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Antiplasmodial activity of compounds isolated from Viburnum nervosum

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ABSTRACT: *The study of the chemical constituents of the whole plant of Viburnum nervosum has resulted in the isolation and characterization of new lupan type triterpene butilinol (1), together oleanolic acid (2), ursolic acid (3), butilin (4), β-sitosterol (5), butilinic acid (6), α-amyrin (7) and germanicol (8). The structural elucidation of the isolated compounds was established based on spectroscopic studies. The in vitro antimalarial activity of compounds 1 to 4 against Plasmodium falciparum shows partial suppression of parasitic growth.*

Keywords: *Viburnum nervosum, triterpenes, anti-malarial activity.*

I. INTRODUCTION

Natural products particularly those obtained from plant species are found to possess biological active compounds. As different plant contains different activity spectrums, they are being used and investigated further to know their pharmacological potential. *Viburnum* is one of the genus found to possess a wide variety of important biological activities. It contains about 200 species, distributed in the temperate and subtropical regions of Asia, North America and Malaysia, only 21 of which have been studied chemically. The six species *Viburnum tinus, Viburnum opulus, Viburnum mullaha D. don, Viburnum cotinifolium D. don, Viburnum cylindricum* and *Viburnum foetns* Dene are found in the Northern Pakistan and in the State of Jammu and Kashmir (1, 2). These species find use in the folk medicine system for their diuretic, antispasmodic and sedative properties mainly on uterine excitability (3-5). *Viburnum* species characteristically contain Irridoids, terpenoids, coumarins and flavones. The rare vibsane diterpenoids e.g. vibsanin B however, have been isolated from the leaves of *Viburnum* species (6, 7). As part of the search for new terpenoids in *Viburnum* species, the chemical constituents of the leaves, bark, stem, and roots of the *Viburnum nervosum* have been studied.

The extract of the plant also showed antioxidant, antimicrobial, antiseptic, antibacterial, anthelmintic, anti-neoplastic, anti-malarial, trypanocidal, anti-rheumatic, anti-inflammatory, anti-hyperlipidemic, anti-estrogenic, hepatoprotective, anti-carcinomic, higher anti-cancerogenic, anti-arthritic, anti-diabetic, anti-nociceptive and antifungal activities (8-11). The methanolic extract of the plant showed strong activity in an antifungal assay. The medicinal importance and bioactivity of *Viburnum nervosum* prompted us to carry out phytochemical investigations on this species.

Malaria is the most important parasitic infections of humans due to its high morbidity and mortality, a threat to over two billion people living in areas of high incidence (Andrade-Nato et al., 2004). *Plasmodium falciparum*, the causative agent of the malignant form of malaria, has high adaptability by mutation and is resistant to various types of antimalarial drugs, a serious setback to antimalarial programs, since it precludes the use of cheap and previously effective drugs like chloroquine (Cragg and Newman, 2001). New families of active compounds are needed as well as polychemotherapy associating molecules with independent mechanism of action, in order to decrease the risk of resistance (Newman et al., 2000). We screened the antimalarial activity of natural products isolated from one of medicinal plant, *Viburnum nervosum*, locally used in Rawalakot, Azad State of Jammu and Kashmir for the treatment of malaria (Eyong et al., 2009; Hussain et al., 2007). We report here the isolation of the compounds 1- 4 from *Viburnum nervosum* and their *in vitro* activity against *P. falciparum* was assessed against chloroquine-susceptible *P. falciparum* NF54 strain.

II. MATERIALS AND METHODS

The UV spectra were recorded on a Shimadzu UV-240 spectrophotometer in MeOH while the IR spectra were recorded on a JASCO A-302 spectrophotometer in CHCl3. High resolution mass spectra were recorded on a Varian MAT-312 mass spectrometer connected to a PDP 11/34 (DEC) computer system. The 1H-NMR spectra were recorded at 300 MHz on a Bruker AM-300 NMR spectrometer. The 13C-NMR spectra were

recorded at 75 MHz on the same instrument. TLC experiments were performed on silica gel pre-coated plates (GF-254, 0.2 mm, E.Merck).

