INTRODUCTION

The genus *XYLOSMA*, a large genus of shrubs and trees is chiefly distributed in most of the tropical and subtropical regions. About four species are found in India. It occurs as shady ravines of the western Himalayas from Kashmir to Kumaon and on low hills up on to an altitude of 1,500 meter. The extract of *XYLOSMA LONGIFOLIUM* (Commonly know as “Khandhara”) leaves is found. Medicinal importance of *X. longifolium* and scanty of work on it encourages us to carry out the comprehensive investigation of the leaves of *X. longifolium*. Earlier investigation on this plant includes the isolation and characterization of β-sitosterol, β- amyrin, friedelin, olean-12-ene-3α,28-en, 3α-D-glucopyranoside. Now we are reporting the isolation and characterization of a novel flavonoid kaempferol-3-β-xylopyranoside-4’-α-rhamnoside along with the known flavonoids kaempferol, quercetin, kaempferol-3-rhamnoside, quercetin-3-rhamnoside which have not been reported so far from this plant.

EXPERIMENTAL

General experimental procedure

The melting points were taken on a Kofler block and are uncorrected. ¹H NMR spectra were recorded on Bruker Avance II 400 MHz with TMS as an internal standard. IR spectra were run on Shimadzu IR-408 Perkin-Elmer 1800 (FTIR). The MS were measured in both EI Mode and Jeol D-300 and FAB mode on Jeol SX 102/ DA-6000 mass spectrometers. The leaves of *XYLOSMA longifolium* collected from Forest Research Institute, Dehradun in winter season and identified by Prof. Wazahat Hussain, Taxonomist, Department of Botany, Aligarh Muslim University, Aligarh, India.

Extraction and isolation

The leaves of *XYLOSMA longifolium* were dried under shade and crushed to make powder. The air dried powdered leaves (2.5 kg) were thoroughly extracted with ethyl acetate-methanol (9:1) and crystallized from chloroform-methanol as colorless needles, m.p. 159-60°C. The compound X1-2 (20 mg) was heated with pyridine (1 ml) and acetic anhydride (2 ml) on water bath for about 4 hours. After usual work up by the procedure described earlier followed by crystallization chloroform-methanol, it afforded yellow colored crystals XI-2Ac, m.p. 194-95°C. The compound X1-2 (20 mg) was heated with pyridine (1 ml) and acetic anhydride (2 ml) on water bath for about 4 hours. After usual work up by the procedure described earlier followed by crystallization chloroform-methanol, it afforded yellow colored crystals XI-2Ac, m.p. 194-95°C.
It was obtained from ethyl acetate-methanol (4:6:1) eluates and crystallized with methanol-chloroform as pale yellow granular crystals (110 mg) m.p. 265-67°C. Analyzed for C_{14}H_{10}O_{5}: Calcd: C, 56.11; H, 3.49%. Found: C, 56.14; H, 3.07%. UV λ_{max} nm: 240sh, 269, 306sh, 345 (MeOH), 245sh, 312, 305sh, 340, 398 (AlCl_3), 277, 304, 396, (AlCl_3/MeOH), 281, 355, 370 (NaOAc), 279, 325sh, 365 (NaOAc/H_2BO_3), 255sh, 296, 355, 405, (NaOMe).

**Acetylation of XI-5:**
The crystalline glycoside (45 mg) was acetylated with dry Ac_2O/py (1:1) at room temperature for 48 hrs. After workup by the procedure described earlier, it was crystallized with chloroform-methanol as cream needles, m.p. 126-28°C. 1H NMR (400 MHz, CDCl_3): δ 6.60 (1H, d, J=2.2 Hz, H-6), 7.10 (1H, d, J=2.2 Hz, H-8), 7.89 (2H, d, J=8.5 Hz, H-2',6'), 2.72 (2H, d, J=8.5 Hz, H-3',5'), 5.15 (1H, d, J=9.5 Hz, H-1'''' xylose), 5.06 (1H, d, J=1.2 Hz, H-1'''''' rham), 1.20 (3OH, d, J=6.1 Hz, rham-CH_2), 3.77-3.60 (10Hn, gly-H), 2.45 (3H, s, Ac-5), 2.34 (3H, s, Ac-5'), 1.98-2.20 (18H, m, aliphatic OAc), Mass m/z: M+ absent, 642 [M+acetylated pentose + H]^+], 628 [M+acetylated hexose + H]^+], 370 [M+acetylated pentose-acetylated hexose + 2H]^+, 286 [M+614]^-, 273 [(rham)Ac^+].

