Supporting Information to:

Studies on the Antihyperlipidemic Properties of *Averrhoa bilimbi* Fruit in Rats

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Preparation of fruit homogenate

The fruits were sliced into small pieces and air-dried at room temperature. The dried pieces were ground with 2% gum acacia in a pestle and mortar to obtain a 10% (W/V) homogenate.

Preparation of extracts

Water extract of the fruit was prepared by extracting the powdered fruit with water (10 g in 100 mL) for 4 hrs at room temperature with constant shaking. The extract was filtered with Whatman No. 1 filter paper. The residue was once again extracted as above and the combined filtrate was freeze-dried. (This two- step extraction was found to be sufficient to extract almost all water-soluble materials). The alcohol extract of the fruit was prepared similarly using ethyl alcohol instead of distilled water. However, in this case, the extract was evaporated to dryness in a rotary evaporator under reduced pressure at 40 ^{0}C. The *n*-hexane extract was prepared using *n*-hexane instead of alcohol. In this case, to ensure complete extraction, 2 g powder was extracted with 100 mL hexane and the process was repeated 3 times. The filtrates from the 3 extractions were combined and dried as above.

Characterization of the active fraction by HPTLC and HPLC

For HPTLC analysis a Linomat V (CAMAG TLC Scanner) automated TLC applicator was used to apply samples on TLC plates (Silica gel 60 F_{254}) under a flow of nitrogen gas. TLC plates were developed in a chloroform: methanol (9:1 V/V) solvent system. Developed plates were dried in a stream of air and spots were scanned using a CAMAG TLC scanner-3 at (maximum absorption) wavelength, 284 nm (scanning speed 20 mm/s) [Photo-documentation by CAMAG repostar-3]. The HPLC system was a Gilson analytical HPLC with a Unipoint LC system software. 5 μL aliquots (1 mg/mL) of the fraction were analyzed using a Kromasil 100 C18 column (256 X 4.6 mm) with a particle size of 5 μm, at a flow rate of 1 mL /minute; solvent: acetonitrile-water (80:20); detector: λ = 254 nm..

Toxicity evaluation in mice

To study short term toxicity, if any, of the fruit, 4 groups each containing 6 male mice were used. One group was kept as control and groups 2, 3, 4 received 250, 500 and 1000 mg/kg of the fruit

homogenate respectively. The fruit in 2% gum acacia was administered daily for 14 days (p. o.). Control group received 2% gum acacia in an identical manner.

The behavior of the animals was observed daily for 1 hr for 14 days. Initial and final body weights, water and food intake, and state of stool were observed. The animals were sacrificed on the 15th day. Liver, heart, spleen and kidneys were weighed and observed for pathological changes. Hematological and serum biochemical parameters were determined using standard methods [1].

Reference

1 Jam NC. Schalm's veterinary haematology, 4th edition. Philadelphia: Lea and Fabiger; 1986: 43-8.

Table 1S Effect of *Averrhoa bilimbi* fruit or its water extract on body weight and weight of organs in high fat diet fed rats

Groups	***Body weight (initial) (g)***	***Body weight (final) (g)***	***Weight of liver (g)***	***Weight of kidneys (g)***	***Weight of spleen (g)***
Normal control	216.0 ± 4.0	303.3 ± 9.0	8.10 ± 0.31	1.78 ± 0.06	0.80 ± 0.03
HFD control	220.3 ± 5.5	358.3± 10.4*	9.50 ± 0.25*	1.80 ± 0.08	0.79 ± 0.05
HFD + *A. bilimbi* fruit (125 mg/kg fruit)	217.0 ± 2.6	306.0 ± 9.8[a]	8.13 ± 0.20[a]	1.78 ± 0.09	0.81 ± 0.07
HFD + *A. bilimbi* fruit (250 mg/kg fruit)	219.3 ± 5.5	290.3 ± 4.5[a]	8.23 ± 0.61[b]	1.81 ± 0.07	0.78 ± 0.04
HFD + *A. bilimbi* Water extract (50 mg/kg)	216.7 ± 5.6	289.0 ± 5.6[a]	8.07 ± 0.21[a]	1.79 ± 0.10	0.79 ± 0.06
HFD + Gemfibrozil (100 mg/kg)	217.9 ± 5.1	318.0 ± 8.1[a]	8.35 ± 0.65[b]	1.91 ± 0.11	0.81 ± 0.07

Values are mean ± SD; n = 6 in each group.

* P<0.001 (compared to normal control).

[a] P < 0.001; [b] P < 0.05 (compared to HFD control).

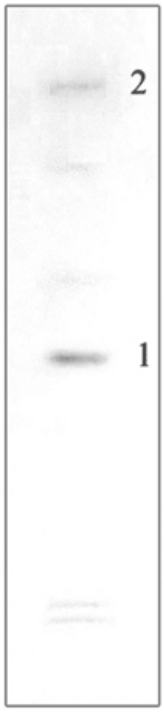

Fig. 1S. Thin layer chromatographic separation on Silica gel 60 F_{254} of the ethyl acetate faction obtained from the water extract of *A. blimbi* fruit. Chloroform:methanol (9:1 V/V) was used as the solvent system. The plate was sprayed with anisaldehyde-sulphuric acid and heated. **1**, **2** are two major components, which showed positive reaction to triterpenoid and steroid, respectively. The triterpenoid positive component (Rf. 0.44).exhibited anti-hyerlipidemic activity.

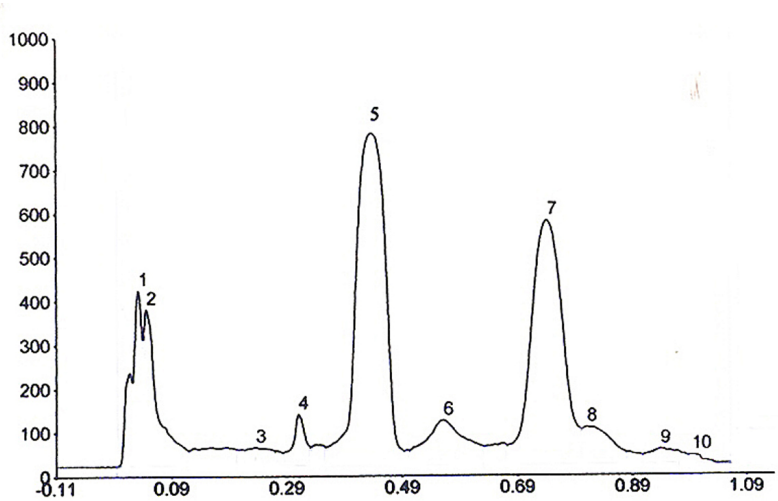

Fig. 2S HPTLC profile of the active fraction (ethyl acetate faction) from the water extract of A. blimbi fruit (λ = 284 nm). Peak 5 is the active component.