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# Characterization of physicochemical properties and thermal analysis of Saja (*Terminalia tomentosa* Roxb. DC) tree gum extracted traditionally and via gum enhancers at Mungeli region of Chhattisgarh

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### Abstract

Saja (*Terminalia tomentosa*) is a member of the Combretaceae family also known as crocodile bark tree and its all part *i.e.* bark and leaves used in medicinal purpose. Various Physico-chemical properties of natural gum exudates are useful in the food industry. As thickening, gelling and emulsifying agents and stabilizers it is also used as adhesives, binding agents, crystal inhibitors, clarifying agents, encapsulating agents, flocculating, foam stabilizers, swelling agents etc. Gum enhancers enhanced the exudation of gum from tree trunk. The exudation of gum was higher in gum enhancers when it was compared with traditionally tapped gum. Physicochemical characteristics were analyzed in saja gums ranges *i.e.* pH (5.24, 5.20), solubility (46.07 and 77.05%), viscosity (933 cPs), moisture content (9.51%, 9.78%), protein (2.02%, 1.78%), fat (1.10%, 1.50%), fiber (84.03, 84.02), tannin (0.544, 0.062), alkaloid (0.607, 0.59), Ash content (3.51%, 4.92%) and acid insoluble ash (6.36%, 6.28%). Major elements (Ca, Mg, and K) were found in gum tapped by traditional method as well as extracted via using gum enhancers. Thermal analysis (DSC and TGA) have shown the exothermic peak at 150  $^{\circ}$ C and TGA analysis indicated three peaks in range up to 900  $^{\circ}$ C.

Keywords: Saja gum, SEM/EDX, DSC, TGA, gum enhancer, tannin, alkaloid

### Introduction

Saja (*Terminalia tomentosa*) is a member of the Combretaceae family. Bark as astringent and used in treatment of ulcer, vitamins, fracture, haemorrhages, bronchitis and diarrheal. Antifungal, antioxidant, anti-hyperglycaemic, antidiarrheal, and antileucorrheal are only a few of the pharmacological effects of the plant. Common polypheolic chemicals reported for various varieties of *Terminalia* are: Ellagic acid, Dimethyl ellagic acid, Penta methyl flavellagic acid, trimethyl flavellagic acid and  $\beta$ -sitosterol (Shinde *et al.* 2011)<sup>[16]</sup>. Although, its bark is used in traditional Indian and traditional medicine for wound healing, gastrointestinal ailments, and anti-inflammatory reasons (Alladi *et al.* 2012)<sup>[1]</sup>, wood is considered sacred for idols in temples. It is having vertical fissures and transverse crack. The chemistry and components of *Terminalia tomentosa* were studied by Row and Rao (1962)<sup>[45]</sup>. Alkaloids, flavonoids, steroids, glycosides, phenolic compounds, resins, phytosterols, volatile oils, gums, tannins, saponins, and lignins, among other active components, provide natural and allopathic medicinal benefit (Krishna and Sujathamma 2019)<sup>[10]</sup>.

Gum trees are economically important found in tropical moist and dry deciduous forests, produce a significant quantity of gum, which are being widely used as industrial, food and medicinal purposes worldwide. In the food industry these are used as thickening, gelling and emulsifying agents and stabilizers. These are also used as adhesives, binding agents, crystal inhibitors, clarifying agents, encapsulating agents, flocculating, foam stabilizers, swelling agents etc. They are insoluble in alcohol and ether but soluble in water. Chhattisgarh State is rich in forest and has vast variety of minor forest products to favorable agro climatic conditions resulting in good forest area *i.e.* 43.6% of total. It is a one of the important non-timber forest produce (NTFP) and viable income sources for thousands of forest dwellers, especially tribal in India. Hence, there is a need to develop scientific and sustainable tapping methods are leading to the death of the tapped trees and erratic supply of gum or resin in the market. Therefore, there a need to develop tapping technology to achieve

potential production of gum and study of characterization of physicochemical properties and thermal analysis for its further use is in various industries. With this view the study has been taken up for studying the physicochemical properties, elemental composition and thermal analysis gum extracted from Saja trees at Mungeli region of Chhattisgarh by traditional tapping and with the use of gum enhancers.

