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EXPERIMENTAL PAPER

Chemical constituents of ethanol extract of leaves and molluscicidal activity of crude extracts from *Vitex trifolia* Linn.

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Summary

β-sitosterol and two triterpenoids: ursolic acid acetate and platanic acid have been isolated from ethanolic extract of *Vitex trifola* leaves. β-sitosterol was previously isolated from the leaves, stem and seeds of *Vitex trifolia*. Ursolic acid acetate has been isolated for the first time in this plant species. Platanic acid has been reported for the first time in *Vitex trifolia* and even in the family of this plant: *Verbenaceae*. These compounds were characterized using spectroscopic methods including 1D-^{1}HNMR, ^{13}CNMR, ESIMS and 2D-NMR (HSQC, HMBC, COSY) experiments and confirmed by comparison of their NMR data with those from the literature. A preliminary molluscicidal test for ethanol, chloroform and n-hexane extracts of leaves of *Vitex trifolia* against *Biomphalaria alexandrina* adult snails showed that ethanol extract of leaves with LC_{50} value 26.42 mg/l (27.92 mg/l – 24.99 mg/l) was more effective than n-hexane extract with LC_{50} value 35.48 mg/l (43.81 mg/l – 28.72mg/l) and chloroform extract with LC_{50} value 46.77 mg/l (53.59 mg/l – 43.81 mg/l) after 24 h exposure.

Key words: *Vitex trifolia, Verbanaceae, β-sitosterol, ursolic acid acetate, platanic acid, 2D-NMR, molluscicidal activity*

INTRODUCTION

Vitex trifolia (Common name: three leaved chaste tree) belongs to *Verbenaceae* family. The plant parts like leaves and flower have a great therapeutic potential in Indian system of medicine [1]. *Vitex trifolia* Linn. is a tropical shrub widespread in pacific Asian countries such as India, Srilanka, China, Phillipines, and French Poloynesia. Among plants of *Vitex* genus that includes approximately 200 species growing in tropical region, *Vitex trifolia* is employed to cure numerous illnesses. The stems of *Vitex trifolia* are used for the treatment of dysentery in Papua New Guinea [2]. In New Caledonia leaves are used to relieve fever, in Samoans to alleviate rheumatic pain and sprained joints when applied topically in New Caledonia.

Abietane-type diterpenes, labdane type diterpenes, rotundifuran, dihydrosolidagenone and abietatriene-2b-ol have been previously isolated from the acetone extract of the fruits of *Vitex* trifolia [3]. Herein we report the isolation of β-sitosterol and two triterpenoids, ursolic acid acetate and platanic acid from the leaves of plant. β-sitosterol has been previously isolated from leaves, stem and seed of *Vitex* trifolia [4-5], ursolic acid acetate and platanic acid are being reported for the first time in this plant species. Platanic acid is being reported for the first time even in *Verbenaceae* family. Ethanol, chloroform and n-hexane extracts of plant leaves were subjected to molluscicidal activity against *Biomphalaria alexandrina* adult snail and LC_{50} with 95% confidence limit calculated. Study aimed at isolation and characterization of plant's active constituents and ascertaining its molluscicidal potential.

MATERIAL AND METHOD

Vitex trifolia (Common name: three leaved chaste tree) belongs to *Verbenaceae* family. It is a up to 6 m high shrub with quadrangular branches. This plant can be commonly seen on the banks of water bodies like channels, rivers and ponds. The leaves are opposite exstipulate, long petioled and 3–5 foliate which are all connected at one point and are elliptical and between 3 and 12 cm long. Flowers are light blue in terminal panicled cymes consist of a tube with five lobes; the central lobes is bigger than the others [6]. Plant leaves (3 kg) were collected from Mandi (H.P.) in July 2009 and air-dried in shade. After complete drying, extraction was carried out in ethanol and n-hexane for separate batches of leaves (2 kg). Thereafter, ethanol extract obtained was partitioned with chloroform, concentrated in a rotary evaporator to give extracts of three types. The extracts of the plant were dissolved in an aqueous solution of dimethyl sulfoxide (DMSO) (0.1%) and series of dilutions that permit the computation of LC_{50} values were prepared.

Crude ethanolic extract (50 g) was adsorbed on silica gel and subjected to column chromatographic separation using $CHCl_3$ followed by increasing proportions of MeOH in $CHCl_3$ (*v/v*) as an eluent. Collection of 150 ml of fractions, $CHCl_3$ 100% eluent gave sample (1). 800 mg (white needles) crystallized from 100% MeOH solvent, $CHCL_3$:MeOH (98:02 *v/v*) eluent gave sample (2). White crystalline, 650 mg, crystallized from 100% MeOH solvent and eluent $CHCL_3$:Methanol (95:05 *v/v*) gave sample (3) white power, 700 mg crystallized from 100% MeOH solvent. Melting points were measured on Kofler block. The ^{1}HNMR spectrum in CD_3OD showed that the samples were not pure compounds, although, TLC showed single spots. For this reason, samples were subjected to further purification by RP-HPLC with water 590 series pumping system equipped with water R401 refractive index detector, a μ-Bondapack C18 column (300×7.8 mm i.d) and a U6k injector using MeOH-H_2O(9:1) as mobile phase (flow rate-2.0 ml/min.) giving pure compound (1) t_R10 min, compound (2) t_R11 min and compound (3) t_R 12.3 min.

