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Antihyperlipidemic Bioactivity Guided Isolation and Structural Elucidation of Isolated Phytoconstituents from *Convolvulus pluricaulis Choisy*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Atherosclerosis is a crucial element in the origination of coronary heart disease (CHD). Lipid aggregation inside the coronary arteries walls lead to the occurrence of hyperlipidemia. Aim: The research was conducted for assessing the lipid lowering effect of ethanol extract and chloroform fraction from *Convolvulus pluricaulis* Choisy. aerial parts in Triton induced hyperlipidemic rat model.
Methodology: The active fraction was obtained from antihyperlipidemic bioactivity. The phytocomponents were separated from the active fraction using flash chromatography technique resulting in the isolation of n-hexatriacontane; 9-octadecenoic acid- octyl ester; 12, 14-heptacosanedione; dodecyl-octadeca-9,12-dienoate; tetracosanyl 9-hexadecenoate; heptacosan-

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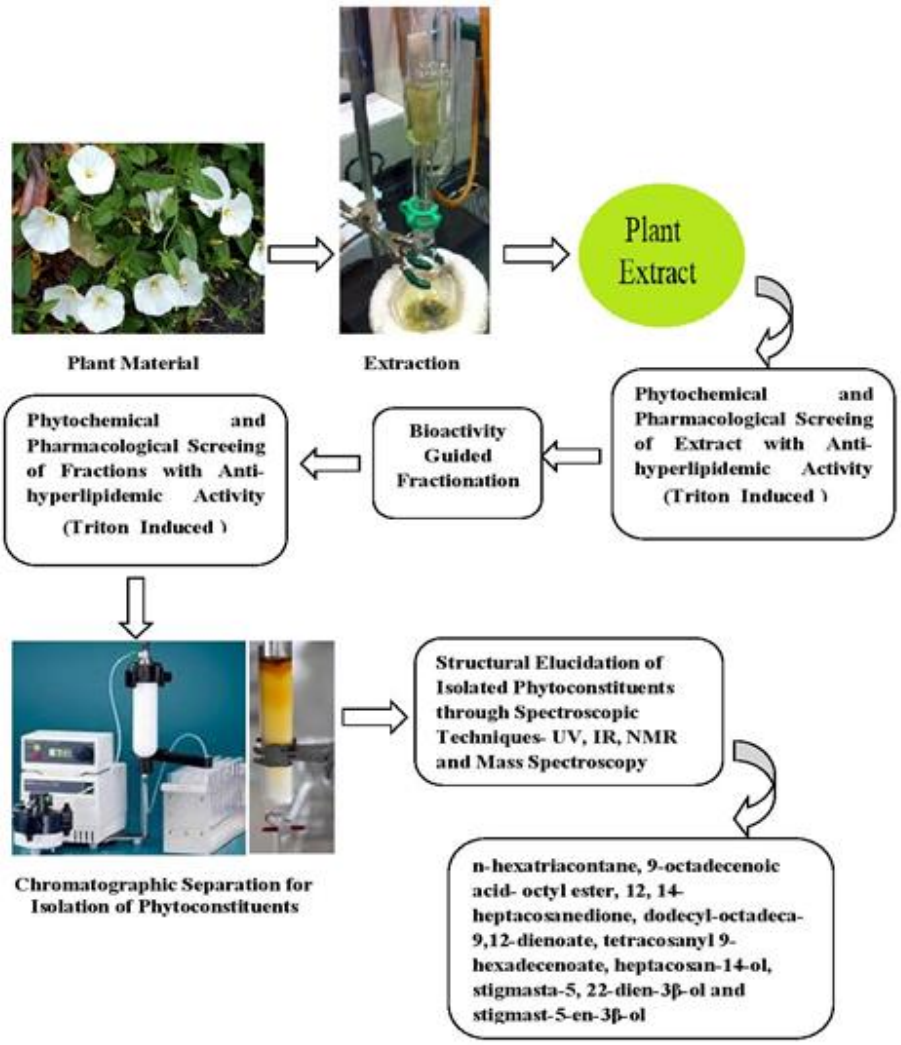
14-ol; stigmasta- 5, 22- dien-3 beta-ol and stigmast-5-en-3 beta-ol. The phytocomponent structures were established by spectral data analysis. Intraperitoneal (i.p.) injection of Triton WR- 1339 at a dose of 400 mg/kg body weight (b.w.) was given to the animals. The ethanol extract at a dose of 200 mg/kg b.w. and chloroform fraction at a dose of 400 mg/kg b.w. were administered orally in rats after 24 hours of the Triton administration.

