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Physicochemical properties and toxicity test of *Prosopis juliflora* (Sw.) DC. and *Balanites aegyptiaca* Del gum exudates from Rajasthan

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Prosopis juliflora (Sw.) DC. (Fabaceae) commonly known as **'**Mesquite' or 'Honey Mesquite' and *Balanites aegyptiaca* Del (Zygophyllaceae) or Desert Date are invasive weeds and have been known for debates on their use as well as harm. The present study reports the phytochemical screening, physico-chemical characterization, thermal stability and toxicity test of gum exudates of *P. juliflora* and *B. aegyptiaca*, collected from Jodhpur (Rajasthan). Standard qualitative chemical tests have revealed the presence of flavonoids, saponins, amino acid / protein and carbohydrate in both the gum exudates. Physicochemically, they were characterized by determining their moisture content (%), colour parameters (L, a, b), ash content (%), elemental (CHNS) analysis, specific rotation [α] in H_2O, deg., heavy metals (mg/g), viscosity (cP) and FTIR. Gums were also thermally characterized using differential scanning calorimetry (DSC), thermo-gravimetric analysis (TGA) and differential thermal analysis (DTA). Thermal studies have shown that the gums are relatively stable up to 220°C, beyond which degradation starts. Short-term, acute oral toxicity test of both the gum exudates was carried out on albino rats of Wistar strain and found that acute toxicity of both the gum exudates by oral route was >2000 mg/kg body weight and no compound related sign, symptoms and mortality were produced in the animals at this permissible limit dose.

Keywords: Desert date, Honey Mesquite, Gum, *Vilayati babool*

Invasive species, in general, are considered a menace, in particular for their dominance over the native species. The former thrive at the cost of the latter. Invariably, invasive species grow faster and survive better (hence called invasive) than their native counter parts despite limited resources. As invasive species thrive better and stay, it will be interesting to study about their phytochemical constituents, properties for possible benefits and try to put them into use. Recently, Shackleton *et al.*[1], in their comprehensive review on *Prosopis juliflora* have highlighted that developed nations adopt mechanical and chemical control measures while developing nations take utilization approaches for managing/controlling invasive species. On these lines, the following two species, *Prosopis juliflora* (Sw.) DC. and *Balanites aegyptiaca* Del. have been studied here for their gum exudates.

The genus *Prosopis* has several species, namely *Prosopis juliflora*, *P. pallida*, *P. chilensis*, *P. alba*, *P. pubescens* and *P. tamarugo*, all native to America, but have now become established in arid and semi-arid Australia, Africa and Asia. *Prosopis cinerarium* is native to India. In India, *Prosopis juliflora*, locally known as '*vilayati babool*', was introduced in the last century. It is a xerophytic evergreen tree which thrives on all kinds of soils under varying climatic conditions. It is seen as an invasive weed but also considered to be one of the most significant tree species of hot arid and semi-arid regions of India[2-6].

Both these trees have been reported to support soil stabilization, lesser soil erosion from windbreaks and within plantations, reduced salinity and alkalinity, improved soil fertility and soil physical characteristics, besides some commercial applications. Adikwu *et al.*[7] reported that the gum obtained from the ripe seeds of *Prosopis africana* contains glucose, fructose, galactose and xylose as the monosaccharide components and more viscous than tragacanth gum at equivalent concentrations. Azero and Andrade[8] have reported that *P. juliflora* seed gum possesses functional properties similar to those of *guar* gum, and therefore, could be used as an alternative gum for industrial applications. *P. juliflora* gum and its spray-dried powders were compared to emulsions prepared with gum *arabic* by Beristain and Vernon-Carter[9].

