boswellic acid 60 %).…… 200 mg
- *Curcuma longa* (Haldi) extract (Standardized for Curcumin 20 %).….100 mg
- *Moringa oleifera* (Sahjan) extract (Standardized for Tannins 1 %).……50 mg
- *Zingiber officinale* (Sunthi) extract (Standardized for Gingerol).…… 35 mg

Excipients ... q.s.

Contudol helps to improve mobility and reduce pain in cases of arthritis and other musculo-skeletal disorders. *Boswellia serrata* (shallaki) has a marked anti-inflammatory and anti arthritic activity<sup>3</sup>. Rheumatic disorders in many situations demand the same anti-inflammatory effect. *Curcuma longa* (haldi) extract shows significant anti-inflammatory activity in acute edema. The alcoholic extract of *Moringa oleifera* (sahjan) root, bark and gingerol from *Zingiber officinale* possesses anti-inflammatory and analgesic properties. This formulation inhibits the production of prostaglandins and leukotrienes that are responsible for pain and inflammation. It shows disease modifying effect, means it slows down the progress of disease, stop further damage to the joint to recover on its own. Quality of this product was checked and for maintaining the same quality, desired test parameters were set by applying various classical and instrumental methods. Qualitative as well as quantitative

estimation of different components adds value to make the quality protocol of the product.

### MATERIAL AND METHODS

All materials required for testing were of AR grade and procured from M/s E. Merck / Qualigens. General Test Parameters of capsules mentioned in Table 2, from S. No. 1 to 5, were carried out as per Indian Pharmacopoeia<sup>4</sup>. Whereas, S. No. 6 to 11, were carried out as per Ayurvedic Pharmacopoeia of India<sup>5</sup>. Content of Boswellic Acid<sup>6</sup> and content of Curcumin<sup>7</sup> were carried out by titration and spectrophotometric method, respectively. Also, Content of Tannins<sup>8</sup> was estimated by titrimetric analysis.

HPTLC technique was applied for determining the presence of gingerol in capsule formulation. The

conditions applied were as follows: Concentration of sample → Blend of 1 capsule/10 ml (methanol), Concentration of Gingerol Reference Standard → 20 mg/10 ml (methanol), Concentration of Sunthi extract → 35 mg/10 ml (methanol), Stationary Phase → TLC Aluminium sheets silica gel 60 F 254, Solvent system used → Diethyl ether : Hexane (7:3), Sample Volume → 10 micro litre, Saturation Time → 30 minutes, Spotting level → 1 cm, Time of Run → 20 minutes, Length of Run → 8.0 cm, Detection → visual detection by spraying Vanillin – Sulphuric acid Reagent (VSR). Heavy Metal testing was carried out on Inductively Coupled Plasma (ICP) spectrometry, to determine the values as per Ayush Guidelines, whereas, microbiological testing was performed as per USP/BP guidelines.

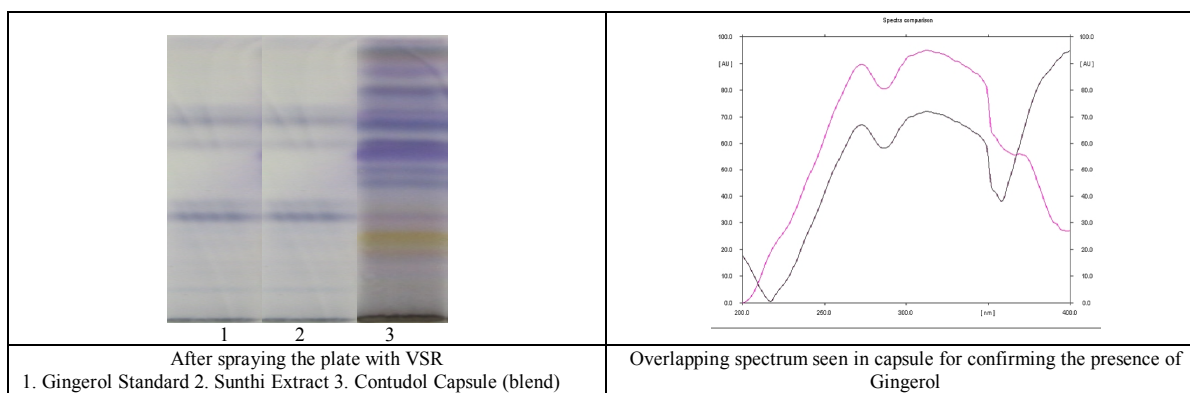
**Table 1: Qualitative and Quantitative Estimation of Active Components in Contudol Capsule**