Results: The study resulted in dose dependent inhibition of triglycerides ($p < 0.01$), total cholesterol ($p < 0.05$), Low density lipoprotein level ($p < 0.05$) while significant increased HDL ($p < 0.01$) level. Alteration in lipid profile in Triton treated rats was restored and found more effective in the chloroform fraction probably due to the presence of phytocomponents. The study elucidated different phytocomponents isolated from the active chloroform fraction of *C. pluricaulis.*

Conclusion: Treatment with extract and fraction manifested significant decrease in triglyceride level. HDL exerts an inhibitory action in the etiology of atherosclerosis. It is considered as a beneficial lipoprotein and its lower level leads to the greater risk of CHD. It may be concluded that the isolated components were responsible for the lipid lowering level of active fraction.

GRAPHICAL ABSTRACT



Keywords: Convolvulus pluricaulis; anti-hyperlipidemic bioactivity; flash chromatography; isolated phytoconstituents.

1. INTRODUCTION

Elevated lipid level in the bloodstream is manifested as hyperlipidemia. Triglycerides, phospholipids, cholesterol and cholesterol esters are well known as lipids that are transported in the blood as lipoproteins. Hyperlipidemia could be either high cholesterol/ triglycerides in the blood or both.

Hyperlipidemia is the manifestation of increased blood lipid levels like total cholesterol [TC], triglycerides [TG] and low-density lipoproteins [LDL] along with the decrease in high- density lipoproteins [HDL]. The state of hyperlipidemia is mainly responsible in initiating the stage of arteriosclerosis. Enervation of the high plasma levels of triglycerides and cholesterol is the basic point in the therapy for hyperlipidemia and arteriosclerosis. Subsequently, the increase in HDL lipid levels is primarily considered as a controlling factor against hyperlipidemia.

Convolvulus pluricaulis Choisy.; Family: Convolvulaceae, commonly known as Shankhpushpi in traditionally based Indian system of medicine. This plant mainly consists of various phytoconstituents like alkaloids e.g. betaine, convolvine, shankhpushpine, phenolic components, flavonoids, coumarins. It is an important herb in the Ayurvedic system of medicine. Its oil is used for promoting hair growth [1]. It is popularly known for its medicinal effect on brain disorders and dysfunctions [2]. The plant is reported to have anxiolytic [3], antiulcer [4], immunomodulatory [5], adaptogenic [6], antioxidant [7] and anthelmintic activities [8].

2. MATERIALS AND METHODS

2.1 Plant Material

Convolvulus pluricaulis aerial parts were procured and authenticated in Botany Department of Saifia College of Science & Education, Bhopal. A voucher specimen of herbarium has been submitted in the botany department.

2.2 Extraction and Fractionation

The dried drug was coarsely powdered and extracted with 90% ethanol in soxhlet apparatus. The ethanol extract was dried to get a dark green colored mass. The yield was found to be 94 g. with a percentage yield of 8.2%. The ethanol extract was then dissolved and extracted by chloroform solvent. The layers were then separated and allowed to evaporate to obtain both the ethanolic and chloroform fractions from the crude extract.

2.3 Phytochemical Profiling

The presence of different phytocomponents was confirmed by various phytochemical analyses. Flavonoids, tannins and alkaloids were found to be present in *C. pluricaulis* ethanol extract while phytosterols were present in the chloroform fraction.

