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Research Article

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Chemical compositions and biological activities of selected exudate gums

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ABSTRACT

Kondagogu, Karaya and Acacia gums are natural products from the medicinal plants namely Cochlospermum gossypium L. De Candole, Sterculia urens Roxb, and Acacia senegal L. Willd. Chemical composition analysis performed by gas chromatographic technique revealed that Kondagogu gum was composed of galactose (14.64%), rhamnose (17.11%) and galacturonic acid (17.24%). Karaya gum contained rhamnose (8.91%), galacturonic acid (9.13%), galactose (14.67%) and glucuronic acid (35.59%) while rhamnose (10.63%), arabinose (21.42%), glucuronic acid (22.54) and galactose (42.57%) were found in Acacia gum. The average molecular weights by SEC analysis revealed the large structures of the studied gums (> 2,350 kDa). The viscosities of Kondagogu, Karaya and Acacia gums were investigated showing 574.08 ± 0.01, 25.02 ± 0.01 and 1.10 ± 0.01 cP respectively. Only Kondagogu gum demonstrated glucose entrapment (39.2 ± 0.2%) from diffusion through dialysis membrane. Kondagogu and Acacia gums decreased cholesterol solubility in single bile micelles (16 ± 0.04 and 23 ± 0.04% respectively). Kondagogu and Karaya gum expressed 11.6 ± 5.5% and 20.2 ± 6.4% DPPH scavenging effects. The studied gums had no inhibitory effect on α-Glucosidase. Concentration dependent reciprocal relationship on pancreatic lipase was revealed among these gums. Kondagogu showed 6% inhibitory activity on mushroom tyrosinase while Karaya and Acacia gums exhibited marginal activation on the tyrosinase enzyme.

Keywords: Plant exudate polysaccharide, glucose entrapment, cholesterol solubility, enzyme inhibition.

INTRODUCTION

Kondagogu, Karaya and Acacia gums from the trees namely *Cochlospermum gossypium* L. De Candolle (COCHLOSPERMACEAE), *Sterculia urens* Roxb. (STERCULIACEAE), and *Acacia senegal* L. Willd. (MIMOSACEAE) are natural products that widely used in pharmaceutical and food industries. The main constituent in gums is hydrophilic heterogenous polysaccharide. The physical properties of polysaccharide gums are used in pharmaceutical necessities as stabilizing, suspending, emulsifying, binding and coating agents. Numerous studies reported the advantage of these polysaccharide gums for drug delivery system [1-2] and laxative effect [3, 4]. Moreover, the epidemiological study evidenced that high consumption of dietary fiber is related to many health advantages [5]. Polysaccharide gums are categorized into soluble dietary fiber, thus the sticky viscous gums may reduce and retard the absorption of glucose, cholesterol and organic compounds in gastrointestinal tract because of their exceptional properties for intrinsic viscosity, water holding capacity, water and nutrients entrapment in the gastrointestinal lumen, gel matrix formation with water [6]. These valuable properties lead the polysaccharide gums to be an interesting topic to study, particularly to explore their biological activities, physical properties and sugar compositions.

EXPERIMENTAL SECTION

Exudate gums preparation

Kondagogu gum (Panacea Biotec Ltd., India), Karaya gum (Sigma–Aldrich Company Co., St. Louis, MO, USA) and Acacia gum (Taian Dingli Gum Industrial Co., Ltd., Shandong, China) were ground to powder by a high-speed mechanical blender. Ultrapure water was mixed with gum powder by homogenizer for gelation.

Effect on glucose diffusion

Glucose diffusion assay was performed by dialysis tubing method. Four milliliters of gum gels (0, 0.5, 1, 2% w/v) with glucose at 2% final concentration were added into the dialysis tubing cellulose membrane (molecular weight cut off = 12,000 Da). The dialysis was performed against 60 ml ringer buffer for 2 h under rotational shaking at 150 rpm. The determination of released glucose was carried out by glucose oxidase - peroxidase reaction using glucose liquicolor kit (Human Gasellsthaft, MBH, Germany). Shortly, 10 ml of the tested sample was mixed with 1 ml of the reagent for 15 min under the room temperature and measured the absorbance at 510 nm.