# **Materials and Methods**

Saja (Terminalia tomentosa) trees of more than 1 m diameters at 1 DBH were selected for experimental purpose and treated traditional method of tapping (Single cut with axe) and by using gum enhancers (ethephon and sulphuric acid) were used *i.e.* during the month of January to June in two consecutive experimental years 2018-19 and 2019-20. The experimental design Randomized Block Design was used in replicated thrice for analysis. The surface microphotography of gum powder was studies by using Scanning electron microscope and elemental composition are analyzed by using EDX system at NIT (National institute of technology) Raipur (CG). The biochemical and physicochemical parameters of gum exudates were analyzed in laboratory of the department of Plant Physiology, Agricultural Biochemistry and Medicinal and Aromatic plants, IGKV Raipur. The physicochemical properties *i.e.* pH, Solubility, viscosity, swelling index, moisture content, protein, fat, fiber, tannin, alkaloid, ash, acid insoluble ash content was determined by using various methods.

The pH value was measured by using 1g of gum powder, it was well combined and dissolved in 100 mL of hot distilled water. The mixture was allowed to remain at room temperature for 5 minutes before the pH and temperature were measured using a precalibrated pH metre (Model ELICO L1614) (Ameh, 2012) <sup>[6]</sup>. The gum's solubility in cold and hot distilled water, acetone, and ethanol was tested (Eddy et al., 2012) <sup>[6]</sup>. The viscosity of the gum exudates was measured using a digital Brookfield DV-E viscometer (FAO, 1990)<sup>[28]</sup>. The Swelling Index was measured by 1g of power gum was placed in a 100 ml stopper measuring cylinder, and added 100 ml of distilled water to create the final capacity 100 ml. The volume of the gum before hydration (V0) was measured. The free water was decanted after 24 hours, and the volume of swollen (hydrated) gum (Vi) was measured (Phani et al., 2011) [33]. Moisture content of gums was determined by following the standard AOAC, 1999 method. The protein content of the gum was determined using the semi-micro Kjeldahl method (AOAC, 1990)<sup>[3]</sup>. Protein content was calculated using the nitrogen conversion factor of 6.25 as proposed by (Rodriguez et al. 2004) [30]. Fat content of the gum samples was determined by employing solvent extraction using Soxhlet extraction unit according to official method (AOAC), while the according to Rao (1993). The total soluble fibre was obtained by subtraction of contents of moisture, ash and protein from 100, as described by Sabah El-Kheir et al. (2008) <sup>[7]</sup>. Total Ash and protein from 100, as described by El-Kheir et al., (2008)<sup>[7]</sup>. The tannin content was measured as per the method described by Markkar (1996). Alkaloid content was measured as per the method described by Harbone (1973) [43]. Ash content of the gum sample was determined by burning 5g of gum sample was in muffle furnace at 600 °C for 4 hour. The ash content was expressed as a% ratio of the mass of the ash to the oven dry mass (Yebeyen et al., 2009)<sup>[24]</sup>. Acid insoluble ash was determined

by using 25 mL dilute HCl was applied to the crucible with total ash. The ashless filter paper (Whatmann number 41) was used to capture the insoluble materials, which was then washed with hot water until the filtrate was neutral. The filter paper containing insoluble matter was transferred to the original crucible and dried on a hot plate and ignited to constant weight. For 30 minutes, the residue was allowed to cool in a suitable desiccators and weighed without delay. The content of the insoluble ash was calculated with reference to the air dried plant material (Mushtaq et al., 2014) [12]. Scanning electron microscopy (SEM) the surface morphology of Gum was observed using SEM. The dried sample of Gum was ground and then coated with gold under vacuum using a sputter coater. The scanning electron microscopy (SEM) was conducted using a ZEISS EVO Series Scanning Electron Microscope Model EVO 18 scanning electron microscope operated at 20.00 kV. EDX analysis: Oxford- Energy Dispersive X-ray system (INCA 250 EDS with X-MAX 20mm Detector).

