



PREPARATION AND EVALUATION OF TRIPHALA GUGGUL TABLET CONTAINING GUGGUL PURIFIED BY DIFFERENT METHODS AND ASSAY OF MARKER COMPOUNDS BY HPLC: AN AYURVEDIC MEDICINE.

Ayurveda

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ABSTRACT

Triphala Guggul is a well-known drug of indigenous system of medicine. Guggul is reported to possess antioxidant, antibacterial, antifungal, cytotoxic, thyroid stimulatory, cardio protective, hypolipidaemic and anti-inflammatory activities. In the present study, three different batches of *Triphala Guggul* tablets (TG1, TG2 and TG3) were prepared by using guggul purified by different methods. Different physicochemical parameters of all three batches of tablets were determined according to the method given in Ayurvedic Pharmacopoeia of India. Level of marker compounds (Guggulsterone E and Z, gallic acid and piperine) in three different batches of tablets were determined by HPLC method. Marked difference in physicochemical parameters were observed for three different batches of tablets. Level of guggulsterone E & Z in three different batches of tablets (TG1, TG2 and TG3) were found to be 0.004 %, 0.0089 % and 0.0036 % respectively. Level of gallic acid and piperine in three different batches of tablets (TG1, TG2 and TG3) were found to be 1.84 %, 1.87 % & 0.92 % and 0.24 %, 0.06 % & 0.02 % respectively. Since biological activities of any formulation depends on the level of chemical constituents present. So, it may be concluded that tablets from TG2 batch will be much better as compared to TG1 and TG3.

KEYWORDS

Triphala guggul, HPLC, guggulsterone, piperine, gallic acid, standardization.

INTRODUCTION:

Triphala Guggul is a traditional Ayurvedic medicine, contains dried fruits of medicinal plants viz. *Terminalia chebula* (Retz.) Lyons (*Combretaceae*), *Terminalia bellerica* (Gaertn.) Roxb. (*Combretaceae*) and *Embolia officinalis* Gaertn. (*Euphorbiaceae*), *Commiphora whitti* (Arn.) Bhandari (*Burseraceae*) and *Piper longum* L. (*Piperaceae*) [1]. It use to treat the many disorders including allergy, sinusitis, fester, costiveness, haemorrhoids, high cholesterol level and as a purgative. Gallic acid, piperine, guggulsterone E and Z are the main key phytochemical constituents of *Triphala Guggul*. Anti-mutagenic, anti-inflammatory and anti-cancers activities were found in gallic acid and act as good antioxidant. Myrrhanol A and myrrhanone A are important triterpenes found in guggul, which shown the very good anti-inflammatory activity [2]. Guggul have hypolipidaemic, anti-inflammatory, antioxidant [3] [4] antibacterial, antifungal [5] [6], cytotoxic [7] [8], thyroid stimulatory [9] [10] as well as cardio protective activities [3] [11]. Its constituents guggulsterone inhibits tumor cells spreading and raised apoptosis [12]. The guggul is good for coronary heart disease enhances fibrinolysis and diminished platelet aggregation [13] [14]. Piperine worked as bio-enhancers for many Allopathic, Ayurvedic and Unani drugs [15]. It interact with drug-metabolizing enzymes, and amenable for oxidation, hydroxylation and glucuronidation of particular drug. Guggulsterone E and Z are the chief constituents of the plant *C. whitti*, which inhibits angiogenesis *in vitro* and *in vivo* [16].

Crude drugs obtained from the natural sources possess several unwanted and toxic materials [17], [18]. Plant materials available in the market are not free from adulteration. Common adulterants in guggul are sand, wood pieces, pieces of bark and gum of shallaki (*Boswellia serrata*) [19]. *Shodhana* is a unique process of detoxification mentioned in Ayurveda which is employed to purify/detoxify as well as to potentiate the effect of various kinds of drugs [20].

