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RESEARCH ARTICLE

Evaluation of Phytochemicals, Physico-chemical Properties and Antioxidant Activity in Gum Exudates of *Buchanania lanzan*

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Abstract Gum of *Buchanania lanzan* Spreng. (Anacardiaceae) is being traditionally used for various medicinal purposes. This study evaluates the phytochemicals as also the physico-chemical properties and antioxidant activity in gum exudates of *B. lanzan* Spreng. Seven samples of gum exudates of *B. lanzan* Spreng. were collected from Bilaspur (Chhattisgarh), Simdega and IINRG farm (Jharkhand), Dindori and Umaria (Madhya Pradesh) and Mirzapur (Uttar Pradesh) for studying variations in their major phytochemicals, physico-chemical properties and antioxidant activity adopting standard procedures. Twenty percent and ten percent concentrations of gum exudates were used for determining their viscosity (cP) at ambient temperature. The antioxidant potential of the gum exudates was evaluated by free radical scavenging activity using 1,1-diphenyl-2-picryl hydrazyl assay. Flavonoids, saponin, amino acid/protein and carbohydrates were found in all the gum exudates. All of them showed intra-specific variation in the physico-chemical properties viz. moisture level, color parameters (L, a, b), ash content, elemental (CHN) level, specific rotation [α] and heavy metals. Tannin was present only in the black gum exudates collected from Madhya Pradesh and Chhattisgarh. *B. lanzan* gum exudates, at twenty percent and ten percent concentrations, exhibited shear thinning/pseudoplastic flow pattern in their viscosity. Antioxidant activity was found only in samples with tannin and the magnitude was related to the tannin level in the gum. The findings show the significant qualitative and quantitative intra-specific variations in *B. lanzan* gum exudates, collected from different places, for their phytochemicals, physico-chemical properties and antioxidant activity.

Keywords *Buchanania lanzan* · Piyar · Char · Chironji · Phytoconstituents · Physico-chemical characterization · Antioxidant activity

Introduction

Buchanania lanzan Spreng. commonly known as char, chironji, piyar and achar, belongs to family Anacardiaceae. It was first described by Francis Hamilton in 1798. The tree is wild and found in the tropical deciduous forests of northern, western and central India, mostly in the states of Chhattisgarh, Jharkhand, Madhya Pradesh and in Varanasi and Mirzapur districts of Uttar Pradesh. Besides India, the plant is also found in other tropical Asian countries, Australia and Pacific islands. Out of seven species reported in India, *B. lanzan* and *B. axillaris* (Syn. *B. angustifolia*) produce edible fruits. Traditional indigenous knowledge reveals the immense value of almost all parts of the plant *i.e.* roots, leaves, fruits, seeds and gum for various medicinal purposes. Unfortunately due to over-exploitation and indiscriminate harvesting (lopping and cutting), considerable reduction in the population of *B. lanzan* has been recorded in the recent past, leading to severe threat to its extinction and needs urgent conservation efforts [1–4]. Bothara and Singh [5] have studied the characterization of thermal property of natural gums obtained from the seeds of *Diospyros melonoxylon* Roxb., *B. lanzan* Spreng. and *Manilkara zapota* Linn. using differential scanning calorimetry (DSC), differential thermal analysis (DTA) and thermogravimetric analysis (TGA) under nitrogen

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atmosphere. They have reported that these gums are thermally stable and can be used as release modifiers in various dosage form. Chirauli nut gum was isolated from the bark of *B. cochinchinesis* (Family: Anacardiaceae) and was used as a release modifier for the preparation of diclofenac sodium spheroids, using the extrusion spheronization technique [6]. A number of research papers are available on the pharmacological potentials of piyar's roots, leaves, bark and kernel [7–15] but data on the phytochemical constituents, physico-chemical characterization and antioxidant activity of piyar gum exudates seems to be scanty. With this view, the present study has been taken up for studying the intra-specific variations amongst the gum exudates collected from the major piyar gum producing states/districts of India.

