

EVALUATION OF PHYSICOCHEMICAL, MICROBIOLOGICAL & TOXICOLOGICAL ACTIVITIES OF SOME NATURAL GUMS

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Abstract: Crude gums such as *Terminalia elliptica*. Willd. (Saj), *Buchanania lanzan*. Spreng. (Chironji) gum and *Albizia lebbeck* (L.) Benth (Siris) were isolated and evaluated for their physicochemical, microbiological, and toxicological characters. The pH result indicates that the three of these isolated gums are fairly acidic. Therefore, it may be necessary to alter the pH when making these gums for an oral or buccal medicine delivery system. The Sirs (*A. lebbeck*) gum had the highest swelling index among the three after a 24-hour examination. Although this gum's swelling index was relatively high, it didn't have the same gel strength as Saj and Chironji gum. All of these gums were of good grade and purity, according to the ash value data. Saj, Chironji, and Siris gums appear to have a minor hygroscopic character and should be stored in an airtight container based on their loss on drying. According to calculations, these gums exhibited good flow properties based on the bulk density, tapped density and Hausner's ratio. Studies indicated for the presence of carbohydrate, protein and absent of alkaloids, tannin, terpenoids, cardiac glycosides in all three gums. In water, each of these gums forms a thick gel but insoluble in organic solvents. Based on the OECD Guideline 425, an oral acute toxicity investigation on albino mice showed that the LD₅₀ of each gum was larger than 2000 mg/kg body weight of animal, making them safe for oral consumption. After one year of preservation, all of these gums were free of any harmful microbes, including E. coli, Pseudomonas aeruginosa, S. aereus, and Salmonella species. These findings pointed to the gums' potential as innovative medicinal excipients as well as their safety and purity.

Keywords: Saj gum, Chironji gum, Siris gum, CFU, OECD, LOD, LD₅₀

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INTRODUCTION

These days natural gums are widely being applied in pharmaceutical sectors due to their biocompatible, bioerodible, non-toxicity, cost-effective, and easy availability from nature [1,2]. The plant species *Terminalia elliptica* Willd., also referred to as "Saj," produces gum. According to a wealth of prior research, the plant's many parts have been used for treating a variety of ailments for a very long time, including diarrhea, fractures, dysentery, pitta, bronchitis, ulcers, haemorrhage etc.[3].

Buchanania lanzan Spreng., is a species that produces gum and is also referred in Oriva as Charu and Chironji or Char in Hindi. It is a member of the genus Buchanania and family Anacardiaceae. [4]. Mostly found in the many states of India including Odisha [5, 6]. All plant parts have been used for a very long time in traditional medicine as expectorants, blood diseases, aphrodisiacs, purgatives, blood purifiers, treatments for digestive problems, wound healing, fever, laxatives, and other things [7]. Another Indian species that produces gum is Albizia lebbeck. Benth., also called "Siris" or Indian walnut. It is a member of the Fabaceae family. In India's tropical and subtropical regions, it is widely cultivated [8]. Many plant parts have historically been used medicinally to treat a variety of conditions, including astringency, cough, eye infection, flu, gingivitis, lung issues, stomach tamers, and inflammation. [9]. Keeping this in view an attempt was made to collect, extract and isolate these gums from the trunk bark of the respective trees and evaluate for their phytochemical properties such as existence of Carbohydrate, protein, alkaloids, tannin, glycosides, flavonoids, steroids and terpenoids etc.

In the present study all three Gums have tested for their solubility, pH, loss on drying (LOD), different ash values, Swelling index, viscosity, bulk density, taped density, hausner's ratio, compressibility index etc. Acute toxicity studies had done on the specified animal model based on the OECD guidelines no 425 to determine LD₅₀. Isolated, old and fresh gums have tested for growth of various pathogenic microorganisms.

