Summary
7. SUMMARY

Guggulu is one of the esteemed products of Ayurveda and enjoys high status for its versatile use in the treatment of varied clinical disorders particularly obesity, lipid disorders, rheumatism, arthritis, cardiovascular and neurological diseases. The natural variability in the composition due to geographic location, age, climate, collection time and genetic variation, needs immediate attention to render reproducibility of therapeutic effect of guggulu formulations. The similarity of guggulu to several other cheap gums allows its easy adulteration which is difficult to detect in the absence of well defined standards for this drug of high repute. Ayurvedic texts describe guggulu purification before incorporating it into various formulations. It is believed that the purification (shodhanavidhi) enhances its therapeutic efficacy and reduces the adverse effects. However, there has been no investigation of purification of guggulu and its effect on chemical composition. The changes that take place during purification are required to be carefully investigated before attempting to standardize guggulu or its Ayurvedic formulations. Furthermore, traditionally guggulu has been administered in the form of chewable vattis (vatti is a crude form of a tablet), which lack accuracy and precision for administering a dose. Numerous variables are responsible for inconsistency of guggulu polyherbal formulations but no effort has been made to study and control these variables to make them acceptable as per modern standards.

The present project was, therefore, planned to carry out the detailed studies on this plant. Guggulu samples from different geographic and market sources were procured.

A total of 12 guggulu samples, coded as, RG-A1, RG-A2, RG-D1, RG-D2, RG-D3, RG-D4, RG-D5, RG-C, RG-Dun, RG-J, RG-L and RG-M were procured. Two samples were collected in two different seasons of summer and winter from guggulu herbal farm, Mangliawas, Ajmer (Rajasthan). The other plant drugs used in the present project were procured from the local market or were collected from the Medicinal Plants Garden of our Institute. The plant drugs were duly
Summary

authenticated by NISCAIR or by comparing them with the monographs given in *The Ayurvedic Pharmacopoeia of India* and were checked for compliance before use. The present work was accomplished in two parts: Part-I & Part-II.

**PART-I: STANDARDISATION STUDIES ON GUGGULU**

**PHYTOCHEMICAL STUDIES**

The outstanding therapeutic profile of guggulsterones conveniently allows these two isomers to be used as markers to standardize this material of great commercial importance. The guggulu was subjected to phytochemical investigations to isolate guggulsterones in the laboratory. The petroleum ether extract of guggulu was chromatographed over silica gel in column chromatography followed by medium pressure liquid chromatography to isolate *E*- and *Z*- guggulsterones. Their identity was confirmed by performing Co-TLC in different solvent systems with authentic samples of guggulsterones procured from Chromadex, USA and through mixed melting points.

**PURIFICATION STUDIES OF GUGGULU**

Ayurvedic method directs that guggulu should be purified before use. There are seven different media (dravyas) that can be used for purification of guggulu as per classical texts of Ayurveda. These all seven media were investigated in present study to purify guggulu and examine their effect. These seven dravyas were triphla kasaya (decoction of fruits of *Terminalia chebula*, *Terminalia bellirica* and *Emblica officinalis*), cow urine, cow milk, vasa swaras (juice of *Adhatoda vasica*), vasa kasaya (decoction of *Adhatoda vasica*), nirgundi swaras with haldi curna (juice of *Vitex negundo* with powder of *Curcuma longa*) and water. The purification studies were conducted using one of the guggulu sample, RG-D5 procured from Delhi, which was shown to be a genuine sample. The differently purified guggulu samples were coded as PG-1 to 7, respectively, and were subjected to chemical, analytical and biological investigations.
CHEMICAL STANDARDIZATION
The fingerprint profile provides an easy tool to identify and ensure quality of a plant drug. The technique was, therefore, used to make comparison of different guggulu samples and to study effect of purification on chemical composition of this drug. Several solvent systems were tried in an attempt to develop a well-resolved TLC fingerprint profile of guggulu. The best chromatogram for cultivated guggulu sample was developed using precoated silica gel TLC plate developed in toluene : acetone (9 : 1) and visualizing the spots under UV light or derivatizing the spots with anisaldehyde-sulphuric acid reagent. The comparison of chromatograms of different guggulu samples revealed that each spot was not seen in all samples but all samples showed the presence of guggulsterones in variable amounts. The effect of purification was apparent in the fingerprint profile. Some of the components had disappeared while spots of few others became more intense including that of E- & Z- guggulsterones. Among the added new spots, some were derived from the medium of purification.