Effect on α-glucosidase inhibition

Ten milliliters of 1 U/ml yeast α-glucosidase was added into 40 µl of various concentrations of the tested samples (0.06, 0.13, 0.25, 0.50, 1 % w/v) at 37 ºC for 10 min. After that, 20 µl of 1 mM *p*-nitrophenyl-α-D-glucopyranoside in 0.1 M sodium phosphate buffer at pH 6.9 was added to start the reaction at 37 ºC for 20 min. The change of pH by 50 µl of 1 M sodium carbonate was used to terminate the enzymatic reaction. The *p*-nitrophenol resulting from the enzymatic reaction was measured in triplicate at 450 nm. In this study, 1-deoxynorjirimycin was used as a positive control.

Effect on cholesterol solubility in single bile micelle

This method was adapted from previous study [7]. Twenty five mM sodium taurodeoxycholate (NaTDC) in 15 mM phosphate buffer saline (pH 7.5) was used for single bile micelle. Three milliliters of bile, 10 mg of solid cholesterol (excess for saturation) and 15 mg of gums powder were mixed together by magnetic stirrer at room temperature for 24 h. The separation of soluble micelles in the mixture was performed by 0.22 µm PES membrane filtration. The cholesterol concentration in the filtrates was detected by cholesterol oxidase method using Cholesterol Liquicolor kit. Shortly, 10 ml of the tested filtrate was mixed with 1 ml of the reagent under the room temperature for 15 min and measured at 510 nm.

Effect on lipase activity

This assay was adapted from previous studies [8]. Ten milligrams of porcine pancreatic lipase was mixed with 1 ml of water, then the solution was centrifuged at 12,000 rpm for 5 min. The reaction mixture contained 400 µl of 0.1 M Tris buffer (pH 8.2), 50 µl of 1% polysaccharide gels or positive control (Oristat) and 150 µl of pancreatic lipase solution was incubated in water baht at 37 ºC for 5 min. Started the reaction by adding 450 µl of *p*-nitrophenyl laurate emulsion, further incubated for 2 hr at 37 ºC then filtered through 0.45 µm PVDF filter. The absorbance of *p*-nitrophenol from enzymatic reaction at 410 nm was measured by spectrophotometer.

Tyrosinase inhibitory activity

Dopachrome method was used to determine the kinetic reaction of tyrosinase activity. The enzyme was prepared by adding 0.5 mg of mushroom tyrosinase enzyme in 5 ml of 15 mM phosphate buffer (pH 7.3). The substrate was prepared by adding 0.8 mg of L-DOPA in 5 ml of 15 mM phosphate buffer (pH 7.3). The mixture assay contained 20 µl of various concentrations of polysaccharide gels (0.25, 0.5 1.% w/v), 40 µl of the enzyme, 140 µl of 15 mM phosphate buffer (pH 7.3) and started the reaction with 20 µl of L-DOPA. The absorbance was measured at 405 nm for 10 min under the room temperature. The positive control was 0.1 M ascorbic acid [9]. The result of kinetic activity was carried out by MikroWin200, version 4.

DPPH radical-scavenging activity

DPPH method was used in this study [10]. The reactive mixture composed of 1.5 ml of 0.004% methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) and 0.5 ml of various concentrations of the studied gums. The reactive mixture was incubated under the room temperature for 30 min without light. Butylated hydroxytoluene was a positive control in this study. The colorimeter was determined at 517 nm. Sigmaplot software was used to calculate the IC_{50} of scavenging activity.

Monosaccharides analysis

One milligram of selected exudate gums power was hydrolyzed in 4 M methanolic HCl at 80 ºC for 24 h in the acid-washed vial and further dried out with nitrogen. The washing of hydrolyzed samples with methanol under nitrogen was repeated twice. The samples were mixed with 0.4 ml of derivatizing agent using trimethylchlorosilane:

hexamethyldisilazane: pyridine in the ratio 1:2:5 at the room temperature for 30 min. Gas chromatographic analysis (Finnigan Trace GC Ultra) was performed using SGC BPX5 capillary column (30m x 0.25mm x 0.25 µm) with helium (carrier gas) at the flow rate of 1.0 ml/min. The temperature of injector and FID detector were 260 and 300 ºC. The initial temperature of column was 140 ºC, then ramped up at the rate of 1 ºC/min to 170 ºC and further ramped at 6 ºC/min to 250 ºC. A set of standard monosaccharides was assayed as the above method except for methanolysis using 1M methanolic HCl instead.