2.4 Instrumentation

C-610 Buchi model flash chromatography system [National Institute of Pharmaceutical Education and Research, Mohali, India] was utilized for the chromatographic separation. Shimadzu UV spectrophotometer 1700 [VNS Faculty of Pharmacy, Bhopal, India] was used to obtain the ultraviolet spectrum. Jasco FT/IR-5300 spectrophotometer [Rajiv Gandhi Technical University, Bhopal, India] was used to record the Infrared spectrum. V 300 and BKS nuclear magnetic resonance spectrometer [National Institute of Pharmaceutical Education and Research, Mohali, India] were used to measure the ^{1}H and ^{13}C NMR spectra using dimethyl sulfoxide and $CDCl_3$ as solvents. Mass spectrum was obtained from JEOL JMS AX-500 mass spectrometer [Rajiv Gandhi Technical University, Bhopal, India].

2.5 Screening for Antihyperlipidemic Activity

Triton loaded albino rats either of male or female sex; 6 - 8 weeks old; 100-120 g weight were selected for evaluating antihyperlipidemic activity.

2.6 Preparation of Test Material

Tween 80 and distilled water combination had used to prepare the test sample of ethanol extract and chloroform fraction.

2.7 Animal Model

Swiss albino rats were chosen and housed in the polypropylene cages. They were maintained under controlled conditions. The animals had access to the food pellets and water *ad libitum*. Prior to experiment, the animals were kept fasting for duration of 12-14 hours and allowed to

the free access to water. Rats either of male or female sex; 6 - 8 weeks old; 100-120 g weight selected for experimentation.

2.8 Measurement of Biochemical Parameters

Albino rats were sorted into 7 groups. One group consisted of 6 rats each. Group I was assigned as a vehicle control group. Group II was administered with Triton only to keep it as a hyperlipidemic group. II-VII group animals were injected intraperitoneally with a dose of 400 mg/kg body weight (b.w.) Triton WR- 1339. Group III animals received atorvastatin with an oral dose of 50 mg/kg b.w., after 24 hours of Triton injection. Group IV and V animals were treated with *C. pluricaulis* ethanol extracts at oral doses of 200mg/kg and 400 mg/kg b.w. Group VI and VII animals were treated with chloroform fraction, by oral doses of 200 mg/kg and 400 mg/kg b.w. The treatment was followed for a period of 5 days to study the effect on blood lipid profile [9].

Ocular puncture technique was used to withdraw the blood samples. The samples were collected in centrifuge tubes. The blood was centrifuged for 20 min. at a speed of 3000 rpm. The supernatant was utilized for the estimation of different parameters. The level of triglycerides, total cholesterol and HDL were determined in the serum by using auto- analyzer according to standard procedures.

2.9 Statistical Analysis

Student's t- test with the help Graph PAD Instat software- Kyplot, was used for statistical evaluation of the data. $p<0.05$ significant value was taken into consideration.

2.10 Chromatographic Studies and Isolation of Compounds

The sample was packed in the silica gel column. The column was eluted by using different solvents in successive manner with increasing polarity. Solvents like Pet. ether, chloroform ($CHCl_3$) and methanol were used to separate phytoconstituents. Recrystallization of all the isolates was done using acetone.

Chloroform fraction sample was subjected to flash chromatography technique. Column elution was done by Petroleum ether: chloroform (1:1). Fractions 1 to 42 were pooled, concentrated to get colorless sticky mass and then recrystallized. The obtained yield was found to be 84 mg. (0.004% yield). $CHCl_3$: Petroleum ether (1:1) was the appropriate TLC mobile phase system showing the best resolution. R_f value of the sample was found to be 0.54.

