

## ANALYTICAL STANDARDIZATION OF *KUKKUTNAKI GUGGULU*

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### ABSTRACT

Herbs are natural resources for a variety on biochemical products and are used medicinally in different countries; they are the source of many potent and effective drugs. According to retrospective literary review, the combination of *Kukkutnaki* (*Aspidium cicutarium* Sw.) and purified *Guggulu* (*Commiphora mukul* Hook. Ex Stocks) was first mentioned in book *Chikitsa pradeep* named as *Kukkutnaki Guggulu*. This *Anubhut* herbal formulation *Kukkutnaki Guggulu* was prepared according to text “*Guggulu kalpana*”. It was documented as a herbal drug which is used for goiter, cysts, tumors, tonsilitis, abscess. The quality test for the finished product were performed in which total ash%  $6.49 \pm 0.553$ , acid insoluble ash%  $1.323 \pm 0.547$ , water soluble extract%  $35.64 \pm 0.590$ , alcohol soluble extract%  $20.45 \pm 0.822$ , pH  $3.81 \pm 0.041$ , moisture content%  $9.63 \pm 0.851$ , were estimated. The tablets or *vati* of the study drug were analysed for hardness, uniformity of weight, friability and disintegration time(DT) in which hardness( $\text{Kg/cm}^2$ )  $1.42 \pm 0.080$ , uniformity of weight %  $4.267 \pm 0.163$ , friability%  $0.566 \pm 0.232$ , disintegration time (min)  $9.35 \pm 0.229$  were estimated. HPTLC analysis of *Kukkutnaki Guggulu* using toluene: ethyl acetate: formic acid(7:3:1) as mobile phase showed 14 peaks for all three batches. The coinciding max Rf values were 0.10, 0.18,0.33,0.42 and 0.80. Hence, the standard parameters were developed for current formulation. So, in the current study, the analytical standard of *Kukkutnaki Guggulu* was developed which will be ready reference for the next researcher.

**Keywords:** *Kukkutnaki*, *Guggulu*, HPTLC, Physico-chemical analysis, *Guggulu Kalpana*

**INTRODUCTION:** In the plant kingdom, there are number of plants that yield medicines useful to mankind. The world population relies mainly on plant and plant extract for health care. Tribal as well as folk sources were utilized by Acharyas of Ayurveda while documenting Ayurvedic materia medica.<sup>1</sup> *Kukkutnaki*, (*Aspidium cicutarium* Sw.) is a herb belong to family dryopteridaceae,<sup>2-6</sup> grown around the monsoon and rainy seasons. It is generally found in *sahyadri* foot hill region. It is also called *Nirvishi*., *Bichava* or *Kombadnaki*<sup>7-8</sup>. It is first mentioned in the book named “*Gharguti Aushadhe*”<sup>9</sup> for the treatment of various types of cysts. As per Charakacharya, different safe combinations can be formulated as

medicinal product based on physicians logic, condition of patient and type of disease.<sup>10</sup> *Kukkutnaki Guggulu* was selected as a study drug according to retrospective literary review from various texts and scientific magazines. The single available reference from *Chikitsa pradeep*<sup>11</sup> was found to be appealing and used by senior *Vaidyas* in clinical practice hence was selected for study. The proprietary drug i.e. *Kukkutnaki Guggulu* can be formulated by using authenticated *Kukkutnaki* (*Aspidium cicutarium* Sw.) and *Guggulu* (*Commiphora mukul* Hook. Ex Stocks)<sup>12-18</sup> in the same proportion for oral administration and it was prepared as per a text “*Guggulu kalpana*”.<sup>19</sup> Formulations develops from exudates of *Commiphora*

*mukul Hook. ExStocks*). Since last 3 decades, it was documented as a herbal drug which is used for diseases of *Mansvaha strosas*, tonsillitis, goiter, cysts, tumors,, abscess.<sup>20</sup> This combination is not mentioned in classical texts of Ayurveda, however considering potency of ingredients of this combinations its standardization on analytical ground is needed