Estimation of the average molecular weights

One milligram of exudate gums were dissolved in 1 ml of ultrapure water (0.1% w/v) to form polysaccharide gels and further filtered the tested solutions through 0.45 µm PVDF centrifugal filters. HPLC with refractive index detector was used for Size exclusive chromatography method in this study. Mobile phase, ultrapure water at a flow rate of 0.5 ml/min through OHpak SB-806 M HQ HPLC column was used for the separation. Ten microliters of the samples was an injection volume. The standard curve of Showa Denko's Pullulans was used to determine the estimate average molecular weights of the polysaccharide gels.

Total protein analysis

The total protein content in selected exudate gums was estimated by folin reaction from Lowry assay [11]. The protein standard was prepared by various concentrations of bovine serum albumin ranging from 0-100 µg/ml. One milligram of the tested sample was dissolved in 0.5 ml of ultrapure water and further mixed to 0.7 ml of biuret reagent, incubated at the room temperature for 20 min. The mixture solution was added with 0.1 ml of folin-ciocalteu in ultrapure water (1:1.2) and incubated under the room temperature for 30 min. All solutions were measured at 750 nm against the reagent blank.

Viscosity

A falling ball viscometer was used to measure the viscosity of selected exudate gums. The sample solutions were prepared by mixing 5 g of exudate gums power in 250 ml of water. The viscous sample solutions further filled into the cylindrical tube with known inner diameter (approx. 15.94 ± 0.001 mm). The standard ball with specific radius and density was placed into the cylindrical tube and the falling time of the ball was recorded for the calculation of viscosity following the equation:

$$\text{Viscosity (in mPa.s)} = K(\rho 1 - \rho 2) \times t$$

Where K is a ball constant (mPa.s. cm3/g.s). ρ1 and ρ2 are a density of the ball and a density of the sample solution (g/cm3), while t is the falling time of the ball in seconds.

RESULTS AND DISCUSSION

Effect on glucose diffusion

At the same concentration (2% polysaccharide gel), Kondagogu, Karaya and Acacia gums showed 60.8 ± 0.2, 95.8 ± 0.2, 93.5 ± 0.1% of glucose releasing respectively (Figure 1). Dialysis tubing is an *in vitro* method that mimics the situation in jejunal lumen [12] by retarding the glucose absorption in gastrointestinal lumen with sticky viscous dietary fiber. The study evidenced the potential of exudate gums to retard glucose absorption across the intestinal lumen.

Effect on α-glucosidase inhibition

The hydrolyzing action of α-glucosidase breaks down polysaccharides at the terminal non-reducing 1, 4 linked alpha bond, yielding monosaccharides for the gastrointestinal absorption. Previous study revealed the ability of various polysaccharide plants to terminate the α-glucosidase enzyme [8]. The *in vitro* study of selected exudate gums had no therapeutic effect on α-glucosidase inhibition comparing to 1-deoxynorjirimycin (IC_{50} = 124.3 µg/ml). Thus, this study evidenced that three studied exudate gums had no effect (0% inhibition) to inhibit α-glucosidase enzyme.

Effect on cholesterol solubility in single bile micelle

Bile acid, an emulsifier is the derivative of cholesterol which plays an important role in lipid aggregation; therefore, lipid metabolism is depended on its solubility. In this *in vitro* study, the competitive mechanism of cholesterol solubilization in micelles (NaTDC) was used to determine the ability of exudate gums to inhibit cholesterol absorption (Figure 2). The *in vitro* study on single bile micelles (NaTDC) with Kondagogu and Acacia gum had the slightly inhibitory effect (16 ± 0.04% and 23 ± 0.04%), while Karaya gum had no inhibitory effect. Previous study at 25 mM NATDC, 0.5% w/v gels of *Ocimum canum* and glucomanan showed 25% and 16% inhibitory effect in cholesterol solubility respectively [13].