III. EXTRACTION PROCEDURE

The dried plant material (4 kg) was extracted with methanol (20 L). The extract was concentrated on a rotary evaporator under reduced pressure. This afforded 125 g of methanolic extract. A portion of the extract (50 g) was subjected to the silica-gel column chromatography. The column was eluted with increasing polarities of petroleum ether/dichloromethane. This afforded 50 fractions. The fractions of similar Rf values were combined which resulted in 3 main fractions, S1 (7 g), S2 (3 g) and S3 (4 g). The same column was also eluted with 100% ethyl acetate. This resulted in 20 fractions. The fractions of similar Rf values were combined to give two main fractions S1 (7 g) and S2 (2 g).

III.1. Isolation of compound 1

The fractions S1 was dried and subjected to the flash silica gel column chromatography. The column was eluted with chloroform/petroleum ether (2.0: 8.0) as the solvent system to afford two fractions, S1a and S1b. The fraction S1a was re-chromatographed on the pre-coated silica-gel (GF-254) plates with chloroform/ pet. ether (5.0: 5.0) as the solvent system. This resulted in the isolation of the pure compound **1** as an amorphous material (10 mg, Rf =0.3).

Spectral data: IR ($CHCl_3$) U_{max} cm-1:3430 (OH), 3070, 1635 and 880; UV (MeOH) λ_{max}, nm: 389, 226, and 200; EI-MS m/z (rel. int. %):456.1 (C31H50O2, M+) (11.29), 248 (14.16), 207 (25.23), 189.1 (54.30), 135 (32.92), 95 (51.15) and 55 (100); HRMS (m/z): 456.1 (C31H50O2). ^{1}H-NMR (CD_3OD, 300 MHz) δ: 4.72 (s, 1H), 4.59 (s, 1H), 4.21 (t, J=12Hz 1H), 3.47 (s, 1H), 3.18 (dd, J1=4Hz, J2=4Hz, 1H), 2.97 (ddd, J1=4Hz, J2=8Hz, J3=4Hz, 1H), 2.26 (s, 1H), 2.25 (s, 1H), 2.23 (d, J=4Hz, 1H), 2.19 (d, J=2.8Hz, 1H), 2.16 (d, J=3.6Hz, 1H), 1.99 (s, 1H), 1.98 (s,1H), 1.97 (s,1H), 1.95 (s,1H), 1.94 (s, 1H), 1.92 (s, 1H), 1.67 (s, 3H), 1.64 (d, J=4Hz, 1H), 1.59 (s, 1H), 1.51 (m, 4H), 1.47 (s, 1H), 1.44 (s, 1H), 1.41 (d, J=4Hz, 1H), 1.38 (s, 1H), 1.35 (s, 1H), 1.30 (s, 1H), 1.28 (s, 1H), 1.24 (s, 1H), 1.20 (d, J=4Hz, 1H), 1.17 (d, J=4Hz, 1H), 1.03 (s, 1H), 1.00 (s, 1H), 0.95 (d, J=4Hz, 1H), 0.92 (s, 1H), 0.90 (s, 1H), 0.88 (d, J=4Hz, 1H), 0.86 (s, 1H), 0.80 (s, 3H), 0.73 (s, 3H), 0.67 (s, 1H) and 0.65 (s, 1H); ^{13}C-NMR (CDCL3, 75 MHz) δ: 150.37 (C-1), 109.69 (C-2), 79.02 (C-3), 68.19 (C-4), 56.21 (C-5), 55.43 (C-6), 50.61 (C-7), 49.35 (C-8), 46.87 (C-9), 42.49 (C-10), 40.77 (C-11), 38.89 (C-12), 38.78 (C-13), 38.41 (C-14), 37.26 (C-15), 37.01 (C-16), 34.40 (C-17), 32.17 (C-18), 30.57 (C-19), 29.73 (C-20), 28.01 (C-21), 27.46 (C-22), 25.57 (C-23), 22.97 (C-24), 20.91 (C-25), 19.39 (C-26), 18.33 (C-27), 16.13 (C-28), 16.05 (C-29), 15.34 (C-30) and 14.71 (C-31).

