



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.¹ 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.² 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-arthritis. 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 activity³. 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 Pharmacopoeia⁴. Whereas, S. No. 6 to 11, were carried out as per Ayurvedic Pharmacopoeia of India⁵. Content of Boswellic Acid⁶ and content of Curcumin⁷ were carried out by titration and spectrophotometric method, respectively. Also, Content of Tannins⁸ 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 _f 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	Heavy Metals (As per department of AYUSH)		
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	Microbiological Testing (As per USP / BP)		
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

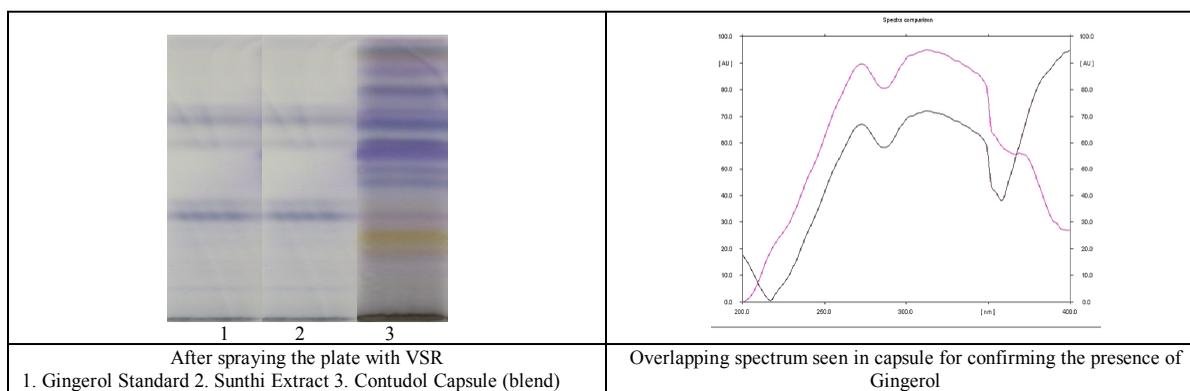


Figure 1: Identification of Gingerol in Capsule Formulation

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*⁹ 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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