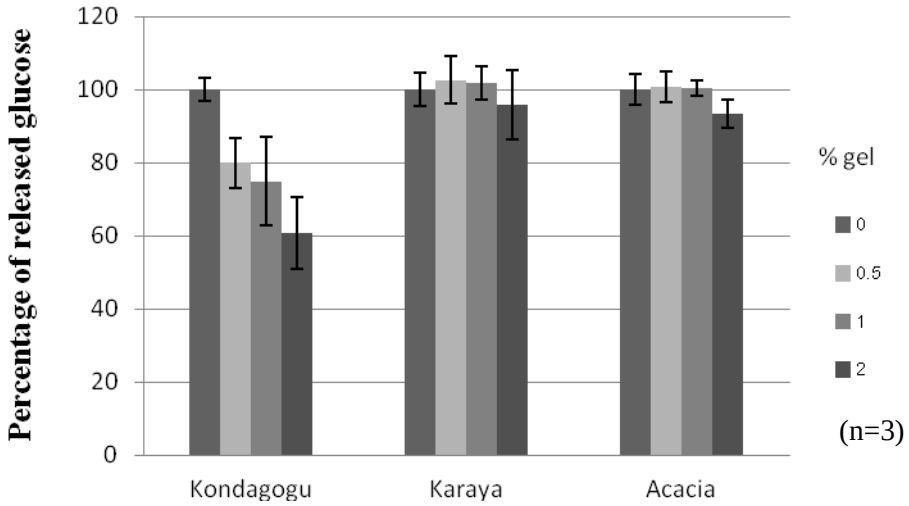

Figure 1: Percentage of glucose releasing from various concentrations of polysaccharide gels (0, 0.5, 1, 2% w/v) with 2% glucose after two hours dialysis (n=3)

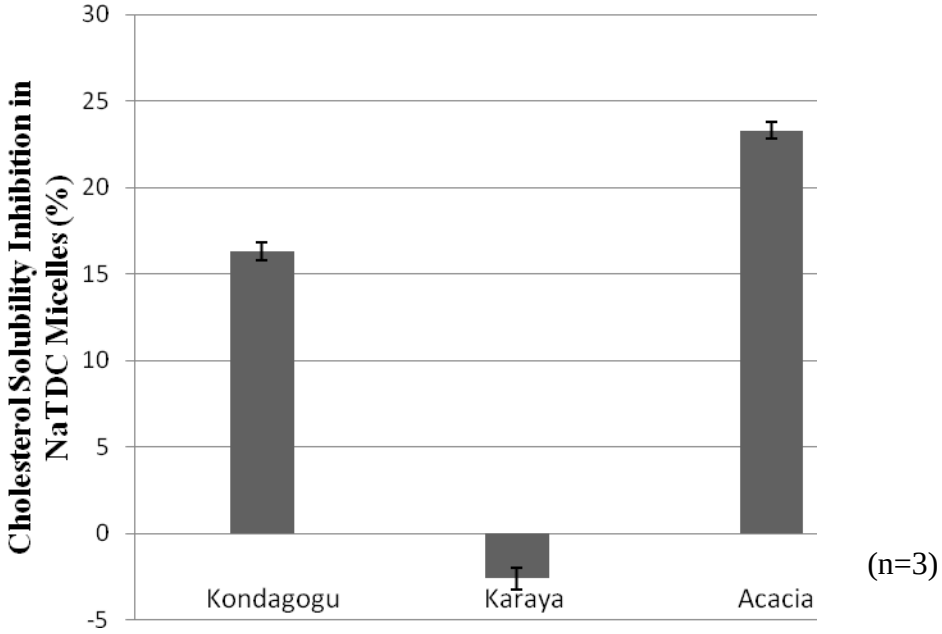

Figure 2: The inhibitory effect in percentage on micellar solubility of cholesterol using NaTDC (n=3)

