



## Research Article

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### HPLC ANALYSIS OF PHENOLIC ACIDS AND ANTIOXIDANT ACTIVITY OF SOME CLASSICAL AYURVEDIC GUGGULU FORMULATIONS

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#### ABSTRACT

Classical guggulu formulations have been used as mainstay therapeutics in chronic inflammatory diseases in Ayurvedic system of medicine since centuries. The present study included six guggulu formulations such as Kaishora guggulu, Kanchanara guggulu, Yogaraj guggulu, Punrnava guggulu, Amritadi guggulu and Trayodasanga guggulu. Phenolic compounds play a vital role as evident from phenolic and flavonoid content in guggulu drugs as well as antioxidant activity assayed by DPPH radical scavenging activity and FRAP antioxidant power. Yograj guggulu was found to have strong reducing ability (1156.76  $\mu$ M Fe(II)/mg) among six drugs chosen in this investigation and Amritadi guggulu has revealed powerful scavenger of DPPH radicals (IC<sub>50</sub>:1.05 mg/ml). Phenolic acid composition for each guggulu drug was analyzed and twelve phenolic acid compounds were identified and estimated by HPLC. Gallic acid (8.29-49.28 mg/g) was found in high concentration and ellagic acid (0.69-6.02 mg/g) was found to the next in all formulations. Other phenolic acid components estimated were protocatechuic acid, p-hydroxybenzoic acid, gentisic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid in all guggulu formulations. A synergistic activity towards therapeutic efficacy was proposed on the basis of phenolic acid composition in each guggulu formulation.

**Keywords:** Ayurveda, Guggulu formulations, Antioxidant, Phenolic acids, Flavonoids, HPLC

#### INTRODUCTION

Guggulu is an oleoresin that obtained as exudates from the bark of *Commiphora mukul* (Syn. *Commiphora wightii*) belongs to the family Burseraceae. It is widely distributed in the tropical and sub tropical areas especially northeastern Africa, southern Arabia, India, Bangladesh and Pakistan. The medicinal importance of guggulu have been classified in Charak Samhita (1000 B.C.), Sushruta Samhita (600 B.C.), Vagbhata Samhita (17th century A.D.) and various Nighantus (Ayurvedic literatures) written in India between 12<sup>th</sup> and 14<sup>th</sup> centuries.<sup>1-2</sup> It is also mentioned in Ayurvedic texts that guggulu formulated with other plant ingredients have been indicated for therapeutic booster. There are several classical guggulu formulations comprised of plant ingredients and generally recognized by unique combination of herbs with definite proportions and named suffixed with guggulu such as Kaishora guggulu. Interestingly, Yogaraj guggulu prepared with maximum number of ingredients i.e., 29, while, Kanchanara guggulu made by only 12 ingredients.<sup>3</sup> Moreover, various purification (shodhana) processes have been described for guggulu preparation to eliminate impurities and improve its bioavailability.<sup>4</sup>

From ancient time to present era, different guggulu formulations have been acclaimed popular practices for the treatment of obesity, inflammatory conditions, joint pains, arthritis, tumours, coronary diseases, hypertension, liver disorder etc.<sup>5-7</sup> Modern research identified guggulu is a complex mixture of several bioactive compounds like steroids (guggulsterones), diterpenoids, triterpenes, aliphatic esters, alcohols, flavonoids

and variety of inorganic compounds.<sup>8-9</sup> Although, oleoresin of *Commiphora mukul* has been chemically analysed exhaustively, chemical composition of guggulu formulations wherein guggulu is an essential compulsory component along with multiple potent herbal ingredients have been evaluated very little. Polyphenolic compounds are known to be common constituent of medicinal plants and ample scientific evidence suggested that they have protective role against many chronic diseases<sup>10</sup>. Therefore, there are possibilities to recognize potent bioactive phenolic compounds from guggulu formulations that will not only be supportive to clarify its therapeutic actions (other than guggulsterones), but also could serve as biomarker for chemical standardization of guggulu formulations. The validation of herbal products is now a major public health concern both in developed and resource-poor countries. In this context, we decided to identify and quantify phenolic compounds in six classical Ayurvedic guggulu formulations, such as Kaishora guggulu, Kanchanara guggulu, Yogaraja guggulu, Punrnava guggulu, Amritadi guggulu and Trayodasanga guggulu, keeping in mind their potent pharmacological actions in inflammatory conditions and oxidative stress.