Material and Methods

All the chemicals, reagents and solvents used for determination of phytoconstituents, physico-chemical characterization and antioxidant activity of piyar gum exudates were of analytical grade and purchased from Rankem, New Delhi and Merck India Ltd., Mumbai, India.

The details of seven gum exudates collected from different places are as under:

Piyar gum 1, exudates collected from Bilaspur (Chhattisgarh);
Piyar gum 2, exudates collected from IINRG farm (Jharkhand);
Piyar gum 3, exudates collected from Simdega (Jharkhand);
Piyar gum 4, exudates collected from Dindori (Madhya Pradesh);
Piyar black gum 5, exudates collected from Umaria (Madhya Pradesh);
Piyar white gum 6, exudates collected from Umaria (Madhya Pradesh);
Piyar gum 7, exudates collected from Mirzapur (Uttar Pradesh).

All the gum exudates after manual cleaning and sorting were converted into fine powder and passed through 0.4 mm mesh sieve and packed in air tight containers for further analysis.

Phytochemical Screening

All the gum exudates after processing were screened for major phytochemicals viz. flavonoids, saponin, alkaloids, steroids, tannin, amino acid/protein and carbohydrate, adopting standard qualitative chemical tests [16].

Physico-chemical Characterization

Physico-chemical characterization of gum exudates was carried out following standard procedures. Each analysis was repeated three times, and values reported are actually the average of three replications.

Moisture level or loss on drying was determined according to the method described by Association of Official Analytical Chemists [17]. Ash content (total ash) was determined following the method of Ohwoavworhua [18] while method of Bowen [19] was adopted for determining swelling index (%, v/v).

A digital Brookfield viscometer (Model: LVDV-II + Pro) was used for the determination of viscosity. It measures the torque required to rotate an immersed spindle in a fluid. The instrument features a rotating spindle with multiple speed transmission and interchangeable spindles that measure a variety of viscosity ranges. Two concentrations (20 and 10 %, w/v) of the gum exudates were prepared in distilled water, appropriate enough to immerse the spindle groove in the fluid. Speed of rotation was made varied (10, 20, 50 and 100 rpm) to determine its effect on the viscosity values as the drag force is known to alter with changes on the spindle size and rotational speed. For each concentration and at each rotational speed, three measurements were taken.

The tri-stimulus values of the colors namely L (white–black), a (green–red) and b (blue–yellow) were measured using a Hunter Colorimeter (Model: LabScan XE, USA), being more straightforward and simpler to perform than visual methods eliminating subjectivity, and more precise and less time-consuming. The values are average of the three replicates. Elemental analysis of carbon, hydrogen and nitrogen was carried out using a Euro EA Elemental Analyzer.

Specific rotation [α] in H_2O, degree of 0.2 % aqueous gum solution was determined using Rudolph Research Analytical Autopol 1, USA, equipped with a sodium lamp and a cell of 10 cm path length at 589 nm.

Presence of heavy metals viz. iron, lead, cadmium and nickel in gum exudates was determined using Atomic Absorption Spectrophotometer.

Determination of Antioxidant Activity

The ability of the gum exudates to scavenge the free radicals was estimated through in vitro method, using a stable nitrogen centered radical viz. 1,1-diphenyl-2-picryl hydrazyl (DPPH) (100 μM in ethanol) [20]. The absorbance was recorded at 517 nm using UV–visible Spectrophotometer (Model: Shimadzu UV-1700). The free radical scavenging activity was expressed as percentage inhibition, calculated using the following formula:

$$\% \text{ Inhibition} = [(\text{Ab control} - \text{Ab sample})/\text{Ab control}] \times 100$$

where Ab control = Absorption of blank sample and Ab sample = Absorption of test sample.

The EC_{50} values (mg/ml) were calculated by linear regression of plots where the abscissa represented the concentration of tested gum exudates and the ordinate the average percent of antioxidant activity from three separate tests.