Effect on lipase activity

Monoacylglycerol and free fatty acids are the products resulting from fat hydrolysis by pancreatic lipase. Thus, the termination of lipase enzyme decreases fat concentration in blood. At 0.25% gel, Kondagogu and Acacia gums had 16.2 ± 4.9% and 20.4 ± 1.1% inhibitory effect on lipase activity respectively. The reciprocal relationship between the inhibitory effect and gel concentration was shown. Karaya gum expressed lipase activation at higher concentration. Surprisingly, at 0.13% gel, kondagogu enhanced lipase activity as well. (Figure 3) Oristat, a positive control showed the IC_{50} of 2.4 µg/ml (Figure 4). Pancreatic lipase is a class of interfacial enzymes that interact only with lipid-water interfaces such as emulsions, micelles or bilayers [14]. Surface active property of polysaccharide gel may play a role on compartmentalization between two phases that affected lipolytic activity.

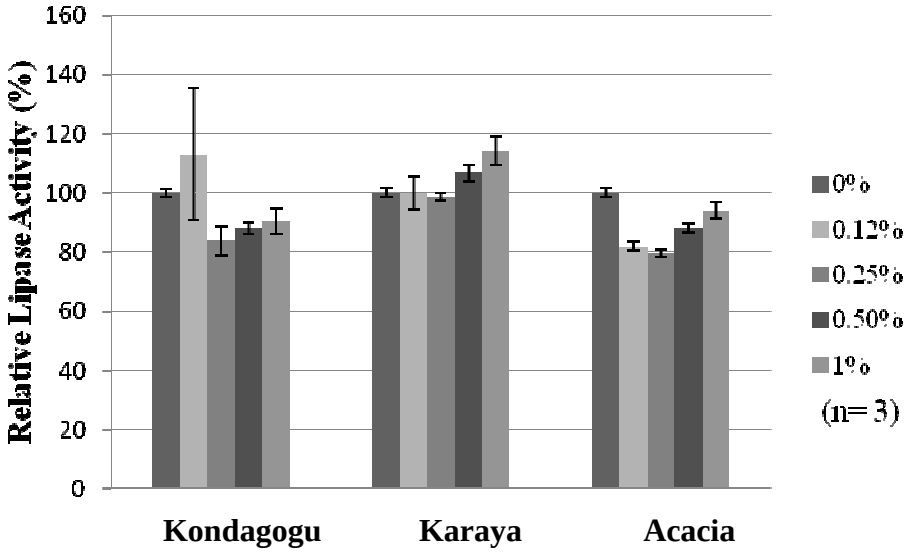

Figure 3: The lipase activity as percentage relative to gel 0 (n=3)

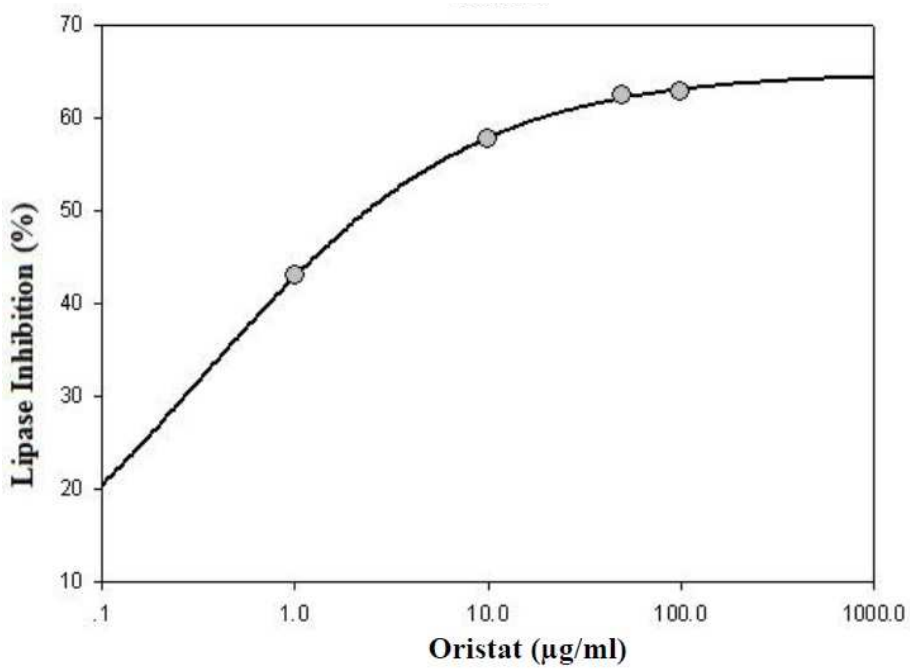

Figure 4: Percentage of lipolytic activity with Oristat, a positive control (IC_{50} = 2.4 µg/ml)