MATERIALS AND METHODS

All the materials used were of pharmaceutical grades. Various reagents for the phytochemical tests were purchased from the CDH Fine Chemical, New Delhi, Specific nutrient Media for the microbial test were purchased from the HiMedia Lab Pvt. Ltd and Other chemicals were purchased from Yarrow Chem Ltd, Mumbai.

Collection of gum

The bark plants *T.elliptica* (Saj), *B. lanzan* (Chironji), and *A. labback* (Siris) was hand-tapped to get the fresh gum exudates throughout the months of January through March in the Sambar Dhara jungle in the Bargarh District. Based on the methods used in earlier studies [10, 11], and were approved by Prof. Mr. Surya Kumar Badapanda, Reader and Head of the Department of Botany at Rampur College in Sonpur, Odisha.

Extraction and Separation of gums

The gums that oozed out from several deep cuts in the bark were gathered and prepared by hand-removing the exterior bark and other unnecessary debris. Finally, it was heated to 50°C in a hot air oven and dried until it was sufficiently brittle. Following suitable trituration in a fast mechanical blender (Bajaj India), mess number 100 was run through the dried gums. A ratio of 1 part powdered gum to 10 parts distilled water is used to dissolve it. The crude powdered gum was dissolved in purified water over the course of 24 hours while being continuously stirred with a rotary shaker (Remi Instrument Ltd, Mumbai, India).

The leftover residue was then thoroughly cleaned with water and added to the supernatant after the supernatant had been filtered through a muslin towel. The previous process was carried out three times [12, 13].

The gum was unglued from the supernatants in the form of a precipitate using two times as much acetone as usual. Centrifugation was used to separate the precipitate. The precipitate was dried at 40 to 50 $^{\rm 0}$ C in hot air oven. Each separated gum was then powdered, put through a sieve with a number of 100, and kept in desiccators for later tests [14,15].

In-Vitro Characterization of isolated gum powders

In-Vitro Characterization of isolated gum powders calculated by measuring Percentage yield [16], Solubility studies [16], Organoleptic properties [13], Loss or loss on drying (LOD) [18,19], Ash value [20], Swelling index[20,6], pH, and Viscosity [21], Bulk density and Tapped density, Hausner's ratio, Compressibility or Carr's Index, Angle of repose [22]as per the standard procedures.

Qualitative assessments of phytochemical constituents

Standard method was tailed for qualitative studyof carbohydrate, protein, alkaloids, tannins & phenols, glycosides, flavonoids, and Steroids & terpenoids of 1% (w/v) of each gum solution [23, 24, 25].

Acute toxicity study of gums:

According to OECD guideline no. 425, exrtracted gums from *Terninaliaelliptica* (Saj), *Buchananialanzan* (Chironji), and *Albizialabback* were examined for acute toxicity. The Institutional Animal Ethics Committee (IAEC), with approval number CPCSEA/ IAEC/JLS/17/03/22/041, gave its support to the current investigation.

By using healthy Albino mice of any sex, the LD_{50} was calculated. For each gum, 35 animals weighing 160–200 g were chosen, and each animal was arbitrarily allocated into 7 groups of five. One of the seven groups was used as a control

group, while the other six were used as test groups. Overnight fasting was imposed on all albino mice in the control and test groups. Each animal's body weight was calculated while fasting. The remaining groups got 100, 200, 500, 1000, 1500, and 2000 mg/kg/body weight of gum dispersion in distilled water, whereas the control group received normal saline (10 ml/kg/body weight per oral dose). For the first 30 min after dosage, the animals were continually monitored for specific behavioural changes and death. They were then periodically monitored for the following 24 hours, with particular focus placed on the first 4 hours, then every day for the following 14 days. After 14 days, the impact of gums on body weight was noticed. [26].