#### MATERIALS AND METHODS

##### Chemicals and Reagents

Ultra-pure standards like, gallic acid, protocatechuic acid, p-hydroxybenzoic acid, gentisic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, ellagic acid, quercetin, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and Aluminium chloride have been

procured from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals and reagents like ascorbic acid, acetic acid, acetonitrile, phosphoric acid, methanol etc. were obtained from Merck (India). All reagents were analytical grade.

### Test Drugs

Six classical Ayurvedic preparations of guggulu formulations, viz., Kaishore guggulu (KSG), Kanchanara guggulu (KNG), Yogaraj guggulu (YG), Punrnava guggulu (PNG), Amritadi guggulu (AMG) and Trayodasang guggulu (TDG) were obtained from the pharmacy department of RKMA Ayurvedic Hospital, Narendrapur. The composition of classical guggulu formulations as described in Ayurvedic literatures was shown in Table 1.

### Test Sample Preparation

1 g of each guggulu formulation was taken separately, refluxed with 20ml of aqueous methanol (20:80, v/v) for 30 min, filtered through Whatmann filter paper no. 41 and this procedure was repeated thrice. The pooled filtrate was concentrated and volume was adjusted to 50 ml with methanol in a volumetric flask.<sup>11</sup>

### Estimation of Total Phenolics

The amount of total phenolics present in each guggulu formulation was determined as described earlier<sup>7</sup>. To 0.02ml of each guggulu extract (10mg/ml), 1.08ml of Folin-Ciocalteu reagent was added, mixed and incubated in the dark for 5min. Thereafter, 0.8ml of sodium carbonate solution (7.5%) was added and further incubated in the dark for 30 min. Finally, 3ml of distilled water was added and the absorbance was measured spectrophotometrically (Shimadzu UV-1800) at 765nm.<sup>12</sup> The total phenolic content was expressed as gallic acid equivalent (GAE) in mg per g of guggulu formulation.

### Estimation of Total Flavonoids

For the estimation of flavonoids, 0.02 ml of each guggulu extract (10mg/ml) was mixed with 0.98 ml methanol, 0.1ml of 10% aluminium chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. After 30 min the absorbance of the reaction mixture was measured spectrophotometrically (Shimadzu UV-1800) at 415nm.<sup>13</sup> The total flavonoid was expressed as quercetin equivalent (QE) in mg per g of guggulu formulation.

### DPPH Radical Scavenging Activity

To 2.5 ml of each guggulu extract at different known concentrations was mixed with 1 ml of 0.3 mM DPPH solution and was allowed to stand in dark for 30 minutes. The absorbance values were measured spectrophotometrically (Shimadzu UV-1800) at 518 nm.<sup>14</sup> The antioxidant activity of each guggulu formulation was expressed as 50% inhibitory concentration or IC<sub>50</sub>. Ascorbic acid was used as standard.

### Ferric Reducing Antioxidant Power (FRAP) Assay

Total antioxidant activity (FRAP assay) was determined in the extract according to method of Benzie and Strain<sup>15</sup> with some modification. The stock solutions included 300 mM acetate buffer (3.1 g sodium citrate trihydrate and 16 ml glacial acetic acid), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl<sub>3</sub>·6H<sub>2</sub>O in deionised water. The fresh working solution was prepared by mixing acetate buffer, TPTZ and FeCl<sub>3</sub>·6H<sub>2</sub>O in the ratio 10:1:1. Guggulu drug extracts (100 µL) were allowed to react with 2ml

of the FRAP solution for 30 min incubated at 37°C. Absorbance of the colored product (ferrous tripyridyltriazine complex) was taken at 593 nm. The standard curve was linear between 10 and 100 µM FeSO<sub>4</sub>. Results are expressed in µM Fe (II)/mg dry material and compared with that of ascorbic acid.