Tyrosinase inhibitory activity

The percentage of tyrosinase inhibitory activity was showed in (Figure 5). Kondagogu at 1% w/v slightly inhibited activity while low concentration of Kondagogu as well as all concentration of Karaya and Acacia activated tyrosinase. Therefore, this study revealed the effect of selected polysaccharides on either inhibition or activation of tyrosinase enzyme. The results are interested for further investigation of the whitening potential of Kondagogu gum as well as the skin coloring (re-pigmentation) potential of Karaya and Acacia gums.

DPPH radical-scavenging activity

DPPH is the simple method for free radical scavenging effect of natural compounds. At 1% w/v of polysaccharide gels, Kondagogu and Karaya gums showed 11.6 ± 5.5% and 20.2 ± 6.4% scavenging activity, while Acacia gum had no effect. These exudate gums exhibited less scavenging effect on free radical than previously reported plant mucilages [8].

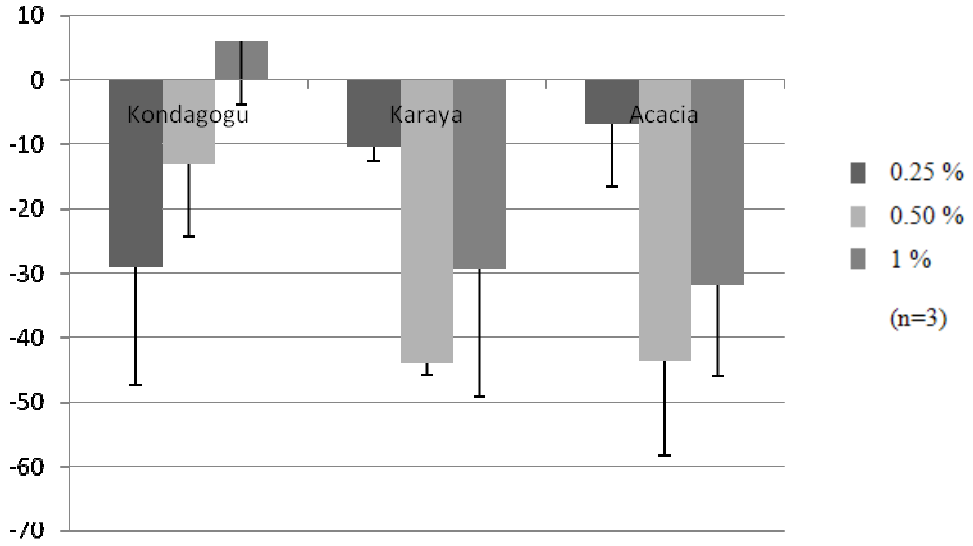

Figure 5: Percentage of tyrosinase inhibitory activity of 0.25, 0.5, 1% w/v of polysaccharide from exudate gums compared to gel 0 (n=3)

