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## Note

# Anticomplementary and hypoglycemic activity of Okra and Hibiscus mucilages*

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Several plant mucilages have been isolated from plants belonging to Liliaceae, Amaryllidaceae, Dioscoreaceae, Orchidaceae, Saxifragaceae, Malvaceae, and Plantaginaceae families, and structural studies on the polysaccharides were carried out by our laboratory. Recently, anticomplementary activity of fifteen plant mucilages[1] and hypoglycemic activity of twenty plant mucilages[2] were reported. In general, the substances obtained from plants of monocotyledons showed insignificant activity. On the other hand, paniculatan from the inner bark of *Hydrangea paniculata* Sieb (ref. 3) and Plantago-mucilage A (ref. 4) from the seed of *Plantago asiatica* L. showed considerable anticomplementary activity. In addition, many mucilages obtained from the plants belonging to the Malvaceae family showed remarkable hypoglycemic activity on administration to normal mice. The effective substances possessed the common backbone structure being represented by the most effective mucilage[5], Abelmoschus-mucilage M, from the root of *Abelmoschus manihot* Medicus.

We report herein the anticomplementary activity and hypoglycemic activity of the plant mucilages obtained from *Abelmoschus esculentus* Moench (*i.e. Hibiscus esculentus* L.; okra), *Hibiscus moscheutos* L., and *Hibiscus syriacus* L.

Okra-mucilages F (ref. 6) and R (ref. 7) were isolated from the immature fruit and the root of *Abelmoschus esculentus,* respectively. Hibiscus-mucilages Mo (ref. 8) and ML (ref. 9) were isolated from the root and the leaf of *Hibiscus*

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TABLE I

COMPOSITION OF OKRA AND HIBISCUS MUCILAGES

| *Mucilage* | *Sugar composition* | | *Other components*[a] *(%)* | |
|---|---|---|---|---|
| | *Components*[a] | *Molar ratio* | *O-Acetyl groups* | *Peptide* |
| Okra F | Gal:Rha:GalA | 1:1:1 | 5.5 | 10.8 |
| Okra R | Gal:Rha:GalA:GlcA | 1.9:1.1:1:1 | 7.4 | 19.3 |
| Hibiscus Mo | Rha:GalA:GlcA | 1:1:1 | 8.0 | 16.9 |
| Hibiscus ML | Gal:Glc:Rha:GalA:GlcA | 12:1:18:12:11 | 1.3 | 8.6 |
| Hibiscus SL | Gal:Rha:GalA:GlcA | 1.1:8:8:4 | | 1.8 |
| Hibiscus SF | Gal:Rha:GalA:GlcA | 36:36:33:22 | 5.9 | 8.1 |

[a]Abbreviations: Gal, D-galactopyranose; Glc, D-glucopyranose; Rha, L-rhamnopyranose; GalA, D-galactopyranosyluronic acid; and GlcA, D-glucopyranosyluronic acid.

*moscheutos,* and Hibiscus-mucilages SL (ref. 10) and SF (ref. 11) from the leaf and the flower bud of *Hibiscus syriacus,* respectively. Data of the composition on these mucilages are given in Table I.

The anticomplementary activities of the mucilages are shown in Fig. 1. Hibiscus-mucilages ML and SF showed remarkable activities. Okra mucilage R and Hibiscus-mucilage SL also had potent activities, which were almost at the same level as that of the positive control, AR-arabinogalactan[12] from the root of *Angelica acutiloba* Kitagawa. The activities of Okra-mucilage F and Hibiscus-mucilage Mo were lower than those of the former four mucilages.

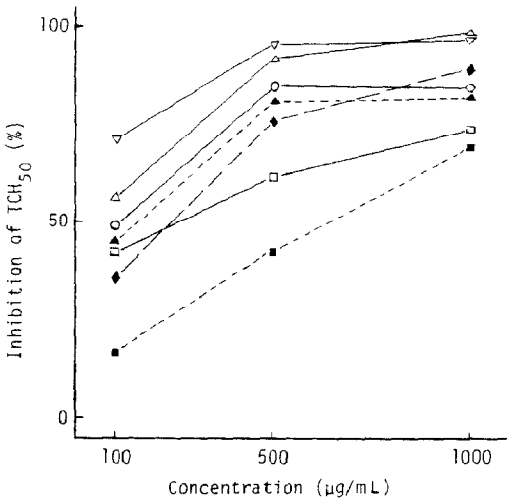



Fig. 1. Anticomplementary activity of Okra and Hibiscus mucilages: (◆) AR-arabinogalactan mixture, (▲) Okra-mucilage R, (■) Okra-mucilage F, (▽) Hibiscus-mucilage SF, (△) Hibiscus-mucilage ML, (○) Hibiscus-mucilage SL, and (□) Hibiscus-mucilage Mo.

