TABLE 2: METHOD VALIDATION PARAMETERS

Parameter	Result
Instrumental precision (% CV) (n=7)	0.043
Repeatability (% CV) (n=6)	0.65
Specificity	Specific
Accuracy (%)	99.05 ± 1.14*
Linearity range (µg/ml)	30-180
Correlation coefficient (r)	0.999

* Mean value ± standard deviation of three determinations

lovastatin (R_f value=0.50) in presence of other excipients in the formulations (fig. 1). The specificity was also confirmed by overlaying the spectra of standard lovastatin with the spectra of sample recorded on TLC scanner in UV range (fig. 2). Linearity range for lovastatin was found to be in the range of 30-180 µg/ml. The correlation coefficient (r) and other method validation parameters are given in Table 2. Precision of the instrument was checked by repeated scanning of the same spot (1200 ng/spot) of lovastatin seven times and the % CV was found to be 0.043. Repeatability of the method was checked by analysing a standard solution of lovastatin (120 µg/ml) after application (10 µl) on a TLC plate (n=6) and the % CV for peak area was found to be 0.65. Accuracy of the method was evaluated by carrying out a recovery study. A known concentration of the standard Lovastatin solution (equivalent to 45 µg) was added to a preanalysed tablet sample solution (45 µg/ml), extracted and quantified as mentioned above. The percentage recovery was found to be 99.05. Thus the method was found to be simple, specific, precise and accurate and can be used for routine quality control purpose.

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Chemical Constituents of the Roots of *Vitex negundo*

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Five compounds were isolated from the methanolic extract of the roots of *Vitex negundo* linn and purified by crystalisation and preparative TLC. They were identified as 2 β, 3α-diacetoxyoleana-5,12-dien-28-oic acid, 2α, 3α-dihydroxyoleana-5,12-dien-28-oic acid, 2α, 3β-diacetoxy-18-hydroxyoleana-5,12-dien-28-oic acid, vitexin and isovitexin by spectral data and chemical conversions.

Vitex negundo Linn (Fam: Verbenaceae), a large aromatic shrub with bluish-purple flowers widely prevalent in North-Western Himalayan region, has been used for various medicinal purpose in the Ayurvedic and Unani systems of medicine[1]. The roots (3 kg) of *V. negundo* were collected from Siripalli, Ganjam District, Orissa and

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coarse powdered and extracted with methanol and the filtrate was concentrated on a water bath to one third volume and suspended in cold water and was fractionated with chloroform and ethyl acetate. The chloroform fraction residue and ethyl acetate residue were column chromotographed over silica gel and eluted with solvents of increasing polarity.

Three compounds A, B, C were obtained from the eluates of chloroform:methanol (99:1), chloroform: methanol (98:2) and chloroform:methanol (95:5) respectively from the chloroform extract residue. The uv spectrum of compound A did not show absorption above 225 nm. The ^{1}H-NMR spectrum of compound A gave signals at δ 5.28 (m, 2H, vinylic protons), 4.72 (1H, H-2α) and 4.634 (1H, H-3β). The sharp three-proton singlets at δ 2.05 and 1.99 revealed the presence of two acetyl groups. The spectrum displayed C-Me singlets at δ 1.26 (3H), 1.07(6H), 0.90(9H) and 0.76 (3H), suggesting an oleanane skeleton. The EIMS of A gave the molecular ion peak at m/z 554 (1%) and other major peaks at m/z 434 [M-2 x HOAc]$^+$ (4%), 419 (20%), 248 (100%), 203 (66%), 189 (23%) and 133 (44%). These data suggested that a C-12 double bond and a C-28 carboxyl group were present in the molecule. Signals at δ 143.8 (C-13) and 122.2 (C-12) in the ^{13}C-NMR spectrum supported the presence of Δ^{12} double bond. The spectrum also indicated the presence of two additional olefinic carbons at δ 138.2 (C-5) and 125.5 (C-6). Singlets at δ 184.0, 171.0 and 170.8 confirmed the presence of a carboxyl group and two acetoxyl groups. Hydrolysis of compound A with ethanolic potassium hydroxide afforded a crystalline material, m.p. 245-246° (dec)[4]. Compound did not form an acetonide, indicating that the hydroxyl groups at C-2 and C-3 were diaxial.

Methylation of A with CH_2N_2 yielded a crystalline compound, mp 205-206° and the EIMS showed the molecular ion peak at m/z 484 (1%). The spectral data of compound A and its derivatives indicate that compound A is 2 β, 3α-diacetoxyoleana-5, 12-dien-28-oic acid.

Compound B did not show absorption above 224 nm in the UV spectrum. Their spectrum showed bands at 3200 cm^{-1} (OH), 1700 cm^{-1} (C=O, -COOH) and 1390 and 1375 cm^{-1} (gem-dimethyl). The ^{1}H-NMR spectrum gave a multiplet at δ 5.27 for two vinylic protons. The multiplet at δ 3.87 and a doublet at δ 3.34 (d, *J*=3.0 Hz), one proton each, were assigned to H-2β and H-3β, respectively. The spectrum also gave singlets at δ 1.14, 1.09, 0.98, 0.96, 0.92, 0.84 and 0.81, corresponding to seven methyl groups, which suggested the oleanane skeleton. The EIMS gave a molecular ion peak at m/z 470 (1%) and fragments at m/z 452 [M-H_2O]$^+$ (1%), 434 [M-2 x H_2O]$^+$ (2%), 425 [M-COOH]$^+$ (2%), 248 (100%), 203 (65%) and 189 (20%). Retro-Diels-Alder fragmentation was observed in the mass spectrum of B, with ions at m/z 248 and 203, indicative of a triterpenoid having Δ^{12} -unsaturation. The higher intensity of peak m/z 203 suggested an oleanane skeleton with a 28-carboxyl group[2,3].