Statistical Analysis

The data reported are the means of triplicate observations excepting specific rotation for which the said instrument itself provides the average of 10 values. Data have been analyzed by non-parametric one -way analysis of variance (ANOVA) using Agri Stat Package with Critical Difference (CD) ($p < 0.05$). When ANOVA indicated significant F values, the multiple sample comparison was also performed for detecting significant differences.

Results and Discussion

All the piyar gum exudates were light brown to dark brown in color and found to be water soluble at room temperature to form viscous solutions, pH ranging from 4.46 to 5.20. Screening of the gum exudates for major phytochemicals showed the presence of flavonoids, saponin, amino acid/protein and carbohydrates in all the seven gum samples, whereas tannin was found in the gum exudates collected from Bilaspur, Dindori and Umaria (black sample) (Table 1). Physico-chemically they were characterized by determining their moisture percentage, color parameters, ash percentage, viscosity, swelling index (%, v/v), elemental (CHN) analysis, specific rotation [α] and heavy metals and the values obtained are given in Tables 2 and 3. The highest viscosity (1914 cP) was displayed by the 20.0 % solution of gum exudate collected from IINRG farm (Jharkhand) at 10 rpm using spindle number LV-2, however, at 20 rpm it could not be sheared with the same spindle size because it was out of range. On the other hand, in case of using spindle number LV-3 at 10 rpm, viscosity was 1902 cP and at 100 rpm it was 1022 cP (Fig. 1). The gum exudates collected from Bilaspur (Chhattisgarh) under the same conditions displayed two-fold decreased viscosity than the gum exudates collected from IINRG farm (Fig. 2).

The antioxidant potential of the gum exudates was evaluated by free radical scavenging activity using DPPH assay. The highest percent inhibition (67.58 %) was shown by the black gum exudates collected from Umaria as compared to the standard ascorbic acid (95.54 %) (Fig. 3) and its EC_{50} value was found to be 16.59 mg/ml (Fig. 4).

The good solubility (up to 40.0 %) of piyar gum exudates indicates that these are natural gums of the hydrophilic colloid group, lacking cross linking between polymeric chains. This is because gums having cross linked polymeric chains only swell in water, without dissolving [21]. The phytochemical screening is a very difficult aspect in standardization and quality control because the constituents vary quantitatively and qualitatively not only from species to species but even within the same species depending upon climate, botanical source, season of collection, harvesting and post-harvest handling. Tannin was found to be nil in the white gum exudates collected from Umaria (Madhya Pradesh), Simdega, IINRG Farm (Jharkhand) and Mirzapur (Uttar Pradesh). The black gum exudates collected from Umaria, had 1.234 mg/g tannin of gum exudates. In the collections from Dindori and Bilaspur, it was 1.092 mg/g and 0.579 mg/g of gum exudates, respectively (Tables 2, 3). Absence of tannin in one of the piyar gum exudates (no. 6) collected from Umaria may be attributed to the genetic variation as the other sample collected from the same district

Table 1 Phytochemical screening of piyar gum exudates

Sl. no.	Tests	Piyar gum 1	Piyar gum 2	Piyar gum 3	Piyar gum 4	Piyar gum 5	Piyar gum 6	Piyar gum 7
1.	Alkaloids (Dragendorff's, Wagner's test)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
2.	Steroids (Lieberman–Burchard, Salkowski test)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
3.	Flavonoids (ammonia, aluminium chloride test)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
4.	Saponins (froth)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
5.	Amino acid/protein (ninhydrin)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
6.	Carbohydrate (Molisch's, Fehling test)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
7.	Tannin (Folin–Ciocalteu)	(+)	(−)	(−)	(+)	(+)	(−)	(−)