### Identification and Quantification of Phenolic Compounds by HPLC

HPLC analysis was performed using instrument Dionex Ultimate 3000, Thermo Scientific, USA equipped with quaternary pump (LPG 3400 SD) for solvent delivery, 20µl loop for injection and PDA detector (DAD 3000) and Chromeleon 6.8 system manager as data processor. The separation was achieved using reverse phase column, Acclaim™ 120 C18 column (250mm x 4.6mm, 5µm)<sup>16</sup>. Individual guggulu extract was further diluted with hydro-methanol (20:80) at a concentration of 1 mg/ml and filtered through 0.2 µm PDVF filter. Standard polyphenols like, gallic acid, protocatechuic acid, p-hydroxy benzoic acid, gentisic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid and ellagic acid were prepared in hydro-methanol (20:80) at concentration 1 mg/ml as stock solution. Further dilutions were made for calibration of each standard. The mobile phase contains methanol (Solvent A) and 1% aq. acetic acid solution (Solvent B), the column was thermostatically controlled at 28° C. The gradient elution was 10 % A with flow rate 1ml/min to 0.7 ml/min in 27 min, from 10 % to 40% A with flow rate 0.7ml/min for 28 min, 40 % A, with flow rate 0.7 to 0.6 ml/min for 5 min, from 40 to 44 % A with flow rate 0.6 to 0.3 ml/min in 5 min, 44 % A with flow rate 0.3 to 0.6 ml/min in 5 min. The mobile phase composition back to initial condition and allowed to run for another 8 min, before another injection of sample. The detection of compounds was performed using detector at 280 nm. Each compound was identified by its retention time and by spiking with standards under the same conditions. The quantification of the sample was done by the measurement of the integrated peak area and the content was calculated using the calibration curve of the respective standard sample.

### Statistical Analysis

The data were represented as mean ± SEM. Descriptive statistics were conducted wherever it was applicable.

## RESULTS

### Total Phenolic and Flavonoid Content

Phenolic content of Ayurvedic guggulu formulation was found considerably high. Amongst six formulations, Yogaraj guggulu (YG) contain highest amount (129.73 mg/g GAE) and the least phenolic content found in Trayodasang guggulu (TDG), 31.74 mg/g GAE (Table 2). The decreasing order of phenolic content was as such YG>AMG>PNG>KSG>KNG>TDG. The flavonoid content in guggulu formulations was quite less in comparison to phenolic content. The highest concentration was found in YG (10.12 mg/g QE) and the decreasing order as revealed from Table 2 was YG>PNG>AMG>KSG>KNG>TDG.

### DPPH Radical Scavenging Activity

The capacity of scavenging DPPH radical was reflected by IC<sub>50</sub> values of respective guggulu formulations (Table 2). The most effective scavenger was AMG (1.05 mg/ml) and the poorest scavenger was TDG (4.09 mg/ml). PNG (1.09 mg/ml) has quite similar activity with AMG. The radical scavenging activity of

different guggulu formulations in decreasing order was AMG>PNG>KSG>YG>KNG>TDG.

#### Antioxidant Power by FRAP

The reducing ability of different guggulu formulations was in the range of 277-1156  $\mu\text{M Fe(II)/mg}$  (Table 2). Although these values were below the value for ascorbic acid (3925  $\mu\text{M Fe(II)/mg}$ ), YG (1156  $\mu\text{M Fe(II)/mg}$ ) was found to have strong reducing ability. The decreasing order of reducing ability was YG>AMG>KSG>PNG>KNG>TDG. Antioxidant power (FRAP) increased proportionally with phenolic content of respective guggulu drugs.

#### Phenolic Acid Analysis by HPLC

Phenolic acids present in different guggulu formulations were analyzed by HPLC-DAD using twelve phenolic acids as external standard for identification and estimation. The results are presented in Table 3. Gallic acid (highest in KSG 49.28 mg/g and lowest in KNG 8.29 mg/g) was found most abundant phenolic acid in all guggulu formulations under investigation. The next abundant phenolic acid, ellagic acid (highest in KSG