**Acid hydrolysis of XI-5:**
The glycoside XI-5 was hydrolyzed with 2N HCl-MeOH (5 ml), (100°C, refluxed for 2 hr). Water was added and the mixture was extracted with ethylacetate. The aqueous hydrolysate was neutralized with Ag_2CO_3 precipitate filtered off and filtrate was evaporated in vacuo giving a residue.

**Identification of aglycone:**
The aglycone in EtOAc fraction was crystallized from CHCl_3-MeOH as yellow needles, m.p. 280-81°C and identified as Kaempferol by spectral and chromatographic comparison with authentic sample.

**Acetylation of XI-5:**
The glycoside XI-5 (I) was acetylated with dry Ac_2O/py (1:1) at room temperature for 48 hours. The contents were filtered and the residue washed with little DMSO. It was obtained from ethyl acetate-methanol (4:6-1:1) and crystallized with methanol-chloroform as pale yellow granular crystals (110mg) m.p. 265-67°C. The anhydrous glycoside (25 mg) was hydrolysed by refluxing with 0.2N HCl. After cooling over night, the aglycone was dried and weighed (11.5 mg), the ratio of aglycone to glycoside is 44.2% indicating the presence of two moles of sugar/mole of aglycone.

**Antimicrobial Activity:**
The in vitro antimicrobial activity was carried out against Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Bacillus subtilis and in vitro antifungal activity was carried out against Candida albicans, Fusarium oxysporum, Penicillium notatum, Aspergillus niger and Trichoderma viridae. The agar well diffusion method was used. 0.1ml of diluted inoculum (10^5CFU/ml) of test organism was spread on Mueller Hinton Agar plates (Hi-Media Pvt. Ltd., Mumbai, India). The wells of 8mm diameter were punched into the agar medium and filled with 100 µl of plant extract and some plant product of 1mg/ml concentration and solvent blank (DMSO) separately. The plates were incubated at 37°C overnight. The antibiotic (Chloramphenicol) and antifungal disc (Nystatin) of 30 mcg potency each were used in the system as positive controls. Zone of inhibition of bacterial and fungal growth around each well was measured in mm.

**RESULT AND DISCUSSION**
The air dried powdered leaves of Xyllosma longifolium (2.5 kg) after being defatted with light petroleum ether (60-80°C) were thoroughly extracted with benzene and methanol successively. The methanolic extract gave a color test for flavonoid and showed a intricate mixture of five major compounds on TLC [benzene-pyridine-formic acid (36:9:5) and toluene-ethyl formate-formic acid (5:4:1)]. It was purified through column chromatography over silica gel.

Identification of sugars:
The neutral hydrolysate was concentrated and chromatographed on whatman No.1 filter paper using n-ButOH-acetic acid-water (4:1:5) and EtOH-Pyridine-H_2O (2:1:2) as solvent systems, employing the descending techniques. Authentic sugars were used as checks. The chromatograms were run for 24 hours and after drying at room temperature, they were sprayed with anilin phthalate and p-anisidine phosphate solutions. The chromatograms on drying at 100-05°C, showed the presence of only rhamnose (0.37, 0.28) and xylose (0.30, 0.22).

**GLC of TMSi ether of sugars:**
The TMSi ether of sugars was obtained by taking 15 mg of sugar in dry 0.5 ml and trimethylsilsilane (0.2 ml) in a 10 ml round bottom flask. To this solution (0.2 ml) of trimethyl chlorosilane was added and flask was stoppered and allowed to stand at room temperature for 45 minutes. The solution was then dried and taken in heptane. The heptane soluble TMSi ether derivatives of sugar were then subjected to GLC (% OV-1, column temp. 150-250°C, 10 min. detec. temp.300°C, N_2 50 ml/min) while the compound XI-5 was found to be a novel compound and characterized as Kaempferol-3-O-pyloside-4'-'a-rhamnose which is discussed below.

**Enzymatic hydrolysis of XI-5:**
A mixture of compound XI-5 (100 mg) and α-rhamnosidase (contain α-pectinase) (10 mg) was incubated in (NH_4)_2SO_4-NaOAc buffer (pH 5.0) at 250°C for 30 hrs and then after addition of water, it was extracted with n-ButOH. The n-ButOH extract was chromatographed on silica gel column to give a partial glycoside, m.p. 254-56°C, identified as kaempferol-3-O xylose. From the H_2O layer, L-rhamnose was identified by PC (n-butanol: acetic acid:water, 4:1:5).