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They have reported that average oil droplet size of the gum capsules was smaller than that of gum *arabic*. Emulsions made with mesquite gum showed better stability than those made with gum *arabic*. Encapsulation capacity of mesquite gum was found to be 80.5% of the starting oil, whereas in case of gum *arabic* it was 93.5%. The rate of release of salicylic acid from gels prepared from *Prosopis* gum was studied by Adikwu and Attama[10] using tragacanth gum gel as standard. They have reported that correlation of the quantity of drug release was dependent on the viscosity of the gels, the highly viscous gels showing slower release rates. The gum exudate of *P. juliflora* is comparable to gum *arabic* and can be used in food and cosmetic industries also. But due to non-availability of the required toxicological data essential for industrial purposes, its use is limited[11]. Wamburu *et al*.[12] have reported the acute and sub-acute toxicological evaluation of ethanolic extract of the leaves of *P. juliflora*. Microscopic study of the films formed from *P. juliflora* and sodium carboxymethyl cellulose are of equal consistency without pores as reported by Ojile *et al*.[13]. The antibacterial activity of various seed extracts of '*Desi babool*' (*Acacia nilotica*), '*Subabool*' (*Lucenea leucocephala*) and *'Vilayati babool'* (*P. juliflora*) was determined *in vitro* against Gram negative and Gram positive bacterial strains. The results revealed that *desi babool* exhibited most significant activity followed by *subabool* and *vilayati babool* and also amongst various extracts, water-extract exhibited the most promising activity as compared to n-hexane, chloroform, acetone and alcohol[14]. Wamburu *et al*. [15] have reported the spectrometric detection of organic compounds and toxicity of ethanolic leaves extracts of *P. Juliflora*. Sharma & Kaur[16] have reported that methanolic extracts of unripe pods of *Prosopis cineraria*, seeds of *Acacia senegal* and dried fruits of *Capparis decidua* (*Trikuta*, traditional food of western Rajasthan) showed antimicrobial activity but in unripe pods of *P. cineraria* the activity was found more pronounced than in *C. decidua*'s dried fruits and *A. senegal* seeds when tested against seven clinical isolates including one Gram positive and six Gram negative bacteria by Agar well diffusion method.

Literature survey reveals that *Balanites aegyptiaca* has a long history of traditional uses for wide range of diseases. It possesses antioxidant, antimicrobial, anticancer, antiviral, antidiabetic, anti-inflammatory, diuretic, hypocholesterolemic, wound-healing, hepatoprotective, mosquito larvicidal, analgesic and anthelmintic properties. Bark, fruits, seeds, and leaves of this plant are widely used in folk medicines[17-21]. Moreover, fruit of *B. aegyptiaca* is also considered as source of *Ingudi* of Ayurveda[22]. Nasser *et al*.[21] have reported that aqueous extracts of fruits of *B. aegyptiaca* and leaves of *Petroselinum sativum* have shown antidiabetic and antioxidant effects on the diabetic rats. They further suggested that these extracts can be potentially used with insulin therapy to minimize its side effects and to improve the treatment of Type 1 diabetes mellitus (T1DM) and probably other oxidative stress-associated diseases. To serve as quality control document for *Ingudi*, quite comprehensive studies of different fruit extracts of *B. aegyptiaca* have been carried out by Kumar *et al*.[22] for their macro & microscopical, physicochemical, phytochemical and HPTLC finger printing profiling for identification purpose.

P. juliflora and *B. aegyptiaca* plants are lesser known species found in Rajasthan. In rural areas, *P. juliflora* is often the only source of fuel and dry season fodder and also is the only source of livelihood for many families[23]. Similarly, *B. aegyptiaca* is an useful plant of the desert regions and is used for fodder, fuel and fencing. At ICAR-CAZRI, Jodhpur (Rajasthan), the gum production of *P. juliflora* is 120 g/tree whereas in case of *B. aegyptiaca*, it is 140 g/tree [23].

In this paper, we studied physicochemical characteristics and thermal stability of the gum exudates of *Prosopis juliflora* and *Balanites aegyptiaca* collected from Jodhpur (Rajasthan) and also their toxicity in albino rats.

Materials and Methods

Chemicals

All the chemicals, reagents and solvents used for phytochemical screening and physicochemical characterization of *P. juliflora* and *B. aegyptiaca* gum exudates were of analytical grade and purchased from Rankem, New Delhi and Merck India Ltd., Mumbai, India. The EC Number of *P. juliflora* is 01214/83 from Mexico, where as *B. aegyptiaca* was a wild plant at ICAR-CAZRI, Jodhpur (Rajasthan)'s research farm. Both the gum exudates after manual cleaning and sorting were converted into fine powder and passed through 0.4 mm mesh sieve and packed in airtight containers for further analysis.

Phytochemical screening

The gum exudates after processing were screened for major phytochemicals *viz*. alkaloids, steroids, flavonoids, saponins, amino acid/protein, carbohydrate and tannin adopting standard qualitative chemical tests (Table 1)[24].

