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Proximate, Antibacterial and Hemolytic Analysis of Some Selected Gums

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The aim of this study was to evaluate proximate, antibacterial and hemolytic potential of some selected gums. Acacia Senegal, Acacia modesta, Sterculia urens, Ferula asafeotida, Dalbergia sissoo and Albizia lebbeck were the selected gums. The crude and purified gums were used. To determine the antimicrobial potential the disc diffusion method was performed followed with their minimum inhibitory concentration. For the in vitro toxicity the hemolytic analysis was carried out. The selected gums possess antibacterial activity against a panel of bacterial species. The hemolytic analysis reveals that the gum samples were non-toxic. These gums contain high amount of carbohydrates determined through proximate analysis. The results of this study indicate that the gums have bioactive properties and can be explored for their different applications.

Key words: Proximate, Antibacterial, Hemolytic, Gums, non-toxic.

Natural gums or carbohydrate biopolymers are promising biodegradable materials for use in drug delivery systems because of their well-known biodegradability and biocompatibility (Sharma *et al*., 2013). Biopolymers are also used as drug carriers, due to their sustainability and biosafety they have been the subject of research (Shahid *et al*., 2013; Prajapati *et al*., 2013). Biopolymers are obtained from natural sources. These polysaccharides are relatively cheap, easily available, non-toxic (Shahid *et al*., 2013).

The spreading of resistance by the existing antibiotics by microorganisms is the reason for the increased development of new antibiotics (Tabasum *et al*., 2013). Different biological and toxicological screenings were reported for different extracts, and compounds of samples. Among these in vitro hemolytic activity of gums was performed to check the possible toxicity mechanism and to check the safety of plant constituents, thus to make then suitable for the preparation of natural extractable drugs (Kalaivani *et al*., 2011; Shahid *et al*., 2013).

The proximate analysis was performed to check inherent potential such as total moisture, Nitrogen, carbohydrates, Nitrogen free extract, ash and total energy content of plant gums.

There is lot of information, data and literature, but there is very limited work reported on the present direction. Thus the present study was aimed to evaluate the proximate, anti bacterial and toxicological potential.

MATERIALS AND METHODS

Purification of gum

The dried gums were identified and authenticated from Department of Botany, University of Agriculture, Pakistan. The selected gum was grinded to powder. These were the crude gums. For the process of purification, the

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dispersion of gum was formed in water and solution was left for a night. The dispersion was filtered and then slowly added to absolute ethanol. White amorphous precipitates were formed when ethanol was added to gum solution. They were collected and washed with ethanol. In hot air oven, at 40 °C the white precipitates were placed to dry. The dried gum was grinded to powder and stored for further use, as purified gum (Shahid *et al*., 2013).

Proximate analysis

For determination of physical parameter of gums proximate analysis was performed following AOAC methods (AOAC, 2011). Moisture, crude fat, crude protein, ash, total carbohydrates and total energy were determined (Galla and Dubasi, 2009).

Antibacterial assay of selected gum extracts

The antibacterial activity of gums was checked against bacterial species by disc diffusion method (Afzal *et al*., 2013). The methanolic gum extracts were prepared. The bacterial strains were Escherichia coli and Staphylococcus aureus. These pathogenic strains were used to determine the antimicrobial activity with reference to the minimum inhibitory concentration (MIC) of the natural polysaccharides extracts (Brudzynski and Kim, 2011).

***In vitro* toxicity by hemolytic activity**

To check the toxicity of selected gums a method reported by Shahid *et al*., 2013, was used to determine in vitro hemolytic activity of crude and purified gum extracts.

RESULTS AND DISCUSSION

Determination of Moisture

The proximate analysis (Table 1) of crude and purified gums is presented in the following tables:

There are diverse natural sources of gum extensive variety of composition and texture. Natural composition and the balance between the compositions of nutrients affect the Value of food. So there was a need of a detailed proximate analysis to find out the composition of natural products. The amount of carbohydrate was found to be high in gum, indicating its purity. Natural products like gums contain excess water which at suitable temperature may lead to activation of enzymes and to the proliferation of living organisms (Rowe *et al*., 2006). There was an increase in carbohydrate amount after the process of purification. The total ash content is designed to measure the total amount of residual material remaining after ignition. The total ash figure is of importance and indicates to some extent the amount of care taken in the preparation of the sample. The maximum limit for total ash for food and pharmaceutical quality gum acacia and gum tragacanth is set at 4.0% (w/w) (Rowe *et al*., 2006). Nitrogen and amino acid contents are useful parameters in distinguishing gums from different species (Brummer *et al*., 2003). The nature and amount of these constituents depend on the composition of soil upon the trees grew (De Paula *et al*., 2001).