NMR experiments, a Bruker DRX-600 NMR spectrometer using the UXNMR software package, NMR 600 MHz for ^{1}HNMR and 150 MHz for ^{13}CNMR, solvent CD_3OD, values relative to TMS reference. Chemical shift expressed in δ (parts per million) values, solvent peak δ_H 3.34 ppm and δc 49.0 ppm for CD_3OD; coupling constant (J) are in Hz. 1D- and 2D-NMR experiments were carried out using conventional pulse sequence[7]. ESIMS was performed on a Finnigan LQ-Q Deca instrument (Thermoquest, San Jose, Ca) equipped with Xcalibur software. Column chromatography was performed on silica gel (Merck) and TLC on Kieselgel 60G (Merck), spot on TLC was visualized by spraying with 20% H_2SO_4 and heating at 120°C for a few minutes.

Compound (1)

Crystallized from methanol as white needles. M.P.153-151°C.UV^{meoh}_{max}nm: 220. IR ν^{max}_{KBr} (cm^{-1}) 3440 (-OH), 2970, 2959, 2859 (C-H streching), 1440 (C=C streching), 1463, 1380 (gem-dimethyl group) and 1055 (C-O streching). ESIMS 413[M-H]$^-$, 415[M+H]$^+$. ^{1}HNMR(CD_3OD): cf. (tab. 1). ^{13}CNMR(CD_3OD): cf. (tab. 1). HSQC correlations H6-C6, H3-C3, H4-C4, H19-C19, H21-C21, H29-C29, H18-C18, H26-C26, H27-C27, H24-C24, H 17-C17, H9-C9, H14-C14. HMBC correlation were established for H4-C5; H6-C4, C7, C10; H19-C5, C9, C10; H29-C25, C26, C28; H21-C17, C20, C22; H26/H27-C25, C24; H18-C12, C13, C17 . The compound (50 mg), acetic anhydride (1 ml) and 2-3 drops of pyridine were mixed together and heated in water bath for ½ h in a 50 ml r.b. flask and then kept overnight. The reaction mixture was poured in cold water bath and product was filtered. It's acetate was crystallized from ethanol.

Table 1.

^{13}C and $^{1}HNMR$ spectroscopic data of compounds (1-3) in CD_3OD[a]

Position	Compound (1)		Compound (2)		Compound (3)	
	δ_C	δ_H(J in Hz)[b]	δ_C	δ_H(J in Hz)[b]	δ_C	δ_H(J in Hz)[b]
1	38.2	1.09, 1.89, m	38.8	1.69,m	38.3	1.37;1.91,m
2	32.2	1.49, 1.79, m	24.9	1.66;1.98,m	27.7	1.56,m
3	72.4	3.37, m	82.2	4.48,dd (4.3,11.3)	80.2	31.3,dd,(3.9,11.1)
4	42.9	2.24, 2.45 m	39.0	–	39.9	–
5	141.2	–	57.3	0.97,m	56.4	0.69,d (9.9)
6	122.1	5.34, brd (4.5)	19.0	1.45;1.56,m	18.9	1.51,m
7	32.6	1.56, 2.00, m	34.1	1.36;1.55,m	33.6	1.30,m;2.28,d(11.2)
8	32.3	1.49, m	40.3	–	40.5	–
9	51.3	0.96, m	48.9	1.61,m	51.4	1.30,m
10	36.9	–	38.8	–	37.2	–
11	21.9	1.56, m	24.5	1.69;1.94,m	21.7	1.42,m
12	40.7	1.21, 2.08, m	126.0	5.24,br,s	27.5	1.30;2.09,m
13	42.8	–	139.8	–	35.2	1.39,m
14	57.9	1.05, m	43.0	–	42.8	–
15	25.0	1.19, 1.63, m	29.0	1.08;2.02,m	29.8	1.30,m
16	29.1	1.33, 1.89, m	25.0	1.66;2.02,m	30.5	1.09;1.56,m
17	57.2	1.17, m	47.3	–	58.1	–
18	12.0	0.73, s	54.6	2.24,d (11.3)	53.9	3.46,m
19	19.7	1.05, s	40.2	1.37,m	50.8	1.97,m
20	37.2	1.40, m	40.2	0.99,m	216.0	–
21	19.1	0.98,d(6.5)	31.7	1.37;1.50,m	28.9	1.13,m
22	34.7	1.07,1.40,m	38.0	1.67;2.17,m	37.9	1.39;2.38,m
23	26.9	1.24,m	28.4	0.90,s	26.9	0.96,s
24	46.9	0.98,m	17.0	0.90,s	15.4	0.74,s
25	30.3	1.70,m	15.9	1.01,s	16.1	0.84,s
26	19.1	0.86,d(6.7)	17.0	0.90,s	16.4	0.98,s
27	20.0	0.86,d(6.7)	23.0	1.15,s	14.2	0.98,s
28	23.8	1.33,m	183.0	–	183.5	–
29	11.7	0.88,t(7.4)	17.0	0.90,m	28.2	2.17,s
30			22.0	0.97,d (6.8)		
1’			172.7	–		
2’			21.7	2.03,s		