| S. No. | Test Parameters               | Source (Active ingredients) of active components                                    | Theoretically calculated value of active component  |
|--------|-------------------------------|---|---|
| 1      | Content of Boswellic acid     | <i>Boswellia serrata</i> (Shallaki) extract. (Standardized for 60 % Boswellic acid) | 200 mg of Shallaki extract in a capsule is equivalent to 120 mg Boswellic acid. 80 % of 120 mg is 96 mg Proposed specification is about 120 mg/capsule (Not less than 96 mg/capsule)                                |
| 2      | Content of Curcumin           | <i>Curcuma longa</i> (Haldi) extract Standardized for 20 % Curcumin                 | 100 mg Haldi extract in a capsule is equivalent to 20 mg Curcumin. 80 % of 20 mg is 16 mg. Proposed specification is about 20 mg/capsule (Not less than 16 mg/capsule)  |
| 3      | Content of Tannins            | <i>Moringa oleifera</i> (Sahjan) extract Standardized for about 1 % Tannins         | 50 mg Sahjan extract in a capsule is equivalent to 0.50 mg Tannins. Proposed specification is about 0.5 mg/capsule. Other herbal ingredients also contribute to some extent   |
| 4      | Presence of Gingerol by HPTLC | <i>Zingiber officinale</i> (Sunthi) extract   | Presence of blue colored spots at R <sub>f</sub> value 0.20, 0.40, and 0.70 proves the presence of Gingerol in active ingredient - sunthi extract and capsule formulation against the respective reference standard |

**Table 2: Physicochemical Test Parameters of Contudol Capsule Formulation**

| S. No. | Test Parameters                                  | Findings of Lot 1   | Findings of Lot 2   |
|--------|--|---|---|
| 1      | Description                                      | “0” size capsule filled with yellowish brown colored powder | “0” size capsule filled with yellowish brown colored powder |
| 2      | Disintegration Time                              | 14 minutes  | 14 minutes  |
| 3      | Average weight                                   | 617.80 mg   | 623.77 mg   |
| 4      | Average filled weight                            | 492.70 mg   | 503.53 mg   |
| 5      | Uniformity of weight                             | + 7.50 % of average filled weight                           | + 7.50 % of average filled weight                           |
| 6      | Loss On Drying at 105°C (blend)                  | 4.649 %w/w  | 4.597 %w/w  |
| 7      | Total Ash (blend)                                | 16.705 %w/w   | 15.952 %w/w   |
| 8      | Acid Insoluble ash (blend)                       | 8.433 %w/w  | 7.343 %w/w  |
| 9      | Water Soluble Extractive (blend)                 | 22.929 %w/w   | 20.891 %w/w   |
| 10     | Alcohol Soluble Extractive (blend)               | 52.900 %w/w   | 50.119 %w/w   |
| 11     | pH of 1.00%w/v solution (blend)                  | 6.82  | 6.50  |
| 12     | Content of Boswellic acid (blend)                | 129.15 mg/capsule   | 131.33 mg/capsule   |
| 13     | Content of Curcumin (blend)                      | 20.806 mg/capsule   | 20.005 mg/capsule   |
| 14     | Content of Tannins (blend)                       | 2.063 mg/capsule  | 1.883 mg/capsule  |
| 15     | Presence of Gingerol (blend)                     | Complies  | Complies  |
| 16     | <b>Heavy Metals (As per department of AYUSH)</b> |   |   |
| i      | Content of Arsenic (as As)                       | 0.291 ppm   | Less than 0.001 ppm   |
| ii     | Content of Mercury ( as Hg)                      | Less than 0.001 ppm   | Less than 0.001 ppm   |
| iii    | Content of Lead (as Pb)                          | 1.138 ppm   | 2.216 ppm   |
| iv     | Content of Cadmium (as Cd)                       | 0.051 ppm   | Less than 0.001 ppm   |
| 17     | <b>Microbiological Testing (As per USP / BP)</b> |   |   |
| i      | Total aerobic microbial count                    | Less than 10 cfu/g  | 20 cfu/g  |
| ii     | Total combined yeast and moulds count            | Less than 10 cfu/g  | Less than 10 cfu/g  |
| iii    | Bile tolerant Gram negative bacteria             | Less than 10 cfu/g  | Less than 10 cfu/g  |
| iv     | <i>Escherichia coli</i>                          | Absent  | Absent  |
| v      | <i>Salmonella spp.</i>                           | Absent  | Absent  |
| vi     | <i>Staphylococcus aureus</i>                     | Absent  | Absent  |
| vii    | <i>Pseudomonas aeruginosa</i>                    | Absent  | Absent  |
| viii   | <i>Clostridium spp</i>                           | Absent  | Absent  |