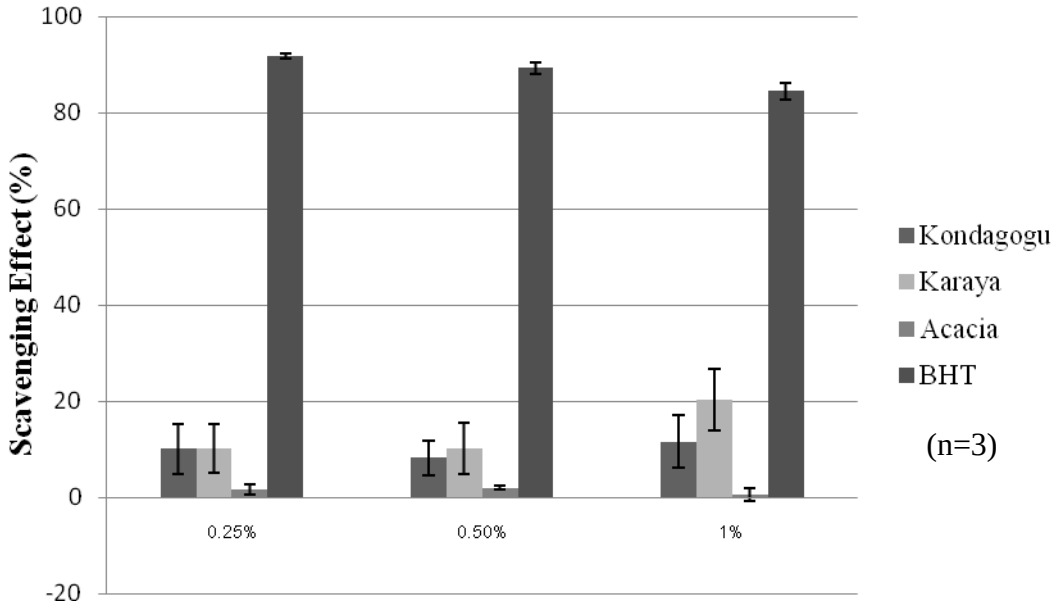

Figure 6: Percentage of scavenging effect of 0.25, 0.5, 1% gels containing 0.004% methanolic solution of DPPH after incubation for 30 min. (n=3).

Monosaccharides and total protein analysis

Qualitative and quantitative of sugar compositions and total protein content of the studied gums were analyzed by GC and Lowry assay respectively (Table 1). Kondagogu gum contained rhamnose, galactose and galacturonic acid (36 : 32 : 32 % mole ratio). Karaya gum contained rhamnose, galactose, galacturonic acid and glucuronic acid (13 : 23 : 12 : 52 % mole ratio). Acacia gum was composed of rhamnose, arabinose, galactose and glucuronic acid (10 : 26 : 43 : 21 % mole ratio). The difference in types of monosaccharide in the study and the literature may be due to the difference in analytical techniques and also the nature of gums. The studied gums were crude unfractionated polysaccharides[15, 16, 17]. The protein contents among these gums were very low compared to other mucilaginous polysaccharides [8].

Estimation of average molecular weights and viscosity

The average molecular weights of the selected exudate gums were much more than the highest molecular weight of standard pullulan (2,350 kDa). By extrapolation of the calibration curve, the estimated average molecular weights of Kondagogu, Karaya and Acacia gums were 9,640 kDa, 11,140 kDa and 4,350 kDa respectively. By means of Falling ball viscometer, Kondagogu gum had a highest viscosity among the studied gums (574.08, 25.02 and 1.10 cP for Kondagogu, Karaya and Acacia gums respectively).

Table 1 Monosaccharide and total protein contents (µg/mg) among selected exudate gums

	Rha	Ara	Gal	GalA	GlcA	TP
Kondagogu	171.141	-	146.378	172.353	-	2.45
Karaya	89.051	-	146.723	91.262	359.790	9.48
Acacia	106.304	214.197	425.735	-	225.398	4.68

CONCLUSION

The selected exudate gums, *Cochlospermum gossypium* De Candole, *Sterculia urens* Roxb. and *Acacia senegal* (Linn.) Willd. are the famous economic natural products for food and pharmaceutical industries. The study evidenced highly viscosity of the exudate gums which related to their molecular weight sizes. The main chemical constituents of the exudate gums are monosaccharides. Gelation of the exudate gums when merged with water revealed their dietary soluble fiber property, thus the studies of their biological activities are investigated, resulting in the studies of effects on glucose entrapment ability, cholesterol solubility, DPPH scavenging activity, α-glucosidase, pancreatic lipase and tyrosinase inhibitory activities. These *in vitro* studies from crude exudate gums provided general knowledge of chemical components, physical properties and biological activities. The simply models, cheap and rapid experiments should expand our knowledge in the field of viscous polysaccharide in herbal medicine application.

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