III.2. Isolation of compound 2

Elution of the column with ethyl acetate/ petroleum ether (3.0: 7.0) also afforded fractions S1c and S1d. The fraction S1c was re-chromatographed on PTLC pre-coated silica gel (GF-254) plates with ethyl acetate/ pet. ether (4.0: 6.0) as the solvent system. This resulted in the isolation of the pure compound **2** as an amorphous material (15 mg, Rf = 0.6).which gave positive color reactions of triterpenes.

Spectral data: IR ($CHCl_3$) U_{max} cm^{-1}: 3420 (OH), 1705 (CO), 2930, 1450, 1250, 690 and 820; EI-MS (m/z) rel. int. %): 456 [C30H50O3, M]+ (2), 248 (100), 207 (17.8), 203 (85), 175 (13.5), 147 (16), 133 (32.8), 119 (28.6), 107 (27), 105 (32.2), 95 (37.9), 69 (93), 57 (70) and 55 (86); ^{1}H-NMR (CDCL3, 300 MHz) δ: 5.28 (t, J=3.5, 1H, H-12), 3.14 (t, J=6.8 Hz, H-3), 2.78 (dd, J=3.5Hz, 14.0Hz, 1H,H-18), 0.75 (s, 3H), 0.86 (s, 3H), 0.96 (s, 3H), 0.91 (s, 3H), 1.04 (s, 3H), 1.12 (s, 3H), 1.23 (s, 3H); 13C-NMR (CDCL3, 75 MHz) δ: 38.41 (C-1), 27.17 (C-2), 79.01 (C-3), 39.25 (C-4), 55.203 (C-5), 18.31 (C-6), 32.67 (C-7), 37.04 (C-8), 47.603 (C-9), 38.74 (C-10), 23.04 (C-11), 122.67 (C-12), 143.53 (C-13), 41.69 (C-14), 27.67 (C-15), 23.04 (C-16), 46.42 (C-17), 41.179 (C-18), 45.87 (C-19), 30.66 (C-20), 33.79 (C-21), 32.39 (C-22), 28.08 (C-23), 15.54 (C-24), 15.3 (C-25), 17.00 (C-26), 25.87 (C-27), 180.06 (C-28), 32. 67 (C-29) and 23.39 (C-30).

III.3 Isolation of compound 3

The fraction S2 was subjected to silica gel column chromatography. The column was eluted with ethyl acetate/ petroleum ether (5.0:5.0) as the solvent system to afford two fractions S2a and S2b. The fraction S2a was re-chromatographed on PTLC pre-coated silica gel (GF-254) plates with ethyl acetate/ petroleum ether (5.0: 5.0) as the solvent system. This resulted in the isolation of the pure compound **3** as an amorphous powder (15 mg, Rf = 0.4).