Considering the pharmaceutical application, an attempt was made for the purification (*Shodhana*) of guggul using three different media (*Triphala* Kwath (Decoction), Cow's urine and *Vasapatra Kwath* (*Adhatoda vasica* Nees., leaf's decoction) of purification mentioned in Ayurvedic texts. Guggul purified by using three different media were used for the preparation of three different batches of *Triphala Guggul*

tablet (TG-1, TG-2 and TG-3) respectively. Level of marker phytochemical constituents (guggulsterone E and Z, gallic acid and piperine) were determined in three different batches of tablet by using high performance liquid chromatography (HPLC) [15].

MATERIALS AND METHODS

Chemicals and solvents

Guggulsterone E & Z, piperine and gallic acid were procured from Sigma-Aldrich Co. LLC., India. All analytical grade chemicals, solvents and reagents were used. Millipore filter used for filtration of distilled water.

Procurement of raw materials

Raw drug guggul was purchased from Radhika Enterprises, New Delhi, India. Remaining raw drugs such as *Haritaki* (*T. chebula*), *Vibhitaki* (*T. bellerica*), *Amalaki* (*E. officinalis*) and dry *Vasapatra* (*Adhatoda vasica* Nees.) were purchased from the local market of Vijayawada, Andhra Pradesh, India. Raw drugs were certified on the basis of macroscopic, microscopic characters and physicochemical parameters as described in the Ayurvedic Pharmacopoeia of India [21]. Cow's urine was freshly collected from Goshala of Vijayawada, Andhra Pradesh, India.

Table 1: Ingredients *Triphala Guggul* and their ratio.

Botanical Name	Family	Sanskrit Name	Part Use	Quantity
<i>E. officinalis</i>	Euphorbiaceae	Amalaki	Fruit	1 part
<i>T. bellerica</i>	Combretaceae	Vibhitaki	Fruit	1 part
<i>T. chebula</i>	Combretaceae	Haritaki	Fruit	1 part
<i>P. longum</i>	Piperaceae	Pippali	Fruit	1 part
<i>C. wightii</i>	Burseraceae	Guggulu	gum	5 part

Shodhana of guggul

Certified each raw drug *Haritaki*, *Vibhitaki* and *Amalaki* were made coarse powder and mixed in equal quantities (*Triphala*). *Triphala* (500 g) was filled into an extraction vessel. Distilled water (8.0 L) was filled into the extraction vessel and mixed properly, Then the mixture was remain stand for 12 hours. The mixture was heated slowly to boil by using gas stove and heating kept continue to water evaporation by gentle boiling until drug-water mixture remains one-fourth of its original volume. After that, mixture was cooled at room temperature. The mixture was strung with muslin cloth and the marc obtained was

washed by hot water. The strained filtrate was two times filtered and concentrated it by rotatory evaporator below 60 °C. This concentrate decant is known as *Triphla Kasaya*.

From raw guggul foreign particles like sand and stone were removed manually and taken this *Guggul* (1 Kg) in the form of *pottali* (is the bundle of cotton cloth contains materials). It was hanged by *Dola Yantra* (an instrument used to stow medicine in liquids media such a manner that it was completely engrossed in liquid media but did not touch to the bottom of the container) in *Triphla Kasaya* (4 L) taken in an iron pot. *Triphla Kasaya* was heated at the temperature between 85 °C- 95 °C until all the guggul passed into the *Triphala Kasaya* through the cotton cloth. The relic remains in the *pottali* was discarded. Fluid acquired was boiled until modified in semi-solid mass. The semi-solid mass was seared in a tray dryer at 60 °C [21], [22]. Alike process was followed for the purification of guggul with Cow's urine and *Vasapatra Kwath*.

100 g of powder of each *Haritaki*, *Bibhitaki*, *Amlaki* and *Pippali* were mixed with the powder of purified guggul (500 g) in three different media separately and mixed well by adding needed quantity of distilled water to make a solid mass. It was cut manually into small pieces and dried in tray dryer at 60 °C and modified into granules by using granulator. Granules are compressed in tablet compression machine to get tablets (500 mg) [23].

Evaluation of *Triphala Guggul* tablets.

Organoleptic properties (colour, taste, odour and appearance) of tablet were evaluated by visual inspection. [23]. Weight variation, disintegration and hardness test (Strong Cobb hardness tester) of tablets were also evaluated.