ANALYTICAL STUDIES
A validated method of estimation of guggulsterones was developed using three analytical tools- TLC-densitometry, HPLC and UPLC. The comparison of results of these analytical techniques was made to highlight advantages and disadvantages of using either of these techniques for the estimation of guggulsterones. The best technique for the estimation of these markers was identified on the basis of ease, economics, efficiency and quality of results. The two samples of guggulu, RG-D6 (Delhi) and RG-Dun (Dehradun) showed the highest content of guggulsterones in all the techniques, whereas, sample procured from Chandigarh, RG-C was the poorest in guggulsterone content. It was interesting to note that out of the two cultivated samples procured from guggulu herbal farm, Mangliawas, Ajmer, the sample collected in summer (RG-A1) showed higher content than the sample collected in winter (RG-A2). The results clearly indicated the natural variability in the composition of gum and the importance of the time of collection.
Summary

The guggulu samples purified using different media, PG-1 to 7, were also analysed for guggulsterone content. The effect of purification on the content of guggulsterones was investigated using TLC-densitometry, HPLC and UPLC. The results of different analytical techniques were also compared. The results showed that purification enriches the content of total guggulsterones, especially, Z-guggulsterone. PG-6 (guggulu purified with nirgundi swaras and haldi) showed the maximum enrichment and PG-7 (guggulu purified with water) showed the minimum and insignificant improvement in guggulsterones content.

The developed TLC analytical method for quantitative estimation of guggulsterones was comparable to that of UPLC method. It was shown to be reproducible, fast and economic method that is ideal for routine analysis of guggulsterones in large number of guggulu samples. But, in terms of maintaining resolution performance and efficiency, the UPLC is the technique of choice. The results of HPLC were compromised despite numerous trials and testing of IP and USP methods as well. Several brands of columns were also tried but HPLC showed limitation in terms of co-elution of peaks with guggulsterones.

Chemometric analysis

The fingerprint profiles and results of marker analysis in guggulu samples were analysed using statistical tools. The data analysis was done using SPSS Software, version 20. The results indicated that the two cultivated samples procured from Ajmer showed complete matching with each other illustrating their close chemical similarity. The guggulu sample, RG-J was closest to genuine samples while RG-L was the most different sample. Based upon their similarity, the samples could be classified into eight different clusters in hierarchical agglomerative cluster (HCA) analysis. The results of HCA analysis were in complete agreement with that of similarity analysis. The RG-J was the most closely related sample to genuine sample while RG-M and RG-L were the least related samples in both chemometric analyses. On the basis of content of guggulsterones, the purified samples were classified in two clusters. The samples were grouped in two categories based on their values in rotated
component matrix scores. The samples which had significant scores for the component were extracted in the respective component.

The results of HCA and PCA producing same inference provided proof to the validity of results in similarity assessment. The scree plot between the two extracted components revealed that the maximum difference lied in the peaks at retention time 1.92, 11.11 and 13.3 min. The last two peaks were of E- and Z-guggulsterones, respectively, which showed that the difference lied in the peak areas of these markers. Purification altered the content of guggulsterones in all the samples.

**BIOLOGICAL STUDIES**

**Anti-inflammatory activity:** The anti-inflammatory activity of raw and purified guggulu samples was determined using carrageenan-induced rat hind paw oedema at three dose levels of 100, 200 and 400 mg/kg. Anti-inflammatory response of purified guggulu samples was better in comparison to raw guggulu at all dose levels. The protection was maximum and significant in comparison to control at a dose of 400 mg/kg. Guggulu purified with nirgundi swaras and haldi was most active and the guggulu purified with triphla kasaya was the least active amongst all purified samples. The results confirmed that purification significantly influences the anti-inflammatory potency of guggulu and medium of purification further has its role.

**Analgesic activity:** The analgesic activity of all guggulu samples was evaluated by two models of nociception, tail-flick test and acetic acid-induced writhing. The results showed better response of purified guggulu than raw guggulu. The guggulu purified with nirgundi and haldi (PG-6) showed the maximum activity amongst all the samples at the highest dose of 400 mg/kg. In acetic acid-induced writhing model, the protection level (66 per cent) of PG-6 was even better than aspirin. The analgesic activity in both the models indicated that guggulu exert its effect through different mechanisms involving both central and peripheral pathways.
Summary

Antioxidant activity: The antioxidant activity was evaluated by DPPH assay. The antioxidant activity of raw guggulu was weak in comparison to ascorbic acid. The simple purification of guggulu using water showed the weakest effect while the strongest effect was observed by purifying the guggulu in juice of nirgundi and haldi.