a – assignments confirmed by 2D COSY, HSQC, and HMBC experiments

b – 1H-1H coupling constants (Hz) were measured from the COSY spectra

Compound (2)

White power crystallized from methanol. Mol. formula: $C_{32}H_{50}O_4$. M.P. 276°C. It gave positive test with Liebermann-Burchard reaction, Noller's reagent and yellow color with TNM. ESIMS 497[M-H]$^-$, 437[M-HCOOCH$_3$]$^-$, 392[M-HCOOCH$_3$-COOH]$^-$. IR: ν_{kBr}^{max}(cm^{-1}): 3440, 3140, 2930, 2880, 2800, 1735, 1680, 1450, 1390, 1325, 1300, 1250, 1190, 1070, 1030, 980, 930, 880 and 745.

^{1}HNMR: cf. (tab. 1). ^{13}CNMR: cf. (tab. 1). HSQC correlations: H12-C12, H3-C3, H18-C18, H9-C9, H2"-C2", H19-C19, H20-C-20, H5-H5, H23-C23, H30-C30, H29-C29, H36-C36, H24-C24. HMBC correlation H2'-C1'; H3-C2, C4, C23, C24, C1'; H12-C9, C11, C12, C14; H18-C11, C12, C13, C17, C28; H23/H24/H26/H29-C3, C4, C5, C18, C19, C20, C23, C24, C30; H25-C5, C9, C10; H27- C8, C13, C14, C15; H30-C17, C19, C20, C21, C29. Compound (10 mg) was heated with 10% KOH on MeOH (5 ml) for 5 h. After concentration, it was crystallized from MeOH to yield colorless needles identified as ursolic acid.

Compound (3)

Crystallized from methanol as white power. Molecular formula: $C_{29}H_{44}O_4$. IR (ν_{max}^{KBr}) cm^{-1}: 3400, 3440, 2930, 2880, 1686, 1070, 1030, 980, 930, 880 and 745.1. ^{1}HNMR: cf. (Table 1). ^{13}CNMR: cf. (tab. 1). ESIMS: 457[M-H]$^-$, 413[M-COOH]. HSQC: H3-C3, H18-C18, H19-C19, H29-C29, H9-C9, H5-C5, H23-C23, H26-C26, H25-C25, H27-C27, H24-C24. HMBC correlation were established for H3-C23, C24, C9; H18-C19, C21; H19-C17, C18, C20, C22, C28; H23-C3, C4, C5, C24; H24-C2, C3, C4, C5, C23; H25 C4, C5, C9, C10, C24; H26/H27-C9, C10, C14, C15; H29-C19, C20, C21. Compound was acetylated with Ac_2O/pyridine at 25°C for 20 h; the resulting viscous solid was crystallized from methanol, m.p. 286°C. IR (ν_{max}^{kBr}) cm^{-1}: 3450 (-OH of COOH), 1735 acetate.

Molluscicidal assay

Adult snails of *Biomphalaria alexandrina* free from trematodes infestation were kept in laboratory conditions for a period not shorter than 3 weeks before usage in toxicity experiments. The procedure applied for screening tests on adult snails is based on the professional testing technique recommended by WHO (1965) [8]. 12 snails (10 mm in size), were put into a nylon mesh bag (mesh size 2 mm) and immersed into 400 ml dechlorinated water solution of a known concentration. Experiments were conducted at 21°C. Each concentration of the plant extracts was tested 3 times. A similar number of control snails was maintained in normal dechlorinated water under the same conditions. The snails were considered to be dead when they were retracted in their shells and discolored. After 24 hour, the

number of deceased snails was counted in each group and data was subjected to the probit analysis, the LC_{50} with 95% confidence limit was calculated. Corrected % mortality was calculated using Abbott's formula [9] and was rounded off to nearest simple whole number:

Corrected mortality (%) = $(M_{obs}\text{-}M_{control})\times 100/(100\text{-}M_{control})$.

Corrected percent of mortality was converted to probits [10-11] (tab. 3-5) by looking up to those corresponding to the percentage which responded in Finney's table (tab. 2). For example, for a corrected 17% response, the corresponding probit would be 4.05. Additionally, for a corrected 50% response (LC_{50}), the corresponding probit would be 5.00.

