ISOLATION AND ANTIDIABETIC ACTIVITY OF NEW LANOSTENOIDS FROM THE LEAVES OF
*PSIDIUM GUAJAVA* L.

PRIYANKA BAGRI, MOHAMMED ALI*, VIDHU AERI, MALAY BHOWMIK

Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi 110062, India

Email: maliphyto@gmail.com

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ABSTRACT

Objective: Diabetes mellitus is a chronic metabolic disease which affects our body's ability to use the energy found in food. Our study was planned to isolate chemical constituents from the leaves of *Psidium guajava* L. (Myrtaceae), to characterize their structures and to investigate their antidiabetic activity.

Methods: The air-dried leaf powder was exhaustively extracted with methanol in a Soxhlet apparatus. The concentrated leaf extract was adsorbed on silica gel (60-120 mesh) for the preparation of a slurry. The dried slurry was chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol, successively, in order of increasing polarity to isolate the compounds. These natural constituents were tested for the antidiabetic activity in STZ-induced diabetic models.

Results: Six new lanosteryl-type triterpenoids characterized as lanost-7-en-3β,12β,29-triol-26-oic acid (2), lanost-7-en-3β,12β,29-triol-26-oic acid (4), lanost-cis-1,7,23-trien-3β,12β,18α,22α-tetraol-26-oic acid (5), lanosteryl-3β-O-D-xylopyranosyl-2′-p-benzaldehyde (7) and lanost-7-en-3β,12β,29-triol-26-oic acid-3β-D-glucopyranoside (8) along with the known compounds arachidic acid (1) and β-sitosterol glucopyranoside (8) were isolated from the leaves. The compounds 2, 3, 4 and 8 exhibited significant antidiabetic activity against streptozotocin-induced diabetic rats.

Conclusion: The leaves of *P. guajava* possessed antidiabetic lanostene-type triterpenoids.

Keywords: *Psidium guajava*, Leaves, Lanostenoids, Structural elucidation, Antidiabetic activity

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INTRODUCTION

Diabetes mellitus (DM) is a chronic, lifelong disease which affects our body's ability to use the energy found in food. It is a group of metabolic diseases characterized by high blood sugar, frequent urination, excessive thirst and increased hunger. DM is due to either the pancreas is not producing sufficient insulin or the cells of the body not responding properly to the insulin produced. At present nearly 387 million people, equal to 8.3% of the adult population with equal rates of males and females, have diabetes worldwide, with type 2 diabetes suffering about 90% of the cases [1]. Presently, herbal medicines are mainly used to control the disease due to less side effects [2]. In recent years, herbal medicines have started to gain importance as a source of hypoglycemic agents. The plant products are rich in phenolic compounds, flavonoids, terpenoids, coumarins, and other constituents which show a reduction in blood glucose levels [3, 4].

*Psidium guajava* L. (Myrtaceae), universally known as guava, is a native to Mexico and Peru and now cultivated in many countries as a shrub or small evergreen tree with many branches [5, 6]. Its fruits, known as the ‘poor man’s apple’ enrich the diets of millions of people for its nutritional value [7]. Presently, *P. guajava* is known as the ‘poor man’s apple’ enrich the diets of millions of people for its nutritional value [7].

During the course of our search for antidiabetic activity, we selected flavinone-2,2′-ene, prenol, dihydrobenzo-phenanthridine and a number of other constituents which show a reduction in blood glucose levels [3, 4].

*MATERIALS AND METHODS*

**General procedures**

Melting points were determined by a thermoelectrically heated Perkin-Elmer melting point apparatus without correction. UV spectra were measured with a Lambda Bio 20 spectrophotometer in methanol. Infrared (IR) spectra were recorded using a Jasco FT-IR-5000 Spectrometer. The 1H (400 MHz) and 13C (100 MHz) NMR spectra were recorded on a Bruker ARX-Spectrometer by using CDCl3 and DMSO-d6 as solvents and TMS as an internal standard. Mass spectrometric detection was carried out on ESI-MS (Q-TOFESI), an electrospray-ionization (ESI) technique with positive ionization mode. Column chromatography was performed on silica gel 60–120 mesh and solvents taken were purchased from Merck Specialties Private Limited. Pre-coated aluminum TLC plates of silica gel 60 F254 were used to run and spots were visualized by exposure to iodine vapor, UV radiations and spraying with an anisaldehyde-sulfuric acid solution.

