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Effect of water extract of *Psidium guajava* leaves on alloxan-induced diabetic rats

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A water extract of *Psidium guajava* leaves was screened for hypoglycemic activity on alloxan-induced diabetic rats. In both acute and sub-acute tests, the water extract, at an oral dose of 250 mg/kg, showed statistically significant hypoglycemic activity.

Psidium guajava Linn. (Myrtaceae), an arborescent shrub or small tree, up to 8 m high; often referred to as the apple of tropics; is native of tropical America and has long been naturalized in India (Anonymous 1998 a). The leaves contain catechol, tannins, wax, resins, sugars, carotene, vitamins B_1, B_2, B_6, niacin (Anonymous 1998b), essential oil, vitamin C (Ambasta 1986), calcium and manganese in combination with phosphoric, oxalic and maleic acids (Nadkarni 1985a). The leaves are used in traditional medicine as astringent, anodyne, febrifuge, antispasmodic, in wounds, ulcers, cholera, diarrhoea, vomiting (Vaidyaratnam 1995; Anonymous 1998c), for swollen gums and ulceration of mouth (Nadkarni 1985b). Biological activities, viz. antidiarrhoeal (Lutterodt 1992), antitussive and antimicrobial (Jairaj et al. 1999), analgesic, anti-inflammatory, CNS depressant (Olajide et al. 1999), topical haemostatic (Jairaj et al. 2000), atiamoebic (Tone et al. 1998) actions of various extracts of leaves of the plant have been reported.

PS. guajava is additionally known for its antihyperglyemic activity which was evaluated in this study. The effect of *P. g*uajava water extract on alloxan-induced diabetic rats (Table 1) has shown statistically significant hypoglycemic effects ($P < 0.001$). In the untreated animals, blood glucose levels did not change significantly. The sub-acute treatment with *P. guajava* extract on alloxan-induced diabetic rats produced consistent reduction in the blood glucose levels (Table 2), as compared with diabetic control.

The results indicated that *P. guajava* leaves posses significant hypoglycemic activity as is evident in acute and sub-acute treatments. It is generally accepted that alloxan treatment causes permanent destruction of β-cells (Pari and Maheshvari 1999). It is, therefore, conceivable that the hypoglycemic principles in the water extract of *P. guajava* exert their effect by an extra pancreatic mechanism in diabetic rats.

Table 1: Effect of acute treatment of *P. guajava* leaf water extract (250 mg/kg, p.o.), on blood glucose level in alloxan induced diabetic rats[a]

Group	Treatment	Blood Glucose (mg/dl)		
		Basal value	1 h	3 h
I	Control (Distilled water only)	75.60 ± 1.24	77.00 ± 1.87	74.80 ± 1.3
II	Diabetic Control (Alloxan only)	353.80 ± 2.85	352.60 ± 2.78	352.00 ± 1.59*
III	Standard (Alloxan + Std. Drug)	329.00 ± 6.49	315.60 ± 5.68 NS	308.20 ± 5.07 NS
IV	Test (Alloxan + extract)	339.60 ± 7.29	254.20 ± 9.27*	239.80 ± 17.65***

[a] Values are means ± S.E.; n = 6, * $p < 0.05$, *** $p < 0.001$, NS, not significant vs. group II.

Experimental

Fresh stem bark of *P. guajava* was collected from Bullandshehar district of Uttar Pradesh, India and authenticated at the Taxonomy division, Faculty of Science. A voucher specimen is deposited in the laboratory of Pharmacognosy. Shade dried, powdered leaves (500 g) were extracted with water and filtered. The filtrate was dried by vacuum rotary evaporation, which yielded a solid residue of 26.5 g (yield, 5.3%).

Wistar rats (180–220 g) of either sex were used. They were obtained from the Central Animal House, Jamia Hamdard and housed in standard environmental conditions at the animal house. The animals were fasted for 16 h prior to experiment, with access to water *ad libitum.* Hyperglycemia was induced by a single i.p. injection of 120 mg/kg alloxan monohydrate in sterile saline. After 5 days of alloxan injection, the diabetic rats (glucose level > 300 mg/dl) were separated and divided into three groups of five diabetic animals each. Group I was previously selected as normal control and received distilled water and no alloxan. Group II served as diabetic control and was received distilled water. Group III received the standard anti-diabetic drug gliclazide at an oral dose of 25 mg/kg (Panacea Biotech Ltd., Batch No. 01030513). Group IV was treated orally with water extract at an oral dose of 250 mg/kg; the dose was selected after preliminary behavioural and acute toxicity tests. Blood samples were collected from the tip