#### **MATERIAL AND METHODS:**

**Material:** Dry Rhizomes of *Kukkutnaki* were collected from *Bhimashankar, Tal-aambegaon*, district- Pune and the sample was authenticated from Agharkar research institute, Pune. Raw *Guggulu* and *triphala* purchased from local market of Masjid bundar, Mumbai and authenticated from Gurunanak khalsa college, Matunga, Mumbai. pH meter, Hot air oven, Muffle furnace, Dessicator, Citizen Electronic balance, Fractional weighing, Hot plate, Viscometer, Pycnometer, Disintegration time apparatus, Monsanto tablet hardness Tester, Digital friability test apparatus, Magnetic stirrer, Glassware's-Petri dishes of capacity 50mL, Silica Gooch Crucibles(capacity of 1000°C, Conical flask of capacity 500mL, Glass measuring cylinder of capacity 25mL, 50mL and 100mL, Glass beaker of capacity 50mL and 100mL, Chemicals-Diluted HCL, 10% Ethanol solution, NaOH solution, Buffer solution of pH 4 and pH 7 , CAMAG TLC

Scanner 3\_131215, HPTLC machine, UV chamber were used for analytical study.

#### **Method:**

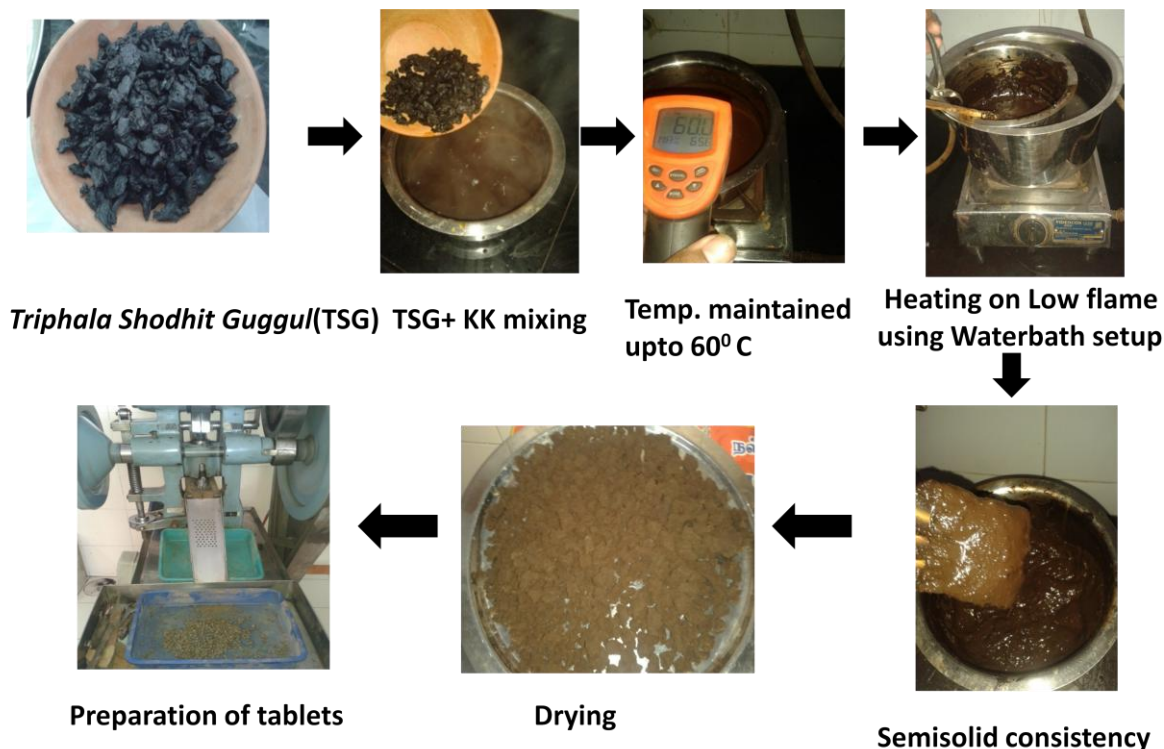
#### **Preparation of Kukkutnaki Guggulu :**