Antibacterial activity

The extracts for gums were prepared in methanol. The antibacterial activity was evaluated against gram negative (Escherichia coli) and gram positive (Staphylococcus aureus). Rifampicin was used as the positive control. The result of antibacterial assay is given in Table 2. The activity was measured by the help of zone reader. The zone of inhibition (mm) exhibited by gum samples was measured. The experiment was performed in triplicate. The mean and S.D was calculated. The antibacterial activity of gums was increased after the purification. As the gums have antibacterial activity against some pathogenic microorganisms so these extracts can be used for medical purpose and for food preservation (Omer *et al*., 2011). These gum samples may be recommended as a new potential source of natural antimicrobial for treatment, prevention and control of bacterial diseases.

Minimum inhibitory concentration (MIC) of gum samples

The gum samples showing antibacterial activity were analyzed to determine their minimum inhibitory concentration. The method used for this purpose was sensitive, simple, robust, reliable and rapid. MIC analysis is used to evaluate the antibacterial properties of the natural products and also used for the study of cell growth. Methanolic extracts were used in this assay as they are effectiveness of this method. This MIC method solves the problem of dilution inaccuracies and this method was easy to carry out. The % growth inhibition of the samples calculated in MIC is presented in the Figure 1.

Table 1. Proximate analysis of crude and purified gums

(a) Proximate analysis of crude gums

S. No.	Moisture (%)	Crude fat (%)	Crude protein (%)	Crude fiber	Ash content (%)	Carbohydrate (%)	Nitrogen free extract	Energy (K cal)/5g
S. urens	7.6 ± 0.012	2.05 ± 0.03	2.19 ± 0.02	0.5 ± 0.02	6.00 ± 0.05	60.96 ± 0.02	50.02	182.2± 0.05
A. modesta	10. 8 ± 0.01	1.28 ± 0.01	2.33 ± 0.015	0.1± 0.02	2.8 ± 0.01	63.78 ± 0.01	45.48	102.5 ± 0.01
A. senegal	14.2 ± 0.030	1.20 ± 0.013	2.33 ± 0.01	0.4 ± 0.03	1.60 ± 0.02	63.67 ± 0.001	31.20	90.6± 0.001
F.asafeotida	11.7 ± 0.020	1.95 ± 0.021	4.74 ± 0.01	0.06 ± 0.05	1.20 ± 0.01	70.31 ± 0.03	42.90	180.99± 0.03
D. sissoo	12.8 ± 0.02	1.95 ± 0.021	3.71 ± 0.01	0.3 ± 0.01	1.2 ± 0.01	60.31 ± 0.03	52.81	180.99 ± 0.03
A. lebbeck	13 ± 0.03	2.11 ± 0.02	3.2 ± 0.2	0.2 ± 0.03	3.2 ± 0.03	68.45± 0.02	43.34	170.78± 0.02

Table 1. Proximate analysis of crude and purified gums

(b) Proximate analysis of purified gums

S. No.	Moisture (%)	Crude fat (%)	Crude protein (%)	Crude fiber	Ash content (%)	Carbohydrate (%)	Nitrogen free extract	Energy (K cal)/5g
S. urens	5.9 ± 0.01	0.99 ± 0.02	0.5 ± 0.02	0.2 ± 0.02	4.1 ± 0.02	69.96 ± 0.02	41.02	100.2± 0.02
A. modesta	9.7 ± 0.02	0.7 ± 0.01	0.03 ± 0.01	0.1± 0.02	1.6 ± 0.02	63.18 ± 0.01	38.48	99.4 ± 0.03
A. senegal	10.1 ± 0.02	0.18 ± 0.01	0.22 ± 0.01	0.3 ± 0.03	1.4 ± 0.02	64.92 ± 0.02	30.12	89± 0.02
F.asafeotida	9.2 ± 0.01	0.11 ± 0.01	0.72 ± 0.02	0.03± 0.01	1.56 ± 0.02	72.21 ± 0.01	35	90.29± 0.01
D. sissoo	8.8 ± 0.01	0.48 ± 0.01	0.43 ± 0.02	0.02 ± 0.02	1.1 ± 0.01	63.21 ± 0.01	44.21	99 ± 0.02
A. lebbeck	10 ± 0.02	0.95 ± 0.021	0.32 ± 0.01	0.19 ± 0.01	2.5 ± 0.01	70.45 ± 0.01	31.33	103± 0.02

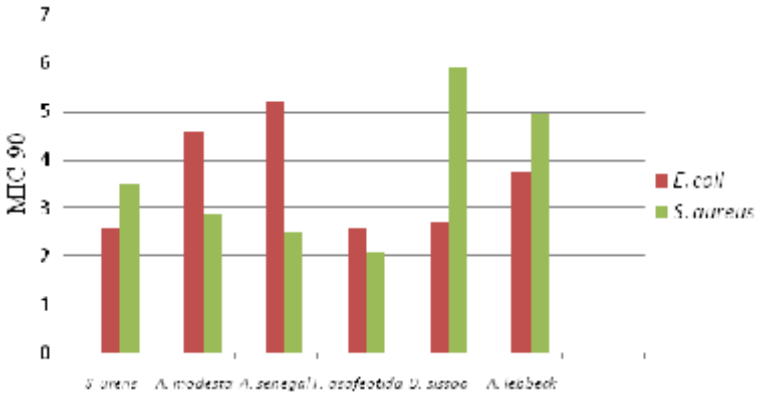
Table 2. Antibacterial activity of selected gums