**Plant material**

*P. guajava* leaves were collected freshly from Faridabad, Haryana, India. The plant was identified by Prof. M. P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. A specimen voucher of the drug was deposited in the Phytochemistry Research Laboratory, Jamia Hamdard with a reference number PRL-JH/2008/05.

**Extraction and isolation**

The air-dried leaves (1.5 kg) were coarsely powdered and exhaustively extracted with methanol in a Soxhlet apparatus. The combined extracts were concentrated to a steam bath under reduced pressure to get 225 g of a dark brown mass. It was dissolved in 250 ml methanol and adsorbed on silica gel (60-120 mesh) for the purification of a slurry. The slurry was dried in air and chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, petroleum ether-chloroform (1:1, 1:3, v/v), chloroform and chloroform-methanol (97:3, 19:1 and 9:1, v/v) mixtures to isolate the compounds 1–9.
Arachidic acid (1)  
Elution of the column with petroleum ether furnished colourless crystals of 1, recrystallized from acetone, 96 mg (0.064% yield); Rf: 0.52 (petroleum ether): m.p. and mixed m. p.: 62-63 °C; +ve FAB MS m/z (rel. int.): 313 [M+H]+[C₇H₁₄O₃] (13:1).

Psidialanostenoic acid (2)  
Elution of the column with petroleum ether-chloroform (1:1) mixture afforded colorless crystals of 2, recrystallized from acetone, 856 mg (0.059% yield); Rf: +ve FAB MS m/z (rel. int.): 341, 335, 293, 285, 1694, 1640, 999 cm⁻¹; IR (KBr): 3410, 3389, 3250, 2924, 1692, 1650, 808 cm⁻¹; +ve FAB MS m/z (rel. int.): 475 [M+H]+[C₃H₅O₃] (2:1).

12β-Hydroxypsidinalanostenoic acid (3)  
Elution of the column with chloroform-methanol (1:3) mixture yielded colourless crystals of 3, recrystallized from acetone, 732 mg (0.046% yield); Rf: +ve FAB MS m/z (rel. int.): 684 (chloroform: methanol-4:1): m.p.: 222-225 °C; UV λ max (MeOH): 212, 277 nm (log ε 5.3, 2.8): IR ν max (KBr): 3419, 3315, 2923, 2855, 1694, 1640, 999 cm⁻¹; +ve FAB MS m/z (rel. int.): 435 [M+H]+[C₃H₅O₇] (2:3).

Trihydroxypsidinalanostenoic acid (4)  
Elution of the column with chloroform-methanol mixture afforded colourless crystals of 4, recrystallized from acetone, 564 mg (0.037% yield); Rf: 0.64 (chloroform: methanol-4:1): m.p.: 222-225 °C; UV λ max (MeOH): 212, 277 nm (log ε 5.3, 2.8): IR ν max (KBr): 3419, 3315, 2923, 2855, 1694, 1640, 999 cm⁻¹; +ve FAB MS m/z (rel. int.): 475 [M+H]+[C₃H₅O₇] (2:3).

Guavalanostenoic acid (5)  
Elution of the column with chloroform-methanol (97:3) mixture furnished colourless crystals of 5, recrystallized from acetone, 462 mg (0.030 % yield); Rf: 0.70 (chloroform: methanol-1:1): m.p.:188-190 °C; UV λ max (MeOH): 211, 298 nm (log ε 5.1, 2.9): IR ν max (KBr): 3410, 3367, 3291, 3294, 2950, 1692, 1603, 990 cm⁻¹; +ve FAB MS m/z (rel. int.): 503 [M+H]+[C₁₃H₁₈O₅] (2:2).

β-Sitosterol xyloside (6)  
Elution of the column with chloroform-methanol (19:1) mixture gave colourless amorphous powder of 6, recrystallized from methanol, 286 mg (0.018% yield), Rf: 0.32 (chloroform: methanol-4:1): m. p.: 270-272 °C; +ve FAB MS m/z (rel. int.): 547 [M+H]+[C₃H₅O₇] (4:3).