Assessment of physicochemical parameters

Assessment of Loss on drying, total ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value and pH according to the procedure mentioned in Ayurvedic Pharmacopoeia of India [24].

Quantitative estimation of guggulsteron E & Z

Essay solutions were made by refluxing of 3.06213 g (TG1), 3.07398 g (TG2) and 3.06581 g (TG3) of sample with 50 mL of acetonitrile on water bath for 30 min. It was cooled and filtered in a volumetric flask by Whatmann filter paper No. 45 and volume was setup to 100 ml by addition of require amount of acetonitrile. Less quantity of sample was filtered through Millipore filter (pore size 0.45 µm) in a vial and kept in sample holder of HPLC instrument [25].

HPLC instrument (Agilent Technologies 1260 infinity) was made up of stainless steel column, which dimension was 250 mm x 4.6 mm and packed with C-18 silica gel (5 µm). Combination of acetonitrile: water (45:55) was used after filtration and degassed as mobile phase with flow rate 2 mL/min. specimen (20 µL) was injected in the column and maintained the temperature as 25 °C.

Specimen was detected at 242 nm by using UV-visible spectrophotometer as detector [25].

Quantification of gallic acid [26]

Coarse powder of TG1 (2.05981 g), TG2 (2.06246 g), and TG3 (2.05291 g) were suspended in 50 ml of distilled water and heated on water bath for 15 minutes. It was cooled and final volume was setup to 100.0 ml with distilled water and filtered. HPLC instrument (Agilent Technologies 1260 infinity) was made up with stainless steel column of dimension 250 mm x 4.6 mm and filled with C-18 silica gel (5 µm). Different ratio of acetonitrile and phosphate buffer was used as mobile phase in gradient manner, started from 100 % acetonitrile to 100 % buffer solution.

Buffer solution was ready by dissolving 0.136 g of potassium di-hydrogen orthophosphate in 500 ml of distilled water. It was added with 0.5 ml of orthophosphoric acid and final volume was set up to 1000 ml with distilled water. Flow rate of mobile phase was kept 1.5 ml per minute. Specimen (20 µL) was injected in the column and temperature was kept at 25 °C. Specimen was detected at 270 nm by using UV-visible spectrophotometer as detector [25].

Quantification of piperine

Test solutions for the estimation of piperine were prepared by

suspending coarse powder of TG1 (2.09410 g), TG2 (2.13882 g) and TG3 (2.0012 g) in 50 mL of methanol and boiled on water bath for 15 minutes. It was cooled and set up to 100 mL. by methanol and filtered. HPLC instrument (Agilent Technologies 1260 infinity) was made up with stainless steel column of dimension 250 mm x 4.6 mm and filled with C-18 silica gel (5 µm).

Different ratio of acetonitrile and phosphate buffer was used as mobile phase. Buffer solution was made by dissolving 0.136 g of potassium di-hydrogen orthophosphate in 900 mL. distilled water, pH was maintained 2.5 with orthophosphoric acid and diluted up to 1000 ml with distilled water. Flow rate of mobile phase was adjusted 1.5 ml per minute. Specimen (20 µL) was injected in the column and set the temperature at 25 °C. Specimen was detected at 340 nm by using UV-visible spectrophotometer as detector [27], [28].

RESULTS

Evaluation of *Triphala Guggul* tablets

Organoleptic evaluation of three different batches (TG1, TG2 and TG3) of tablets represented in (Table-1). Total 20 tablets were taken for weight variation study from all three batches, out of which one tablet shown deviation from permissible limit ($\pm 5\%$) in weight [23], (Table 2). Disintegration time for tablets was found to be 28, 31 and 29 min. for TG1, TG2 and TG3 respectively.

Hardness of tablets for three different batches (TG1, TG2 and TG3) were found to be 4.9, 5.2, and 5.1 (Kg/cm²) respectively (Table 2). Results of different physico-chemical parameters of all three batches of tablets represented in (Table 3).

