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Preliminary Phytochemical Standardization of Tree Exudates from India: Gum Kondagogu and Gum Ghatti

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ABSTRACT

Tree exudates (Gums) have a history of 5000 years and well established as an inert pharmaceutical excipients, which are polysaccharides in nature perform versatile functions in the development of controlled drug delivery systems. In the present study tree exudates gum kondagogu (GKG) obtained from the bark Cochlospermum gossypium (DC) belongs to family Bixaceae, and gum ghatti (GG) obtained from the bark Anogessius latifolia (Roxb) belongs to family Combretaceae were standardized. Proximate analysis of gums indicates they have low total ash, high methanolic extractive values, and low moisture content. The nature of gums was confirmed by phytochemical screening and with Thin Layer chromatography (TLC), and High Performance Thin Layer chromatography (HPTLC) which indicated distinct finger prints with methanol extract. In order to prevent adulteration and substitution, the above mentioned parameters were set for GKG and GG, which can be a useful tool in identification of gums.

Key words: Gum Ghatti, Gum Kondagogu, Proximate analysis, Standardization, Tree exudates.

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INTRODUCTION

Gums and mucilages are used as inert pharmaceutical excipients and well known for their medicinal applications in India. They are widely used in food and pharmaceutical industry as thickening, stabilizing, emulsifying and disintegrating agents. Today, gums are an ideal excipient comply to perform multitask functions like, control physicochemical properties, enhancing the solubility of poorly soluble drugs, to achieve desired drug release at required site in the form of controlled drug delivery systems. One of the most commonly used natural polymers are obtained from trees in the form of gums known as tree exudates. Tree exudates gums have a history of about 5000 years. Tree exudates gums are polysaccharides with high molecular weight mainly composed of monosaccharide units, uronic acids, and to some extent proteins and fibres. Most common tree exudates commonly used in pharmaceutical industry are gum acacia, gum tragacanth, and gum arabia with various food and pharmaceutical application.[1,2,3] Tree exudates are characterized by low toxicity, abundantly available, biocompatible, biodegradable, inert, and cheap compared to that of synthetic polymers.

Gum kondagogu (GKG) is gummy exudates obtained from the tree bark Cochlospermum gossypium belonging to genus Cochlospermum and family Bixaceae, distributed throughout India, in dry forest and sub-Himalayan region. It is a small deciduous tree, 2.4- 5.3 m in height, deeply furrowed bark containing gum. It is composed of major neutral sugars like arabinose, galactose, rhamnose, mannose, α-D-glucose, and sugar acids like D-glucouronic acid, and D-galactouronic acid. Dried leaves and flowers are used as a stimulant. Gum is sweet, cooling and useful in diarrhoea, dysentery, cough, pharyngitis, and also used as a pharmaceutical aid. GKG is been used as substitute for gum tragacanth. [4]

Gum ghatti (GG) is gummy exudates obtained from the tree bark of Anogeissus latifolia (Roxb.) belongs to the family Combretaceae, commonly known as 'Dhawara'**.** It is a small to medium sized tree, distributed throughout India and ascending up to 1200 m in the hills of south India. [5] Roots are astringent, acrid and stomachic. The bark is used as cooling, anti-inflammatory, and urinary astringent. A part of tree finds its use in the treatment of piles, diabetes, and anaemia. [6] The bark has been evaluated scientifically for its antioxidant and wound healing activity. It is a complex polysaccharide of high molecular weight. It occurs in nature as a mixed calcium, magnesium, potassium, and sodium salts. It is composed of L-arabinose, D-galatose, D-mannose, D-xylose and D-glucouronic acid. GG can be used as substitute for gum arabic. Surface morphology, chemical and structural assignment and rheological properties of GKG and GG have been well established thus enabling its commercial exploitation in food and pharmaceutical industry. [7, 8, 9] However to our knowledge standardization and evaluation of GKG and GG so far has not been carried out. Present article, is an attempt to establish proximate studies for gum kondagogu and gum ghatti in order to set the standardization parameters.

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MATERIAL AND METHODS

Collection of Gums

GKG and GG samples were collected from Girijan Co-operative Corporation, Government of Andhra Pradesh Undertaking, Hyderabad, India. Gums are available in three grades. Grade I, Grade II, and Grade III. Grade I, handpicked, freshly collected during April to June, free from any extraneous material like sand, bark were selected and used in the experimental analysis. Gum samples were stored in polypropylene air-tight containers under desiccated conditions.

