PHYSICO-CHEMICAL CHARACTERIZATION AND MICROBIOLOGICAL EVALUATION OF GUM GHATTI AS POTENTIAL FOOD ADDITIVE

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Abstract:
The paper deals with the in depth analysis of physico- chemical and microbiological properties of gum ghatti (Anogeissus latifolia Wallich) in response to the call from European Union demanding detailed evaluation of the safety of this gum as food additives. The parameters studied following standard methods and modern analytical instrumentsinclude ash%, acid insoluble ash%, cold water soluble %, pH, acidity (as HCl) % by mass, BFOM % by mass, viscosity, elemental and heavy metals analysis and FT-IR.Presence of E. coli and Salmonella spp. in the gum was also evaluated after standardizing microbial criteria. The study findings established gum ghatti as potential food additive.
Key words: *Dhawda, Gum ghatti, viscosity, food additive, E. coli and Salmonella spp.*

Introduction:

Gum ghatti is a dried exudate obtained from the bark of a dhawra tree,*Anogeissus latifolia* Wallich (family- Combretaceae), commonly found in the dry, deciduous forests in India. Chemically it is a polysaccharide consisting of arabinose, galactose, mannose, xylose, rhamnose, and glucuronic acid and occurred in nature as calcium and magnesium salt [1, 2]. As mentioned in the standard specification for gum ghatti by 29th JECFA (1985), water soluble fraction (approximately 90%) has a molecular weight of about 12,000 Da. The gum when freshly exuded is in a soft plastic form and is collected in the form of glassy nodules after drying. Gum ghatti is generally subjected to a series of processes, such as dissolution, filtration, sterilization, and spray drying, to obtain refined gum ghatti powder [7].

and then used as an additive in many food products [3]. It was originally considered as a substitute for gum Arabic in the early 1900s but was not commercially developed because of batch-to-batch variability [4]. Commercially it is available in market in three different grades i.e. Grade I, II & III, depending on its color and purity.

Gum ghatti has some physical properties superior to other gum products including greater acid resistance, salt tolerance, and oil binding capacity, resulting in excellent emulsification properties, even at considerably lower concentrations than gum Arabic [5, 6]. It is cost-effective emulsifier that can be used at concentrations as low as 25% of those of gum Arabic concentrations necessary for stable emulsions

Figure1.Appearance of grade I, II and III of gum ghatti

Gum ghatti is now emerging as one of the important emulsifying materials for foods [8, 9]. It is approved for use as an food additive in Japan [10, 11]. It is generally recognized as safe (GRAS) since 1976 and is approved for use as an emulsifier and stabilizer in the USA [12]. But the European Union subsequently demanded more detailed evaluation of the safety of this gum as food additives, and lack of the required information has resulted in the deletion of gum ghatti from European lists of approved additives [13, 14]. Hence to generate information on physico- chemical and microbiological properties of gum this study was undertaken. In this study, to set quality standards for different grades of gum ghatti, in depth data on physico-chemical parameters of different grades of gum ghatti was generated by using standard methods prescribed by Bureau of Indian Standards (BIS) and modern analytical instruments.

Materials and Methods:

Gum ghatti sample Grade I, II & III were purchased from Bahubali Udyog, Bilaspur, Chhattisgarh. Procured Gum ghatti samples of different grade was vacuum dried in desiccator in small batches. ghatti Gum was tested according to BIS procedure IS 7437: 1974 for following tests- Ash%, Acid insoluble ash%, Cold water solubles, pH, Acidity (as HCl) % by mass and BFOM % by mass. pH of Gum ghatti sample Grade I, II and III, was determined as prescribed in BIS procedure IS 7437: 1974. Viscosity of 5 % Gum ghatti solutions of Grade I, II and III, was determined by using BrookField viscometer (LV-3, 20 rpm). Color values of dried Gum ghatti samples Grade I, II and III, were determined by Hunter colorimeter, LabScan-SE. FTIR analysis of gum ghatti (Grade I, II and III) was done by Shimadzu, IR-Prestige-21.Elemental analysis of gum ghatti samples was carried out. Based on the Nitrogen percentage obtained from elemental analysis of the different samples, protein content was calculated by multiplying with protein factor, 6.25. To detect *E. coli* and *Salmonella* in different grades of gum ghatti samples, the BIS standards, IS:5887 (Part I)-1976 and IS:5887 (Part3)-1999; ISO:6579- 1993,respectively,were followed after due standardization.