Microbial studies of gums:

Studies were conducted on isolated gum powder samples from *Terninaliaelliptica* (Saj), *Buchananialanzan* (Chironji), and *Albizialabback* that were both fresh and one year old (Siris). By using the pour plate method, the samples were inspected for the incidence of specific microbial species and the total number of viable aerobic microorganisms [27]. Potato dextrose agar medium was used to calculate the total fungal count, and the plates were cultured for 48 hours at room temperature (23–25 °C). Using certain media such as mannitol salt agar medium (Staphylococcus aureus), cetrimide agar medium (Pseudomonas), MacConkey agar medium (Escherichia coli), and deoxycholate citrate agar medium (Salmonella sp.), the presence of selected microbial species in the gum sample was assessed [27].

RESULTS AND DISCUSSION

Qualitative test results of isolated gum powders

During the summer months of April and March, gums were harvested from the bark of the plants Terimnaliaelliptica (Saj), Buchananialanzan (Chironji), and Ablizialebbeck (Siris). Using distilled water, gums were removed from the raw gums. Pure gums were separated by precipitation with acetone after extraction. For Saj, Chironji, and Siris gums, the percentage yield was discovered to be 32%, 6.57%, and 7.12%, respectively. In order to form a gel-like mass in cold water and transform into a colloidal solution in warm water, all of these gums were found to be sparingly soluble, but they were practically insoluble in organic solvents such ethanol, methanol, benzene, acetone, and petroleum ether. As shown in Figures 1, 2, and 3, the isolated Saj, Chironji, and Indian Siris gums had dark brown, light brown, and beige appearances, respectively. All three of these gums had an uneven texture, a distinctive smell, and a sweetish-sour taste.

Fehling's test and Benedict's test both show that all 3 gums contain carbohydrates (Table 1). The findings of protein assays such as the Ninhydrin and Biuret tests demonstrated that all of these gums contained protein (Table 1). The findings of the Dragendorff, Mayer, and Hager tests indicated that none of the three gums contained any alkaloids (Table 1). Tannin was discovered to be lacking in each of these gums, as reported in Table 1. The findings of the Borntrager's test and the Keller Killaine test demonstrated that none of these gums contained cardiac glycosides (Table 1). Table 2 demonstrates that only Chironji gum contained flavonoids, which was supported by assays like the alkaline reagent test and the lead acetate solution test. The findings of the Liebermann-Burchard

& Salkowaskitest, which are presented in Table 1, did not reveal the presence of tri-terpenoids or steroids in these gums.



Figure 1: Crude gum &Isolated gum powderof Saj (Terminalia elliptica)



Figure 2: Crude gum &Isolated powder ofChironji (Buchananialanzan)

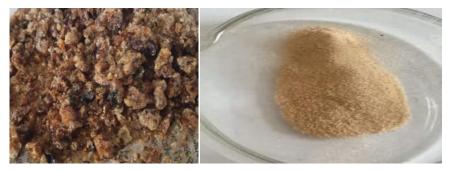


Figure: 3: Crude gum & Isolated gum powder of Indian Siris (Albizialebbeck)

Table 1.Qualitative test for isolated gums powders

SL.No.	Tests	Terminalia	Buchanania	Albizia						
		elliptica gum	lanzan gum	labback gum						
1	For Carbohydrates:									
	Fehling's test	+	+	+						
	Benedict's test	+	+	+						
2	For proteins									
	Biuret test	+	+	+						
	Ninhydrin test	+	+	+						
3	For Alkaloids									
	Mayer's test	-	-	-						
	Dragendorff 's test	-	-	-						
	Hager's test	-	-	-						
4	For Tannin									
	Ferric chloride test	-	-	-						
5	Cardiac Glycosides									
	Keller-Killiani test	-	-	-						
	Borntrager's test	-	-	-						
6	For Flavonoids:									
	Alkaline reagent test	-	+	-						

		Lead acetate solution test	-	+	-
I	7	For Steroids and terpenoi	ds		
ı		Liebermann Burchard test	-	-	-
ı		Salkowski test	-	-	-

(+)sign stand forpresence and (-) sign stand for absence of phytoconstituents.