In *Kukkutnaki Kwath(KK)*, 300gm of purified *Guggulu (triphala shodhit)*<sup>21-24</sup> was mixed properly & kept overnight. Next day, the mixture was heated on low flame with constant stirring with wooden spatula. Then the mixture was kept on water bath to avoid the charring and the temperature was maintained upto 60<sup>0</sup>C, till the mixture attains consistency of *Gudapaka* i.e. semisolid thickness. Then the mixture was removed from the gas burner. Then 1 part (300gm) of *Kukkutnaki churna* was added immediately into the mixture. The mixture was triturated well in *Khalva yantra* for two days to get a homogeneous mixture in soft solid form. The mixture was spread over the drying tray of the dryer and kept at 40<sup>0</sup>c for 48 hrs. After mixture was well dried then it was subjected for granulation in pulverizer at 40 mesh, then sieved. The dry powdered mixture was then transferred to the tablet punching machine & the obtained granules were further compressed by 8mm 7 punch. Tablets of weight 250 mg was prepared and the final product was kept in sunlight to dry. The dried tablets were preserved in glass bottles. The 3 separate batches were prepared & subjected for physicochemical analysis.

**Table no. 1: Contents of Kukkutnaki Guggulu for each batch (Total 3 batches)**

Sr. no.	Contents	Proportion	Quantity
1	<i>Kukkutnaki Bharad</i>	1part	300 gm
2	Distilled water	16 parts	4.8L
3	Purified <i>Guggulu</i>	1 parts	300 gm
4	<i>Kukkutnaki Churna</i>	1 parts	300 gm

**Fig. No. 1: Preparation of Kukkutnaki Guggulu and its tablets**



**Analytical study:** The three batches of *Kukkutnaki Guggulu* were subjected for Organoleptic and physico-chemical analysis in order to develop its standard. The following parameters were carried out in this phase- Organoleptic characteristics: Colour, odour, touch and taste and Physicochemical analysis:<sup>25-26</sup>Loss on drying at 110°, pH value, Total Ash%, Acid Insoluble Ash%, Water soluble extract%, Alcohol Soluble Extract%, Moisture content%, Hardness(Kg/cm<sup>2</sup>), Uniformity of weight%, Friability%, Disintegration time(min). HPTLC profile:<sup>27-30</sup> HPTLC analysis of was carried out using stationary phase, 10X10 cm sized silica gel plates and Toluene: Ethyl acetate: Formic acid- 7:3:1 as solvent system(mobile phase) through trial and error method. The TLC developed was

scanned by CAMAG TLC Scanner 3\_131215. The developed plate was visualized under visible day light, short UV (254 nm), long UV (366 nm) and the Rf values were recorded.

**OBSERVATIONS AND RESULTS:**

**Mass balance of *Kukkutnaki Guggulu* and tablet:** Weight of *Kukkutnaki Guggulu* observed after *gudpak*, drying, granulation and tableting process of three batches was noted. Loss in weight were observed during each of these process. The average loss % of three batches after drying process in dryer was found 40.93%. The average % loss observed after granulation process of three batches was found 10.40% and average % loss observed after tableting process of three batches was 3.75%.

**Table No. 2: Loss % of *Kukkutanakhi Guggulu*:**

Step	Batch-1	Batch-2	Batch-3	Mean
Loss % after drying (in dryer)	38.05	40.24	44.50	40.93
% Loss after granulation	9.61	12.24	9.37	10.40
% Loss after tableting	3.19	3.48	4.58	3.75

**Physico-chemical analysis of *Kukkutnaki Guggulu*:** In organoleptic analysis the colour of the product was found dark

brown, *Rasa*(taste) was *Tikta, Katu, Kashaya* and *odor(gandha)* was specific *Guggulu gandhi*. (Table no.1)The quality

test for the finished product were performed in which total ash% 6.49 ±0.553, acid insoluble ash% 1.323± 0.547, water soluble extract% 35.64±0.590, alcohol soluble extract% 20.45±0.822, pH 3.81±0.041, moisture content% 9.63±0.851, were estimated. The *tablets* or

*vati* of the study drug were analyzed for hardness, uniformity of weight, friability and disintegration time(DT) in which hardness(Kg/cm<sup>2</sup>) 1.42±0.080, uniformity of weight % 4.267±0.163, friability% 0.566±0.232, disintegration time (min) 9.35±0.229 were estimated.(Table no. 2)