6.02 mg/g and lowest in TDG 0.69 mg/g), ferulic acid (highest in AMG 0.17 mg/g and lowest in KSG 0.03 mg/g) and chlorogenic acid (highest in KNG 6.06 mg/g and lowest in KSG 0.14 mg/g) were also found in all formulations. Syringic acid (highest in YG 5.58 mg/g and lowest in KG 0.41 mg/g) and p-coumaric acid (highest in YG 0.79 mg/g and lowest in PNG 0.02 mg/g) were present in all guggulu formulations except TDG. Caffeic acid (highest in PNG 0.22 mg/g and lowest in AMG 0.06mg/g) and p-hydroxybenzoic acid (highest in KSG 2.92 mg/g and lowest in KNG 0.07 mg/g) was found in four guggulu formulations. Other phenolic acids such as protocatechuic acid (highest in AMG 0.48 mg/g and lowest in KNG 0.07 mg/g) was found in three formulations, gentisic acid (highest in PNG 5.64 mg/g and lowest in AMG 4.18 mg/g) and vanillic acid (highest in KNG 0.82 mg/g and lowest in KSG 0.06 mg/g) were found in two formulations and sinapic acid (0.46 mg/g) was present only in TDG. The distribution of phenolic acids in each formulation was also different as revealed from Table 3 as well as HPLC chromatograms in Fig. 1-6. Maximum ten components were identified in KSG whereas at least five components were detected in TDG.

Table 1: Composition of Ayurvedic Guggulu Formulations

Name of test drug	No. of ingredients	Important ingredients	Reference
Kaishore Guggulu	17	<i>Tinospora cordifolia</i> (Willd.)Miers. (Guduchi , St.), <i>Commiphora wightii</i> (Arn.) Bhand(Guggulu, Exudate)	Bhaisajyaratnavali, Vataraktadhikara; 97-101 AFI* Part-I, 5:2
Kanchanara Guggulu	12	<i>Bauhinia variegata</i> Blume.(Kanchanara,St. bk.), <i>Commiphora wightii</i> (Arn.) Bhand (Guggulu, Exudate)	Sarangadharasamhita, Madhyamakhandha Adhyaya 7; 95-98 AFI Part-I, 5:1
Yogaraj Guggulu	29	All equal part	Bhaisajyaratnavali, Amavatadhikara; 90-93 AFI Part-I, 5:7
Punarnava Guggulu	21	<i>Boerhaavia diffusa</i> Linn.(Punarnava Rt.), <i>Ricinus communis</i> Linn. (Eranda.Rt.), <i>Zingiber officinale</i> Roxb.(Sunth, Rz), <i>Commiphora wightii</i> (Arn.) Bhand (Guggulu, Exudate), <i>Operculina terpehthum</i> (L.)Silva Manso(Trivrit,Rt.)	AFI Part-II, 5:2
Amritadi Guggulu	15	<i>Tinospora cordifolia</i> (Willd.)Miers. (Guduchi,St.), Triphala, <i>Trianthema portulacastrum</i> Linn.(Varsavhu,Rt.), <i>Commiphora wightii</i> (Arn.) Bhand (Guggulu, Exudate)	Bhavaprakasa, Vatarakta-chikitsa Prakarana; 183-190 AFI Part-III, 5:4
Trayodasanga Guggulu	14	All equal part	Bhaisajyaratnavali, Vataavyadhyadhikara; 89-89 <sup>1/2</sup> AFI Part-I, 5:4

\*AFI means Ayurvedic Formulary of India

Table 2: Antioxidant Potential of Ayurvedic Guggulu Drugs

Name of the Drug	Total phenolic content (mg/g GAE)	Total flavonoid content (mg/g QE)	FRAP value ( $\mu\text{M Fe(II)/mg}$ )	DPPH radical scavenging capacity ( $\text{IC}_{50}$ mg/ml)
Kaishore Guggulu (KSG)	90.12 $\pm$ 0.03	6.89 $\pm$ 0.010	797.82 $\pm$ 3.59	1.30 $\pm$ 0.004
Kanchanara Guggulu (KNG)	57.90 $\pm$ 0.02	5.44 $\pm$ 0.001	494.31 $\pm$ 25.85	2.11 $\pm$ 0.009
Yogaraj Guggulu (YG)	129.73 $\pm$ 0.02	10.12 $\pm$ 0.004	1156.76 $\pm$ 22.35	1.54 $\pm$ 0.006
Punarnava Guggulu (PNG)	93.41 $\pm$ 0.01	7.84 $\pm$ 0.011	776.17 $\pm$ 21.88	1.09 $\pm$ 0.003
Amritadi Guggulu (AMG)	100.90 $\pm$ 0.04	7.18 $\pm$ 0.003	846.80 $\pm$ 9.13	1.05 $\pm$ 0.005
Trayodasanga Guggulu (TDG)	31.74 $\pm$ 0.04	3.40 $\pm$ 0.001	277.15 $\pm$ 17.57	4.09 $\pm$ 0.008
Ascorbic acid			3925.75 $\pm$ 308.59	0.21 $\pm$ 0.008