PART-II: DEVELOPMENT AND EVALUATION OF ANTI-INFLAMMATORY PREPARATIONS OF GUGGULU

FORMULATION DEVELOPMENT

In search of a new polyherbal formulation, an initiative was taken to develop a formulation which met all essential requirements of being a quality formulation with proven therapeutic efficacy for the prevention and treatment of inflammation. The designed formulations used six Ayurvedic plants Commiphora wightii, Barleria prionitis, Asparagus racemosus, Amaranthus viridis, Stellaria media and Piper nigrum. The selection of plants was based on a survey of commercial and proprietary polyherbal anti-inflammatory formulations with detailed analysis of collected data and on the basis of chemical constituents and therapeutic profile of these plants. Two polyherbal guggulu tablet formulations, F-VI and F-VI A, were developed using approved excipients under Rule 169 vide Government of India notification 'Drugs and Cosmetics (Vth Amendment) Rules, 2005'. Formulation, F-VI contained guggulu purified with triphla kasaya and formulation, F-VI A used guggulu purified with nirgundi swaras and haldi. The guggulu purified with nirgundi swaras and haldi had shown maximum enrichment of guggulsterones and most potent anti-inflammatory activity amongst all purified guggulu samples.

FORMULATION EVALUATION

Physical and chemical evaluation: The designed formulations complied with pharmacopoeial limits for different parameters of uniformity of weight, disintegration time, hardness and friability. TLC fingerprint profile of all the plant materials and final formulations were also developed. Assay of finished formulations was developed using TLC-densitometry for guggulsterones content.
The total content of guggulsterones was 0.13 and 0.23 per cent in formulation, F-VI and F-VI A, respectively.

**Biological evaluation:** Both the formulations were evaluated for anti-inflammatory (acute, sub-chronic and topical models of inflammation), analgesic (tail-flick test and acetic-acid induced writhing), antioxidant (DPPH assay) and antiulcer (pyloric ligation-induced ulcers) activity.

**Anti-inflammatory activity:** Both formulations were evaluated in carrageenan-induced paw oedema model and the results were compared with standard drug ibuprofen. Both the formulations showed promising anti-inflammatory activity. Most significant activity was exhibited by 200 mg/kg dose. Another interesting information was that both formulations showed good protection during first 6 h, followed by a transient dip and then surge in protection at 24 h which was better than ibuprofen. It was observed that anti-inflammatory response elicited by designed polyherbal formulations in terms of per cent protection of paw oedema and the pattern of activity is in close similarity to the response of ibuprofen. Therefore, based on this information it is postulated that the designed formulations act via non-steroidal anti inflammatory pathway.

The designed formulations were further evaluated for their anti-inflammatory activity in sub-chronic (cotton pellet granuloma) and topical (croton oil ear oedema) models of inflammation. The significant protection of inflammatory response in acute / sub-chronic / topical models of inflammation explains the rational combination of selected ingredients. The formulation, F-VI A, was found to be better than formulation F-VI in ameliorating the symptoms of inflammation. It showed highly significant protection in all models of inflammation at a dose of 200 mg/kg.

**Analgesic activity:** The two formulations were evaluated for analgesic activity in two different models. They showed good analgesic activity in both the models. The analgesic activity of formulation, F-VI A was even better than the standard drug, ibuprofen in the peripheral model, whereas, it was comparable to standard
Summary

pentazocin in the central model of analgesia. This suggests that these formulations act through different mechanisms involving both central and peripheral pathways. The antinociceptive and anti-inflammatory response was variable at different doses. Such an association is known for various non-steroidal anti-inflammatory drugs (NSAIDs), especially salicylate by-products.

**Antioxidant activity**: The two formulations exhibited weak antioxidant activity, in comparison to the standard ascorbic acid. The formulation, F-VI A, showed better activity than F-VI.

**Antiulcer activity**: The effect of two formulations on gastric mucosa was evaluated using pyloric ligation-induced gastric ulcer model. These formulations are intended for long-term treatment. It is highly significant to note that these formulations, contrary to ulcerogenic response of NSAIDs, produced protection of gastric mucosa by showing reduction in ulcer index by 65 and 71 per cent, respectively for formulation F-VI and F-VI A. The gastric secretions and total acidity was also significantly decreased.

**Stability studies**: The tablet formulations were subjected to accelerated stability studies. After 1 month, various parameters were re-evaluated and no significant change in any of the parameters of the designed formulations were noted.