Table 2.

Finney table

%	0	1	2	3	4	5	6	7	8	9
0	–	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

Table 3.

Probit calculation table for ethanol extract of the plant leaves

Concentration [mg/l]	Log_{10} concen.	Total no of snails	No. of dead	Corrected % mortality	Probits
0	–	12	0	0	0
20	1.301	12	1	8	3.59
22.3	1.348	12	2	17	4.05
24.6	1.390	12	3	25	4.33
26.4	1.421	12	5	42	4.8
28.7	1.452	12	7	58	5.2
29.35	1.467	12	9	75	5.67
31.01	1.491	12	11	92	6.13

Table 4.

Probit calculation table for chloroform extract of the plant leaves

Concentration [mg/l]	Log_{10} concen.	Total no of snails	No. of dead	Corrected % mortality	Probits
0	–	12	0	0	0
25	1.397	12	1	8	3.59
35	1.544	12	3	25	4.33
41	1.613	12	4	33	4.56
47	1.672	12	5	42	4.8
56	1.748	12	8	67	5.44
69	1.838	12	10	83	5.95
75	1.875	12	11	92	6.41

Table 5.

Probit calculation table for n-hexane extract of the plant leaves.

Concentration [mg/l]	Log_{10} Concen.	Total no of snails	No. of dead	Corrected % mortality	Probits
0	–	12	0	0	0
30	1.477	12	1	8	3.59
32.3	1.509	12	3	25	4.33
34.45	1.537	12	5	42	4.8
37.53	1.574	12	7	58	5.2
39.63	1.598	12	10	83	5.95
41.97	1.622	12	11	92	6.41

Calculation of LC_{50}.

Graph Probit values (Y-axis) against log10 concentration (X-axis) and draw a straight line of best line through plotted points, then use this line to estimate the log_{10} concentration associated with a Probit of 5. (fig. 1-3).

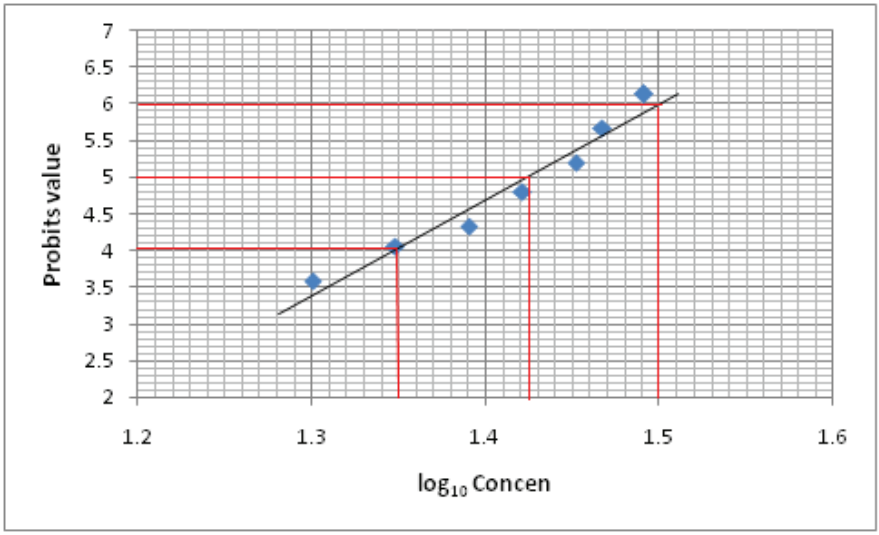


Figure 1.

Calculation of LC_{50} value for ethanol extract
(LC_{50} = antilog 1.422=26.42 mg/l)

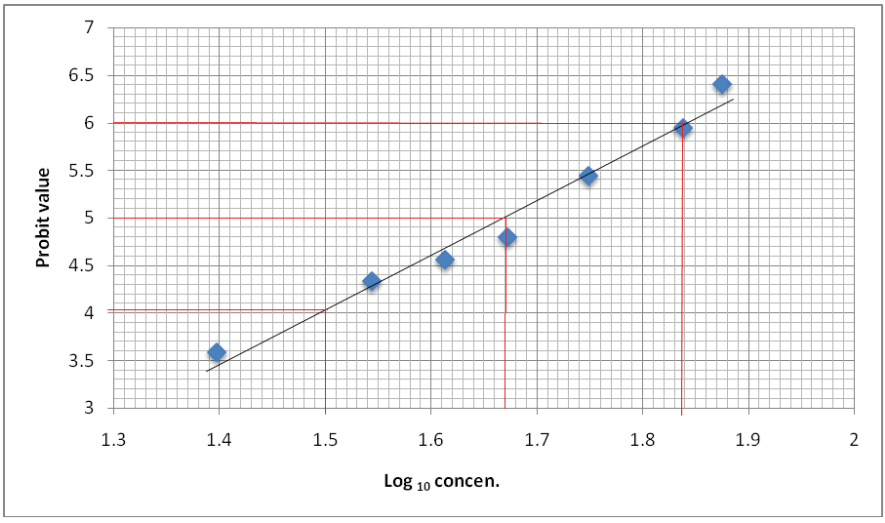


Figure 2.