N=6 in each test; results are Mean $\pm$ SEM; GAE means gallic acid equivalent; QE means quercetin equivalent

Table 3: Phenolic Acid Composition of Different Ayurvedic Guggulu Drugs Analyzed by HPLC

Guggulu Drugs/Phenolic acid	GA	PCA	BA	GNA	CGA	VA	CA	SGA	p-CA	FA	SA	EA
KSG	49.28 ± 0.023	0.37 ± 0.002	2.92 ± 0.006	ND	0.14 ± 0.003	0.06 ± 0.001	0.07 ± 0.001	0.41 ± 0.004	0.17 ± 0.002	0.032 ± 0.006	ND	6.02 ± 0.004
KNG	8.29 ± 0.014	0.07 ± 0.001	ND	ND	6.06 ± 0.018	0.82 ± 0.007	ND	2.34 ± 0.006	0.26 ± 0.001	0.04 ± 0.001	ND	4.55 ± 0.013
YG	40.83 ± 0.061	ND	0.19 ± 0.007	ND	1.63 ± 0.002	ND	0.12 ± 0.001	5.58 ± 0.011	0.79 ± 0.006	0.12 ± 0.002	ND	5.28 ± 0.004
PNG	34.82 ± 0.088	ND	0.07 ± 0.002	5.64 ± 0.006	0.22 ± 0.003	ND	0.22 ± 0.001	2.29 ± 0.008	0.02 ± 0.001	0.06 ± 0.001	ND	4.14 ± 0.003
AMG	32.83 ± 0.072	0.48 ± 0.007	ND	4.18 ± 0.008	0.22 ± 0.002	ND	0.06 ± 0.001	3.20 ± 0.009	0.32 ± 0.002	0.17 ± 0.001	ND	4.19 ± 0.012
TDG	18.26 ± 0.015	ND	0.34 ± 0.006	ND	0.30 ± 0.001	ND	ND	ND	ND	0.09 ± 0.001	0.46 ± 0.006	0.69 ± 0.003

N=3 for each test; results are Mean ± SEM; ND= not detected (<0.01); values are expressed in mg/g; GA= Gallic Acid, PCA= Protocatechonic Acid, BA= p-Hydroxy Benzoic Acid, GNA= Gentisic Acid, CGA= Chlorogenic Acid, VA= Vanillic Acid, CA= Caffeic Acid, SGA= Syringic Acid, p-CA= p-Coumaric Acid, FA= Ferulic Acid, SA= Sinapic Acid, EA= Ellagic Acid, KSG= Kaishore Guggulu, KNG= Kanchanara Guggulu, YG= Yograj Guggulu, PNG= Punarnava Guggulu, AMG= Amritadi Guggulu, TDG= Trayodasang Guggulu