Physicochemical and bulk characterization of isolated gum powders

Saj, Chironji, and Siris gums' 1% (W/V) solutions were found to have pH values between 4.4 -5.9, 4.3-4.9 and 4.3-4.8respectively (Table 2). This finding suggests that all three of these isolated gums have a somewhat acidic pH. Therefore, when making these gums for an oral or buccal medication delivery system, the pH may essential to be changed. After a 24-hour study, the swelling indices of Saj, Chironji, and Siris gums were determined to be 17.00 ± 1.69 %, 15.42 ± 1.93 % and 22.94± 2.02 % respectively (Table 2). This suggests that all three gums have exceptional swelling characteristics and could be utilized as a drug release modifier in the formulation of pharmaceutical dosage forms. The Sirs (Albizialebbeck) gum was shown to have the highest swelling index of the three. Despite having a relatively high swelling index, the gel strength of this gum was not as strong as that of Saj and Chironji gum. For Saj, Chironji, and Siris gum, the 4% W/V solutions' respective viscosities were measured to be 42±0.98 cps, 38% \pm .61cps and 77% \pm 0.23. The total ash, acid insoluble and water-soluble ash was found to be 3.23 ± 0.61 %, $1.21\pm 0.79\%$ and $1.72\pm 0.31\%$ for Saj gum, $4.53\pm 0.02\%$, $1.15 \pm 0.63\%$ and $1.61\pm 0.64\%$ for Chironji gum, $3.54\pm$ 0.69%, $1.24 \pm 0.81\%$ and $1.76 \pm 0.27\%$ Siris gum respectively (Table 2). The ash value data showed that all of these gums were of good grade and purity. The loss on drying for Saj, Chironji, and Siris gums 10.31 ± 0.73 , $8.41 \pm 0.31\%$, $6.8 \pm 0.50\%$ respectively (Table 2). This implies that these gums have a mild hygroscopic character and should be stored in an airtight container.

According to calculations, the bulk density and tapped density of three gums were $0.476\pm~0.02~gm/cm^3$, and $0.588\pm~0.06~gm/cm^3for~Saj~gum,~0.525\pm~0.03~gm/cm^3and~0.623\pm~0.02~gm/cm^3for~Chironji~gum,~and~0,501\pm~0.03~gm/cm^3and~0.575\pm~0.01~gm/cm^3~for~Siris~gum,~respectively.~All~of~the~Saj,~Chironji,~and~Siris~gums~had~a~Hausner's~ratio~1.23\pm~0.06~\%,~1.12\pm~0.03~\%,~1.18\pm~0.05~\%$ respectively, according to Table 2, which shows that these gums had good flow characteristics.

The compressibility Index were calculated as $19.04\pm1.01~\%$, $15.73\pm0.96~\%$ and $12.86\pm0.94~\%$ respectively for Saj,Chironji, and Siris gum (Table 2).The compressibility characteristics of Indian Siris gum were lower than those of the other two gums, whereas the Saj gums were more compressible than the other two gums(Table 2).Saj gum, Chironji gum, and Siris gum all had angles of repose that were $23.37\pm0.03~^0$, $16.04\pm0.15~^0$, and 13.37 ± 0.21^0 , respectively (Table 2). This data suggests that these separated gum powders have good to exceptional flow qualities.

Table 2. Physicochemical & bulk characterizations of three gum powders

Properties of gums	Terminalia elliptica gum (Saj) gum	Albizia Lebbeck (Siris) gum				
pH of gums	4.23 - 5.58	(Chironji) gum 4.20- 5.05	4.89-5.29			
(1% W/V solution)						
Swelling index %	17.00± 1.69 %	15.42± 1.93 %	22.94± 2.02 %			
Viscosity of	42±0.98%	38% ±0.61	$77\% \pm 0.23\%$			
(1% W/V) cps						
Total ash %	3.23± 0.61 %	4.53 ± 0.02	3.54 ± 0.69			
Acid insoluble ash %	1.21± 0.79	$1.15 \pm 0.63\%$	1.24 ± 0.81			
Water soluble ash %	1.72 ± 0.31	1.61± 0.64 %	1.76 ± 0.27			
Loss on Drying %	10.31 ±0.7	$8.41 \pm 0.31\%$	6.8 ± 0.50			
Bulk Density (gm/cm ³)	0.476 ± 0.02	0.525 ± 0.03	$0,501 \pm 0.03$			
Tapped Density	0.588 ± 0.06	0.623 ± 0.02	0.575 ± 0.01			
(gm/cm ³)						
Hausner's Ratio (%)	1.23± 0.06	1.18 ± 0.05	1.12± 0.03			
Compressibility index(%)	19.04± 1.01	15.73 ± 0.96	12.86± 0.94			
Angle of repose (0)	23.37±0.03	16.04±0.15	13.37±0.21			