Spectral data: UV (MeOH) *λmax*, nm: 202; The IR (CHCl3) U_{max}, cm^{-1}: COOH (3400 cm^{-1}-2650 cm^{-1}), (2900-2810 cm^{-1}) and tri-substituted double bond (1690 cm^{-1}, 1640 cm^{-1} and 108 cm^{-1}) EIMS (m/z) rel. int. %: 456 [C30H48O3, M+] (6.1), 438 (4.3), 300 (5.3), 248 (100), 235 (6.2), 291 (14.6), 203 (79.3), 189 (22.8), 175 (12.9), 161 (7.6), 147 (13.8), 133 (47.8), 119 (18.3), 105 (13.5), 95 (16.1), 81 (12.6), 79 (7.2) and 55 (12.9); 1H-NMR (C_2D_5N, 300 MHz) δ: 5.13 (t, J= 4HZ, 1H), 3.08 (t, J= 8HZ, 1H), 3.26 (s, 1H), 3.28 (s, 1H), 3.30 (s, 1H), 3.41 (bs, OH), 3.80 (s, 1H), 4.00 (s, 1H), 4.02 (s, 1H), 4.10 (s, 1H), 1.15 (s, 1H), 1.17 (s, 1H), 1.21 (s, 1H), 1.23 (s, 1H), 1.26 (s, 1H), 1.29 (s, 1H), 1.37 (s, 1H), 1.39 (s, 1H), 1.40 (s, 1H), 1.41 (s, 1H), 1.48 (d, J= 4HZ, 1H), 1.50 (s, 1H),1.52 (s, 1H), 1.53 (s, 1H), 1.55 (s, 1H), 1.58 (s, 1H), 1.73 (ddd, J1= 4HZ, J2= 8HZ, J3= 12HZ, 1H), 1.80 (t, J= 4HZ, 1H), 1.89 (ddd, J1= 4HZ, J2= 8HZ, J3= 12HZ, 1H), 2.08 (d, J= 8HZ, 1H), 0.60 (s, 1H), 0.62 (s, 1H), 0.66 (s, 3H), 0.70 (s, 3H), 0.74 (s, 3H),0.76 (s, 3H), 0.81 (s, 3H), 0.84 (s, 3H), 0.87 (s, 3H) and 0.97 (s, 3H); 13C-NMR (C_2D_5N, 75 MHz) δ: 180.51 (C-1), 138.061 (C-2), 77.20 (C-3), 63.37 (C-4), 47.68 (C-5), 41.93 (C-6), 39.33

(C-7), 36.79 (C-8), 125.35 (C-9), 78.72 (C-10), 55.12 (C-11), 52.70 (C-12), 47.43 (C-13), 38.95 (C-14), 38.76 (C-15), 38.53 (C-16), 36.68 (C-17), 32.90 (C-18), 30.53 (C-19), 26.71 (C-20), 24.07 (C-21), 23.12 (C-22), 18.16 (C-23), 27.89 (C-24) 23.34 (C-25), 20.95 (C-26), 16.81 (C-27), 16.72 (C-28), 15.42 (C-29), 15.22 (C-30).

III.4. Isolation of compound 4

The evolution of the column with ethyl acetate/ petroleum ether (5.0: 5.0) as the solvent system affords two fractions namely, S2a and S2b. The fraction S2b was re-chromatographed on PTLC pre-coated silica gel (GF-254) plates with ethyl acetate/ petroleum ether (5.0: 5.0) as the solvent system. This resulted in the isolation of the pure compound **4** as reddish powder (13 mg, Rf = 0.7).

Spectral data: IR (KBr) U_{max} cm^{-1}: 3430, 3070, 1635 and 880; UV (MeOH), λ_{max} nm (logε): 200 (0.574), 204 (1.043); HRMS: 442.379 [M]+ (C30H50O2), 427 (C29H47O2), 424.36 (C30H48O1), 411 (C29H47O1) and 409 (C29H45O1); EIMS m/z (rel.intensity in %): 442 [M]+ (21), 424 [M-H2O]+ (10.6), 411 (52), 409 (7.3), 3.99 (11.7), 3.85 (10.9), 257 (9.8), 234 (23.8), 220 (16.2), 207 (60.3), 203 (100), 189 (84), 175 (35), 161 (25.4), 147 (29.0), 135 (49.0), 121 (37), 107 (37.9), 95 (50.1), 81 (36.9), 69 (35.01), 55 (28.5) and 44 (45.4); ^{1}H-NMR (300MHz), (CDCL3) δ : 4.66 (s, 1H), 4.56 (s, 1H), 3.78 (d, 1H, J=12Hz), 3.31 (d, 1H, J=12Hz), 3.17 (m, 1H), 2.37 (ddd, 1H, J1=8Hz, J2=4Hz), 1.93 (m, 1H), 1.83 (m, 1H), 1.66 (s, 3H), 1.63 (m, 1H), 1.59 (m, 1H), 1.56 (m, 1H), 1.51 (s, 6H), 1.35 (S, 3H), 1.40 (m, 1H), 1.23 (m, 1H), 1.15 (m, 1H), 1.05 (m, 1H), 1.00 (s, 3H), 0.96 (s, 3H), 0.95 (s, 3H), 0.80 (s, 3H) and 0.74 (s, 3H); ^{13}C-NMR (75 MHz, CDCL3) δ: 38.73 (C-1), 27.08 (C-2), 78.89 (C-3), 38.87 (C-4), 55.3 (C-5), 18.31 (C-6), 34.26 (C-7), 40.95 (C-8), 50.43 (C-9), 37.18 (C-10), 20.08 (C-11), 25.24 (C-12), 37.34 (C-13), 42.74 (C-14), 27.42 (C-15), 29.20 (C-16), 28.009 (C-17), 47.81 (C-18), 48.80 (C-19), 150 (C-20), 29.78 (C-21), 33.98 (C-22), 37.99 (C-23), 15.34 (C-24), 16.10 (C-25), 15.99 (C-26), 14.77 (C-27), 60.59 (C-28), 19.09 (C-29), 110 (C-30).