Calculation of LC_{50} value for chloroform extract

(LC_{50} = antilog1.67=46.77 mg/l)

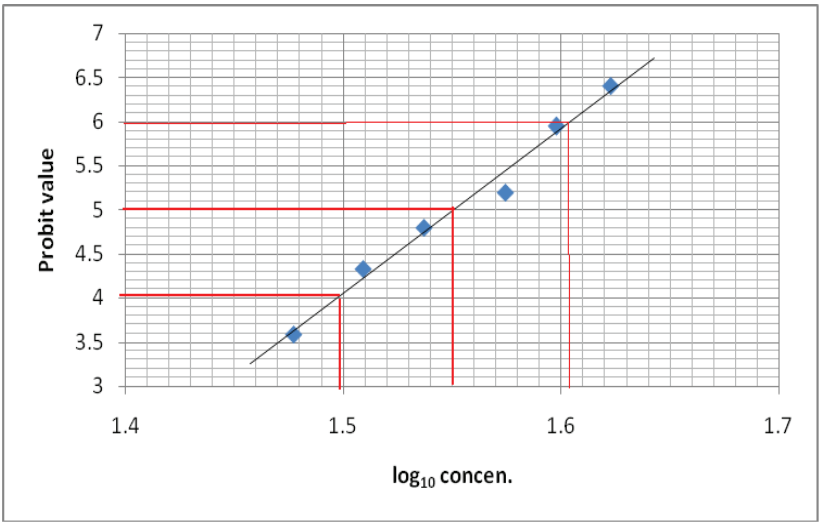


Figure 3.

Calculation of LC_{50} value for n-hexane extract
(LC_{50} = antilog 1.55=35.48 mg/l)

Calculation of 95% Confidence Limits [12] of LC_{50}.

1. Inverse prediction was used from the graph to estimate the $\log_{10}$ of the LC_{84}, LC_{16}, and LC_{50} then "un-log" the values and express as mg/l.
2. S and $\text{Log}_{10}(S)$ (use the 'un-logged' dose/conc. values) was calculated.

$$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2}$$

$$\text{Log}_{10}(S) = x$$

3. Determination of N
   N = total number of individuals tested between the range of dosages that correspond to the LC_{16} to the LC_{84}.
4. Calculation of $\log_{10}(f)$ and f.

$$\log_{10}(f) = \frac{\{2.77\}}{\sqrt{N}} \times \text{Log}_{10}(S)$$

$$\text{Log}_{10}(f) = \frac{\{2.77\}}{\sqrt{N}} \times x$$

$$f =$$

5. Calculation of upper and lower 95% confidence limits (multiply or divide 'un-logged' concentration/dose values by f):
   Upper limit = $LC_{50} \times f$ =
   Lower limit = LC_{50}/f =

RESULTS

Compound (1)

It was found to be sterol as it gave positive Liebermann-Berchard test [13] and Noller test [14]. It also responded positively to TNM test [15] for unsaturation. The IR spectrum showed characteristic absorption at ν_{KBr}^{max} (cm^{-1}) 3440 (-OH), 2970, 2959, 2859 (C-H streching), 1440 (C=C streching), 1463, 1380 (gem-dimethyl group) and 1055 (C-O streching). The interpretation of the proton spin-spin coupling pattern was done with the aid of 2D NMR viz. HSQC experiments and the ^{1}HNMR and ^{13}CNMR. On the basis of aforementioned spectral data, the structure of compound (1) was assigned as β-sitosterol (fig. 4) which was further confirmed by direct comparison with an authentic sample.

Compound (2)

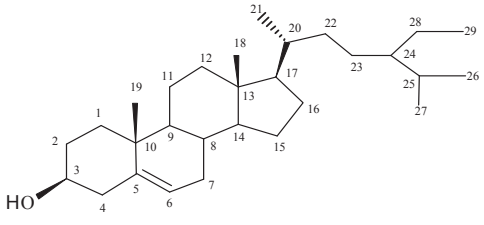
Molecular formula was established as $C_{32}H_{50}O_4$ on the basis of elemental analysis and molecular weight determination. In the ESIMS peaks observed at 497$[M-H]^-$. Other prominent peaks were observed at 437$[M-HCOOCH_3]^-$ and 392$[M-HCOOCH_3-COOH]^-$. It gave positive test with Liebermann-burchard [13] and Noller's reagent [14] and developed yellow color with TNM [15], thereby indicating it to be a triterpenoid compound with an unsaturation in it. The characteristic absorption bands in it's IR spectrum were observed at ν_{max}^{KBr} (cm^{-1}) 3440 (OH stretching of –COOH group), 2930, 2880 (-C-H stretching) with a broad spectrum range of 3400-2800, 1680 (C=O stretching of COOH group), 1260 (acetate), 1450 (-CH_2 deformation), 1390, 1360, 1325, 1300, 1250 (characteristic of Ursane series).