Psidilanosteroside (7)  
Elution of the column with chloroform-methanol (9:1) mixture furnished pale yellow crystals of 7, recrystallized from methanol, 210 mg (0.014% yield); Rf: 0.64 (toluene: ethyl acetate: formic acid): m. p.: 58-59 °C; UV λ max (MeOH): 212, 278 nm (log ε 4.9, 4.6); IR ν max (KBr): 3411, 3386, 2921, 2837, 1703, 1644, 950, 888 cm⁻¹; +ve FAB MS m/z (rel. int.): 313 [M+H]+[C₇H₁₄O₃] (13:1).
RESULTS
Elution of the column with petroleum ether gave arachidic acid [1]. The compounds 2 and 3 were obtained in petroleum ether-chloroform mixtures. Further elution of the column with chloroform and methanol mixtures afforded compounds from 4 to 8. All the phytoconstituents were obtained in crystalline forms. Diabetes was induced in rats by intraperitoneal injection of STZ solution. Aqueous solutions of the tested compounds were administered orally once a day. The \(^{13}C\) NMR spectra of the compounds exhibited signals for vinylic carbons from \(\delta 143.92\) to 115.81, carboxyl carbons near \(\delta 178.33\), C-3 carbinol or oxygenated methine carbons between \(\delta 76.53\) and \(\delta 178.33\) and methyl carbons in the upfield region from \(\delta 28.87\) to 1.79. The \(^{13}C\) NMR spectrum of 7 displayed C-3 carbinol signal at \(\delta 77.85\), anomic carbon at \(\delta 106.32\) (C-1’), aromatic signals at 168.41 (C-1’), 127.40 (C-2’), 125.51 (C-3’), 130.06 (C-4’), 124.07 (C-5’). 128.4 (C-6’), sugar carbons between \(\delta 73.52\) and 67.16 and aldehydic C-7’ carbon at \(\delta 192.19\). The \(^1H\) NMR spectrum of 8 also exhibited signals for anomic carbon at \(\delta 101.53\) (C-1’) and other sugar carbons resonated between \(\delta 76.53\) and 62.99. The \(^1H\) and \(^{13}C\) NMR spectral data of the isolated compounds were compared with the lanosterol-triterpenoids [18-20]. The \(^1H\)-H COSY spectra of the triterpenoids showed correlations of the adjacent protons. The HMBC spectra of these compounds exhibited interactions of protons with the adjacent carbons. On the basis of spectral data analyses and chemical reactions, the structures of the isolated phytoconstituents were elucidated as lanost-7-en-3β,12β,29-triyl triol-26-oic acid [21].

Compoounds 2-8 responded positively to Liebermann-Burchard test and yielded effervescences with sodium bicarbonate solution indicating triterpenic acid nature of the molecules. Their IR spectra displayed characteristic absorption bands for hydroxyl functions between \(\delta 1.22\)–0.83, all attached to the saturated carbons.

DISCUSSION
Compounds 1 and 6 are the known compounds characterized as arachidic acid and \(\beta\)-sitosterol xylopyranoside [17]. Compounds 2-8 responded positively to Liebermann-Burchard test and yielded effervescences with sodium bicarbonate solution indicating triterpenic acid nature of the molecules. Their IR spectra displayed characteristic absorption bands for hydroxyl functions (3410-3275 cm\(^{-1}\)), carboxylic groups (1692-1688 cm\(^{-1}\)) and unsaturation (1603-1650 cm\(^{-1}\)). The molecular ion peaks were determined on the basis of mass and \(^1C\) NMR spectra and the position of vinylic bonds and functional groups were established on the basis of mass ion fragments. The \(^1H\) NMR spectra of the compounds displayed signals for vinylic protons from \(\delta 6.79\) to 5.03, oxygenated H-3α methine protons from \(\delta 4.49\)-3.72 and methyl protons between \(\delta 1.22\)–0.83, all attached to the saturated carbons. The \(^{13}C\) NMR spectra of the compounds exhibited signals for vinylic carbons from \(\delta 143.92\) to 115.81, carboxyl carbons near \(\delta 178.33\), C-3 carbinol or oxygenated methine carbons between \(\delta 83.61\) and 77.85 and methyl carbons in the upfield region from \(\delta 28.87\) to 1.79.