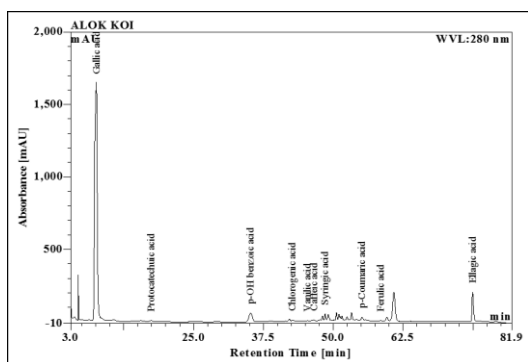


Figure 1: HPLC chromatogram of Koishore Guggulu

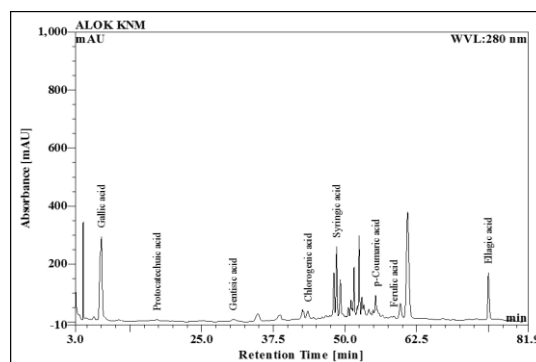


Figure 2: HPLC chromatogram of Kanchanara Guggulu

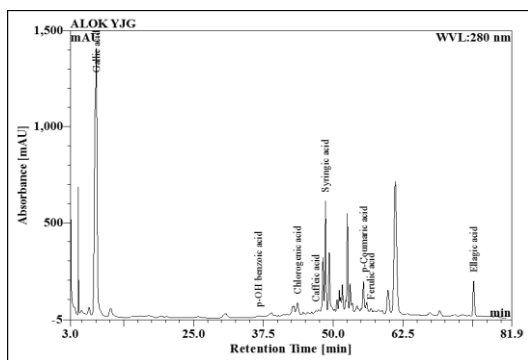


Figure 3: HPLC chromatogram of Yograj Guggulu

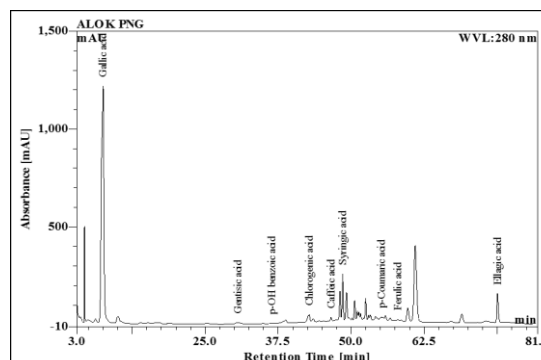


Figure 4: HPLC chromatogram of Punarnava Guggulu

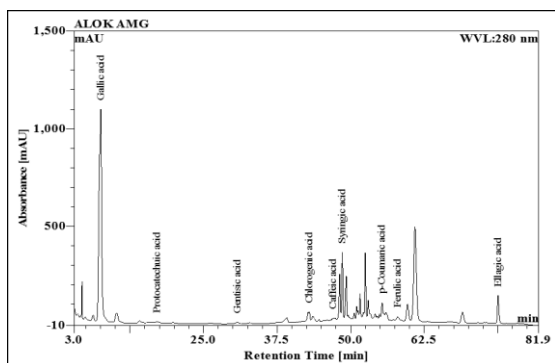


Figure 5: HPLC chromatogram of Amritadi Guggulu

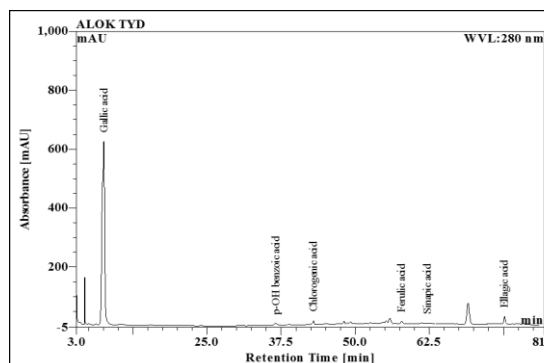


Figure 6: HPLC chromatogram of Trayodasang Guggulu

## DISCUSSION

Guggulu (*Commiphora wightii* (Arn.) Bhand) have been used in Ayurvedic therapeutics for years and till date it is successfully employed in the treatment of several disease conditions such as cardiovascular diseases including hypercholesterolemia and atherosclerosis. Apart from its individual role, several other guggulu preparations in combination with other herbs have been formulated in Ayurvedic literatures<sup>6</sup>. The concept behind guggulu kalpana has target specific efficacy towards particular disease conditions in consequence of inflammatory pathogenesis. Another important reason is that guggulu combined with herbs in a formulation might have synergistic effect and thus promote healing fast. The guggulu preparations taken under this investigation were Kaishore guggulu (KSG), Kanchanara Guggulu (KNG), Yograj guggulu (YG), Punaranava guggulu (PNG), Amritadi guggulu (AMG) and Tryodasang guggulu (TDG). Sodhita guggulu (purified guggulu as described in Ayurvedic literature) was common prominent ingredient in these formulations and the herbal constituents were unique for each formulation that conferred desired therapeutic efficacy. These guggulu drugs have been used in chronic degenerative diseases with inflammatory conditions. For example KSG is used as antiallergic and have important role in ameliorating fibromyalgia, gout<sup>17</sup>. KNG is an efficient drug in fibroids, <sup>18</sup> PNG used for renal dysfunction,<sup>19</sup> YG for all different types of gouty conditions<sup>20</sup>, AMG and TDG for osteoarthritis.<sup>21</sup> It is evident that active natural products present in these guggulu formulations having significant anti-inflammatory activity were responsible for therapeutic efficacy.