Compound B formed an acetonide and its EIMS gave peaks at m/z 510 [M]$^+$ (1%), 464 [M-H-COOH]$^+$ (1%), 452 [M-MeCOMe]$^+$ (1%), 406 (5%), 248 (100%) and 203 (48%). Formation of an acetonide supported the cis configuration or the diequatorial conformation of the hydroxyl groups.

Acetylation of compound B yielded a gum. The occurrence of two sharp three-proton singlets at δ 2.11 and 1.96 in the ^{1}H-NMR spectrum revealed the presence of two acetyl groups. Other signals were observed at δ 5.28 (2H, vinylic protons), 4.98 (H-2) and 4.70 (H-3). The ^{1}H-NMR spectrum also showed seven C-Me singlets at δ 1.25, 1.18, 1.12, 1.04, 0.97, 0.87 and 0.76. The similarity in chemical shifts of the seven methyl groups in the ^{1}H-NMR spectrum of B and its diacetate suggested that the two oxygen functionalities in the molecule were both α. If the hydroxyl groups were β, the chemical shifts of the C-24 and C-25 methyls of B would appear downfield in comparison to those of the diacetate[4,5]. Methylation of B with CH_2N_2 yielded a crystalline methyl ester, mp 285-286°. Thus compound B was shown to be 2α,3α-dihydroxyoleana-5,12-dien-28-oic acid.

Compound C showed absorption at 225 nm in the UV spectrum. The ^{1}H-NMR spectrum exhibited signals at δ 5.41 (m, 2H, vinylic protons), 5.13 (dd, 1H, J=10.0, 4.0 Hz, H-2β), 4.76 (d, 1H, *J*=10.0 Hz, H-3α), 2.06 (3H, s, OAc) and 1.98 (3H, s, OAc). These values are in close agreement with known 2 α, 3β-diacetoxy triterpenoids. A one-proton singlet at δ 2.55 may be due to a hydroxyl group, the presence of which was indicated in their spectrum. The hydroxyl group appears to be tertiary as it could not be acetylated. The spectrum showed seven C-Me singlets at δ 1.24, 1.21, 1.06, 0.99, 0.96, 0.93 and 0.73 consistent with an oleanane skeleton. The EIMS showed the olecular ion at m/z 570 (1%). These data revealed the presence of a C-12 double bond, a C-28 carboxyl group and a C-18 hydroxyl group in the molecule. Thus,

compound C has been identified as 2α,3β-diacetoxy-18-hydroxyoleana-5,12-dien-28-oic acid.

The ethyl acetate extract residue did not give any crystalline compound. The residue was chromatographed over silica gel and yellow coloured compound obtained from the eluates of ethyl acetate: methanol (98:2). The compound was heterogeneous and gave two spots in TLC. The mixture of compounds is separated by preparative PC (n BuOH:HOAC:H_2O: : 4:1:5) to yield compounds D and E.

Compound D crystallised by MeOH as yellow needles (20 mg), m.p. 223-24° (dec.) It was purple under UV and light yellow under UV/NH_3 and had λ_{max} (MeOH) 272, 338 nm and diagnostic shifts typical of apigenin[6].Its R_f 0.67 (PC, BAW, 4:1:5), 0.55 (PC, 15%, HOAc) and 0.48 (TLC, cellulose, 15% HOAc), non-hydrolisability with 7% H_2SO4 and two characteristic IR absorption bands at 1036 and 1010 cm^{-1} indicated it to be a flavonoid-C-glycoside. On hydrolytic fission with HI[7], it yielded apigenin and on $FeCl_3$ oxidation[8] gave glucose. The mass spectrum of permethyl-ether gave an intense parent peak (M+530, 80%) typical of apigenin-6-C-glycoside[9]. From these data it was identified as isovitexin and the identity was further confirmed by direct comparison with an authentic sample.

Compound E was crystallised from MeOH as yellow needles (5 mg), m.p. 258-59° (dec.) R_f 0.51 (PC B:A:W::4:1:5) 0.24 (PC, 15% HOAC) and 0.29 (TLC, cellulose, 15% HOAC). The compound gave Shinoda positive test for flavonoids. It had the molecular formula $C_{21}H_{20}O_{10}$. Its UV spectra in methanol had maxima at 270 and 331 nm which shifted to 279, 303 (sh) and 378 nms on addition of $NaOCH_3$. The mass spectrum of compound E showed a peak at m/z 4.14 (20%). The base peak occurred at m/z 283. Other prominent peaks were seen at m/z 270 (49%), 165(36%) and 121(28%). From the above data compound D was identified as vitexin and this is further confirmed by m.m.p. and chromotographic comparision[10,11] of vitexin.

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