The \(^{13}C\) NMR spectrum of 7 displayed C-3 carbinol signal at \(\delta 77.85\), anomic carbon at \(\delta 106.32\) (C-1’), aromatic signals at 168.41 (C-1’), 127.40 (C-2’), 125.51 (C-3’), 130.06 (C-4’), 124.07 (C-5’), 128.4 (C-6’), sugar carbons between \(\delta 73.52\) and 67.16 and aldehydic C-7’ carbon at \(\delta 192.19\). The \(^1H\) NMR spectrum of 8 also exhibited signals for anomic carbon at \(\delta 101.53\) (C-1’) and other sugar carbons resonated between \(\delta 76.53\) and 62.99. The \(^1H\) and \(^{13}C\) NMR spectral data of the isolated compounds were compared with the lanosterol-triterpenoids [18-20]. The \(^1H\)-H COSY spectra of the triterpenoids showed correlations of the adjacent protons. The HMBC spectra of these compounds exhibited interactions of protons with the adjacent carbons. On the basis of spectral data analyses and chemical reactions, the structures of the isolated phytoconstituents were elucidated as lanost-7-en-3β,12β,29-triyl triol-26-oic acid [21], lanost-7-en-3β,12β-diol-26-oic acid [3], lanost-7-en-3β,12β,29-triol-26-oic acid [4], lanost-3-cis-1,7,23-trienyl-3β,12β,29-triol-26-oic acid [5], lanosteryl-3β-D-xylopyranosyl-2′-p-benzaldehyde [7] and lanost-7-en-3β,12β,29-triyl triol-26-oic acid-3-β-D-glucopyranoside [8].

Compound 2, 3, 4 and 8 were tested for the antidiabetic activity in STZ-induced diabetic model [21]. Table 1 shows the levels of blood glucose level in normal and experimental animals in each group.
The isolated phytoconstituents have been found to show significant antidiabetic activity. In all the groups prior to STZ administration, the basal levels of blood glucose of the rats were not significantly different.

However, after STZ administration, significant \( (P<0.001) \) elevation in blood glucose level was observed in diabetic control rats (group II) when compared with normal control rats (group I). Oral administration of the selected isolated compounds at a dose of 50 mg/kg for one week significantly \( (P<0.001) \) reduced the blood glucose levels in diabetic rats as compared with diabetic control rats (table 2). This implies that the compounds can prevent or be helpful in controlling blood glucose level near to normal levels, a key for preventing or reversing diabetic complications. In conclusion, the present study demonstrates that the isolated compounds from the leaves of *P. guajava* at tested dose level exhibit potent antidiabetic potential in STZ-induced diabetic rats.

Further studies are warranted in this area to outline precise mechanism behind the antihyperglycemic property of these compounds.

**Table 2: Effect of isolated compounds on blood glucose level in diabetic rats**

| Groups                      | Blood glucose (mg dl\(^{-1}\)) | 3 d after STZ | First week |
|-----------------------------|---------------------------------|---------------|------------|
| Normal control              | 90.34 ± 8.89                    | ---           | 87.66 ± 8.89 |
| Diabetic control            | 83.66 ± 11.62                   | 311.50 ± 8.24 | 327.33 ± 6.41* |
| Diabetic + compound 2 (50 mg/kg) | 100.67 ± 10.71               | 309.50 ± 8.57 | 172.17 ± 6.41** |
| Diabetic + compound 3 (50 mg/kg) | 87 ± 11.78                     | 310.16 ± 8.86 | 154.50 ± 6.06** |
| Diabetic + compound 4 (50 mg/kg) | 84.50 ± 12.42                | 315.50 ± 6.38 | 142.50 ± 12.03** |
| Diabetic + compound 8 (50 mg/kg) | 90.33 ± 12.88               | 325.66 ± 8.71 | 165.67 ± 8.73** |

The data are expressed in mean ± SD; \( n=6 \) in each group. *\( P<0.001 \) compared with the corresponding values for glibenclamide [22] treated animals.
CONCLUSION

Six new lanosterol-type triterpenoids, arachidic acid, and β-sitosterol xylopyranoside were isolated from the leaves of *Psidium guajava* for the first time. The triterpenic constituents exhibited significant antidiabetic activity against streptozotocin-induced diabetic rats.

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CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

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