ppm: parts per million Cfug: Colony forming unit/gram



**Figure 1: Identification of Gingerol in Capsule Formulation**

## RESULTS

Table 1 summarizes major specific quality parameters. Theoretical values of active components in the active ingredients used in the formulation. Calculation was shown on the basis of label claiming of active raw materials. Qualitative and quantitative specifications were proposed accordingly. Table 2 gives actual findings of all general as well as specific test parameters for two different lots of Contudol capsule. Findings of all general test parameters from description to pH were complying as per the general pharmacopoeial specifications. Quantitative active components like content of Boswellic acid was found to be 129.15 mg/capsule and 131.33 mg/capsule in respective lots, which comply with the proposed specification mentioned in Table 1. Similarly content of Curcumin was found to be 20.806 mg/capsule and 20.005 mg/capsule in corresponding lots against the expected value 20 mg/capsule. Content of Tannins was found to be 2.063 mg/capsule and 1.883 mg/capsule in two individual batches. These findings seem to be bit higher than the theoretically calculated value, but it might be because other herbal ingredients also contribute tannins to some extent in different concentration. Presence of Gingerol in the capsule formulation was proved by applying HPTLC technique, where *Zingiber officinale* (Sunthi) extract and Contudol capsule formulation clearly showed blue colored spots at  $R_f$  about 0.20, 0.40 and 0.70 against the gingerol reference standard after spraying the plate with VSR. Also, respective overlapping spectrum gives the confirmation, as shown in Figure 1. Mansoor Ahmad *et al*<sup>9</sup> has also applied this HPTLC technique for estimating poly-herbal drug named JPR-1. Heavy metals like Arsenic, Mercury, Lead and Cadmium were found to be well within specifications as per the guideline proposed by AYUSH. Similarly findings of specified parameters mentioned under microbiological testing also complied with the limits mentioned in the pharmacopoeia, which proves our formulation is safe to consume with respect to microbial load.

## DISCUSSION

It is necessary to apply appropriate classical and instrumental methods to determine and maintain the

quality of the final formulation, as cited in above mentioned formulation, by taking into consideration precision and ease of testing, we have applied classical as well as UV visible spectrophotometry for estimating active functional groups like boswellic acid, curcumin and tannin content respectively. However, the most well versed HPTLC technique was used to carry out qualitative estimation of one of the ingredients namely, gingerol in *Zingiber officinale*. Accordingly, we can set various test parameters as per the active raw materials used in the formulation. Application of these test parameters will definitely add the value to maintain the quality level of the polyherbal capsule formulation.

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## REFERENCES

- Mukherjee PK. Quality Control of Herbal Drugs. Role of Traditional System of Medicine in Primary Health Care. 1<sup>st</sup> ed. Kolkata (India): Business Horizons Pharmaceutical Publishers; 2002. p. 22.
- Soni A, Gupta SJ, Kumar A, Tripathi YB. Qualitative and Quantitative Estimation of a Poly-herbal Compound Dashang Lepa. Int. J. Res. Ayurveda Pharm 2013; 4(5): 719-722. <http://dx.doi.org/10.7897/2277-4343.04520>
- Wyk B, Wink M. Medicinal Plants of The World. The plants in alphabetical order. South Africa: Briza Publications; 2004. p. 69.
- Indian Pharmacopoeia. Vol. 2. Govt. of India. Controller of Publications. New Delhi; 2007. p. 633, 634.
- The Ayurvedic Pharmacopoeia of India. Part 1. Vol. 1. Govt. of India. Controller of Publications. New Delhi; 1989. p. 143, 156.
- Rajpal V. Standardization of Botanicals. Volume 1. *Boswellia serrata*. New Delhi (India): Eastern Publishers; 2002. p. 51.
- Rajpal V. Standardization of Botanicals. Volume 1. *Curcuma longa*. New Delhi (India): Eastern Publishers; 2002. p. 126, 127.
- Ranganna S. Handbook of Analysis and Quality Control for Fruit and vegetable Products. Polyphenols. 2<sup>nd</sup> ed. New Delhi (India): Tata McGraw-Hill Publishers; 1977. p. 80, 81.
- Ahmad M, Saeed F, Mehjabeen, Jahan N. Studies on Polyherbal Drug (JPR-1) Used for Arthritis. Unique Journal of Ayurvedic and Herbal Medicines 2013; 1(2): 31-40.

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