**Table No. 3: Organoleptic analysis of Kukkutnaki Guggulu:**

Sr.no	Batches	Shabda	Sparsha	Roopa	Rasa	Gandha
1	Batch-1	Not significant	Shlakshana	Dark Brown	Katu, tikta, kashay	Specific Guggulu gandhi
2	Batch-2	Not significant	Shlakshana	Dark Brown	Katu, tikta, kashay	Specific Guggulu gandhi
3	Batch-3	Not significant	Shlakshana	Dark Brown	Katu, tikta, kashay	Specific Guggulu gandhi

**Table no.4 Physico-chemical analysis of Kukkutnaki Guggulu:**

Parameters	Results			Mean	S.D
	Batch-1	Batch-2	Batch-3		
Total Ash%	7.12	6.08	6.27	6.49	0.553
Acid Insoluble Ash%	1.08	1.95	0.94	1.323	0.547
Water soluble extract%	35.82	34.98	36.12	35.64	0.590
Alcohol Soluble Extract%	20.23	19.76	21.36	20.45	0.822
PH	3.78	3.86	3.80	3.81	0.041
Moisture content%	8.94	10.58	9.38	9.63	0.851
Hardness(Kg/cm <sup>2</sup> )	1.41	1.35	1.51	1.42	0.080
Uniformity of weight%	4.241	4.442	4.118	4.267	0.163
Friability%	0.48	0.83	0.39	0.566	0.232
Disintegration time(min)	9.40	9.10	9.55	9.35	0.229

**HPTLC analysis of Kukkutnaki Guggulu:** There is no standard reference data of HPTLC available for *Kukkutanakhi Guggulu*, hence this analysis was done. Chromatogram of Raw *Guggulu*, purified *Guggulu*, Raw *Kukkutnaki*, three batches

*Kukkutnaki Guggulu* (KG) in Figure no. 2 and tracks of three batches of *Kukkutnaki Guggulu* has been shown in figure no. 3, 4 and 5 respectively.(Fig. 3-5)