(+) present; (−) absent

Table 2 Physico-chemical characterization of piyar gum exudates

Sl. no.	Piyar gum exudates	Moisture level (%)	Ash content (%)	Color parameters			Specific rotation [α] in H_2O, deg.	Tannin (mg/g)[a]
				L	a	b		
1.	Piyar gum 1	3.18 ± 0.41e	1.88 ± 0.11e	29.93 ± 3.87ab	5.67 ± 1.52a	9.13 ± 0.86a	(+) 14.99	0.579 ± 0.12c
2.	Piyar gum 2	3.85 ± 0.26de	4.09 ± 0.10b	25.00 ± 5.83bc	3.61 ± 1.50b	7.42 ± 0.74b	(−) 6.94	Nil d
3.	Piyar gum 3	9.65 ± 0.39a	3.31 ± 0.06c	14.29 ± 3.03d	1.80 ± 0.46c	1.28 ± 0.32e	(−) 16.74	Nil d
4.	Piyar gum 4	5.29 ± 0.35b	4.08 ± 0.07b	16.96 ± 2.88d	0.64 ± 0.11c	0.76 ± 0.15e	(+) 7.13	1.092 ± 0.06b
5.	Piyar gum 5	4.65 ± 0.24bc	4.82 ± 0.19a	17.84 ± 3.09 cd	1.18 ± 0.04c	2.44 ± 0.43d	(+) 16.11	1.234 ± 0.14a
6.	Piyar gum 6	4.48 ± 0.45cd	3.02 ± 0.25d	36.82 ± 6.06a	3.86 ± 0.15b	7.54 ± 0.45b	(+) 23.70	Nil d
7.	Piyar gum 7	3.75 ± 0.43e	4.63 ± 0.34a	25.29 ± 1.06b	4.13 ± 0.16b	6.38 ± 0.17c	(+) 9.36	Nil d
	CD (0.05)	0.706	0.283	7.445	1.517	0.982		0.053

Swelling index (%, v/v) absent in all gum exudates. Alphabets indicating the comparison of critical difference amongst treatment means. Means sharing the same alphabets within a column were not significantly different ($p < 0.05$) ($n = 3$)

[a] Data are square root transformed

Table 3 Elemental and heavy metals analyses of piyar gum exudates

Sl. no.	Piyar gum exudates	Elemental analysis			Heavy metals (ppm)			
		C%	H%	N%	Fe	Cd	Pb	Ni
1.	Piyar gum 1	30.89 ± 0.77e	4.94 ± 0.14c	0.31 ± 0.03a	0.43 ± 0.10b	0.22 ± 0.10	0.38 ± 0.11	Nil
2.	Piyar gum 2	34.04 ± 0.12d	5.78 ± 0.21ab	0.23 ± 0.04b	1.12 ± 0.12a	0.21 ± 0.03	0.42 ± 0.12	Nil
3.	Piyar gum 3	35.87 ± 0.13b	6.06 ± 0.18ab	0.06 ± 0.01d	0.98 ± 0.08a	0.19 ± 0.07	0.36 ± 0.03	Nil
4.	Piyar gum 4	26.17 ± 0.09f	4.29 ± 0.03d	0.20 ± 0.02b	1.14 ± 0.12a	0.26 ± 0.02	0.38 ± 0.14	Nil
5.	Piyar gum 5	35.03 ± 0.14c	5.64 ± 0.53b	0.05 ± 0.01d	1.08 ± 0.12a	0.19 ± 0.10	0.25 ± 0.11	Nil
6.	Piyar gum 6	36.16 ± 0.06b	5.99 ± 0.37ab	0.25 ± 0.04b	0.34 ± 0.12bc	0.21 ± 0.04	0.23 ± 0.03	Nil
7.	Piyar gum 7	37.27 ± 0.17a	6.20 ± 0.03a	0.11 ± 0.02c	0.22 ± 0.04c	0.27 ± 0.14	0.19 ± 0.04	Nil
	CD (0.05)	0.558	0.502	0.044	0.186	NS	NS	–

Alphabets indicating the comparison of critical difference amongst treatment means

Means sharing the same alphabets within a column were not significantly different ($p < 0.05$) ($n = 3$)