In ^{1}HNMR spectrum, cf. (tab. 1) the presence of seven methyl groups for 21H was observed in the region of 0.88–1.25. Two secondary methyl group were observed at δ 0.68 (d, J=60Hz) whereas five tertiary methyl at δ 0.76 (2×CH_3), 0.82, 0.89, 1.25(s, 3×CH_3). A multiplet at δ 1.5-3.2 with side bands indicating the presence of 23H accounting for –CH_2- and –CH- protons. A signal at δ 2.03 (s) is due to the presence of methyl protons of acetyl group. The esterified methine was observed at δ 4.48 indicating the presence of a pentacyclic triterpenoid skeleton. Through HSQC spectrum it was possible to assign the chemical shifts to some methine proton at C-5(δ_H 0.97, m), C-9 (δ_H 1.61, m), C-18(δ_H 2.24, d), C-19 (δ_H 1.37, m), C-3(δ_H 4.48, dd), C-18 (δ_H 2.24, d), C-20(δ_H 0.99, m) and to methyl proton at C-23 (δ_H 0.90), C-24(δ_H 0.90), C-25(δ_H 1.01), C-26(δ_H 0.90), C-29(δ_H 0.90). HMBC correlations established for H2'-C1'; H3-C2, C4, C23, C24, C1'; H12-C9, C11, C12, C14; H18-C11, C12, C13, C17, C28; H25–C5, C9, C10; H23/H24/H26/H29–C3, C4, C5, C18, C19, C20, C23, C24, C30; H27-C8, C13, C14, C15; H30-C17, C19, C20, C21, C29 helped in assigning quaternary carbons atoms chemical shift for C-1'(δc 172.7), C-20(δc 40.2), C-28(δc 183), C-14(δc 43.0), C-10(δc 38.8), C-4(δc 39) and C-20(δc 40.2).

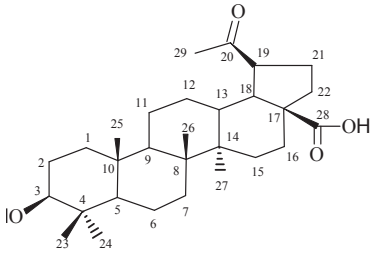
On the basis of comparison of ^{1}HNMR and ^{13}CNMR spectral data with the data available in literature [16], compound was assigned as triterpenoid ursolic acid acetate (fig. 1). It was further confirmed by deacetylation to give ursolic acid, m.p.266-267°C (m.m.p, co-tlc).

Compound (3)

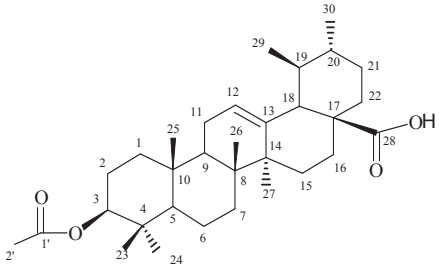
It gave positive Liebermann-Burchard test [13] and Noller's Test [14] thereby indicating to be a triterpenoid compound. The characteristic absorption bands in it's IR spectrum were observed ν_{max}^{KBr}(cm^{-1}) 3400 (-OH stretching), 3440 (OH stretching of –COOH group) with a broad spectrum range of 3440–2820, 1680 (›C=O stretching of –COOH group). In the ESIMS various peaks were obtained at 457[M-H]$^-$, 413[M-COOH]$^-$. The triterpenoid structure was confirmed by the ^{1}HNMR and ^{13}CNMR experiments which exhibited the presence of one acetyl group (δ_C 216 and δ_C 28.2) and δ_H 2.17 (s, H-29) and twenty seven other carbon atoms. The ^{1}HNMR spectrum showed the presence of six methyl singlets (δ_H 0.96, δ_H 0.74, δ_H 0.84, δ_H 0.98, δ_H 0.98 and δ_H 2.17), a highly deshielded proton (δ_H 31.3) at C-3 due to presence of hydroxyl group. The HSQC spectrum was one of the key steps in the analysis. It was possible to assign the chemical shifts to some methine proton at C-5(δ_H 0.69), C-9(δ_H 1.30), C-18(δ_H 3.46),C-19 (δ_H 1.97) and methyl proton C-23(δ_H 0.96), C-24(δ_H 0.74), C-25(δ_H 0.84), C-26(δ_H 0.98), C-27(δ_H 0.98), C-29(δ_H 2.17). HMBC correlations established for H3-C23, C24, C9; H18-C19, C21; H19-C17, C18, C20, C22, C28; H23-C3, C4, C5, C24; H24-C2, C3, C4, C5, C23; H25–C4, C5, C9, C10, C24; H26/H27-C9, C10, C14, C15; H29-C19, C20, C21 helped in assigning quaternary carbons atoms chemical shift for C-17(δc 58.1), C-20(δc 216.0), C-28(δc 183.5), C-14(δc 42.8), C-10(δc 37.2), C-4(δc 39.9) and C-20(δc 216.0). On the basis of aforementioned spectral data and its comparison with literature[17,18] the compound (3) was assigned as triterpenoid platanic acid (fig 4).