Sample		Zone of inhibition (mm)	
		E. coli	*S. aureus*
S. urens	Crude	12 ± 0.3	12 ± 0.1
	Purified	14 ± 0.2	13 ± 0.1
A. modesta	Crude	12.5 ± 0.2	12 ± 0.1
	Purified	13.33 ± 0.2	13 ± 0.1
A. senegal	Crude	10 ± 0.2	12 ± 0.2
	Purified	11 ± 0.1	12 ± 0.3
F.asafeotida	Crude	12 ± 0.0	12 ± 0.2
	Purified	14 ± 0.1	13 ± 0.1
D. sissoo	Crude	13 ± 0.1	12.6 ± 0.5
	Purified	14.6 ± 0.2	12.5 ± 0.5
A. lebbeck	Crude	9 ± 0.1	10 ± 0.1
	Purified	13 ± 0.2	12 ± 0.1
Positive control		21 ± 0.1	20 ± 0.2

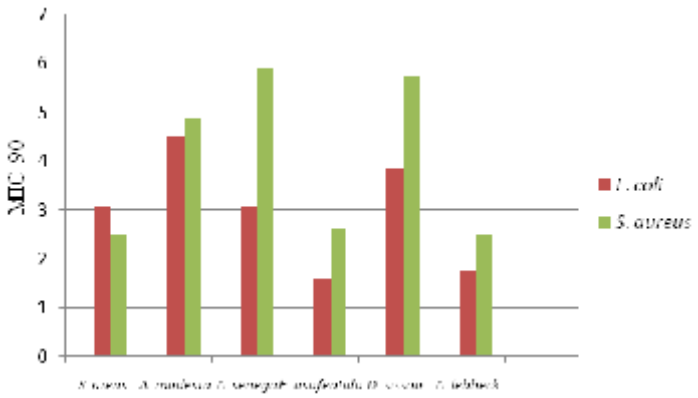
The values are mean ± S.D (n=3)

In vitro toxicity by hemolytic activity

According to our literature survey and best of knowledge, no information was available about the toxicity of the selected gums. The hemolytic activity of crude and purified gum samples were tested by the help of a rapid assay against human erythrocytes. The negative control (PBS) shows no hemolytic activity, whereas, triton-X-100 gives 99.9 % of lysis of human erythrocytes. The results of hemolytic activity are presented in Table 3. All used gum samples were non-toxic, as very low hemolytic activity was observed by them and therefore these gums can be considered for medication use in treatment of various diseases. Same analysis was performed using guar gum that was also non-toxic (Shahid *et al*., 2013; Tabasum *et al*., 2013).



(a)MIC of crude gums



(b)MIC of purified gums

Fig. 1. Minimum inhibitory concentration of crude and purified gums against *Escherichia coli* and *Staphylococcus aureus*

Table 3. *In vitro* toxicity by hemolytic activity of crude and purified gums

Sample	2%	4%	6%	8%	10%
(a) Hemolytic activity of crude gums					
S. urens	1.23±0.01	2.23±0.01	2.34±0.02	2.29±0.04	3.45±0.02
A. modesta	3.12±0.04	3.11±0.01	2.12±0.06	2.78±0.03	3.55±0.02
A. senegal	4.11±0.02	3.11±0.04	2.13±0.08	3.89±0.03	3.55±0.03
F.asafeotida	1.23±0.03	2.45±0.02	3.19±0.01	2.10±0.02	3.89±0.02
D. sissoo	1.24±0.02	2.11±0.01	3.57±0.02	2.23±0.02	3.68±0.02
A. lebbeck	2.03±0.03	2.45±0.03	4.25±0.14	5.60±0.06	6.57±0.09

The values are mean ± S.D (n=3)

Table 3. *In vitro* toxicity by hemolytic activity of crude and purified gums

Sample	2%	4%	6%	8%	10%
(b) Hemolytic activity of purified gums					
S. urens	1.14±0.01	1.34±0.02	1.98±0.02	2.30±0.01	2.22±0.01
A. modesta	2.33±0.01	2.18±0.02	1.23±0.03	1.83±0.02	2.13±0.02
A. senegal	1.85±0.02	2.23±0.01	1.80±0.02	2.01±0.02	2.47±0.02
F.asafeotida	1.11±0.01	1.66±0.01	2.89±0.01	1.40±0.02	2.72±0.03
D. sissoo	1.20±0.02	1.32±0.02	2.35±0.03	1.12±0.02	2.11±0.03
A. lebbeck	2.01±0.02	2.03±0.04	2.66±0.02	3.21±0.01	2.98±0.03

Triton-X-100: 99.9 %; Phosphate buffer saline: 0 %

CONCLUSION

It can be concluded that all the selected gum samples used were non-toxic. The gum samples exhibited moderate antibacterial activities. The proximate analysis reveals high amount of carbohydrates. It is concluded from the results that these gums can be a good candidate for the use in pharmaceutical industry as natural bio-binder.

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