Purification of *Guggul* by *Ayurvedic* process (*Shodhana*), estimation of Guggulsterone E & Z before and after purification by HPLC analysis

Prince Kumar Pal¹, Goli Penchala Prasad¹, Gajji Babu¹, Dev Nath Singh Gautam² & Narendra Kumar Singh³⁴*

¹Regional Ayurveda Research Institute for Skin Disorders (Under CCRAS, Ministry of Ayush, Govt. of India), Vijaywada-520 015, Andhra Pradesh, India;
²Department of Rasa Shastra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221 005, Uttar Pradesh, India;
³Pharmacy Ayurveda Research Laboratory, Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur-231 001, Uttar Pradesh, India.

E-mail: narendra_pharma1982@rediffmail.com

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*Guggul* is one of the *Ayurvedic* product in great demand, used for over thousands of years and high status for its versatile use in several ailments. The two most important pharmacological properties of *guggul* are its anti-inflammatory and hypolipidaemic actions. The present study was undertaken to find out the effect of three different media of purification (water, cow’s urine and *Triphala Kashaya* (decoction of three myrobalans)) of raw *guggul* on its markers Guggulsterone E & Z at different heating temperature during *Shodhana* (purification) process. The *guggul* purified by different methods was found to be quite variable in reference to physico-chemical parameters and its marker compounds Guggulsterone E & Z by HPLC analysis at similar chromatographic conditions. The Guggulsterone E & Z was found in the order: water > cow’s urine > *Triphala Kashaya* (0.29 w/w, 0.24 w/w and 0.16 w/w, respectively at 85 °C to 95 °C). But the same raw *guggul* purified with same media like water, cow’s urine and *Triphala Kashaya* at temperature 60 °C to 70 °C showed the presence of high quantity of Guggulsterone E & Z (0.49 w/w, 0.45 w/w and 0.30 w/w for water, cow’s urine and *Triphala Kashaya*, respectively), as compared to the quantity of Guggulsterone E & Z found at temperature 85 °C to 95 °C.

**Keywords:** *Guggul, Shodhana, Guggulsterone E & Z, HPLC.*

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*Guggul,* an oleo-gum-resin is obtained from *Commiphora wightii* (Arnott.) Bhandari (syn. *Commiphora mukul*) of family Burseraceae. It is mentioned in classic *Ayurvedic* texts to be highly efficacious in the treatment of various disorders particularly obesity, lipid disorders, rheumatism, arthritis, cardiovascular and neurological diseases. The hypolipidaemic, anti-atherosclerotic and anti-inflammatory activities of *guggul* have been investigated in detail and gugulipid (an ethylacetate extract of *guggul*) was commercially marketed in India in 1988 as a hypolipidaemic drug. The effectiveness of *guggul* for treating arthritis has been well demonstrated. It has been effectively used to reduce the possible cause of inflammation by removing deposits of waste and toxic material from the body including mineral deposition from the joints, thereby acting as anti-rheumatic and anti-inflammatory agent. Two triterpenes, myrrhanol A and myrrhanone A of *guggul* have been shown to possess potent anti-inflammatory activity. In addition to its hypolipidaemic and anti-inflammatory activities, *guggul* has been reported to possess antioxidant, antibacterial, antifungal, antitumor, thyroid stimulatory and cardioprotective activities. *Guggul* is also known to inhibit tumor cell proliferation and induced apoptosis. The beneficial effect of *guggul* in coronary heart disease has been associated with increased fibrinolysis and reduced platelet aggregation.

Though, *guggul* used for the treatment of several diseases, yet it is mentioned in Ayurvedic texts that administration of raw *guggul* may sometimes lead to skin rashes, irregular menstruation, diarrhoea, headache, mild nausea and very high doses cause liver toxicity. In order to overcome the several unwanted effects of raw *guggul,* a number of traditional purification processes (*Shodhana*) have been described.

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³Corresponding author
in Ayurveda with different ‘dravyas’, i.e., fluids. These purification processes not only remove the adverse effects of guggul but also enhance the therapeutic activity. It is also mentioned in Ayurvedic texts, that guggul must be purified before being incorporated into herbal formulations. There are large numbers of commercial polyherbal anti-inflammatory formulations available in the market in which guggul is a chief ingredient. However, no study has been done to explore the possible changes during the purification process and its probable effect on level of key constituents, Guggulsterone E & Z. Therefore, the present study was carried out to purify raw guggul by using three different Shodhana Dravyas (purifying substances), viz. water, cow’s urine and Triphla Kashaya as mentioned in Ayurvedic texts and to evaluate the possible changes in the level of key constituents Guggulsterone E & Z during its purification process.