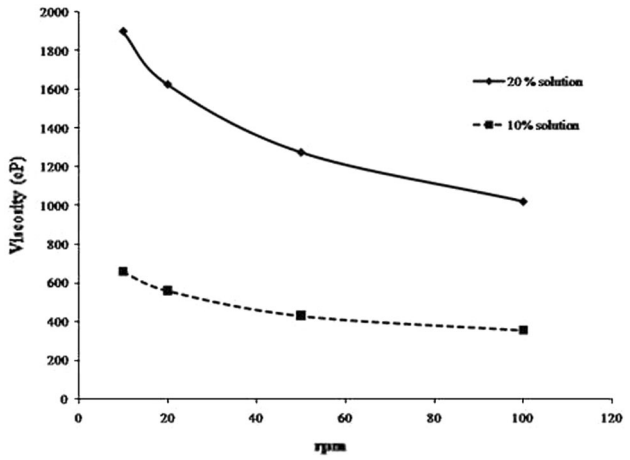

Fig. 1 Viscosity (cP) values of piyar gum exudates collected from IINRG farm

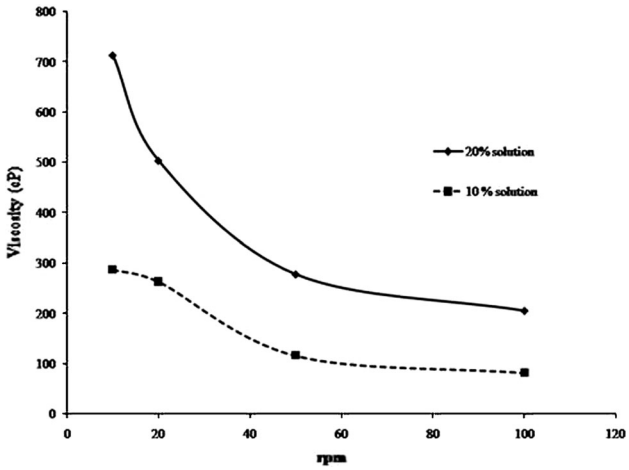

Fig. 2 Viscosity (cP) values of piyar gum exudates collected from Bilaspur

shows the presence of significant tannin in it though the period of collection of both the gum exudates was the same. The highest moisture level (9.65 %) was found in the gum exudates collected from Simdega and the lowest (3.18 %) in the collection from Bilaspur. The highest L value (36.82) was found in the white gum exudates from Umaria and the lowest