# Result and Discussion

# **Collection of gum exudates**

The gum exudates were obtained by tapping techniques via use of gum enhancers and traditionally tapped since January to June in year 2018-19. The gum tapping through gum enhancers was initiated via use after different gum enhancer *i.e.* Ethrel (trade name) contains ethephon (2-chloro-ethyl-phosphonic acid) 39%, Dilute Sulphuric acid applied by battery operated drill machine and hand girmit to induce gummosis. The gum enhancer (10 mm diameter and 1 to 1.5" deep) was injected by syringe in 4 ml at one treatment. It is an mentioned by Bhatt (1987) <sup>[5]</sup> and also by Vashishth (2017) <sup>[29]</sup> used sulphuric acid as a gum enhancer in *Lannea coromandelica*. Collected gum exudates were stored in dry place described by Bhushette and Annapure (2017) <sup>[32]</sup>.

# Physicochemical characteristics

All the biochemical and physicochemical analysis result as shown in Table 1, the average solubility of the Saja gum was higher in cold water and hot water 46.07 and 77.05% respectively and it least soluble in Ethanol and not soluble in acetone. Meer and Davidson (1980) <sup>[26]</sup> reported that the natural gums (natural plant gum) are hydrophilic carbohydrate polymers of high molecular weights, generally composed of monosaccharide units joined by glucocidic bonds.

The natural pH of the Saja (*Terminalia tomentosa*) gum, measured in an 1% w/w aqueous solutions, with an average value 5.24 and 5.20 in gum exudates tapped by using of gum enhancers and traditionally tapped respectively. With a reported value 4.7 and 5.4 due to the generally macromolecules acids presence in gum, the pH of raw and purified were indicating mild acidity in Gum Arabic. The acidity of plant gums is obvious since most of them are known to contain salts (Ca, Mg, K, Na, and Fe) of acidic polysaccharides, the acidity of which is due to uronic acids in their structure (Odeku and Fell, 2004) <sup>[13]</sup>.

The average solubility of tapped gum samples was depicted in table 1. Comparative study of solubility of experimental gum in cold water during the experimental year indicated that there was no significant difference in traditional methods as well as use of gum enhancers. The solubility was maximum in cold water (77.05%) and lower in hot water (46.07%) and least soluble in ethanol (0.98%) while no solubility was observed

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in acetone. Gums was either water soluble or absorb water and swell up or disperse in cold water to give a viscous solution or jelly. The good solubility gum indicated the absence of cross linking between polymeric chains. This is because gums having cross linked polymeric chains only swell in water without dissolving.

Analysis		Traditionally method	Gum enhancers	
Solubility	Hot water	$46.07\pm0.64$	-	
	Cold water	$77.05 \pm 1.196$	-	
	Ethanol	$0.98\pm0.13$	-	
	Acetone	0	0	
pH		$5.20\pm0.058$	$5.24 \pm 0.070$	
Viscosity	(cPs)	933	-	
Moistur	e (%)	$9.51 \pm 0.73$	$9.78\pm0.63$	
Swelling index (%)		$5.14\pm0.06$	$5.89 \pm 0.72$	
Protein (%)		$2.02\pm0.015$	$1.78\pm0.468$	
Fat (%)		$1.10 \pm 0.57$	$1.50 \pm 0.39$	
Fiber		$84.03 \pm 0.023$	$84.02\pm0.004$	
Tannin		$0.544 \pm 0.013$	$0.062\pm0.004$	
Alkaloid		$0.607 \pm 0.026$	$0.59\pm0.02$	
Ash		$3.51\pm0.316$	$4.92\pm0.645$	
Acid insoluble ash		$6.36 \pm 0.11$	$6.28\pm0.16$	
Values - Maar	C (D)			

 Table 1: Physicochemical analysis of Saja (*Terminalia tomentosa*)

 gum exudates by various different tapping techniques.

Values = Mean  $\pm$  SD, n = 3

Murwan and Asma (2008)<sup>[7]</sup> reported that the gum from *Acacia senegal* is a water soluble polysaccharide of the hydrocolloid group and comprised mostly of *arabino galactan* and protein, in addition to some mineral elements. It is insoluble in most organic solvents; however, limited solubility can be also obtained in ethanol (up to 60%) glycerol and ethylene glycol. Similar results were also reported by Williams *et al.* (2000)<sup>[27]</sup>.