**Methylation of Partial glycoside:**
CH_3I (1 ml) and Ag_2O (30 mg) were added to a solution of partial glycoside (30 mg) in DME (3 ml). The mixture was stirred in dark at room temperature for 48 hours. The contents were filtered and the residue washed with little DME. The filtrate was evaporated to dryness and the syrup residue was hydrolysed with ethanol (25 ml). The alcohol was recovered and the syrup residue was hydrolysed with 2N HCl. On usual workup it gave 3-OH, 5,7,4'-trimethoxy flavone (14 mg) m.p. 135-36°C. Analyzed for C_{14}H_{10}O_5: Calcd: C, 65.85; H, 4.87. Found: C, 65.91; H, 4.92.

**Quantitative estimation of sugars:**
The anhydro glycoside (25 mg) was hydrolysed by refluxing with 0.2 N HCl. After cooling over night, the aglycone was dried and weighed (11.5 mg), the ratio of aglycone to glycoside is 44.2% indicating the presence of two moles of sugar/mole of aglycone.
Enzymatic hydrolysis of the parent glycoside XI-5 gave conclusive evidences of the position of attachment of two sugars and nature of their linkage. Hydrolysis of XI-5 with α-rhamnosidase (containing α-pectinase) gave L-rhamnose indicating α-nature of sugar and a partial glycoside of (Ie), which gave a bathochromic shift of +60 nm with NaOMe in band I without a decrease in intensity (absent in glycoside). Thus showing that C-4' hydroxyl which was glycosylated in XI-5 had become free. The partial glycoside of XI-5 was identified as kaempferol-3-xylloside (Ie), m.p., 224-26°C by UV diagnostic shift reagents and co-chromatography with authentic sample.\(^\text{14}\) Methylation of partial glycoside (Ie- 5e) followed by hydrolysis with 2N HCl gave a partial methyl ether (1d), m.p.134-35°C characterized as 3-OH, 5,7,4'-trimethyl ether by spectral and chromatographic comparison with authentic sample.\(^\text{15}\) The methylated sugar were identified as 2,3,4-tri-O-methyl-xylose by SiO\(_2\) TLC according to Petek.\(^\text{16}\) This finally established that L-rhamnose was α-linked at C-4’ while D-xylose was β-linked at 3-position. Quantitative estimation of sugar by Somogyi copper micro method\(^\text{17}\) indicated the presence of two moles of sugar/mole of aglycone.

On the basis of above results, compound XI-5 was identified as Kaempferol-3-β-xyllopyranoside-4′-α-rhamnoside. To the best our knowledge it is being reported for the first time.

The antimicrobial activity of aqueous and alcoholic extracts of Xylosma longifolium, extract was investigated against bacterial and fungal strains. It was seen that all the extracts exhibited a low, moderate or high activity against gram positive and fungal strains. The aqueous extract showed high activity against Staphylococcus aureus (IAO-SA-22) and Candida albican (IAO-109) and moderate activity against Bacillus subtilis (MTCC-121) and Trichoderma viridae (lab isolate). The alcoholic extract found to show high activity against Staphylococcus aureus (IAO-SA-22) and Candida albican (IAO-109) and moderate activity against Bacillus subtilis (MTCC-121), Salmonella typhimurium (MTCC-98), Aspergillus brassicola.

Table 1. Antimicrobial activity of Xylosma longifolium aqueous and alcoholic extracts.

<table>
<thead>
<tr>
<th>Stains</th>
<th>Aqueous extract</th>
<th>Alcoholic extract</th>
<th>Standards</th>
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<tbody>
<tr>
<td>Staphylococcus aureus (IAO-SA-22)</td>
<td>11</td>
<td>14</td>
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<td>Escherichia coli (K-12)</td>
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<tr>
<td>Salmonella typhimurium (MTCC-98)</td>
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<td>18</td>
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<tr>
<td>Bacillus subtilis (MTCC-121)</td>
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<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Candida albican (IAO-109)</td>
<td>11</td>
<td>15</td>
<td>20</td>
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<td>Fusarium oxysporum (lab isolate)</td>
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<tr>
<td>Trichoderma viridae (lab isolate)</td>
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<td>16</td>
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<tr>
<td>Aspergillus brassicola</td>
<td>--</td>
<td>8</td>
<td>18</td>
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Low activity (1-5), moderate activity (6-10), high activity (11-15), very high activity (16-20), no activity (--)