GKG freshly handpicked are cream white to brown vermiform tears about 1-2 cm in size with pungent odour and acidic taste. GG is white to light brown translucent round tears or vermiform masses about 1-2 cm in size, odorless in nature with blend mucilaginous taste. Specimens of gums and trees of GKG and GG are shown in Figure 1 to 4.

Figure 1: Specimen of Grade I Gum GG
Figure 2: Tree Anogessius latifolia (Roxb. Ex DC)
Figure 3: Specimen of grade I GKG
Figure 4: Tree Cochlospermum gossypium (DC)

Preparation of Gum Sample [7]

GKG and GG were pulverized using a high speed blender (Kenstar, India), and subjected for sieving with mesh size 250 microns, in order to obtain uniform sample. 5 g of gum GKG and 10 g of gum GG was accurately weighed and dispersed in one litre of Millipore water. The gum dispersed solution was subjected to stirring using mechanical stirrer for about 12 h at room temperature and allowed to stand overnight in order to separate any undissolved material. The gum solution was filtered through a sintered glass funnel. The clear solution so obtained was used for experimental analysis.

Proximate Studies [10]

Solubility test

Purified GKG and GG were evaluated for solubility in water, chloroform, acetone, and methanol in accordance with I.P. specifications.

Ash values

Ash values are indicative of extent of care taken in collection, preparation of crude drug for market and of foreign matter content of natural drug. The main object is to determine traces of organic materials interfering with analysis of inorganic elements. Hence water soluble ash, total ash, and acid insoluble ash were obtained by reported methods.

Determination of Extractive Values

Extractive value determines amount of active constituents present in specified plant material in a given solvent. Extracts were prepared with various solvents by standard methods. Percentage yield for extracts was obtained.

Determination of Moisture content

Moisture content was determined as per I.P. specifications. 2 g of gum samples were transferred uniformly in the weighed Petri dishes and then dried in hot air oven at 105 ^{0}C until a constant weight was obtained. The moisture content was determined as a ratio of weight of moisture loss to weight expressed as a percentage.

Determination of Foreign Organic Matter

5 g of air dried coarsely powdered gums were spread in a form of thin layer. The gum samples were inspected under microscopic lens (6X). Foreign organic matter was separated manually. After complete separation, matter was weighed. Percentage of foreign organic matter was determined from the weight of the gums taken.

Fluorescence Analysis [11, 12]

Powdered gum samples were screened for fluorescence characteristic with or without chemical treatment. The observation pertaining to their colour under ultra-violet light and in day light were noticed and reported.

Phytochemical analysis [13]

The pet ether, chloroform, ethyl acetate, methanol and aqueous extracts of GKG and GG were subjected to preliminary phytochemical testing for the presence of different classes of compounds.

Thin Layer Chromatography [14]

TLC studies of different extracts of gums were carried out.

Sample preparation: 1 mg of pet. Ether, ethyl acetate chloroform, acetone, and methanol extracts were dissolved in 10 ml of respective solvents.

Stationary Phase: Pre-coated silica gel plates, Merck 60 F254

Chamber saturation: 30 min

Mobile phase and spraying reagents used for visualization of different phytochemical are given in the table.

High Performance Thin Layer Chromatography [14, 15, 16]

Chromatographic Conditions

Sample preparation: 1 mg of methanol extract was dissolved in 10 ml Methanol

Stationary phase: Pre-coated silica gel plates, Merck 60 F254

Mobile phase: 1) Acetone: water: chloroform: methanol (7.5:0.5:1:1)
2) Ethyl acetate: acetic acid: methanol: water (6:1.5:1.5:1)

Lamp: Deuterium

Wavelength: 254 nm

Application mode: CAMAG Automatic TLC Sampler III

Development mode: CAMAG Twin Trough Chamber

Scanner: CAMAG TLC Scanner 3 and WinCATS software

RESULTS AND DISCUSSION

As gums are unorganised plant exudates, Pharmacognistical studies, physico-chemical, phytochemical analysis, chromatography techniques like TLC and HPTLC are required for standardization of gums. TLC has been widely applied for the analysis of extractives and most ideal tool for identification and control of purity in plant derived drugs systematically.