Elution of the column with the same mobile phase i.e. Pet. ether: chloroform (1:1) was further carried out. Pooling of fractions from 43 to 61 yielded amorphous powder; 130 mg. (0. 005% yield). The R_f value was found to be 0.78. Moving further with the same solvent system of Pet. ether: Chloroform (1:1) and pooling fractions from 62 to 81 yielded amorphous powder; 142 mg. (0. 008% yield).The R_f of the component was 0.75. Column elution with Pet. ether: $CHCl_3$ (1:3) and pooling fractions from 82–112 yielded colorless amorphous powder; 175 mg. (0.008% yield). The R_f value of the component was found to be 0.70, determined by $CHCl_3$: Pet. ether (3:1) TLC mobile phase system.

Column was further eluted with Pet. ether: $CHCl_3$ (1:3). Pooled fractions from 114–128 yielded amorphous powder; 138 mg. (0.006% yield) having R_f: 0.61. Column elution with $CHCl_3$ and pooled fractions from 129–156 yielded amorphous powder; 203 mg. (0.01% yield) having R_f: 0.55.

Column elution with Chloroform: methanol (99:1) and pooled fractions from 157–190 yielded colourless amorphous powder; 240 mg (0.011% yield). The R_f value of the component was found to be 0.45 (determined by Pet. ether: chloroform: methanol, 7:1:2, TLC mobile phase system) and column elution with chloroform: methanol (24:1) and pooled fractions from 191–201 yielded amorphous powder; 215 mg. (0.009% yield). The R_f value of the phytocomponent was 0.47, determined by Pet. ether: chloroform: methanol, 1:4:1, TLC mobile phase system.

2.11 Characterization of Compound I

The phytocompound has melting point.: 74–76 °C; UV (λ_{max}) : 245 nm; IR [KBr]: 2924, 2852, 1461, 1378, 1020, 723 cm^{-1}; ^{1}HNMR [DMSO]: δ 1.31 (8-H, broad singlet, 4 × -CH_2-), 0.94 (56-H, broad singlet, 28 × -CH_2-), 0.98 (4-H, m, 2 × -CH_2-), 0.92 (8-H, b broad singlet, 4 × -CH_2-), 0.79 (6-H, broad singlet, Methyl-1, Methyl-36); ^{13}CNMR [DMSO]: *δ* 29.16 (32 × -CH_2-), 31.59 (-CH_2-), 21.52 (-CH_2-), 13.72 (Methyl-1, Methyl-36); Positive ion FAB-MS (*m*/*z*): 506.985 $[M]^+$($C_{36}H_{74}$).

2.12 Characterization of Compound II

The phytocompound has melting point: 60–62 °C; UV (λ_{max}): 251 nm; IR [KBr]: 2914, 2852, 1725, 1639, 1330, 1115, 781, 749, 703 cm $^{-1}$; ^{1}HNMR [DMSO]: δ 2.45 (2-H, broad singlet, H_2-2), 5.18 (1-H, multiplet, H-10), 4.98 (1-H, multiplet, H-9), 4.02 (2-H, broad singlet, H_2-1′), 1.32 (4-H, broad singlet, H_2-8, H_2-11), 1.27 (34-H, broad singlet, {17 × -CH_2-}), 0.88 (6-H, broad singlet, Methyl-18, Methyl-8′); ^{13}CNMR [DMSO]: δ 175.05 (C 1), 130.24 (C 9), 126.20 (C 10), 63.48 (C 1′), 35.91 (-CH_2-), 30.93 (-CH_2-), 34.89 (-CH_2-), 23.47 (-CH_2-), 33.41 (-CH_2-), 28.65 (-CH_2-), 25.11 (-CH_2-), 19.07 (-CH_2-), 24.16 (-CH_2-), 21.66 (-CH_2-), 20.30 (-CH_2-), 13.29 (Methyl-18), 10.82 (Methyl-8); Positive ion FAB-MS (m/z): 394.7 $[M]^+$($C_{26}H_{50}O_2$).