Results and Discussion:

Viscosity of 5 % Gum ghatti solutions of Grade I, II and III, was determined by using BrookField viscometer (LV-3) at different spindle speed. As shown in fig. 2, with increase in speed (rpm) of the spindle, reduction in viscosity of gum ghatti was observed which confirmed shear thinning / thixotrophic nature of gum ghatti. As viscosity is greatly influenced by the polar ionic groups in the gum solution, such as free carboxylic groups in form of uronic acids, the

decrease in viscosity with ageing may be due to decomposition of uronic acids present in the gum following action by temperature and moisture present in the atmosphere.

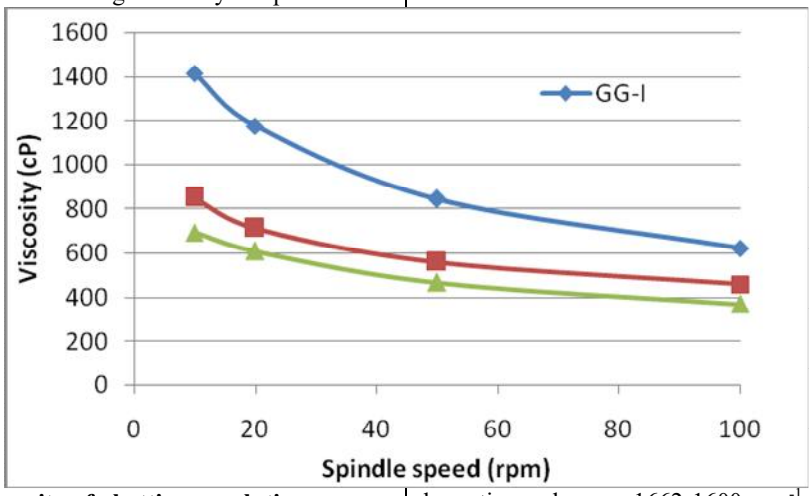

Figure2. Trend in viscosity of ghatti gum solutions

FTIR spectrum of different grades of gum ghatti (fig.3) revealed the broad areas of absorption between 3560 and 3155 cm^{-1} corresponding to O-H stretching absorption due to inter and intra-molecular hydrogen bonds. Bands around 2930 cm^{-1} refer to C-H stretching absorption whereas 1662-1600 cm^{-1} bands are due to the free carboxylate groups from uronic acid present in the gum. Since there was no absorption in the region 1760-1730 cm^{-1}, this indicated no esterified carboxyl groups were present .

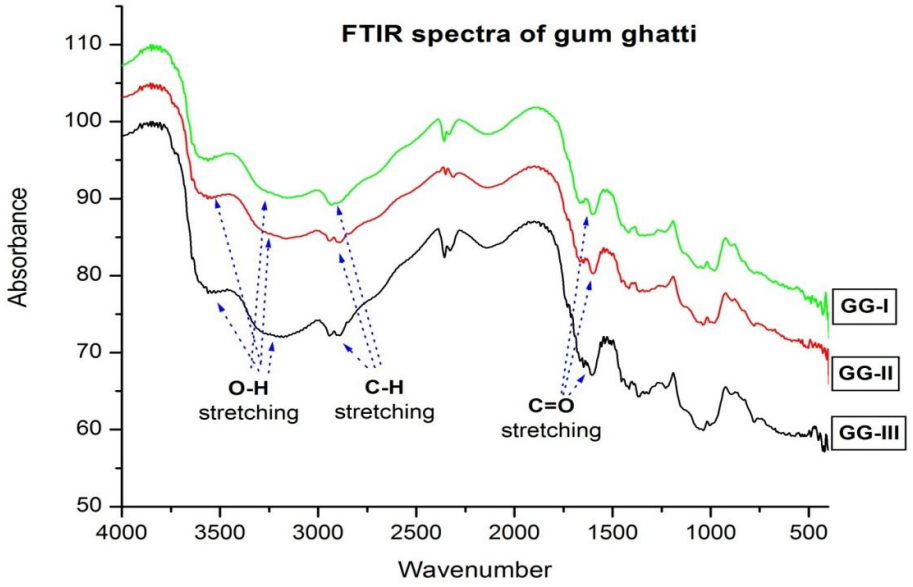

Figure 3. FTIR spectrum of gum ghatti (Grdae-I)

Gum ghatti (grade I, II and III) samples were analyzed for their properties ash%, acid insoluble ash%, cold water soluble %, pH, acidity (as HCl) % by mass and BFOM % by mass, viscosity, elemental and heavy metals analysis, etc.