Physicochemical characterization

Physicochemical characterization of gum samples *viz*. solubility, pH, moisture level (%), ash content (%), colour parameters (L, a, b), specific rotation [α] in H_2O, deg., elemental (CHNS) level (%), heavy metals (mg/g), tannin content (mg/g), viscosity (cP value) and FTIR was studied. Gums were thermally characterized using differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and differential thermal analysis (DTA) following standard procedures.

For determining all the physicochemical properties and the toxicity test, fine powder of both the gum exudates were used. Moisture level or loss on drying was determined according to the method described by the Association of Official Analytical Chemists[25]. Ash content (total ash) was determined following the method of Ohwoavworhua[26] while Bowen method[27] was adopted for determining swelling index (%, v/v). The tristimulus values of the colours, namely L (white-black), a (green-red) and b (blue-yellow) were measured using a Hunter Colorimeter (Model: LabScan XE, USA), based on the principle of CIE (The Commission Internationale de l' Eclairge), simpler, more precise and less time-consuming. Elemental analysis of carbon, hydrogen, nitrogen and sulphur was carried out using a Euro EA Elemental Analyzer. Accurately weighed (*P. juliflora*, 1.380 mg and *B. aegyptiaca*, 1.548 mg) gum samples in a tin capsule were heated to 1150°C with constant helium (carrier gas) flow and the corresponding element was determined by using the elemental analyzer. Specific rotation [α] 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 was determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)[28] (Model: Optical 2100 DV). Digital Brookfield viscometer (Model: LVDV-II+Pro) was used for determination of viscosity. Viscosity as a function of concentration (10 and 20 %) of gum exudates was determined with varying speed of rotations (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. FTIR spectra of gum samples were recorded in Shimadzu, IR Prestige-21 in the range 4000 to 500 cm^{-1} in KBr.

The data reported are the means of triplicate determinations excepting specific rotation and heavy metals. For specific rotation, the instrument used itself provides the average of 10 values and the values obtained for heavy metals are very low (Table 2).

Table 1—Phytochemical screening of gum exudates

Tests	*Prosopis juliflora*	*Balanites aegyptiaca*
Alkaloids (Dragendorff's, Wagner's test)	(-)	(-)
Steroids (Lieberman-Burchard, Salkowski test)	(-)	(-)
Flavonoids (Ammonia, Aluminium chloride test)	(+)	(+)
Saponins (Froth)	(+)	(+)
Amino acid/protein (Ninhydrin)	(+)	(+)
Carbohydrate (Molisch's, Fehling test)	(+)	(+)
Tannin (Folin-Ciocalteu)	(-)	(-)

(+) present; (-) absent

Thermal properties

Thermal properties of gum samples were characterized using a Q20-TA DSC. Nitrogen at the rate of 50 mL/min was used as purge gas. Five milligram of powdered gum sample was sealed in an aluminium pan and heated up to 300°C @10°C/min followed by cooling cycle at the same rate. TGA/DTA study was done by Shimadzu, Japan, DTG-60.

Acute oral toxicity study

The acute oral toxicity study of *P. juliflora* and *B. aegyptiaca* was carried out at CSIR-Indian Institute

Table 2—Physico-chemical characteristics of gum exudates

Gum exudate	Moisture* Level (%)	Ash* content (%)	Colour parameters*			Specific rotation [α]#	Elemental analyses* (%)				Heavy metals (mg/g)				
			L	a	b		C	H	N	S	Co	Cr	Fe	Ni	Pb
P. juli-flora	2.86 ± 0.07	1.29 ± 0.03	21.12 ±0.11	3.87 ±0.05	7.38 ±0.05	(+) 47.66	38.93 ±0.06	1.06 ±0.13	1.00 ±0.09	6.97 ±0.05	BDL	0.0005	0.0095	0.0005	0.0065
B. aegyp-tiaca	2.85 ± 0.08	2.53 ± 0.07	29.95 ±0.05	5.69 ±0.07	9.08 ±0.07	(+) 1.45	23.28 ±0.04	3.59 ±0.06	0.99 ±0.09	Nil	0.0005	0.001	0.0405	0.0005	0.006

in H_2O, deg.*Values are means ± SD of three observations. BDL, Below detection limit.

of Chemical Technology, Hyderabad on albino rats of Wistar strain weighing 110-150 g. The study was conducted as per IICT DOP No. TOX/R-8 which is a modified version of OECD guidelines 420 (2001). The animals were maintained in a room of controlled temperature (22 ± 3°C), RH 30-70% and lighting of 12 h light:dark throughout the experiment; and fed with standard rat feed and water *ad libitum*. The gums were administered in a single dose to the rats by gavage using a stomach tube.