III.5 Anti-malarial test

Compounds **1 to 4** separately were dissolved in a solution of water + DMSO 0.02% v/v (Brown et al., 1975). The compounds were administered over a period of four days to the culture and the number of parasites determined daily. An untreated culture of plasmodia served as a control.

Culturing of *P. falciparum NF54 Strain:* *P. falciparum* isolate NF54 and R strain were maintained in small petri dishes (5 cm) according to a protocol from Moloney et al. (1990) and Trager and Willaims (1992) in a gaseous phase of 90% N2, 5% CO2 and 5% O2. Parasites were cultured in human erythrocytes (blood group A+) in RPM1640 medium (Sigma) supplemented with 25 µM HEPES, 20 mM sodium bicarbonate and 10% heat inactivated human A+ plasma at 10% (v/v) hematocrit. The parasitemia of infected erythrocytes was determined by light microscopy and estimated by Giemsa stained smears. Parasitemia detected in the cultures were scored visually with a 100-fold oil immersion objective, counting at least 1000 infected erythrocytes to determine the parasitemia.

Inhibitor experiments by monitoring multiplication and growth of plasmodia: Cultures were adjusted to a parasitemia of 0.5%. Aliquots were diluted 1:10-fold in RPMI-medium, dispensed into 12-well micro culture trays and incubated at 37°C in a gaseous phase of 90% N2, 5% CO2 and 5% O2. Thereafter, growth medium was changed once a day for four days and inhibitors were added to the media in the concentration of 20 µM as indicated. Each substance was analyzed in four independent wells of the micro culture tray. Parasitemia were estimated as triplicates daily in each of the four independent wells from Giemsa stained smears by counting 1000 erythrocytes.

IV. RESULTS AND DISCUSSION

IV.1. Butilinol (1)

The IR spectrum **(**$CHCl_3$) showed intense U_{max} absorptions at 3430 (OH), 3070, 1635 and 880 cm^{-1}. The UV (Methanol) spectrum of the compound (1) showed λmax absorptions at 389 nm, 226 nm and 200 nm characteristics for lupan class of triterpenes. The IR spectrum showed absorptions at 3430 (OH), 3070, 1635 and 880 (terminal methylens).

The molecular ion peak was established by HRMS at 456.10 m/z corresponding to the molecular formula $C_{30}H_{50}O_2$, indicating seven degrees of un-saturations in the molecule. Besides the molecular ion peaks, the EIMS showed other prominent fragmented ion at m/z 423.1, 302.0, 248.0, 207.0, 189.1, 135.0, 95.0 and 55.0, characteristics of lupan group of triterpenes.

The ^{1}H-NMR (300 MHz, CD_3OD) spectrum of (1) showed 50 proton resonances in the molecule. Two singlets at δ 4.72 and δ 4.59 ascribable to an endomethylene proton. This showed α-stereochar of the side chain, as in case of β-stereochar, the two methylenes protons should have appeared as two singlets at δ 3.47 and δ 4.21.