Level of guggulsteron E & Z in three different batches of tablets (TG1, TG2 and TG3) were found to be 0.004 %, 0.0089 % and 0.0036 % respectively (Fig. 1 A-C). Level of gallic acid and piperine in three different batches of tablets (TG1, TG2 and TG3) were found to be 1.84 %, 1.87 % & 0.92 % (Fig. 2 A-C) and 0.24 %, 0.06 % & 0.02 % respectively (Table 4 and Fig. A-C).

Table- 1 Organoleptic properties of tablets from three different batches-

Organoleptic appearance	Tg1	TG2	TG3
Colour	Black Shiny	Light brown	Dark brown
Taste	Kasaya (Astringent), Katu (Bitter)	Katu, Tikta (Acrid)	Katu, Tikta
Odour	Triphala smell	Go-mutra smell	Vasa smell

Table- 2 Evaluation of different parameters for tablets from three batches.

Parameters	Tablets		
	TG1	TG2	TG3
No. of tablets showing deviation from average weight by more than $\pm 5\%$	1	1	1
No. of tablets showing average weight $\pm 5\%$	19	19	19
Disintegration test (min.)	28	31	29
Hardness test (Kg/cm ²)	4.9	5.2	5.1

Table.3 – Various physico-chemical parameters of three different batches of tablets

Physico-chemical parameters	Tablets		
	TG1	TG2	TG3
Loss on drying (%w/v)	7.71	8.06	7.96
Total ash (% w/w)	7.62	6.89	7.23
Acid insoluble ash (%w/w)	4.03	2.96	2.63
Alcohol soluble extractive (% w/v)	38.21	31.83	32.09
Water soluble extractive (% w/w)	50.41	46.37	45.98
pH (1% solution)	5.3	5.9	5.6

Table.4 HPLC analysis of the tablets from three different batches for the estimation of the levels of marker compounds.

Marker compounds	Quantity (% w/w)		
	TG1	TG2	TG3
Guggulsteron (E&Z)	0.0040	0.0089	0.0036
Gallic acid	1.84	1.87	0.92
Piperine	0.24	0.06	0.02

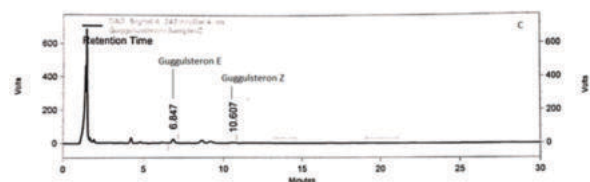


Figure. 1 (A-C) HPLC analysis of guggulsteron *E* & *Z* in three different batches of tablets TG1, TG2 and TG3 respectively.

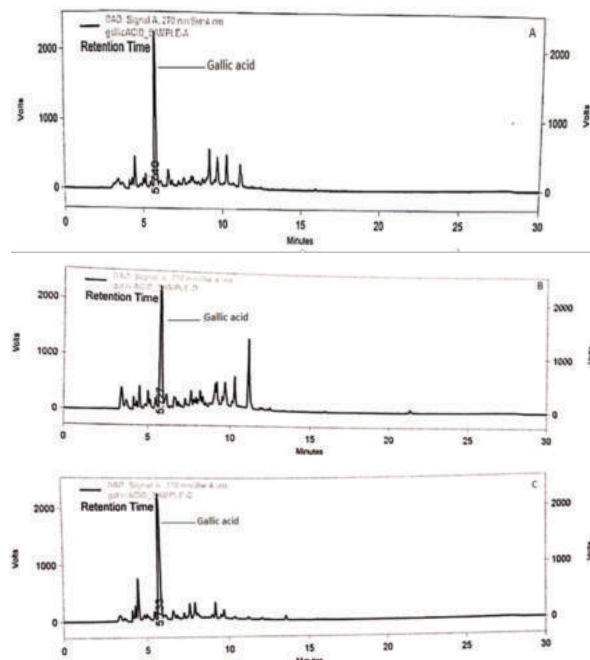


Figure. 2 (A-C) HPLC analysis of gallic acid in three different batches of tablets TG1, TG2 and TG3 respectively.