Results and Discussion

P. juliflora gum, dark brown in colour, was found to be highly soluble in water whereas *B. aegyptiaca* dissolves after overnight swelling and having neutral pH. Screening of the gum exudates for major phytochemicals showed the presence of flavonoids, saponins, amino acid/protein and carbohydrate in both the gum samples (Table 1). The determined physicochemical parameters of *P. juliflora* and *B. aegyptiaca* gum exudates have been given in Table 2. Total ash is normally composed of inorganic mixtures of carbonates, phosphates, silicates and silica and is a useful parameter for determining the characterization and purity of gum. This parameter gives an indication of the degree of mineral interaction in the structure which contributes to the functional properties of the polysaccharide gum[29]. The lower ash content is associated with higher purity degree. *P. juliflora* gum sample has 1.29 ± 0.03 % and *B. aegyptiaca* 2.53 ± 0.07% ash content, both of them having ash % within the prescribed limit[30] and are dextrorotatory. The quantitative elemental (CHNS) analysis showed the presence of sulphur in *P. juliflora* and absent in *B. aegyptiaca*. The low level of nitrogen in both the gum samples is suggestive of amino acid (peptide) crosslink and the ratio of carbon to hydrogen is indicative of good number of unsaturation in the polysaccharide composition. Amongst heavy metals, cadmium was below detection limit (BDL) in both the gum samples and also cobalt in *P. juliflora* (Table 2). Both the gum exudates showed Newtonian behavior with viscosity being shear rate independent and concentration dependent, as viscosity of 20.0% solution was more than 10.0% solution. *P. juliflora* gum exudates displayed less viscosity (Fig. 1A) than *B. aegyptiaca* gum exudates using spindle number LV-2 at 28.5°C (Fig. 1B). Increase in viscosity with concentration may be due to increasing number of high molecular weight polymeric chains of the gums per unit volume and increased interaction between these polymeric chains in aqueous solution, responsible for increase in cohesive density and, therefore, greater resistance to flow. Siddiqui *et al*.[31] have reported physicochemical properties and protein profiling of gum exudates of *Acacia nilotica* collected from different agroclimatic zones in India and have observed that the solubility, viscosity, specific rotation and other values obtained in case of *P. juliflora* gum exudates collected from Rajasthan are alike as also commercially available gum *acacia* from Merck India Ltd. and Himedia, so, *P. juliflora* gum exudates can be exploited in the same manner as the commercial *acacia* gums.

The FTIR spectra exhibiting strong vibrational modes located at 1600 and 1604 cm^{-1} are assigned to the stretching vibrations of the C=O bond of carboxylate group. The vibrational modes located at 1423 and 1427 cm^{-1}, with relatively low intensity, are assigned to the stretching vibrations of the C-O bond. The peaks at 2927, 2943 and 2889 cm^{-1} are characteristics of methyl C-H stretching associated with the aromatic ring. The absorption bands located at 2137 and 2144 cm^{-1} usually assigned to the CO_2 vibration (Fig. 2). Structural and