GKG and GG were standardized for their various parameters as part of evaluation and results are reported as follows.

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Solubility test

Practically GKG and GG were insoluble in chloroform, acetone, and methanol. In water, GKG was insoluble but swells to 60-100 times to their original volume. About 90 % of gum GG was soluble in water and colourless viscous mucilage was obtained.

Ash values [17]

The quality of the plant derived drug can also be determined by ash left after ignition. This parameter helps to judge the identity and purity of plant derived drugs. It is commonly applied parameter for detection of impurities, adulteration and substitution of drug. Inorganic variables like silica, phosphates, silicates, and carbonates are removed by treating with acid. GKG and GG showed low levels of total ash and acid insoluble ash which indicates low levels of contamination during collection and handling of gums. The total ash, water soluble ash, and acid insoluble ash of GKG and GG was presented in the Table 1.

Table 1 : Ash values of GKG and GG

Parameters	GKG	GG
Total ash	7.90±0.022	3.51±0.055
Acid insoluble ash	1.65±0.112	1.95±0.117
Water soluble ash	3.65±0.067	2.05±0.066

The values are expressed as mean± SD, n =4.

Extractive Values [18, 19]

Extractive values in different non-polar to polar solvents are based on the quantity, in which specific constituents are soluble. It is valuable parameter to check the quality of the plant derived drug. In case of substitution or adulteration it clearly indicates change in the extractive values. An extractive value indicates an approximate measure of the chemical constituents present in the plant derived drug. GKG and GG showed high methanol extractive value which indicates the presence of semipolar-polar chemical constituents. The extractive values of GKG and GG was presented in Table 2.

Table 2: Extractive values of GKG and GG

Extracts	GKG	GG
Petroleum ether	0.105±0.140	0.965±0.015
Chloroform	0.182±0.205	1.752±0.105
Ethyl acetate	0.517±0.149	0.853±0.210
Acetone	0.965±0.113	2.587±0.413
Methanol	1.895±0.125	3.424±0.004

The values are expressed as mean± SD, n =4.

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Moisture Content [20]

Moisture content is a key parameter which determines the quality, efficacy and shelf life of the plant derived drug and was confirmed by Loss on drying. Moisture content of GKG and GG were found to be 13.17 ± 0.112% w/w and 10.70 ± 0.015% w/w respectively.

Foreign Organic Matter

As gums are obtained from the outer bark of the tree during picking and collection, traces of dried bark, sand, and dust particles may be present. The foreign organic matter was found to be 1 %w/w in case of GG and completely free from foreign organic matter in case of GKG.

Fluorescence Analysis

To avoid substitution or adulteration, gums were examined under day light (254 nm) and Ultra violet light (365 nm), which is helpful in identifying plant derived drugs from substitution or adulteration. The results of fluorescence analysis for GKG and GG were presented in the Table 3.

Table 3: Fluorescence analysis of GKG and GG

Treatment	Visible Light		UV light			
			Short wavelength		Long wavelength	
	GKG	GG	GKG	GG	GKG	GG
Powder	Cream	Cream	Cream	Cream	Cream	Cream
Powder + 5 % KOH	Colorless	Colorless	White	White	White	White
Powder + 5 % NaOH	Brown	Brown	Light green	Bottle green	Green yellow	Dark green
Powder + 5 % $FeCl_3$	Black	Black	Light brown	Brown	Brown	Bluish brown
Powder + I_2 solution	Brown	Brown	Brown	Brown	Dark brown	Dark brown
Powder + Dil. H_2SO_4	Brown	Brown	Pale Yellow	Faint brown	Yellow	Yellow
Powder + Conc. H_2SO_4	Dark Brown	Brown	Yellow	Dark green	Golden yellow	Dark yellow
Powder + Dil HCl	Colorless	Colorless	Pale white	White	Cream	Cream white
Powder + Conc. HCl	Colorless	Colorless	Whitish cream	Pale white	Cream	White
Powder+NaoH in methanol	Colorless	Colorless	White	White	White	White

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Phytochemical Analysis

Proximate Phytochemical Analysis

GKG and GG showed absence of glycosides, alkaloids, flavonoids, steroids and tannins. The gum sample of GKG and GG showed the presence of carbohydrates and results were presented in Table 4.