In traditionally tapped gums the average viscosity was 933 cPs observed in 10 rpm at room temperature 30°C. Increased in rpm decreased the viscosity in all the gums spindle size used 62. The moisture content was 9.51% in gum samples tapped by traditional method of tapping (single cut with axe) and it was significantly decreased when using gum enhancer ethephon 9.78% moisture content in gum sample. Moisture content is inversely proportional to shelf life. The lesser the moisture content more is shelf stability of gum by inactivating enzymes and decrease microorganism activity (Bashir and Haripriya, 2016; Mirhosseini and Amid, 2012)<sup>[35, 36]</sup>.

The Swelling index was  $5.14 \pm 0.06$  in traditionally tapped gum and the sample were having non-significant difference in swelling index in general swelling tapped by in both the experimental year. While the various tapping treatment were having significant difference in swelling index in gum sample. Shah *et al.* (2011)<sup>[11]</sup> reported that excellent swelling ration in all distilled water (14.5) as compared to HCl buffer (5.0). Swelling index indicated that gum is ionic in nature and due to presence of ions swelling ratio remains lower as compare to distilled water.

The protein content of traditionally tapped gum are (2.02%) it was higher as compared to tapped by gum enhancer (1.78%).

The protein content of gum is mainly responsible for its emulsion, foaming and film forming capacity and also helps as an indication of different species (Sherahi *et al.* 2017)<sup>[41]</sup>. Similar report was found in *Prunus cerasus* gum and Apricot gum its protein percent is 2.40% and 2.25-2.91% respectively (Fathi *et al.*, 2016; Hamdani *et al.* 2017)<sup>[46, 47]</sup>. Gums polysaccharides show remarkable ability to bind with protein food protein are essential component of the diet that provide adequate amount of amino acid need to survival (Friedman, 1996)<sup>[42]</sup>.

The crude fat content in gum enhancer was found to be 1.50% higher as compared to traditionally tapped gum 1.10%. There was no significant impact observed in gum sample tapped by various method of tapping. Vinod *et al.* (2010) <sup>[19]</sup> was found that the Fat content of plant gums may include saturated as well as unsaturated fatty acids. Further, these gums showed non-significant differences in the quantity of fiber tapped *via* traditionally and use of gum enhancers *i.e.* 84.03% and 84.02% respectively. Similar report was observed by Janaki and Sashidhar (1997) <sup>[8]</sup> that the Total fiber 87% and 80% in Gum karaya and Kondagogu gum respectively.

The tannin content of gum Saja has been analyzed (table 1). It was observed that the tannin content of gum tapped via using of gum enhancer (0.62%) is higher than that of traditionally tapped gum (0.544%) in Saja. Janaki and Sashidhar (1997)<sup>[8]</sup> reported that the tannin content of Gum Karaya (0.027%) is lesser than that of gum kondagogu (0.073%). Possibility indicates the gum quality it can be used as one of biochemical parameters to check the quality of the gum kondagogu for grading and also to differentiate it from gum karaya. The Alkaloids content of Saja gums were observed to be in a similar range (0.607 to 0.59%) gum tapped by traditionally and using of gum enhancer. They are small organic molecules, secondary metabolites of plants, containing nitrogen usually in a ring; about 20% of plants species consist of alkaloid (Amirkia and Heinrich, 2014; Khan, 2016)<sup>[39, 40]</sup>. These are stored in different parts of the plant. It is one of most known alkaloid which had been used and still for medicinal purposes. This alkaloid is powerful narcotic which is used for the relief of pain, but its usefulness is limited because of addictive properties.

The ash content of gum tapped by gum enhancer (4.92%) and traditionally tapped gum (3.51%) was significantly differed and that gum tapped by using gum enhancers had a higher inorganic matter that of traditionally tapped gum of Saja (Table 1). Siddique *et al.*, (2015) <sup>[38]</sup> reported that total ash content is normally composed of inorganic carbonates and phosphate. Presence of minerals like Ca, Mg, Zn, Mn and Pb have been reported in plant gums including those obtained from *Acanthophyllum bruteatum* gum (Wani *et al.*, 2019) <sup>[23]</sup>. Moreover, the acid insoluble ash (AIA) of both gums is no significantly differed 6.36 and 6.28% in traditionally tapped gum and via using of gum enhancers respectively. It is important find out the content of the sample which is not hydrolyzed by 72% H<sub>2</sub>SO<sub>4</sub> and is not sub sequentially or volatilized upon incineration of this acid insoluble residue.