Table 1: Physico-chemical parameters of different gum ghatti samples

Sl No	Parameters	Grade I	Grade II	Grade III	BIS Limit (IS 7395: 1989) reaffirmed 2001		
					Grade I	Grade II	Grade III
1	Viscosity (cP)	1176	713	611	1000	900	800
	Ash %	1.97	2.75	3.51	2.2	3.0	4.0
2	Acid Insoluble ash%	0.15	0.55	0.70	0.2	0.3	0.8
3	BFOM	0.39	1.09	3.71	1.3	2.5	5.0
4	Moisture % (Volatile Matter)	12.66	13.32	12.64	14	14	15
5	Cold water solubles	87.75	82.87	82.02	-	-	-
6	Gum Content (%)	83.18	79.76	79.26	-	-	-

7	pH	4.92	5.11	5.07	-	-	-
8	Acidity %	0.196	0.265	0.325	-	-	-
9	N%	0.327	0.58	0.615	-	-	-
10	C%	36.754	38.144	37.68	-	-	-
11	H%	5.309	5.582	5.555	-	-	-
12	O%	57.61	55.694	56.15	-	-	-
13	S%	Nil	Nil	Nil	-	-	-
14	Protein %	2.044	3.625	3.844	-	-	-
15	*E. coli*	negative	negative	negative	negative	negative	negative
16	*Salmonella spp.*	negative	negative	negative	negative	negative	negative

Color values of dried Gum ghatti samples Grade I, II and III, were determined by Hunter colorimeter, LabScan-SE. 'L values' measured (58 for grade I, 40 for grade II and 34 for grade III) confirmed increasing dark color from Grade I to III. Presence of tannin in gum ghatti was confirmed by Follin Ciacalteau reagent. Total color difference (ΔE) for gum ghatti grade-II and III was calculated by Hunter colorimeter, considering grade-I as a standard. Calculated values were, 17.82 for grade-II and 24.21 for grade-III, which confirmed darker color of grade II and further darker for grade III as compared to grade-I.

Heavy metals present in grade I gum ghatti was determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The results obtained confirmed that the gum carbohydrates are present in their Ca (5.615 mg/g) and Mg (1.656 mg/g) salts. Heavy metals concentrations like Lead, Chromium and Copper were found 0.007, 0.0085 and 0.0005 ppm, respectively. And for other metals like Arsenic, Cadmium, Nickel and Cobalt, the values were below detection limit of the instrument, i.e. less than 1ppb. The values found for the heavy metals are found within the safe limits prescribed for consumption.

For detection of *E. coli*, twenty five grams of different grades of gum ghatti samples were suspended in pre enrichment medium (peptone water) and incubated at 37 ˚C for 4 hours. Then the selective enrichment was done in Mac Conkey broth for 18 hours at 37 ˚C at 1 in 10 dilutions. The enriched samples were serially diluted and plated on selective plating out media (EMB Agar, Tergitol Agar and Mac Conkey Agar) and incubated at 37 ˚C for 18 hours. Representative samples of presumptive *E. coli* colonies obtained were tested for biochemical parameters following different biochemical tests *viz*., Indole test, Urease test, TSI test, Voges-Proskauer test, Methyl Red test, Gram staining, Motility and Citrate utilization tests. All the experiments were repeated twice. Based on the biochemical tests, it was confirmed that the presumptive colonies so obtained were not *E. coli*.