**Figure no. 2. Chromatogram-**

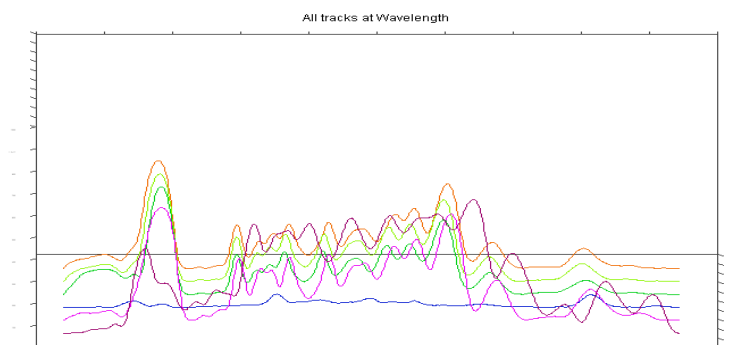


Figure no.3 : Batch 1-KG1

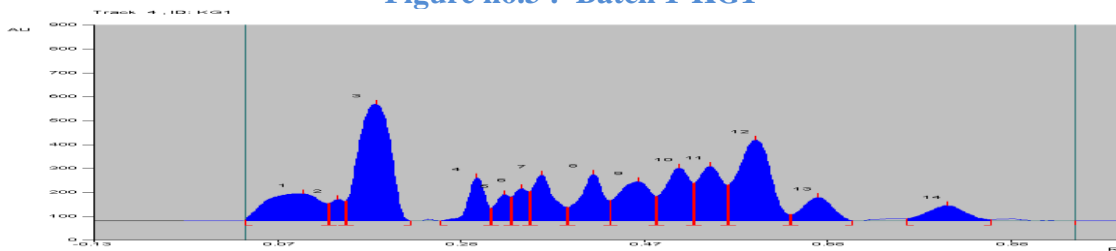


Figure no.4 Batch 2- KG 2

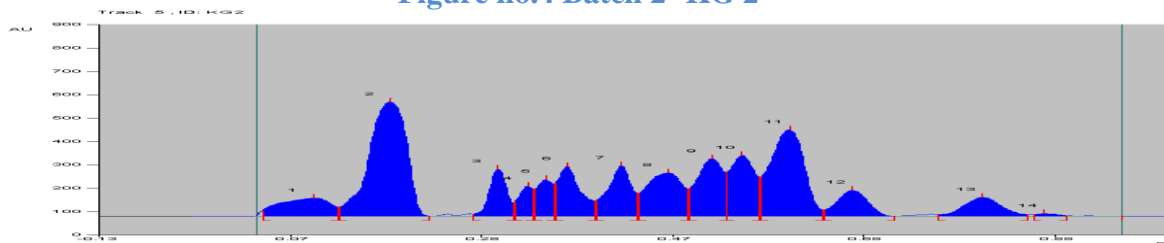


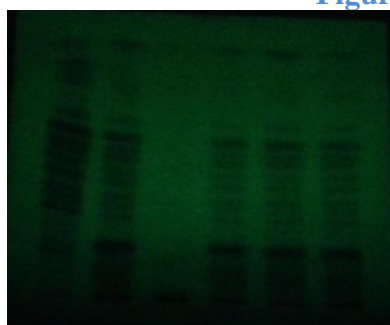
Figure no.5 Batch 3-KG 3



Table no. 5 Estimated max Rf values of three batches of *Kukkutanakhi Guggulu* and its contents:

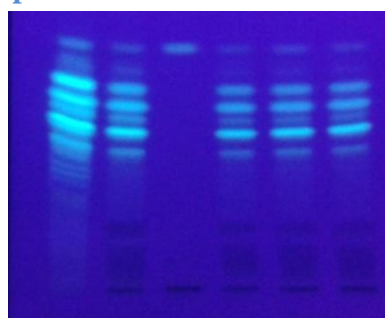
Sr. no.	Purified Guggulu	Raw Kukkutnaki	Batch-1	Batch-2	Batch-3
1	0.11	-	0.10	0.10	0.10
2	0.18	0.18	0.18	0.18	0.18
3	0.33	-	0.32	0.33	0.33
4	0.43	0.42	0.42	0.42	0.43
5	0.81	0.81	0.81	0.80	0.80

Figure of HPTLC plate under UV:



At 254 nm

Figure no. 6



At 366 nm

Figure no. 7

**DISCUSSION:** Plants are natural resources for a variety of biochemical products and can be used for medicinal purpose. Some herbal products are a

source of many traditional medicines. According to retrospective literary review, the combination of *Kukkutnaki* (*Aspidium cicutarium* Sw.) and purified *Guggulu*

(*Commiphora mukul* Hook. Ex Stocks) was mentioned in “*Chikitsa pradeep*” named as *Kukkutnaki Guggulu*<sup>11</sup>. But the proportions of ingredient and preparation method was not mentioned. The therapeutic use of this drug was mentioned in *gandamala*, *galganda*, *apchee* and *arbuda*. In the text of “*Guggulu kalpana*”, the traditional method and proportion of its ingredients is clearly mentioned.<sup>[19]</sup> So for the current study the same formulations was selected to develop its analytical standard. To develop the analytical standard of *Kukkutnaki Guggulu*, the three batches of product prepared were tested for physical and chemical parameters. The organoleptic parameters form the basic criteria for selecting a raw drug and also to confirm the finished product. In organoleptic analysis the colour of the product was found dark brown, rasa (taste) was *Tikta*, *Katu*, *Kashay* and odour was specific representing smell similar to *Guggulu*. This is due to presence of *Guggulu* as a major ingredients which contain few volatile phyto-constitutes having its specific odor. [Table No. 3]

The quality tests for the finished product were performed. Total ash is inclusive of presence of heat stable materials such as metals/minerals particles, sand, soil content etc which either are adhering to the herbal drug or presence as constitute of that herb. Thus it is utilized as criteria for identifying the purity of the drugs based on created standards. Ash value of final product was found %  $6.49 \pm 0.553$ . Acid insoluble ash represents matter which is not soluble in acid, in other word this test is indicator of inorganic material present in a sample. Such inorganic entities are not expected in herbal formulation. The obtained value of Acid insoluble ash in all the batches is negligible and it was found %  $1.323 \pm 0.547$ . Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying, or storage or formulating. The water soluble extract %  $35.64 \pm 0.590$  w/w of water soluble and alcohol soluble

extract%  $20.45 \pm 0.822$  w/w were present in *Kukkutnaki Guggulu* indicating that the drug is having good solubility in water. The pH conventionally represents the acidity and alkalinity. The finished product has pH  $3.81 \pm 0.041$  which indicates that *Kukkutnaki Guggulu* is acidic in nature. Loss on drying at  $105^{\circ}\text{C}$  indicates presence of moisture content. If moisture content % is more than permissible limit then the formulation is more likely to get infected by fungal growth. In the prepared batches moisture content % were  $9.63 \pm 0.851$  which is much less i.e. this formulation has sufficient stability is estimated.