Compound 1 (*β*-sitosterol)



Compound 3 (platanic acid)



Compound 2 (ursolic acid acetate)

Figure 4.

Structures of compounds

Molluscicidal activity

A preliminary molluscicidal test for ethanol, chloroform and n-hexane extracts of leaves of *Vitex trifolia* against *Biomphalaria alexandrina* adult snails showed that the ethanol extract of leaves with LC_{50} value 26.42 mg/l (27.92 mg/l–24.99 mg/l) was more effective than chloroform extract with LC_{50} value 46.77 mg/l (53.59 mg/l-40.81 mg/l) and n-hexane extract with LC_{50} value 35.48 mg/l (43.81 mg/l–28.72 mg/l) after a 24 h exposure.

DISCUSSION

Herbal remedies used in traditional folk medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs but it is necessary to reveal the active principles by isolation and characterization of their constituents and to validate their possible toxicity. The chemistry of *Vitex trifolia* has not been thoroughly analysed. Previous studies on the chemical composition of this genus indicate predominant constituents of *Vitex* species are flavonoids, diterpenoids, iridoids and ecdysteroids [19].The genus is characterized by the presence of poly-methoxylated flavonoids and C-glycoside flavonoids, which usually co-exist within the same species [20]. In its leaves extracts compounds such as vitexilactone, rotundifuran, vititrifolin D and vitetrifolin E have been identified as cell cycle inhibitors inducing apoptosis in mouse tsFT210 cancer cell line [21]. Several studies have shown that *Vitex trifolia* exhibits interesting activities in relation to the modulation of the inflammatory process. Indeed the antipyretic activity of *Vitex trifolia* seeds has been reported in rabbits [22]. Considering multiple biological activities of *Vitex trifolia* and its wide use in traditional medicines, numerous molecules have been isolated. Isolation and characterization of ursolic acid acetate and platanic acid in *Vitex trifolia* Linn. confirms the presence of triterpenoids also in *Vitex* genus and *Verbenaceae* family. Platanic acid has been already reported as a potential anti-HIV [23] agent. The isolation of this triterpenoid from *Vitex trifolia* indicates a new source for this compound. The natural occurrence of these compounds can be conclusive for the chemotaxonomic characterization of this plant.

Schistosomiasis, caused by the parasite Schistosoma, is a widespread disease in many tropical countries [24]. The life cycle of this parasite involves an intermediate host, represented by snails of the *Biomphalaria* genus, thus, apart from chemotherapy of infected people, one of the strategy to combat this disease is to interrupt the parasites life cycle in endemic areas via the control of the snails population. Considerable molluscicidal activity exhibited by ethanolic extract of leaves suggest that active molluscicide principles were present in higher polarity

ethanol extract which could be isolated and studied for their toxicity. It can validate plant as a potent molluscicide which can be a step ahead in the search for new molluscicides in the process of combating schistosomiasis.

CONCLUSION

1. Ethanolic extract of *Vitex trifolia* have triterpenoids ursolic acid acetate and platanic acid.
2. Ursolic acid acetate is isolated and characterized for the first time in this plant species. Platanic acid is reported for the first time in *Vitex trifolia* and even in the *Verbenaceae* family.
3. Ethanol extract has considerable molluscicidal activity higher than chloroform and n-hexane against *Biomphalaria alexandrina* snails which validates *Vitex trifolia* leaves as a potent molluscicide.