Materials and methods

Chemicals and solvents

Guggulsterone E & Z were purchased from Sigma-Aldrich Co. LLC., India. All other solvents, chemicals and reagents used were of analytical grade. Distilled water wherever used was filtered from Millipore filter.

Procurement of raw material

Crude drug guggul gum was procured from Radhika Enterprises, New Delhi, India and authenticated on the basis of macroscopic, microscopic characters and physico-chemical parameters as described in the Ayurvedic Pharmacopoeia of India. Other crude drugs such as Haritaki (Terminalia chebula Retz.), Vibhitaki (Terminalia bellirica Gaertn Roxb.) and Amalaki (Emblica officinalis Gaertn. syn: Phyllanthus emblica L.) (these three drugs are constituents of Triphla in equal proportions) were procured from the local market of Vijayawada, Andhra Pradesh, India and authenticated as per the parameters described in the Ayurvedic Pharmacopoeia of India. Cow’s fresh urine was collected from Goshala of Vijayawada Andhra Pradesh, India.

Shodhana of guggul

Authenticated crude drugs Haritaki, Vibhitaki and Amalaki were coarsely powdered and mixed in equal quantities. This mixed powder called ‘Triphla’ (500 g) was transferred to an extraction vessel. Potable water (8.0 L) was added to the extraction vessel and mixed thoroughly. The mixture was allowed to stand for next 12 h. The content was then heated using gas stove and gentle boiling was maintained until drug-water mixture got reduced to one-fourth of its original volume. Afterwards boiling mixture was cooled at room temperature and solid residue was allowed to settle down. The mixture was strained through muslin cloth and the solid residue was rinsed with boiling potable water. The strained mixture was once again filtered and filtrate was pooled to get ‘Triphla Kashaya’.

The foreign matter like sand and stone were removed from guggul. The guggul (1 Kg) was broken into small pieces and taken in the pottali (bundled in cotton cloth). It was hung in Dola Yantra (an instrument used to impregnate medicine in liquids) in Triphla Kashaya (2 L) taken in an iron vessel. Triphla Kashaya was heated at a temperature between 85 °C- 95 °C until all the guggul passed into the fluid through the cotton cloth. The residue in the pottali was discarded and fluid was filtered and boiled until it was converted in form of semi-solid mass. The mass was dried in tray dryer at 50 °C and pounded with a pestle in a stone mortar. The same procedure was followed for the purification of guggul with cow’s urine and water at different temperature for comparative analytical determination.

Determination of physico-chemical parameters

Loss on drying, total ash, acid insoluble ash, water soluble extractive value and resin content were determined before and after the purification of guggul as per the Ayurvedic Pharmacopoeia of India.

Quantitative estimation of Guggulsterone E & Z

Test solution was prepared by refluxing of 3.0 g of sample with 50 mL of acetonitrile on water bath for 30 min. It was cooled and filtered through Whatman filter paper No. 45 in a volumetric flask and volume was made up to 100 mL by adding sufficient amount of acetonitrile. Small amount of sample was filtered through millipore filter (0.45 µm pore size) in a vial and placed in sample holder of HPLC instrument.

HPLC instrument (Agilent Technologies 1260 infinity) was equipped with stainless steel column of dimension 25 cm x 4.6 mm and packed with C-18 silica gel (5 µm). A filtered and degassed mixture of 45 volume of acetonitrile and 55 volume of water was used as mobile phase with flow rate 2 mL/min. Samples (20 µL) were injected in the column and temperature was maintained at 25 °C. UV-visible
spectrophotometer was used as detector and sample was detected at 242 nm.31.

**Results**

In this study, an attempt has been made to purify the guggul by using three different media as described in ancient treatise and to assess the difference in terms of physico-chemical evaluation and HPLC studies. Evaluation of the effect of different temperature during the Shodhana process was also assessed.