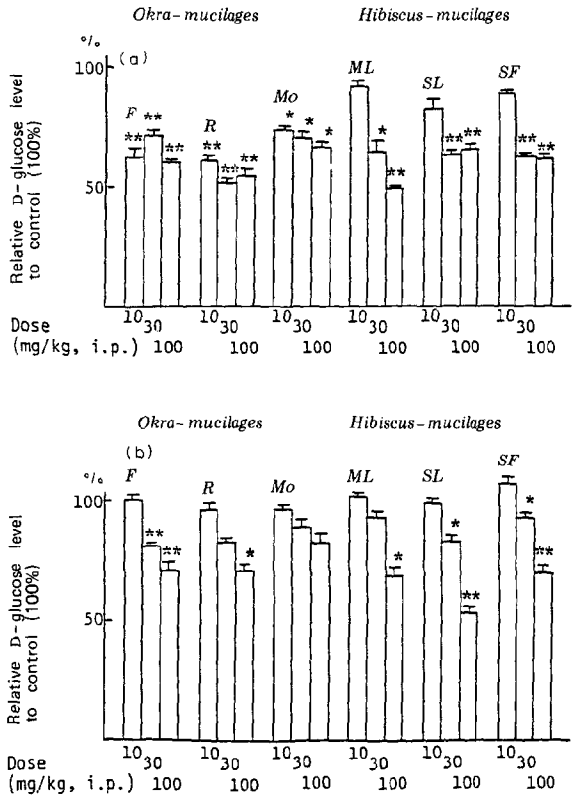


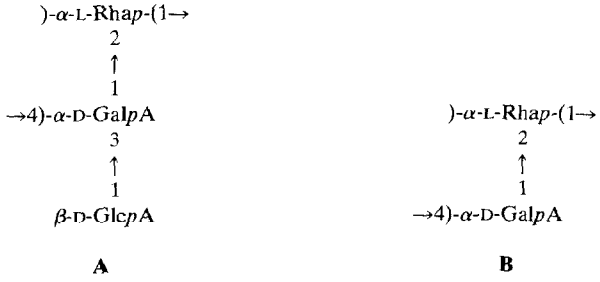

Fig. 2. Effect of Okra and Hibiscus mucilages on plasma D-glucose level. Significantly different from the control (*P<0.05 or **P<0.01): (a) 7 h after administration; (b) 24 h after administration.

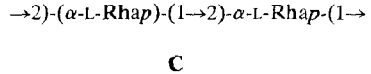
The hypoglycemic activities of Okra-mucilages F and R and of Hibiscus-mucilages Mo, ML, SL, and SF are shown in Fig. 2. Most of these mucilages exhibited significant hypoglycemic activity, though the effect of Hibiscus-mucilage ML was dose dependent.

The structural units of polysaccharides of Okra and Hibiscus mucilages are shown in Scheme 1, and their constitutions in Table II.

Okra-mucilage R and Hibiscus-mucilages Mo and ML have the repeating unit, (1→4)-[*O*-β-(D-glucopyranosyluronic acid)-(1→3)]-*O*-α-D-galactopyranosyluronic acid-(1→2)-*O*-α-L-rhamnopyranose, in the main part of their backbone chains, whereas the repeating unit (1→4)-*O*-α-D-galactopyranosyluronic acid-(1→2)-*O*-α-L-rhamnopyranose occurs preponderantly in the backbone chains of Hibiscus-mucilages SL and SF. In Okra-mucilage F, the latter repeating unit is the sole component of the backbone. On the other hand, every mucilage but Hibiscus-mucilage Mo has a neutral-sugar branch linked to a relatively high degree to O-4 of the L-rhamnose residues of the backbone.