Values set as mean \pm Standard Deviation (SD), (Number of observation, n=3)

Acute toxicity study of isolated gum powders

According to OECD standards number "425," a study on the acute toxicity of all three gums was conducted by giving albino mice a little amount of each gum orally. Tables 3 and 4 provide summaries of the findings. The outcome of gums on the animal body weights did not appear to have any discernible negative effects. When related to the control group of animals, every animal in the test group shown a typical gain

in body weight (Table 4).Both the test and control groups' observational health indicators and behavioural changes, such as changes in the corneal reflex, pupils, salivation, lacrimation, skin fur, pain, grooming, torch reaction, hyperactivity, sleep, alertness, pinna reflex, tremors, grip strength, etc., were noted (Table 4).

The observational results show no substantial change between the test and control groups, demonstrating the safety of these gums. Since no deaths have been reported during the testing period, these gums are safe and harmless(Table 4). Because no death was seen at higher dosages of 2000 mg or 2 g/kg body

weight, all three gums had LD50 values larger than 2 g/kg body weight of animal (Table 4).

Table 3.Outcome of gums on the body weight after 14 days.

Group	Treatment	Body weight (g)	
		Before treatment (Mean ± SD)	After treatment (Mean ± SD)
Control	Normal Saline (10 mL/kg per oral)	169 ±2.72	178 ± 2.45
Treated 1	Terminalia elliptica gum dispersion in distilled water (2000 mg/kg)	185 ± 1.89	192 ±1.67
Treated 2	Buchananialanzan (Chironji) gum dispersion in distilled water (2000 mg/kg)	168 ±1.22	182 ±1.56
Treated 2	Albizialabback (Indian walnut) gum dispersion in distilled water(2000 mg/kg)	172 ±1.37	179±2.39

Table 4. Observations of the treated and control animals' behavioral indicators

	30	Min			4 F	Iours			1 D	ay			2 d	lays			7 d	ays			14	Days		
	С	Те	Bl	Al	С	Те	Bl	Al	С	Te	Bl	Al	С	Те	Bl	Al	С	Те	Bl	Al	С	Те	Bl	Al
Mortality	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corneal reflex	-	•	•	•	-	•	•	•	-	-	•	•	-	•	•	•	•	•	-	•	-	•	•	•
Pupils			-		-						-				-									
Salivation	-															-								
Lacrimation	-	-	-		-			-	-			-		-	-	-	-			-	-	-		
Hyper activity																								
Pain																								
Grooming																								
Torch	•	-	-	-	-	-	-	-	•	•	•	-	-	-	-	-	-	-	-	-	•	-	-	
response																								
Skin fur	-		•	•	•		•				•				•		-			-	•	•		
Alertness	-		•	•	•		•				•				•		-			-	•	•		
Tremors																								
Pinna reflex	-	-	-		-	-	-			-	-	-	-		-	-	-	-	-	-	-	•	-	
Gripping strength	•	-		-	•	-	•	-	•		•	-		-		•		-	•				•	
Sleep	•	•	-		-		•	•		•	•		-		-	•	-	•	-	-	-		•	
Urination	•		-	•	-		•	•		•	•	•		-	-	•	-				-	•	•	

C-Control group given normal saline, □-Absent, ■-Usual, Te-Terminalia elliptica(Saj) gum, Bl-Buchananialanzan (Chironji) and Al-Albizialebbeck (Siris)

Microbial Studies of isolated gum Powders

The gums and mucins have a high moisture content and are hygroscopic, making them more prone to microbial development when kept for a long time. Keeping these in mind, studies on isolated gum microbes Powder tests were conducted on freshly obtained powders as well as powders older than a year, and the results are presented in Tables 5, 6, and 7 for Saj gum, Chironji gum, and Siris gum, respectively.