Table 2: Effect of sub-acute treatment of *P. guajava* leaf water extract (250 mg/kg, p.o., once daily), on blood glucose level on alloxan induced diabetic rats[a]

Group	Treatment	Blood glucose (mg/dl)				
		Basal value	Day 1	Day 3	Day 7	Day 10
I	Control (Distilled water only)	75.60 ± 1.24	93.80 ± 6.08	92.00 ± 3.96	91.00 ± 5.79	92.20 ± 4.19
II	Diabetic Control (Alloxan only)	353.80 ± 2.85	358.40 ± 4.91	353.60 ± 3.17	354.40 ± 4.05	354.80 ± 4.64
III	Standard (Alloxan + Std. Drug)	329.00 ± 6.49	303.80 ± 8.167 NS	306.40 ± 7.16 NS	304.40 ± 6.67 NS	304.60 ± 7.94 NS
IV	Test (Alloxan + extract)	339.60 ± 7.29	218.80 ± 6.70***	208.80 ± 7.47***	200.00 ± 6.55***	184.60 ± 5.75***

[a] Values are means ± S.E.; n = 6, *** $p < 0.001$, NS, not significant vs. group II

of tail just prior to and 1 and 3 h after the extract/standard drug administration. In sub-acute treatment, the administration of extract/standard drug was continued for 10 days, once daily. Blood samples were collected from the tip of the tail just prior to and on days 1, 3, 7 and 10 of the extract/standard drug administration. The blood glucose levels were determined for all the samples by the glucose-oxidase method (Varley et al. 1967). Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnet's t-test. $P < 0.05$ indicates significant differences between group means.

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A new homotriterpene from the roots of *Marsdenia tenacissima* Wight and Arn.

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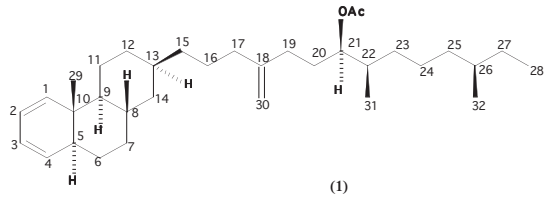
A new homotriterpene isolated from the roots of *Marsdenia tenacissima* Wight et Arn. has been characterized as 13-(31,32-dimethyl-30-methylene-21α-acetoxy-tetradecanyl)-29-methyl-perhydrophenanthr-1,3-diene on the basis of spectral analyses.

Marsdenia tenacissima Wight et Arn. (Asclepiadaceae), popularly known as Safed Nishoth, is a large and stout twinning shrub, with grey or pale brown bark. It is an important herbal drug used in the indigenous system of medicine as a purgative, antipyretic, antifilarial, bitter stomachic and uterine sedative (Anonymous 1966; Kirtikar and Basu 1994). It has also been found to contain antimutagenic factors (Nadkarni 1954; Lee H and Lin JY 1988). The presence of steroidal glycosides (Chen et al. 1999; Yang et al. 1981) and pregnane derivatives (Singhal and Khare 1980) from the various part of the plant has been reported.

Marsdemene (**1**) was isolated from a petroleum ether-chloroform (1 : 1) mixture as a colourless crystalline solid. It responded positively to the tetranitromethane and bromine water tests for unsaturation. Its IR spectrum exhibited absorption bands for ester group (1751 cm^{-1}) and unsaturation (1662 cm^{-1}). The FAB mass spectrum of **1** exhibited a molecular ion peak at m/z 496 corresponding to the molecular formula of tricyclic homotriterpene acetate, $C_{34}H_{56}O_2$, which was supported by its ^1H, ^{13}C and DEPT NMR spectra. The spectra indicated seven double bond equivalents; three of these were adjusted in a tricyclic carbon framework, three in olefinic linkages and the remaining one in the ester group.



(1)

The MS showed diagnostically important fragments at m/z 444 $[C_{1,10}–C_{4,5}$ fission$]^+$, 384 [444-AcOH]$^+$, 106, 390 $[C_{6,7}–C_{9,10}$ fission$]^+$, 120 $[C_{7,8}–C_{9,10}$ fission$]^+$, 146, 350 $[C_{8,14}–C_{9,11}$ fission$]^+$, 160 $[C_{8,14}–C_{11,12}$ fission$]^+$ and 174