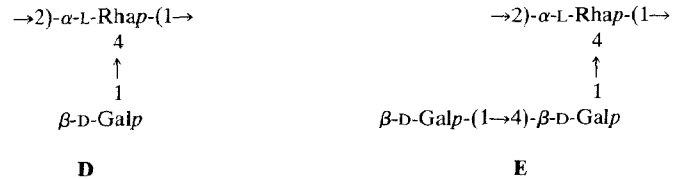
*Backbones and acidic branches*

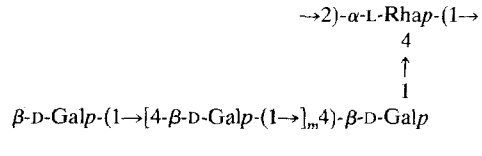


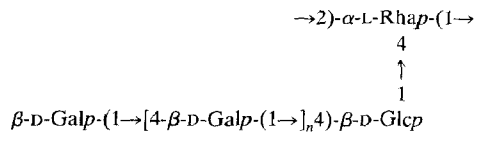

**C**

*Neutral side-chains*




**F**




**G**

Scheme 1. Structural units of polysaccharide components ($m + n = 2$–9).

TABLE II

CONSTITUTION OF THE STRUCTURAL UNITS IN OKRA AND HIBISCUS MUCILAGES

| *Mucilage* | *Structural units* | *Ratio of units* | |
|---|---|---|---|
| Okra F | **B E** | **B:E** | 2:1 |
| Okra R | **A C F** | **A:C:F** | 60:3:40 |
| Hibiscus Mo | **A** | | |
| Hibiscus ML | **A B C F G** | **A:B:C:F:G** | 11:1:3:2:1 |
| Hibiscus SL | **A B D** | **A:B:D** | 4:4:1 |
| Hibiscus SF | **A B C E** | **A:B:C:E** | 44:22:3:36 |

Okra-mucilage R and Hibiscus-mucilages ML, SL, and SF showed remarkable anticomplementary activity, which may result from the highly branched structure. Okra and Hibiscus mucilages which showed extensive hypoglycemic activity have a backbone chain consisting of almost only the repeating unit L-rhamno-D-galacturonan. The D-glucuronic acid side-chain groups at O-3 of the D-galacturonic acid residues in the backbone are not essential for the hypoglycemic effect, as Okra-mucilage F showed a significantly activity. Okra-mucilage R especially showed a remarkable activity. Both Okra-mucilage R and Hibiscus-mucilage Mo possess the trisaccharide repeating unit, (1→4)-[β-D-Glc*p*A-(1→3)]-α-D-Gal*p*A-(1→2)-α-L-Rha*p*, as the main part of their backbone, but the former polysaccharide showed a higher hypoglycemic effect than the latter. Okra-mucilage R possesses side-chains composed mainly of β-(1→4)-linked D-galactopyranose residues, and (1→2)-linked L-rhamnopyranosyl residues in part of the backbone. Therefore, the presence of these side-chains and the units in minor proportions may contribute to the activity of the mucilage.

## EXPERIMENTAL

*Isolation of mucilages.* — Okra-mucilage F was isolated from the immature fruit of *Abelmoschus esculentus* by a precipitation method with cetyltrimethylammonium bromide[6] and Okra-mucilage R from the root of the same plant[7]. Hibiscus mucilage Mo was isolated from the root of *Hibiscus moscheutos* by precipitation with cetyltrimethylammonium bromide[8] and Hibiscus-mucilage ML from the leaf of the same plant by chromatography[9] with DEAE-Sephadex A-25. Hibiscus-mucilage SL was isolated from the leaf of *Hibiscus syriacus* by a similar chromatography[10], and Hibiscus-mucilage SF from the white flower bud of the same plant by precipitation with sodium lauryl sulfate and acetone[11].

*Measurement of anticomplementary activity.* — Gelatin–veronal-buffered saline solution (pH 7.4) contained[12] 500μM $Mg^{2+}$ and 150μM $Ca^{2+}$ ($GVB^{2+}$), and normal human serum (NHS) was obtained from a healthy adult. Various dilutions of the samples of mucilage in water (50 μL) were incubated with NHS (50 μL) and $GVB^{2+}$ (50 μL) at 37° for 30 min, and the residual total hemolytic complement

($TCH_{50}$) was determined by a method using IgM-hemolysin-sensitized sheep erythrocytes at $1 \times 10^8$ cells/mL. Control was provided by incubation of NHS with water and $GVB^{2+}$. The activity of the sample was expressed as the percentage inhibition of the $TCH_{50}$ of the control.

*Measurement of hypoglycemic activity.* — Male mice (Std:ddY strain, 25–30 g) were used in groups of five and given food and drinking water freely. Each sample of mucilage was dissolved in physiological saline solution and injected (i.p.). Blood was drawn periodically from the orbial sinus by micro haematocrit-tubes. The D-glucose level of the plasma obtained by centrifugation of blood was measured with a D-glucose analyzer by the D-glucose oxidase method. The results were evaluated by one-way analysis of variance.

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