REFERENCES

1. Kirtikar K R, Basu B D. Indian Med Plants 1991:1935.
2. Holdsworth DK. Medicinal plants of Papua New Guinea. South pacific commission technical paper. Noumea, New Caledonia 1977; 175:65.
3. Masateru O, Hiromi S, Yasuyuki I, Koichi M, Toshihiro N. Diterpenoids from fruits of *Vitex trifolia*. Phytochemistry 2000; 55:873-877.
4. Yan L, Zhang Q, Wang Z, Xu L, Yang S. Chemical constituents in *Vitex trifolia*. Zhongcaoyao 2010; 41(10):1622-1624.
5. Gu Q, Zhang X I, Jiang Z, Chen J, Zhou J. Chemical constituents in *Vitex trifolia*. Zhongcaoyao 2007; 38(5):656-659.
6. Kannathasan K, Senthilkumar A, Venketsalu V. Mosquito larvicidal activity of methyl-p-hydroxybenzoate isolated from the leaves of *Vitex trifolia* Linn. Acta Tropica 2011;120:115-118.
7. Davis D G, Bax A. Assignment of Complex 'H NMR Spectra via Two-Dimensional Homonuclear Hartmann-Hahn Spectroscopy. J Amer Chem Soc 1985; 107:2820-2821.
8. WHO. Molluscicide screening and evaluation. B. World Health Organ 1965; 33(4):567-581.
9. El Kamali HH. Larvicidal activity of crude aqueous extracts of *Solenostemma argel* against mosquito larvae. J Herb Spices Med Plants 2001; 8(4): 283-286.
10. Finney D. Probit Analysis. Cambridge 1952.
11. Finney DJ, Stevens WL. A table for the calculation of working probits and weights in probit analysis. Biometrika 1948; 35(1-2):191-201.
12. Matsumura F. Toxicology of Insecticides. 2nd ed. New York, London 1985:4-16.
13. Liebermann C. A textbook of physiological chemistry. Ber Deutsch Chem Gesellsch 1885; 18:1804.
14. Noller CR, Smith AR, Morris GH, Walker JW. Saponins and sapogenin.xx.some coloured reactions of triterpenoid sapogenins. J Am Chem Soc 1942; 12(64):3047.
15. Shachi S, Jagdamba S, Shiv K. New Terpenoid from the rhizomes of *Cyperus Scariosus*. International J Chem Eng App 2010; 1(1):25.
16. Taketa A, Breitmaier E, Schenkel EP. Triterpenes and triterpenoidal glycosides from the fruits of *Ilex paraguariensis* (Maté). J Brazilian Chem Soc 2004; 15(2):205-211.
17. Dorninic YA, Subbaian M. Studies on mitochondrial lipid peroxidation in tumour-bearing rats treated with ursolic acid and ursolic acetate. Med Sci Res 1993; 21(5):213-215.

18. Sukumar E, Erande CM, Chelladurai Ali S. Botanical identity and phytochemistry of a commercial drug-jungle pepper. Indian J Pharm Sci 1988; 50(1):55-56.
19. Li CZ, Su YF, Jin XJ. Advances in studies on chemical constituents from plants of Vitex and their bioactivities. Chin Trad Herb Drugs 2005; 36:930–938.
20. Chen SN, Friesen JB, Webster D, Nikolic D, Van BR, Wang ZJ, Fong HS, Farnsworth NR, Pauli GF. Phytoconstituents from *Vitex agnus-castus* fruits. Fitoterapia 2011; 82(4): 528-533.
21. Li WX, Cui CB, Cai B, Yao XS. Labdane type diterpenes as new cell cycle inhibitors and apotosis inducers from *Vitex trifolia* L. J Asian Nat Prod Res 2005; 7:95-105.
22. Ikram M, Khattak S G, Gilani SN. Antipyretic studies on some indigenous Pakistani medicinal plants:II. J Ethnopharmacol 1987; 75:249-256.
23. Toshihiro F, Kashiwada Y, Robert E, Kilkuskie L, Cosentino M, Lawrence MB, Jack BJ, William PJ, Chen IS, Lee KH. Anti-AIDS Agents, 11. Betulinic acid and platanic acid as anti-HIV principles from *Syzigium claviflorum*, and the anti-HIV activity of structurally related triterpenoids. J Nat Prod 1994; 57(2):243–247.
24. Davis A. Schistosomiasis, In: GC Cook. Manson's Tropical Diseases, 20th ed. WB Saunders 1996:1413-1456.

SKŁADNIKI CHEMICZNE ETANOLOWEGO WYCIĄGU Z LIŚCI I DZIAŁANIE PRZECIWŚLIMAKOWE NIEOCZYSZCZONEGO WYCIĄGU Z *VITEX TRIFOLIA* LINN.

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Streszczenie

Z etanolowego wyciągu z liści *Vitex trifola* wyizolowano *β*-sitosterol i dwa triterpenoidy: octan kwasu ursolowego i kwas platanowy. Poprzednio z liści, łodyg i nasion *Vitex trifola* był wyizolowant *β*-sitosterol. Octan kwasu ursolowego został wyizolowany po raz pierwszy z rośliny tego gatunku. Występowanie kwasu platanowego w *Vitex trifolia* oraz w rodzinie *Verbenaceae* zostało opisane po raz pierwszy. Składniki te scharakteryzowano, używając metod spektroskopowych, stosując badania 1D-^{1}HNMR, ^{13}CNMR, ESIMS and 2D-NMR (HSQC, HMBC, COSY) oraz potwierdzono porównaniem ich danych NMR z danymi literaturowymi. Wstępny test działania przeciwślimakowego wyciągów etanolowego, chloroformowego i n-heksanowego z liści *Vitex trifolia* przeciw dorosłym ślimakom *Biomphalaria*