2.13 Characterization of Compound III

The phytocompound has melting point : 70–72 °C; UV (λ_{max}): 248 nm; IR [KBr]: 2921, 2853, 1736, 1638, 1463, 1377, 1260, 1080, 970, 804, 721 cm^{-1}; ^{1}HNMR [$CDCl_3$]: δ 4.98 (1-H, multiplet, H-9), 5.11 (1-H, broad singlet, H-10), 3.94 (2-H, broad singlet, H_2-1′), 2.65 (2-H, broad singlet, H_2-2), 1.69 (2-H, broad singlet, H_2-8), 1.62 (2-H, broad singlet, H_2-11), 0.97 (6-H, broad singlet, 3 ×-CH_2-), 1.25 (30-H, broad singlet, 15 × -CH_2-), 0.83 (3-H, triplet, J = 5.8 Hz, Methyl-9′); 0.81 (3-H, triplet, J = 5.2 Hz, Methyl-18); ^{13}CNMR [$CDCl_3$]: δ 174.33 (C 1), 124.51 (C 9), 120.08 (C 10), 64.17 (C 1′), 31.44 (-CH_2-), 27.89 (20 × -CH_2-), 14.05 (Methyl-18, Methyl-9′); Positive ion FAB-MS (m/z): 408.7 $[M]^+$($C_{27}H_{52}O_2$).

2.14 Characterization of Compound IV

The phytocompound has melting point.: 77-79 °C; UV (λ_{max}): 244 nm; IR [KBr]: 2916, 2850, 1640, 1705, 1463, 1379, 720 cm^{-1}; ^{1}HNMR [$CDCl_3$]: δ 4.92 (2-H, multiplet, H-9, H-13), 5.09 (2-H, multiplet, H-10, H-12), 2.28 (2H, multiplet, H_2-11), 3.63 (2-H, multiplet, H_2-1′), 2.14 (2-H, broad singlet, H_2-2), 1.54 (2-H, broad singlet, H_2-8), 1.67 (2-H, broad singlet, H_2-14), 1.05 (30-H, broad singlet, 15 × -CH_2), 0.90 (4-H, multiplet, 3 × -CH_2-), 0.84 (3-H, triplet, J = 6.1 Hz, Methyl-18), 0.81 (3-H, triplet, J = 5.9 Hz, Methyl-12′); ^{13}CNMR [$CDCl_3$]: δ 177.11 (C 1), 131.88 (C 12), 128.81 (C 13), 37.24 (C 8), 124.43 (C 9), 118.10 (C 10), 62.10 (C 1′), 38.7 (C 11), 32.79 (C 14), 31.88 (-CH_2-), 30.46 (-CH_2-), 30.64 (12 × -CH_2), 25.03 (-CH_2-), 24.75 (-CH_2-), 23.82 (-CH_2-), 24.45 (-CH_2-), 23.68 (-CH_2-), 21.14 (-CH_2-), 20.06 (Methyl-18), 14.28 (Methyl-12′); Positive ion FAB-MS (m/z): 448.8 $[M]^+$($C_{30}H_{56}O_2$).

2.15 Characterization of Compound V

The phytocompound has melting point: 86–88 °C; UV (λ_{max}): 241 nm; IR [KBr]: 2816, 2050, 1723, 1630, 1452, 1284, 1146, 728 cm^{-1}; ^{1}HNMR [$CDCl_3$]: δ 5.48 (1-H, multiplet, H-9), 4.97 (1-H, m, H-10), 3.15 (2-H, broad singlet, H_2-1′), 2.26 (1-H, doublet, J = 6.9 Hz, H_2-2a), 2.14 (1-H, doublet, J = 7.2 Hz, H_2-2b), 1.73 (2-H, broad singlet, H_2-8), 1.58 (2-H, broad singlet, H_2-11), 1.49 (2-H, multiplet, -CH_2-), 1.08 (2-H, broad singlet, -CH_2-), 1.30 (2-H, broad singlet, -CH_2-), 1.18 (56-H, broad singlet, 28 × -CH_2), 1.04 (3-H, triplet, J = 6.7 Hz, Methyl-16), 0.083 (3-H, triplet, J = 6.8 Hz, Methyl-24′); ^{13}CNMR [$CDCl_3$]: δ 174.23 (C 1), 121.39 (C 9), 118.14 (C 10), 60.25 (C 1′), 37.37 (-CH_2-), 36.64 (-CH_2-), 39.72 (-CH_2-), 34.39 (-CH_2-), 33.44 (-CH_2-), 36.11 (-CH_2-), 30.16 (-CH_2-), 28.54 (18 × -CH_2-), 24.16 (-CH_2-), 26.74 (-CH_2-), 26.02 (-CH_2-), 23.45 (-CH_2-), 21.68 (-CH_2-), 22.03 (Methyl-16), 16.20 (Methyl-24′); Positive ion FAB-MS (m/z): 591 $[M]^+$ ($C_{40}H_{78}O_2$).