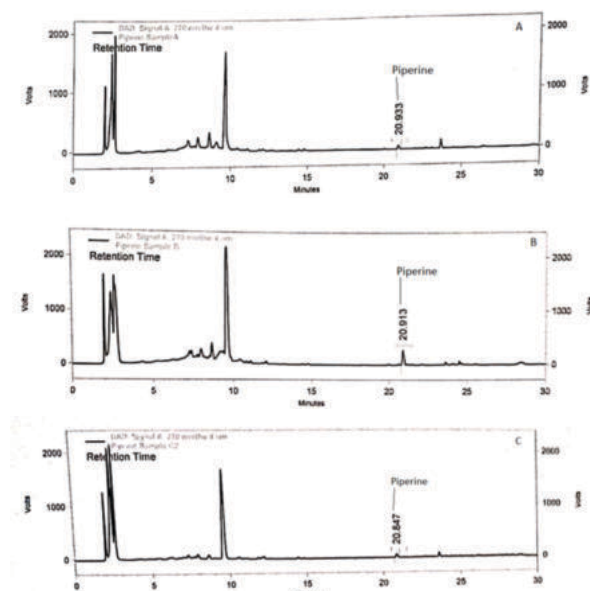


Figure. 3 (A-C) HPLC analysis of piperine in three different batches of tablets TG1, TG2 and TG3 respectively.

DISCUSSION

Triphala guggulu is a traditional poly-herbal formulation in the *Ayurvedic* system of medicine. It contains purified *guggul* (*Commiphora wightii* Arn.), long pepper (*Piper longum* L.) and *Triphala* (fruits of *Phyllanthus emblica* L., *Terminalia chebula* Retz, and *Terminalia bellirica* Gaertn.) [29].

Triphala Guggulu mostly used to manage *Vatarakta* (gout),

Bhagandar (fistula), *Gandamala* (goitre), *Vranaropana* (wound healing), *Kushtha* (leprosy), *Pakvavidradhi* (abscess), and *Asthibhagna* (fractures) [30].

In this study we have sensible to demarcated the effect of three various media, viz. *Triphala Kashaya* (Decoction), cow's urine and *Vasa patra kashaya* (Leaves decoction) used for the purification of raw *guggul* and evaluate the physico-chemical parameters of these formulation (TG1, TG2, TG3) which was made from three purified *guggul*. *Triphala guggul* tablets made by different purified *guggul* was found to be lesser different with context to its physico-chemical parameters and Level of guggulsteron *E* & *Z* in three different batches of tablets (TG1, TG2 and TG3) were found to be 0.004 %, 0.0089 % and 0.0036 % respectively. Level of gallic acid and piperine in three different batches of tablets (TG1, TG2 and TG3) were found to be 1.84 %, 1.87 % & 0.92 % and 0.24 %, 0.06 % & 0.02 % respectively. This study is extent to prove the modification in three batches of *Triphala guggul* tablets which made by three different purification media of raw *guggul* in context to their physicochemical and HPLC studies. The purification procedures and media are presumptive to enhance medicinal characters of the formulation which have *guggul* as a key ingredient and also for removal of its unwanted impact in order to texture it secure for human consumption. This outflow can be described with further experimental and clinical studies to negotiate the advance probability and security in various disease conditions.

CONCLUSION

The present study exhibited that purification media like *Triphala kasay*, *Cow's urine*, and *Vasapatra Kasaya* prominently make an impression on the organoleptic characters, physico-chemical parameters and quantity of its active markers guggulsteron *E* & *Z*, gallic acid, and piperin in *Triphala Guggul* tablets. The quantity of marker compounds were varies in all three different batches of tablets which were prepared by using purified *guggul* in three different media of purification. Quantity of guggulsteron *E* & *Z*, gallic acid and piperine were found maximum in TG2 tablet as compared to TG1 and TG3. Hardness and disintegration time of three different batches of tables were found more or less similar. Since biological activities of any formulation depends on the level of chemical constituents present. So, it may be concluded that tablets from TG2 batch will be much better as compared to TG1 and TG3, Purification media play an important role to varies the quality of final product and its therapeutic value.

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