The ^{13}C-NMR spectrum of the compound showed 31 carbon resonances in the molecule. The multiplicity of carbon signals was determined by the DEPT experiments with the pulse angle 45°, 90° and 135°. On the basis of the DEPT experiments six signals were found to be of methynes, thirteen of methylenes and six of methyls. The remaining six signals in the Broad Band spectrum were assigned to the quaternary carbons in the molecule. The carbon chemical shifts were compared with the betulinic acid. Most of the carbon resonances were found to be 5 similar. However the acid carbon signal of acid was absent. Therefore number of quaternary carbons is reduced to be six. This shows that the compound in hand does not have acid functionality in it. The methyl signals of both the molecules were same in number and in the value of chemical shifts. The comparison of the methylenes signals showed that our molecule possess two methylenes signals δ 68.9 and δ 22.97 which were not present in butilinic acid molecule. The methylene signal at δ 28.01 was another difference in our molecule.

The compound (1) was searched in the library available at HEJ institute of chemistry Karachi including science finder in digital library at LEJ and in dictionary of natural products but found no reference of the data and structure, so it is believed that the compound (1) is a new compound.

IV.2. Oleanolic Acid (2)

The UV (MeOH) spectrum of compound (2) showed λ_{max} absorptions at 203 characteristic of oleanane class of triterpenes. The IR spectrum showed absorption bands for hydroxyl group (342 cm^{-1}) and of acid carbonyl function (1705 cm^{-1}).

The molecular ion peak was established by HRMS at m/z 456.343 corresponding to the molecular formula C30H48O3 indicating seven degrees of un-saturation in the molecule. Besides the molecular ion peak, the EIMS showed other prominent fragment ions at m/z 248, 203, 175, 133, 95 and 69, which were characteristic for Δ12-amyrin skeleton (20, 21). The mass fragmentation indicated that the hydroxyl group was located in ring A instead of in ring B, C, D or E as fragment of m/z 248 in that case would not have been obtained.

The carboxy group was also present in this fragment and the ready loss of this group from the fragment A was in agreement with its positioning at C-17. Another fragment at m/z 189 was also 6 formed by the fragmentation of A; probably involve the loss of carbon17 along with the COOH group with a double hydrogen transfer to afford the conjugated allylic cation.

The ^{1}H-NMR spectrum (CDCl3, 300MHz) of compound (2) showed the presence of H-12 olefinic proton resonating as 1H triplet at δ 5.25(J=3.5 Hz,). Another downfield triplet at δ 3.14 (J=6.8 Hz) corresponds to H-3α [35-40]. A 1H double doublet at δ 2.78 was assigned to H-18 on the basis of its chemical shift as well as multiplicity pattern reported for H-18 with β-configuration. The spectrum also showed signals for seven methyl groups. The tertiary natures of these methyl groups were evident from their sharp singlet, in the spectrum.

The ^{13}C-NMR (CDCl3, 75MHz) of the compound (2) showed a total of thirty carbon resonances. These signals were classified into methyls, methylenes and methines on the basis of DEPT experiments. The quaternary carbons were identified from broad band decoupled ^{13}C-NMR spectrum. The structure (2) was further established by comparison of spectra and physical data to the literature (22).

IV.3. Ursolic Acid (3)

The HRMS indicated the molecular ion peak at 456.000 consistent with the molecular formula C30H48O3 indicating seven degrees of un-saturations in the molecule.

The UV (MeOH) spectrum of compound (3) showed λ_{max} absorptions at 202 characteristic of ursane class of triterpenes. The IR (CHCl3) showed absorption bands for COOH (3400 cm^{-1}-2650 cm^{-1}), (2900-2810 cm^{-1}) and tri-substituted double bond (1690 cm^{-1}, 1640 cm^{-1} and 108 cm^{-1}) C30H48O3, (M+ 456), m.p. 285°C, [α] D+ 70° ($CHCl_3$) is a triterpenic acid which forms a methyl ester, C31H50O3 (M+ 470), m.p. 168°C, [α]D+ 58° ($CHCl_3$), with diazomethane and a monoacetate, C32H50O4 (M+ 498), m.p. 290°C, [α]D+ 70° (CHCl3), withAC_2O/Py. It was identified as ursolic acid by direct comparison of the compound and it's above derivatives with the respective authentic samples (23, 24).