2.16 Characterization of Compound VI

The phytocompound has melting point.: 110–112 °C; UV (λ_{max}): 241 nm; IR [KBr]: 3420, 2925, 2854, 1461, 1382, 1050 cm^{-1} ; ^{1}HNMR [DMSO; d_6]: δ 2.63 (2-H, broad singlet, -CH_2-), 4.56 (1-H, broad singlet, $w_{1/2}$ = 16.3 Hz, H-14α), 1.48 (2-H, broad singlet, -CH_2-), 2.11 (2-H, broad singlet,-CH_2-), 1.25 (42-H, broad singlet, 21 × -CH_2-), 0.89 (6-H, broad singlet, Methyl-1, Methyl-27); ^{13}CNMR [DMSO]: δ 72.94 (C-14), 30.65 (-CH_2-), 33.32 (-CH_2-), 27.35 (20 × -CH_2-), 21.16 (-CH_2-), 22.48 (-CH_2-), 15.24 (Methyl-1, Methyl-27); Positive ion FAB-MS (m/z): 396.7 $[M]^+$ ($C_{27}H_{56}O$).

2.17 Characterization of Compound VII

The phytocompound has melting point: 168–170 °C; UV (λ_{max}): 215 nm; IR [KBr]: 3409, 2936, 2850, 1638, 1453, 1371, 1168, 967 cm^{-1}; ^{1}HNMR [DMSO]: δ 5.42(1-H, broad singlet, H-6),5.04(1-H, multiplet, H-22),4.96 (1-H, multiplet, H-23),1.38 (3-H, broad singlet, Methyl-19),0.97(3-H, doublet, J = 7.4 Hz, Methyl-21),0.89(3-H, doublet, J = 6.2 Hz, Methyl-26),0.86(3-H, doublet, J = 6.5 Hz, Methyl-27),0.83(3-H, doublet, J = 5.9 Hz, Methyl-29),0.70(3-H, broad singlet, Methyl-18); ^{13}CNMR [DMSO]: δ 38.33 (C 1), 30.09 (C 2), 67.74 (C 3), 42.92 (C 4), 139.14

(C 5), 120.83 (C 6), 33.26 (C 7), 35.97 (C 8), 52.18 (C 9), 37.28 (C 10), 20.09 (C 11), 38.73 (C 12), 43.92 (C 13), 55.96 (C 14), 23.30 (C 15), 28.29 (C 16), 56.33 (C 17), 12.48 (C 18), 20.33 (C 19), 35.10 (C 20), 19.65 (C 21), 134.36 (C 22), 128.69 (C 23), 46.38 (C 24), 28.53 (C 25), 25.71 (C 26), 17.50 (C 27), 22.50 (C 28), 19.71 (C 29); Positive ion FAB-MS (m/z): 412.7 $[M]^+(C_{29}H_{48}O)$.