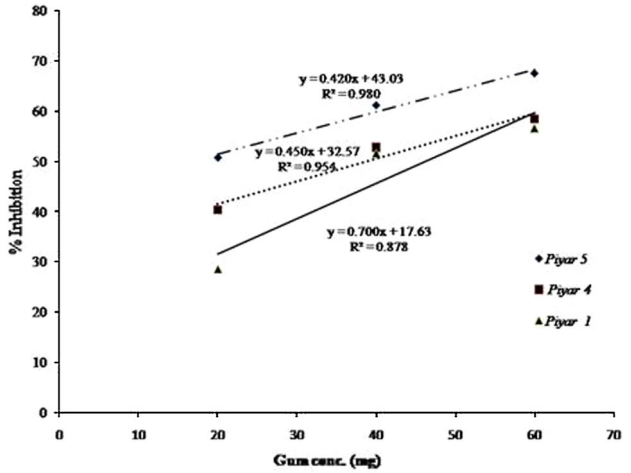

Fig. 3 Percent inhibition of piyar gum exudates

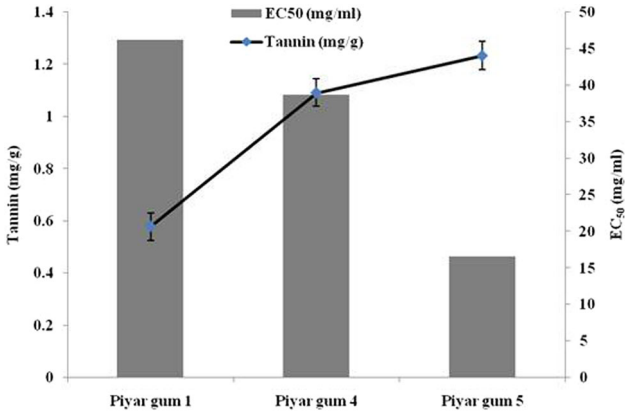

Fig. 4 Co-relation of tannin and EC_{50} values of piyar gum exudates

(14.29) in the one from Simdega. The highest 'a' and 'b' value was obtained in gum samples from Bilaspur and the lowest in gum sample from Dindori. Total ash is normally composed of inorganic mixtures of carbonates, phosphates, silicates and silica. The lowest values of total ash (1.88 %) obtained in the gum sample collected from Bilaspur indicate low levels of carbonates, phosphates, silicates and silica. Almost all the gum samples have ash percentage within the prescribed limit [22]. Swelling index (%, v/v) of all the gum exudates was determined in distilled water. None of them showed any swelling property. The viscosity of piyar gum exudates was found to be dependent on concentration at the same shear rate. It increases with the increase in gum concentration (Figs. 1, 2). The variation in cohesiveness of the gum exudates is apparent as viscosity of the gum is more at 20.0 % (w/v) solution as compared with 10.0 % solution. Generally, molecules in a fluid have different shapes and sizes and the force required to move these molecules in the fluid is determined by their type of bond, shape and size. Piyar gum exudates, at both the concentrations, exhibited shear thinning/pseudoplastic flow pattern on using spindle number LV-2 and LV-3. Amongst all the collected gum exudates, sample No. 6 (white exudates of Umaria) showed the least viscosity under same conditions. The quantitative elemental (CHN) analysis showed that the gum sample collected from Dindori contained the lowest percentage of carbon (26.169 %), whereas the gum sample collected from Mirzapur (U.P.) contained the highest carbon percentage (37.268). Briefly, the low level of nitrogen in all the gum samples is suggestive of amino acid (peptide) crosslink. The ratio of carbon to hydrogen is just over 7:1, indicating good number of unsaturation due to aromatic rings and/or polysaccharide composition. The intraspecific variation in respect of optical rotation is more pronounced. The gum exudates collected from IINRG farm and Simdega (Jharkhand) were found to be levorotatory, whereas the others were dextrorotatory. Amongst heavy metals, Ni was found to be absent in all the gum exudates, whereas other metals viz. Fe, Cd and Pb were found to be within the prescribed limit. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the typical purple color of free DPPH radical decays, and the absorbance change at $\lambda = 517$ nm is measured. The test provides information on the ability of a compound to donate a hydrogen atom [23]. The percent inhibition of free radical/scavenging activity shown by the gum exudates collected from Bilaspur, Dindori and Umaria (black sample) may be due to the presence of flavonoids and tannin in them. Tannins, the water-soluble polyphenols present in the bark, gum and other parts of the plants, inhibit the hydroxyl radical formation. The antioxidant activity of polyphenols is mainly due to their redox properties, which allows them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelaters etc. [24]. The present study indicated that the piyar gum exudates collected from three places possessed tannins and, therefore, exhibited antioxidant activity, which might be helpful in preventing or slowing down the incidence of various oxidative stress-related diseases. Emerging trends in antioxidant research point out the fact that low levels of phenolics and other phytochemicals as also the low value of antioxidant indices in plants do not necessarily translate into poor medicinal properties. Further investigations for isolation and identification of antioxidant component(s) may lead to chemical entities with potentials for pharmaceutical applications. The intraspecific variations are presumably due to effects of difference in geographical locations and age of the tree as has also been reported earlier [25, 26].

Conclusion

The findings of the present study clearly demonstrate the pronounced qualitative and quantitative intra-specific variation in piyar gum exudates collected from different places

in respect of their phytochemicals, physico-chemical parameters and antioxidant activity, which may categorically be attributed to their different geographical locations and climatic conditions around the resource gum tree, its age and nature of soil etc. The presence of major phytochemicals in the gum exudates would make the gums quite useful for treating various ailments/maladies.

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