Table 2: SEM analysis of gum samples tapped by gum enhancers and traditional method of tapping Saja (Terminalia tomentosa).

		Gum enhancer			Traditional method	
S. N.	Element	Weight %	Atomic %	Element	Weight %	Atomic %
1	Ca	25.29	31.15	Ca	39.2	38.83
2	K	26.9	33.97	K	23.9	24.27

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3	Mg	5.16	10.48	Mg	8.02	13.09
4	Cu	7.36	5.72	Cu	8.38	5.24
5	Fe	1.72	1.52	Fe	4.02	2.85
6	Cr	3.42	3.25	Cr	1.91	1.46
7	Ni	2.26	1.9	Ni	1.55	1.05
8	Pd	20.64	9.58	Al	3.48	5.12
9	Cd	3.31	1.45	Со	2.87	1.94
10	Hg	3.93	0.97	Cl	2.31	2.59
11				Na	1.6	2.76
12				Hg	1.11	0.22
13				Pb	1.03	0.2
14				Zn	0.49	0.3
15				Mn	0.14	0.1

# Structural microscopy by SEM and thermal properties of gum (DSC and TGA)

Scanning electron microphotographs (SEM) of Saja gum

exudates obtained is represented in (Figure 1) at different magnifications (550x, 1500x, 2000x, 3000x, 5000x and 6000x).

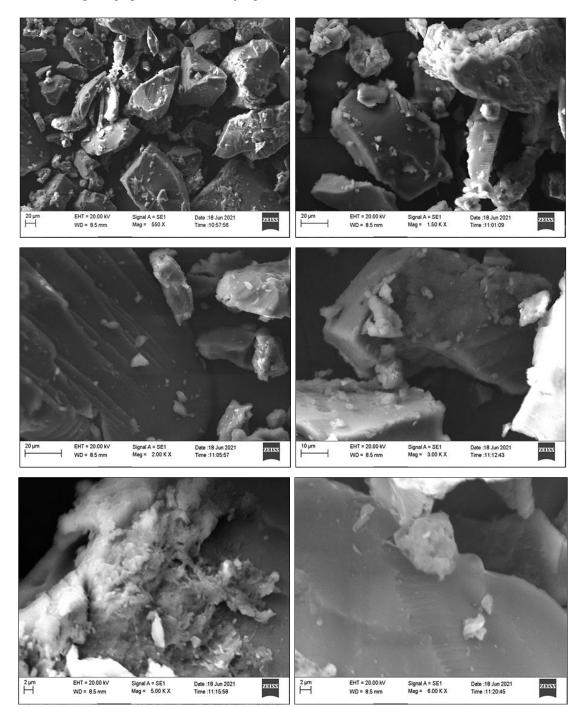


Fig 1: SEM images of gum sample tapped by gum enhancer of Saja (Terminalia tomentosa) at different magnifications.

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The microphotographs of Saja (Terminalia tomentosa) (Figure 1) are indicative of sharp lucent crystalline flanks with relative smooth surface. The particles are mostly seen as aggregates of irregular shapes and dimensions which were fibrous in nature. The SEM results of the present study suggested that, hydration capacity of gums depends on the surface property. The shape and structure or surface topography of Saja (Terminalia tomentosa) gum may be affected by the method of extraction and purification or preparation of the product (Qian et al., 2009)<sup>[34]</sup>. Wang, Ellis, Ross-Murphy (2002, 2003 and 2006) [21, 22] had reported that, particle size and specific surface area influence the hydration behaviour of gums, which in turn influence their intrinsic viscosity and molecular mass. The rough surface and irregular shape of gum Pithecellobium dulce (Roxb.) gum revealed the amorphous nature of gum (Chaudhri and Annapure, 2020)<sup>[2]</sup>.