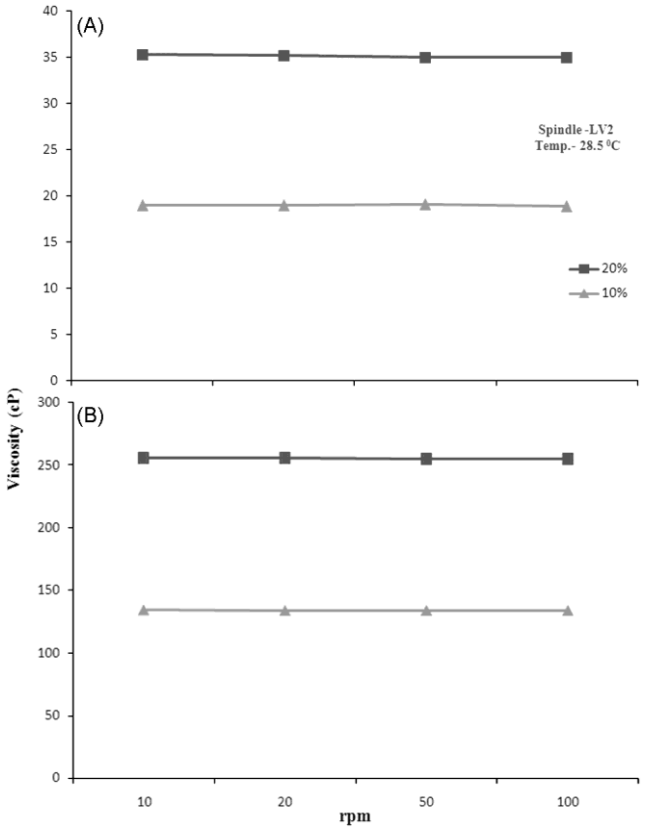

Fig. 1 — Viscosity as a function of concentration of (A) *Prosopis juliflora*; and (B) *Balanites aegyptiaca* gum exudate

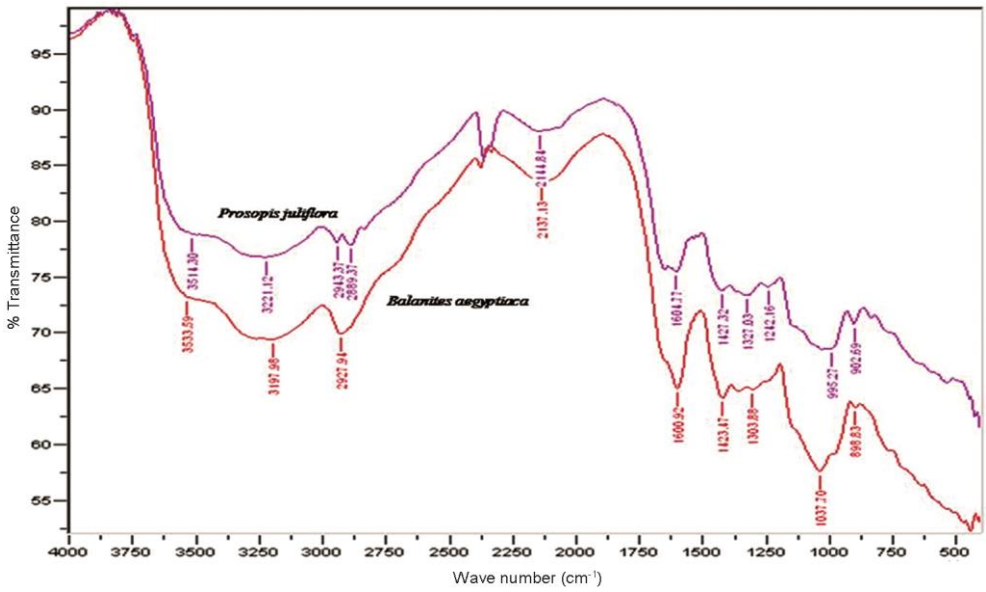

Fig. 2 — FTIR spectra of *Prosopis juliflora* and *Balanites aegyptiaca* gum exudates

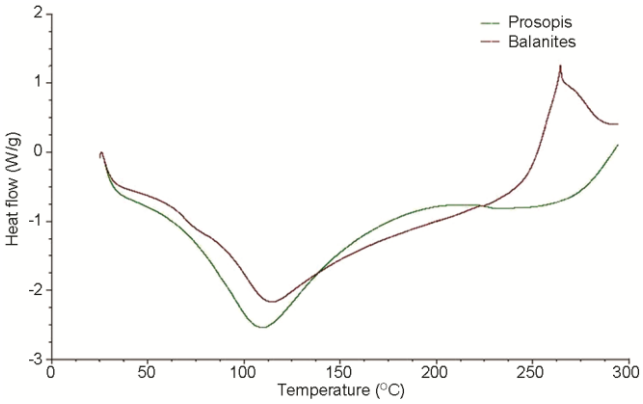

Fig. 3 — DSC chromatogram of *Prosopis juliflora* and *Balanites aegyptiaca* gum exudates

functional group differences in polysaccharide gums influencing the thermal behavior and affecting the transition temperature is generally studied by TGA/DTA and DSC. DSC is used to measure the occurrence of exothermal and endothermal changes with increased temperature. Because of its sensitivity and accuracy, it is being extensively used for studying the phase transition of polymers[32,33]. DSC of *P. juliflora* and *B. aegyptiaca* gum exudates showed presence of a broad endothermic peak at around 95-120°C, which may be attributed to moisture sorption and occurrence of glass transition (Tg) temperature in the range of 70.29-85.82°C and 63.26-69.43°C, respectively. Further, *B. aegyptiaca* gum exudates showed an additional exothermic peak around 270°C (Fig. 3). TGA/DTA thermogram