In physico-chemical evaluation at 85 °C to 95 °C and 60 °C to 70 °C, it was observed that loss on drying is high in raw guggul which is comparatively less in the purified guggul with different media like water, cow’s urine and Triphala Kashaya (Tables 1 & 2). Acid insoluble ash is less in guggul purified in cow’s urine at 85 °C-90 °C range (Table 1) and guggul purified in water in the temperature range of 60 °C to 70 °C (Table 2). Water soluble extractive value is low in guggul purified in cow’s urine in both the range of temperatures (Tables 1 & 2). Resin content is low in guggul purified in Triphala Kashaya in both the range of temperatures (Tables 1&2). The quantity of Guggulsterone E & Z is more in guggul treated with water (Fig. 1a &b) followed by cow’s urine (Figs. 2a & b) and Triphala Kashaya (Fig. 3a & b) at both the range of temperatures (Table 3). The quantity of Guggulsterone E & Z was found less when purification process done in different media at temperature 85 °C to 95 °C (Figs. 1a, 2a & 3a) as compared to the quantity of Guggulsterone E & Z found at temperature 60 °C to 70 °C (Figs. 1b, 2b & 3b) (Table 3). Guggulsterone Z was found to be more in guggul purified with water (Fig. 1a) while Guggulsterone E was found to be more in guggul purified with cow’s urine (Fig. 2a) at temperature 85 °C to 95 °C. At 60 °C to 70 °C temperature range Guggulsterone E was found to be more in guggul purified with water (Fig. 1b) and Guggulsterone Z was found to be more in guggul purified with cow’s urine (Fig. 2b).

**Discussion**

Guggul is used widely in Ayurvedic system of medicine since time immemorial. Ayurveda advocates that guggul must be administered only after its purification (Shodhana). There are seven different media (dravyas), viz. nirgundi swaras with haldi churna, vasa Kashaya, cow’s milk, vasa swarasa, water, cow’s urine and Triphla Kashaya which have been reported in different classical texts of Ayurveda for purification of guggul.27. These purification processes not only potentiate the medicinal properties of the guggul but also detoxify it, thus making it safer for human consumption.28

In the present study we have tried to determine the effect of three different media, viz. water, cow’s urine and Triphala Kashaya used for the purification of raw guggul on its physico-chemical parameters (Tables 1&2) and on the level of Guggulsterone E & Z at different heating temperature during Shodhana process (Table 3). The level of Guggulsterone E & Z was determined by HPLC analysis (Figs. 1-3). The
guggul purified by different methods was found to be quiet different with reference to its physico-chemical parameters and level of Guggulsterone E & Z. The level of Guggulsterone E & Z found in guggul purified with three different Shodhana media was found in the following order: water (0.29 w/w) > cow’s urine (0.24 w/w) > Triphla Kashaya (0.16 w/w) at 85 °C to 95 °C temperature of Shodhana process while water (0.49 w/w) > Cow’s urine (0.45 w/w) > Triphla Kashaya (0.30 w/w) at temperature 60 °C to 70 °C of Shodhana process (Table 3 & Figs. 1-3).

The quantity of Guggulsterone E & Z increases with decrease in the temperature of purification process except Guggulsterone E in Triphla Kashaya (Table 3). It was also found that temperature of purification process also affects the physico-chemical parameters of guggul (Tables 1&2).

Previous studies reported the anti-inflammatory activity of raw and purified guggul in different media. It has been clearly revealed that all the methods of purification enhanced the anti-inflammatory activity of guggul. The anti-inflammatory activity of guggul purified by different methods was in the order of nirgundi swaras with haldi curna > vasa Kashaya > cow milk > vasa swarasa > water/cow’s urine > Triphla Kashaya. Guggul purified by nirgundi swaras with haldi curna at a dose level of 400 mg/kg showed significant protection (50.16 %) at 3 h against 49.7 % protection exhibited by ibuprofen at the same time interval32. The minimum protection observed was 36.72 % with Triphla Kashaya guggul at 3 h. Present study is limited to prove the variation in purified guggul of different media in respect to their physico-chemical and HPLC studies. The purification processes is presumed to potentiate the medicinal properties of the guggul and also for detoxification of its unwanted effects in order to make it safer for human consumption. Present findings can be correlated with further experimental and clinical studies to determine the high potentiality and safety in various disease conditions.

**Conclusion**

The present study demonstrates that purification media like water, cow’s urine and Triphala Kashaya significantly affect the physico-chemical parameters of guggul and quantity of its active markers Guggulsterone E & Z separately as well as combined. The quantity of marker compounds mainly decreases when purification temperature increases in all three purification media. The result of the present study was found comparable with the methods mentioned in the classical texts as mild heat is better for guggul paka.
So it may be concluded that all the three media used for the purification of guggul are effective at low temperature but water was found to be the best media.

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References