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Element composition analysis using EDX system through microstructure of powder gum sample: The elemental composition result was shows in Table 2. The gum resin extracted by gum enhancer get more production as compared to traditional and other gum enhancer method of tapping. Therefore, the quality of gum were analyzed for physicochemical properties as well as SEM (Scanning electron microphotographs) and EDS to find out the impact of method of tapping on quality characteristics of gum. It was observed that the impact of gum enhancers and traditional method of tapping on elemental composition of tapped gum exudates. It was observed that in traditionally (single cut with axe) tapped gum having Al, Co, Cl, Na, Pb, Zn, and Mn element additionally while in chemically tapped gum having Cd and Pd in gum sample. However, remaining Ca, Mg, K, Cu, Fe, Cr, Ni and Hg were same. The present investigation revealed element compositions that were identical to those previously reported for gum Kondagogu by Vinod et al. (2008)<sup>[20]</sup>.

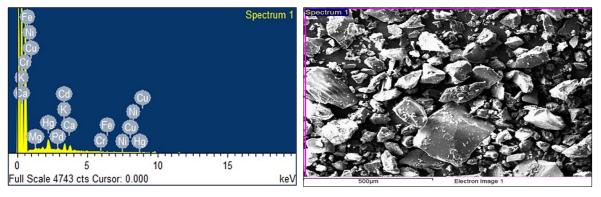


Fig 2: EDX analysis of gum exudate of Saja (Terminalia tomentosa).

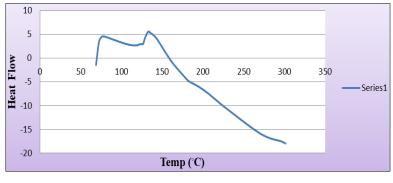


Fig 3: DSC analysis of Saja (Terminalia tomentosa) gum exudates.

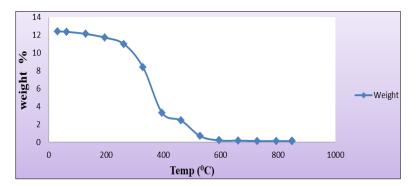


Fig 4: TGA analysis of Saja (Terminalia tomentosa) gum exudates.

# Thermal analysis

**DSC** (Differential scanning calorimetry): The thermal analysis of gum helps in understanding the temperature influence on the process of gum. The DSC analysis results

(Fig. 2) showed initial broad exothermic peak at 150°C caused by crystallization. Similar results have been obtained by Singh *et al.* 2010 <sup>[37]</sup> in *mangifera indica* gum, Singh and Bothara (2014) <sup>[31]</sup> in *Diospyros Melonoxylon* Roxb.

# TGA (Thermo gravimetric analysis)

The thermo grams of gums are given in (Fig. 3) which shows a weight loss of gums with respect to temperature. The gums losses their weight in three steps, first weight loss was a result of dehydration of gum (30 to 262 °C). Second weight loss of gum was attributed due to dehydration of polysaccharides and observed between 262 to 462 °C. The second weight loss event may be attributed to the polysaccharide decomposition (Zohuriaan, Shokrolahi 2004; Varma *et al.*, 1997) <sup>[18, 25]</sup> and is described by a weight loss. While the third step start at 462 and 463 °C and end ends at 900 °C. The third weight loss might be the result of the conversion of gum into carbon residues.

# Conclusion

- 1. The Saja gum more soluble in cold water and hot water indicated less or no resin content present in gum exudates.
- 2. The physicochemical properties *i.e.* hydrophobic Protein, tannin, acid insoluble ash content decreased non significantly. While ash content, moisture, ash content increased significantly with use of gum enhancers. However, there was no change in pH, alkaloid, fiber content in exudates.
- 3. SEM analysis results showed the gum exudates which were fibrous and hydrophilic in nature. EDX analysis denotes that the use of gum enhancers having no change in the major elements of the gum *i.e.* Ca, Mg and K. TGA (Thermo gravimetric analysis) indicated that the exudates of experimental trees shown the presence of volatile compounds in biopolymers and having three peaks range in between (30-900 <sup>0</sup>C).

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