The tablets or *vati* of the study drug were analysed for hardness, uniformity of weight, friability and disintegration time (DT). The average hardness of tablets was  $1.42 \pm 0.080$  kg/cm<sup>2</sup> which indicates that *Kukkutnaki Guggulu* tablets were not brittle in nature. Weight variation of tablet causes variation of active medicament which changes the bioavailability after administration. Uniformity of weight is determined to eliminate variation in dose. All the tablets were within acceptable range of weight variation as for natural herbal products.<sup>31</sup> Friability test is a method to determine physical strength of uncoated tablets upon exposure to mechanical shock and attrition. It is used to test the durability of tablets during transit. The friability %  $0.566 \pm 0.232$  was observed of this formulation is within acceptable range.<sup>32</sup> The disintegration time of tablets plays an important role in quality assessment. Rate of drug dissolution depends upon the time of disintegration which affects the absorption rate of drug. Both hardness and disintegration time interfere with the bioavailability of drug. *Kukkutnaki Guggulu* was found to have  $1.42 \pm 0.080$  kg/cm<sup>2</sup> hardness and  $9.35 \pm 0.229$  min disintegration time which was noticed with in accepted limits. [Table No. 4] As there are no pharmacopoeial standards available for *Kukkutnaki Guggulu*, hence the values estimated in present study are compared with previous

reported work on *Guggulu* formulations as well as with API. Such comparison is not completely applicable to *Kukkutanakhi Guggulu*, however it helps to judge the quality of the product upto expected level. In HPTLC analysis of *Kukkutnaki Guggulu*, total 14 peaks for each of the three batches of *Kukkutnaki Guggulu* were observed and highest peak were seen at Rf value 0.18 and as per table no. 3 the coinciding max Rf values were 0.10, 0.18, 0.33, 0.42 and 0.80. Hence, it can be said that standard parameters were developed for current formulation since all the ingredients used in the preparation has shown equal distribution in all three batches. [Figure no. 2 to 7, Table no. 5]

**CONCLUSION:** Standardized *Kukkutnaki Guggulu* was having total ash%  $6.49 \pm 0.553$ , acid insoluble ash%  $1.323 \pm 0.547$ , water soluble extract%  $35.64 \pm 0.590$ , alcohol soluble extract%  $20.45 \pm 0.822$ , pH  $3.81 \pm 0.041$ , moisture content%  $9.63 \pm 0.851$ , hardness ( $\text{Kg/cm}^2$ )  $1.42 \pm 0.080$ , uniformity of weight %  $4.267 \pm 0.163$ , friability%  $0.566 \pm 0.232$ , disintegration time (min)  $9.35 \pm 0.229$ . HPTLC analysis of *Kukkutnaki Guggulu* using toluene: ethyl acetate: formic acid (7:3:1) as mobile phase showed 14 peaks for all three batches. According to the properties of the contents of *Kukkutnaki Guggulu*, the probable *Rasa*, *Virya* and *Vipaka* are *Tikta*, *Katu*, *Kashaya Rasa*; *Ushna Virya* and *Katu Vipaka*. So in the current analytical study, the analytical standard of *Kukkutnaki Guggulu* was developed which will be ready reference for the next researcher.

**Scope for further research:**

*Kukkutanakhi Guggulu* is not mentioned in classical texts however it is mostly utilized formulation by Ayurveda physicians for the management of manifestations which are similar to cancer. This study provides analytical standards for *Kukkutanakhi Guggulu* which are needed to prepare effective formulation. Hence the current study will be pioneer research source for the further anticancer research on plant originated products, to study its effect on

targeted cancers, specific in vivo scientific studies and human clinical trials which are needed to be carried out by further researchers.

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