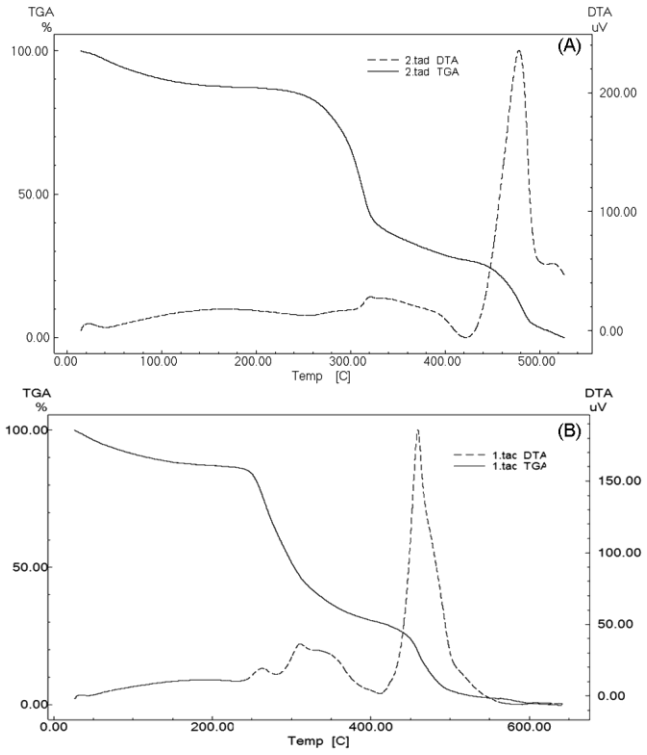

Fig. 4 — TGA/DTA thermogram of (A) *Prosopis juliflora*; and (B) *Balanites aegyptiaca* gum exudate

of *P. juliflora* gum sample revealed that the weight loss occurred in five zones/steps. In the second zone, i.e. the degradation zone at 235-335°C, where a major weight loss (48.06%) was observed, can be attributed to decomposition of gum and 100 % weight loss occurred at 500°C (Fig. 4A). Whereas, in case of

Table 3—Acute oral toxicity test of *Prosopis juliflora* and *Balanites aegyptiaca* gum exudates

Dose (mg/kg)	Number of rats		Percentage mortality	Signs & Symptoms	LD_{50} ± S.E. mg/kg
	Used	Dead			
5.0	1	0	0.00	No adverse signs & symptoms were observed	
50.0	1	0	0.00	No adverse signs & symptoms were observed	
300.0	1	0	0.00	No adverse signs & symptoms were observed	> 2000
2000.0	5	0	0.00	No adverse signs & symptoms were observed.	

B. aegyptiaca, 100 % weight loss occurred at 600°C involving three zones (Fig. 4B).

In preliminary sighting study, the various doses (5, 50, 300 and 2000 mg/kg) of *P. juliflora* and *B. aegyptiaca* gum exudates were administered to single animal of female sex in a sequential manner. The sighting study yielded information on the dose-toxicity relationship. Mortality was not observed with any of the doses 5, 50, 300 and 2000 mg/kg. Therefore, in main study (Limit test) the gums were administered to four female rats with 2000 mg/kg dose. The test animals were kept under observation for a period of 14 days after dosing. The food intake and body weight monitored daily for the observation period, whereas, mortality, signs and symptoms, if any, were recorded twice on the day of dosing and once each day thereafter. Since compound related sign, symptoms and mortality were not produced at the 'Limit' dose of 2000 mg/kg dose, the acute toxicity of *P. juliflora* and *B. aegyptiaca*, in rats by oral acute was >2000 mg/kg (Table 3).

Conclusion

The phytochemical screening of the gum exudates of *Prospis juliflora* and *Balanites aegyptiaca* demonstrated the presence of flavonoids, saponins, amino acid/protein and carbohydrate. The important physicochemical characteristics, *viz*. solubility, viscosity, specific rotation, etc. of *P. juliflora* gum exudates revealed that these are at par with the commercially available gum *acacia* from Merck India Ltd. and Himedia and can be exploited in the same manner as the commercial *acacia* gums. *P. juliflora* and *B. aegyptiaca* gum exudates have LD_{50} values greater than 2000 mg/kg and can be used by both human and animals, with a degree of safety and tolerance.

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