Table 4: Preliminary Phytoconstituents of GKG and GG

Test	GKG	GG
Test for Carbohydrates (Molisch's test)	+	+
Test for Gums (Ruthenium red)	+	+
Test for Reducing sugars (Fehling's test)	+	+
Test for Glycosides(Killer-Killani test)	_	_
Test for Alkaloids (Wagner test)	_	_
Test for Steroids (Salkowski test)	_	_
Test for Flavonoids (Shinoda test)	_	_
Test for Tannins (ferric chloride test)	_	_
Treatment with iodine solution	_	_

+ Present, - Negative

Thin Layer Chromatography [21]

All the extracts of gums were subjected to thin layer chromatography. The R_f values were recorded for GKG and GG which showed presence of polar compound. Detailed results were presented in the Table 5.

Table 5: TLC Pattern of GKG and GG

Extracts	Mobile phase	Visualizing agent	GKG		GG	
			No. of spots	Rf values	No. of spots	Rf values
Pet. ether	Pet. ether: diethyl ether (8:2)	40% Perchloric acid	1	0.91	4	0.77 0.86 0.90 0.96
Chloroform	Chloroform : Benzene (6:4)	Vanillin-H_2SO_4 acid	3	0.82 0.94 0.97	2	0.37 0.95
Ethyl acetate	Chloroform Ethyl acetate : (8:2)	Vanillin-H_2SO_4 acid	2	0.67 0.85	2	0.25 0.95
Acetone	Acetone : Methanol	Vanillin-H_2SO_4 acid	2	0.42 0.75	2	0.90 0.97

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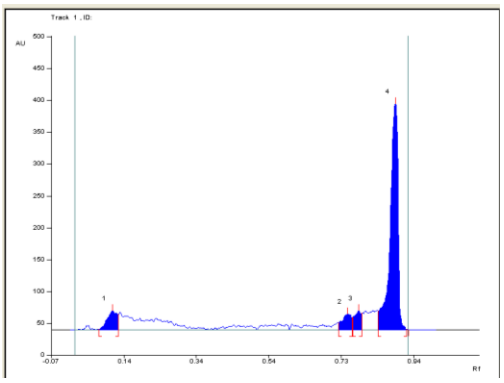
	(8 :2)					
Methanol	Acetone : Water : Chloroform : Methanol (7.5 : 0.5 : 1:1)	Anisaldehyde-sulphuric acid	3	0.55 0.73 0.91	4	0.22 0.77 0.85 0.90
	Ethyl acetate : Acetic acid : Methanol : Water (6 :1.5 : 1.5 :1)		3	0.61 0.82 0.95	4	0.35 0.80 0.91 0.97

High Performance Thin Layer Chromatography [22]

It is the most valuable quality assessment tool for the evaluation of plant derived drugs. Broad number of compounds can be analyzed effectively. Chromatographic fingerprint gives an ideal idea about possible active constituents present in the plant derived drug. High performance thin layer chromatography (HPTLC) is a recent, powerful analytical method with the superior separation power, reproducibility, and performance greater to classic TLC and also suitable for comparison of the fine difference among components with identical plant resource from different geographic locations. HPTLC fingerprint pattern of GG with mobile phase 1 showed 4 spots with different Rf values (Figure 5 and Table 6), and GKG showed 5 spots with mobile phase 1(Figure 6 and Table 7), where as GG showed 5 spots with mobile phase 2 (Figure 7 and Table 8) and GKG showed 6 spots with mobile phase 2 (Figure 8 and Table 9) indicated the presence of various chemical constituents.