The presence of microorganisms was examined in the medication powder samples from each gum. According to the results, none of these gums—Saj gum, Chironji gum, or Siris gum—supported the growth of any harmful microorganisms, including *E. coli, Pseudomonas aeruginosa, S. aereus,* and Salmonella species.

Table 5.Study of microbial load of Saj gum (dry powder)

Parameters	Stored gum (1 year)	Fresh gum	Standard limit as per I.P.
Total fungal count	60 CFU/gm	57 CFU/gm	Not more than 100 CFU/gm
Total aerobic microbial count	41 CFU/gm	38 CFU/gm	Not more than 100 CFU/gm
Species of microbes			
E. coli	Not found	Not found	Should absent/gm
P.aeruginosa	Not found	Not found	Should be absent/ gm

S.aureus	Not found	Not found	Should be absent/ gm
Salmonella species	Not found	Not found	Should be absent/gm

CFU=Colony forming Units, gm-gram

Table 6.Study of microbial load of Chironji gum (dry powder)

Parameters	Stored gum(1 year)	Fresh gum	Standard limit as per I.P.		
Total fungal count	68 CFU/gm	64 CFU/gm	Not more than 100 CFU/gm		
Total aerobic microbial count	51 CFU/gm	47 CFU/gm	Not more than 100 CFU/gm		
Species of microbes					
E. coli	Not found	Not found	Should absent/gm		
P.aeruginosa	Not found	Not found	Should be absent/ gm		
S.aureus	Not found	Not found	Should be absent/ gm		
Salmonella species	Not found	Not found	Should be absent/gm		

CFU=Colony forming Units, gm-gram

Table 7.Study of microbial load of Siris gum (dry powder)

Parameters	Stored gum(1 year)	Fresh gum	Standard limit as per I.P.	
Total fungal count	45 CFU/gm	41 CFU/gm	Not more than 100 CFU/gm	
Total aerobic microbial count	29 CFU/gm	25 CFU/gm	Not more than 100 CFU/gm	
Species of microbes				
E. coli	Not found	Not found	Should absent/gm	
P.aeruginosa	Not found	Not found	Should be absent/ gm	
S.aureus	Not found	Not found	Should be absent/ gm	
Salmonella species	Not found	Not found	Should be absent/gm	

CFU=Colony forming Units, gm-gram

CONCLUSION

Terminalia elliptica (Saj),Buchananialanzan(chironji) andAlbizialebbeck(Indian siris) are among the gum yielding plant species in our locality. It is clear from the numerous reviews of the literature that human beings have long employed all the parts of these plants as traditional medicines for a variety of acute and chronic illnesses.Many works in the pharmacological and pharmaceutical field already have been done by taking most parts of these plants including gums. Natural gums have advantages over synthetic polymers from a pharmaceutical perspective because they are commonly available, biodegradable, inexpensive, harmless, and chemically inert.

Keeping this in view Gums were successfully collected extracted and isolated from their crude source and tested for physicochemical, microbiological and toxicologicalactivities. The resultsconclude the safety, purity and good flow properties of these gums in its isolated form. Hence if properly fabricated, these gums could be more beneficial in the form of pharmaceutical excipients in the future.

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Abbreviations

CFU=Colony forming Units, gm-gram

OECD= Economic Co- operative and Development

LOD= Loss on drying

LD= Lethal Dose

Te= Terminalia elliptica

Bl- Buchananialanzan

Al = Albizialebbeck

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