The carbon chemical shifts of ursolic acid and oleanolic acid differs significantly in respect of their C-11, C-12, C-13, C-27 and the carbon concerning their ring E, while remaining carbon atoms have almost identical shift values. Thus the signals for C-11, C-13, C-19, C-21, C-27, C-29 and C-30 of ursolic acid are shifted up field by δ 6.3, δ 5.3, δ 6.5, δ 3.3, δ 2.7, δ 9.9 and δ 2.4, respectively compared to those of the corresponding carbon atoms of the oleanolic acid. On the other hand C-12, C-16, C-17, C-18, C-20 and C-22 of the compound appear at low fields by δ 2.7, δ 1.0, δ 0.8, δ 11.8, δ 8.3 and δ 4.2, respectively. Such differences of shift values to be diagnostic of isomeric pairs of ursane and oleanane derivatives and the above results provide further corroboration (confirmation) of the above generalization (25, 26).

IV.4. Betulin (4)

The UV spectrum (MeOH) of the compound exhibited λmax absorptions at 200 nm and 204 nm, indicating the presence of lupeol skeleton. The IR spectrum (KBr) showed absorption bands at 3430 cm^{-1} (OH), 3070 cm^{-1}, 1635 cm^{-1} and 880 cm^{-1} (terminal methylene group).

The HRMS exhibited the molecular ion peak at m/z 442.3790, corresponding to the molecular formula $C_{30}H_{50}O_2$ indicating six degrees of un-saturation in the molecule.

The EIMS of the compound (4) exhibited diagnostic peaks at m/z 442[M] +, 424[M-H2O] +, 411, 234, 220, 207, 203, 189, 175 and 161, which were characteristic of pentacyclic triterpenes with an isopropenyl group. The 1H-NMR spectrum of the compound (4) displayed signals due to six tertiary methyls at δ 1.35, δ 1.00, δ 0.96, δ 0.95, δ 0.80 and δ 0.74. It also showed a ^{1}H doublet (J=12.0 Hz) due to carbinolic proton at δ 3.31. Its chemical shifts and coupling constant led us to assign β-configuration to the hydroxyl group at C-3. Another 1H doublet at δ 3.78 (J=10.69 Hz,) could be assigned to the methylene carbon attached to the hydroxyl group. The proton signals of δ 4.66 and δ 4.56 indicated the exomethylene protons.

The ^{13}C-NMR assignments of various carbon atoms were clarified by DEPT experiments which revealed the presence of six methyl, twelve methylene, five methane carbon and six quartnary carbon atoms in the molecule (25).

IV.5. β-Sitosterol (5)

The compound **5** was identified as *β*-sitosterol on the basis of comparison of the structural data with those reported for the compound (27, 28).

IV.6. Betulinic acid (6)

The compound **6** was identified as betulinic acid on the basis of comparison of structural data with those reported for the compound (29).

IV.7. α-Amyrin (7)

The compound **7** was identified as α-amyrin on the basis of comparison of structural data with those reported for the compound (30).

IV.8. Germanicol (8)

The compound **8** was identified as germanicol on the basis of comparison of structural data with those reported for the compound (31).

Antiplasmodial activity of the compounds: *In vitro* treatment of the chloroquine sensitive (CQS) strain of *P. falciparum* NF54 with 3 µM of the compounds **1-4** resulted in a partial inhibitory effect. The best antiplasmodial activity was seen for **3** (Figure 1). This difference was significant when compared to untreated controls on day 4 after start of treatment. However, the effect was only moderate in comparison to chloroquine, which leads already at day 2 after the beginning of the treatment to a complete growth inhibition of the parasites. *In vitro* treatment of the CQS *P. falciparum* strain NF54 with the *Ursolic acid* **(3)** with concentrations ranging from 2 to 8 µM (Figure 2) resulted in a partial inhibitory effect at 4 µM and even stronger at 8 µM. This difference was significant in comparison to untreated controls at day 4 after the start of treatment (Figure 3). However, the effect was only moderate in comparison to chloroquine, which showed a complete growth inhibition of the parasites at a concentration of 0.032 µM.