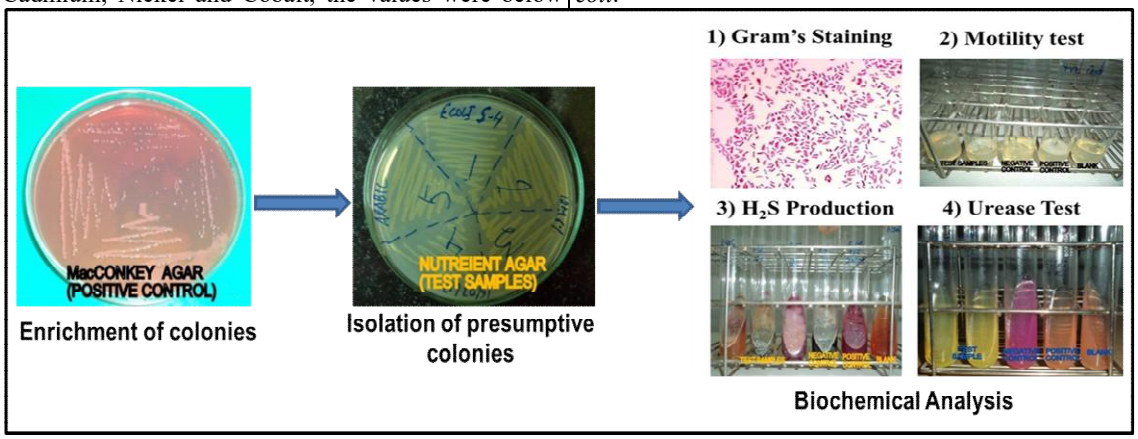

Figure4. Typical procedure for testing presence of microbes

For detection of *Salmonella*, twenty five grams of different grades of gum ghatti samples were suspended in pre enrichment medium (peptone water) and incubated at 37 ˚C for 20 hours. Then the selective enrichment was done in selenite cystine medium for 24 hours at 37 ˚C at 1 in 10 dilutions and also in RV medium at 42 ˚C for 24 hours at 1 in 100 dilutions. The enriched samples were serially diluted and plated on selective plating out medium (Brilliant green agar medium) and incubated at 37 ˚C for 24 hours. The presumptive colonies of *Salmonella* obtained were tested biochemically. Indole test, Urease test, TSI test, L-Lysine decarboxylation test, β-galactosidase test and Voges-Proskauer test had been done for representative colonies from each plate. All the experiments were done in two replications. Based on the biochemical tests, it seems that the presumptive colonies so obtained may not be *Salmonella*.Overall all samples from different grades of gum ghatti were found negative for *E. coli* and *Salmonella* both.

Conclusion:

Various commercially important parameters were analysed which will help in setting quality standards for different grades of gum ghatti. The values determined for the heavy metals in gum were found well within the safe limits prescribed for consumption.Absence of *E. coli* and *Salmonella* implies that the gum is not interfering with the human digestive system and can be considered as consumable without harm. Overall, the study findings established gum ghatti as potential food additive.

Acknowledgement:

The authors earnestly express their immense gratitude to Dr. KKSharma, Director, Indian Institute of Natural Resins & Gums, Namkum,Ranchi (Jharkhand), India, for his continuous encouragement and support.They also thankfully acknowledge the financial assistance fromIndian Council of Agricultural Research, New Delhi, India.

References:

Aspinall G, Hirst E, Wickstrom A (1955) Gum ghatti (Indian gum) the composition of the gum and structure of two aldobiouronic acids derived from it. *J Chem Soc* 1160–1165

Aspinall G, Auret B, Hirst E (1958) Gum ghatti (Indian gum), Part III. Neutral oligosaccharides formed on partial acid hydrolysis of the gum. *J Chem Soc,* 221:4408–4414

Al-Assaf S, Phillips G, Amar V (2009) Handbook of hydrocolloids second edition. Gum ghatti 17:477–494

Kaur L, Singh J, Singh H (2009) Characterization of gum ghatti (Anogeissus latifolia): a structural and rheological approach. *J. Food Sci.* 74, E328–E332

Al-Assaf S, Amar V, Phillips GO (2008) Characterisation of gum ghatti and comparison with gum arabic. In: Williams, P.A., Phillips, G.O. (Eds.), Gums and Stabilisers for the Food Industry 14. Royal Society of Chemistry, Cambridge, pp. 280–290

Al-Assaf S, Phillips GO., Amar V (2000) Gum ghatti, Handbook of hydrocolloids, pp. 477–494

Kang J, Cui S W, Chen J, Phillips G O, Wu Y, Wang Q (2011) New studies on gum ghatti (*Anogeissus latifolia*) part I. Fractionation, chemical and physical characterization of the gum Food Hydrocolloids 25, 1984-1990

Ido T, Ogasawara T, Katayama T, Sasaki Y, Al-Assaf S, Phillips G (2008) Emulsification properties of GATIFOLIA (gum ghatti) used for emulsions in food products. Foods Food Ingred J Jpn 213(4):365–371

Castellani O, Gaillard C, Vie V, Al-Assaf S, Axelos M, Phillips G, Anton M (2010) Hydrocolloids with emulsifying capacity. Part 3-adsorption and structural properties at the air-water surface. Food Hydrocoll 24:131–140

Manual Specification and Standards for Foods, Food Additives, etc., Under the Food Sanitation Act (Abstract) 2010 (2011) Japan External Trade Organization, pp 156

Japan's Specification and Standards for Food Additives (8th edn.) (2007), pp 279

Sakai E, Katayama S, Ogasawara T, Mizuno M (2013) Identification of *Anogeissus latifolia* Wallich and analysis of refined gum ghatti, *J Nat Med*, 67:276–280

BeMiller J N (Ed.) (1973) Quince seed, psyllium seed, flax seed, and okra gums, Industrial Gums. New york: Academic Press

Glicksman M (Ed.) (1983) Gum ghatti (Indian gum). Boca Raton: CRC Press Amar V, Al-Assaf S & Phillips G (2006) An introduction to gum ghatti: another proteinaceous gum. Foods & Food Ingredients Journal of Japan, 211, 275–279