Phenolics, especially phenolic acids and flavonoids are common natural products found in plants that have significant antioxidant and anti-inflammatory activity.<sup>22</sup> Phenolic acids are subclassified as benzoic acid and cinnamic acid backbone structure containing seven (C<sub>6</sub>-C<sub>1</sub>) and nine (C<sub>6</sub>-C<sub>3</sub>) carbon atoms respectively. Hydroxybenzoic acid derivatives such as gallic acid (GA), protocatechuic acid (PCA), gentisic acid (GNA), vanillic acid (VA), p-hydroxy benzoic acid (BA), syringic acid (SGA), ellagic acid (EA) and cinnamic acid derivatives such as caffeic acid (CA), chlorogenic acid (CGA), ferulic acid (FA), sinapic acid (SA), p-coumaric acid (p-CA) are predominantly available in plants. Ayurvedic guggulu drugs also contain significant amount of phenolic compounds as evident from estimated phenolic and flavonoid content (Table 2) and obviously the source is herbal ingredients of guggulu formulations. The availability of phenolic acids was more pronounced in guggulu formulations than flavonoids. Therefore further analyzes were performed by HPLC for phenolic acid composition in guggulu drugs that provided distributive pattern of different hydroxybenzoic acid and cinnamic acid derivatives of phenolic compounds. The link between chronic inflammation and various chronic diseases such as cardiovascular complications, cancer, metabolic disorders like diabetes, arthritis, autoimmune diseases, pulmonary diseases and neurological diseases is well known.<sup>23</sup> Gallic acid (GA) was most abundant phenolic acid in guggulu drugs and the main contributory herb might be Amlaki (*Phyllanthus emblica*) used almost all guggulu formulations. Ellagic acid (EA) was next to GA with respect to availability in all guggulu formulations and the source might be guggulu used in the preparation of drugs under investigation. Gastroprotective effect of GA and EA <sup>24-25</sup> was shown in peptic ulcer models and attenuated elevated levels of inflammatory mediators. Reactive oxygen species (ROS) is produced by various metabolic activities and are major contributor of many chronic and degenerative diseases which include various type of arthritis. Phenolic acids have been demonstrated as powerful scavenger of ROS. It was observed (Table 2) that antioxidant power

estimated by FRAP assay was significant in guggulu formulations. The positive correlation between phenolic content and FRAP was an important indicator to inhibit ROS and consequently inflammatory chronic diseases like arthritis. In this respect YG was most potent drug amongst the six formulations studied. Especially antioxidant properties of FA have been studied in cardioprotection<sup>26</sup> and the role of PCA, VA in reducing lipid peroxidation<sup>27</sup> has been well established. Production of NO and PGE<sub>2</sub> were suppressed by CGA via down-regulation of iNOS and COX-2<sup>28</sup> and have shown anti-osteoarthritis properties. CA has also been showed for attenuating NO production and thus inhibited inflammatory cascades.<sup>29</sup> EA and p-CA have been shown to decrease oxidative stress and exert apoptosis leading to inhibition of carcinogenesis. Although phenolic acids of different molecular structure induce antioxidant activity and inhibit inflammatory processes in different degree, the potential effect of the combination of individual compounds has been little investigated. The synergistic or antagonistic effect has been studied for GA, PCA, CGA and VA<sup>30</sup> and the combination of these four compounds exhibited synergistic effect. We found that each guggulu drug has particular phenolic acid profile which includes twelve compounds of different concentrations. Therefore synergism could not be excluded among phenolic acids in guggulu drugs which are reflected in their antioxidant activity and therapeutic efficacy. Moreover contributory effect of phenolic acids investigated in guggulu drugs consolidated anti-inflammatory effect and therapeutic efficacy of the respective guggulu formulations as prescribed in Ayurvedic system of medicine.

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