V. CONCLUSION

Viburnum nervosum is an important medicinal plant available in various parts of Pakistan especially, in the State of Jammu and Kashmir. The selection of *Viburnum nervosum* for phytochemical investigations was due to its use in traditional medicines. The phytochemical studies of the plant resulted in few compounds with significant antiplasmodial activity.

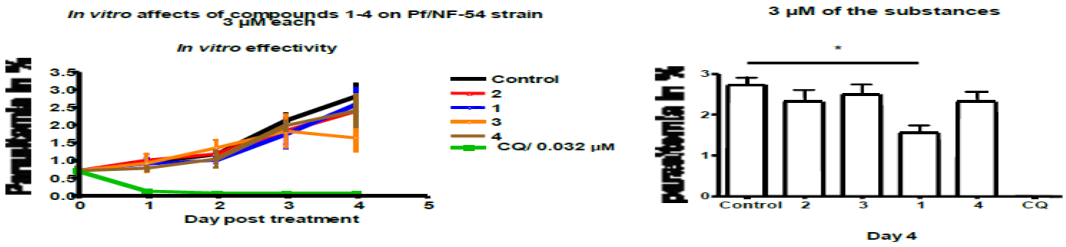


Figure 1. Antiplasmodial activity of compound 1, 2, 3 and 4.

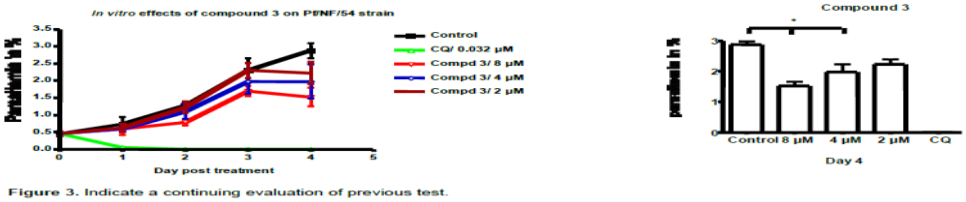
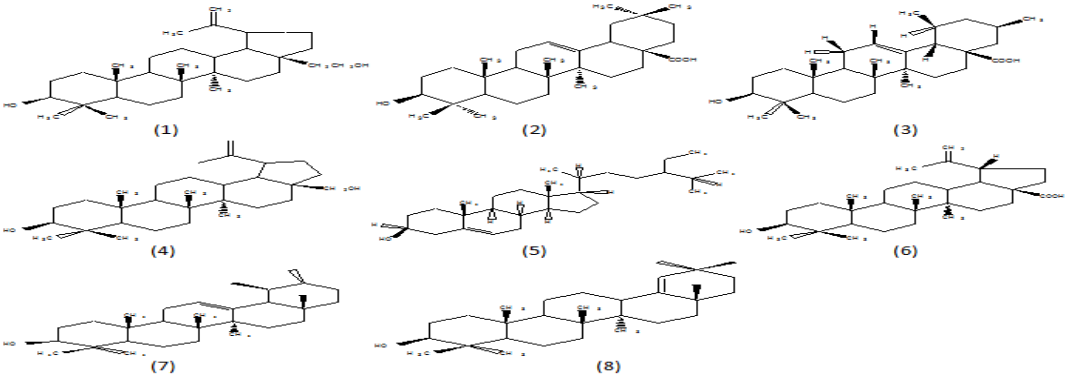


Figure 3. Indicate a continuing evaluation of previous test.

Figure 2. Structures of compounds 1-8 isolated from Viburnum nervosum



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