Table 6: Peak values of GG with mobile phase 1



Peak	Rf	Height (AU)	Area (%)
1	0.10	30.3	8.52
2	0.75	25.6	6.64
3	0.78	30.7	6.15
4	0.89	354.3	78.70

Figure 5: Densitometry scan of GG with mobile phase 1

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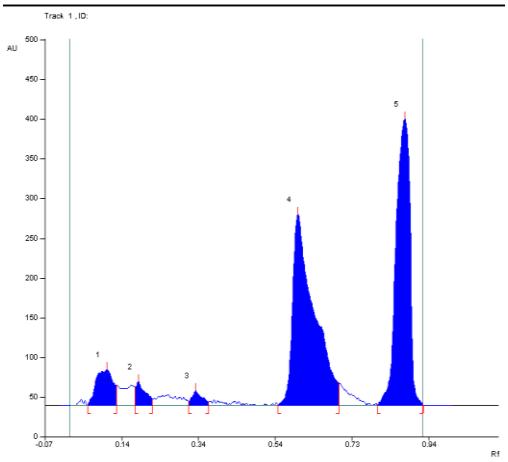

Figure 6: Densitometry scan of GKG with mobile phase 1

Table 7: Peak value of GKG with mobile phase 1

Peak	Rf	Height (AU)	Area (%)
1	0.09	45.1	6.69
2	0.18	29.9	2.48
3	0.33	18.5	1.63
4	0.59	239.9	45.23
5	0.87	360.2	43.97

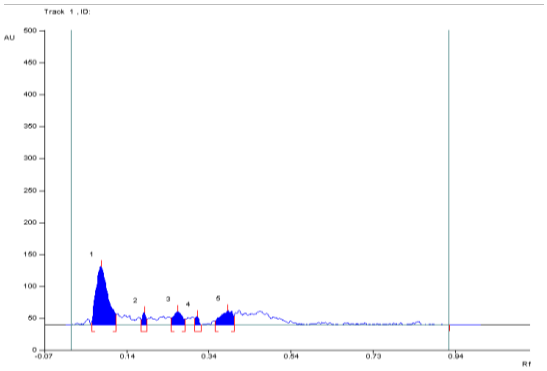

Figure 7: Densitometry scan of GG with mobile phase 2

Table 8: Peak value of GG with mobile phase 2.

Peak	Rf	Height (AU)	Area (%)
1	0.07	91.8	64.84
2	0.18	19.2	4.47
3	0.26	20.8	11.47
4	0.31	13.6	3.14
5	0.38	22.6	16.08

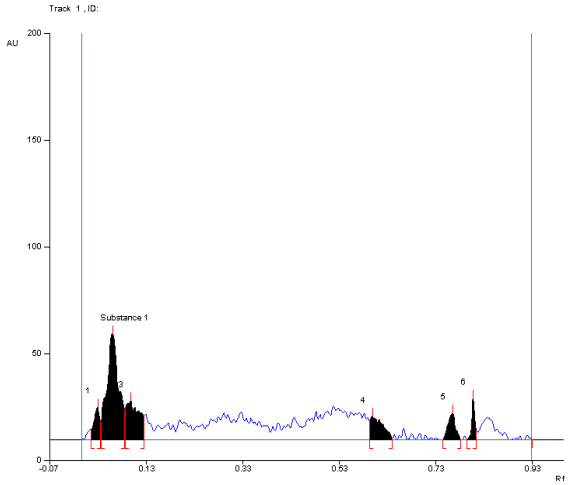

Figure 8: Densitometry scan of GKG with mobile phase 2

Table 9:Peak value of GKG with mobile phase 2.

Peak	Rf	Height (AU)	Area (%)
1	0.03	15.3	7.42
2	0.06	49.8	48.52
3	0.10	18.4	19.91
4	0.60	11.0	11.53
5	0.77	12.4	7.62
6	0.81	19.7	5.01

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CONCLUSION

GKG and GG tree exudates confirms positive tests for carbohydrates and reducing sugars and negative for glycosides, alkaloids, flavonoids, steroids, and tannins. Moisture content of GKG and GG were found to be low which would not support microbial growth and ensures longer shelf life of gums. Ash values of gums showed minimum organic contamination. GKG and GG showed high extractive values with methanol indicating the presence of polar compounds and it is justified by TLC and HPTLC results. Fluorescence analysis showed more or less similar report in case of both the gums. TLC with different organic solvents indicated the presence of various chemical constituents, more specifically with methanol extract. HPTLC of GKG and GG showed very distinct peaks with both the mobile phases which helps to differentiate gum exudates.

Results obtained in the above study are established for the first time. The proposed work can be a useful tool to minimise adulteration and substitution and to ensure high quality plant derived gums.

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