2.18 Characterization of Compound VIII

The phytocompound has melting point: 137–138 °C; UV (λ_{max}): 210 nm; IR [KBr]: 3461, 2955, 2845, 1640, 1475, 1365, 1210, 1108 cm^{-1}; ^{1}HNMR [$CDCl_3$]: δ 5.18 (doublet, J = 5.8 Hz, H-6),3.51(1-H, broad singlet, $w_{1/2}$ = 15.8 Hz, H-3α), 1.15 (3-H, broad singlet, Methyl-19), 0.95 (3-H,doublet, J = 6.8 Hz, Methyl-21), 0.83 (3-H, doublet, J = 6.4 Hz, Methyl-29), 0.90 (3-H, doublet, J = 6 Hz, Methyl-27), 0.87 (3-H, triplet, J = 6 Hz, Methyl-26), 0.62 (3-H, broad singlet, Methyl-18); ^{13}C NMR [$CDCl_3$]: 39.28 (C 1), 32.14 (C 2), 68.41 (C 3), 44.57 (C 4), 142.19 (C 5), 120.92 (C 6), 32.12 (C 7), 30.87 (C 8), 48.64 (C 9), 38.71 (C 10), 20.42 (C 11), 40.81 (C 12), 42.79 (C 13), 58.14 (C 14), 22.18 (C 15), 26.55 (C 16), 51.48 (C 17), 10.29 (C 18), 20.34 (C 19), 38.75 (C 20), 19.30 (C 21), 34.29 (C 22), 26.43 (C 23), 46.24 (C 24), 30.18 (C 25), 21.39 (C 26), 19.32 (C 27), 24.57 (C 28), 14.15 (C 29); Positive ion FAB-MS (m/z): 414.7 $[M]^+(C_{29}H_{50}O)$.

3. RESULTS AND DISCUSSION

The rats showed an increase in the serum cholesterol level after their treatment with triton compared to the initial cholesterol level. Ethanol extract and chloroform fraction at a particular dose of 200 mg/kg b.w. decreased the serum total cholesterol (TC) level by 33.59% ($p<0.05$) and 37.14% ($p<0.05$). While ethanol extract and chloroform fraction at a particular dose of 400 mg/kg b.w. decreased serum total cholesterol level by 35.09% ($p<0.05$) and 39.15% ($p<0.05$) accordingly (Table 1). Ethanol extract and chloroform fraction at a particular dose of 200mg/kg b.w. decreased serum triglyceride (TG) level by 36.44% ($p<0.05$) and 41.80% ($p<0.01$) accordingly. While ethanol extract and chloroform fraction at a particular dose of 400 mg/kg b.w. decreased serum triglyceride level by 45.80% ($p<0.01$) and 52.63% ($p<0.01$) accordingly (Table 2). Ethanol extract and chloroform fraction at a particular dose of 200 mg/kg b.w. increased the serum level of high density lipoproteins (HDL) by 8.97% ($p<0.05$) and 11.66% ($p<0.05$). While ethanol extract and chloroform fraction at a particular dose of 400mg/kg b.w. increased the serum level of HDL cholesterol by 12.44% ($p <0.01$) and 14.79% ($p <0.01$) (Table 3). The reduction in serum low density lipoproteins (LDL) level by ethanol extract and chloroform fraction at the dose of 200mg/kg b.w. found to be 68.41% ($p<0.05$) and 70.64% ($p<0.05$). While ethanol extract and chloroform fraction at a particular dose of 400mg/kg b.w. decreased serum LDL cholesterol by 72.90% ($p<0.05$) and 76.88% ($p <0.05$) (Table 4).

Bioactivity guided fractionation and isolation was carried out for the most active chloroform fraction using flash chromatography technique to separate the phytocomponents. The structures of phytocompounds achieved from the chloroform fraction of *C. pluricaulis*, were characterized or elucidated as n-hexatriacontane (Compound I) - +ve ion FABMS m/z:506.985[M]+ ($C_{36}H_{74}$) ; 9-octadecenoic acid - octyl ester (Compound II) - +ve ion FABMS m/z : 394.7 [M]+ ($C_{26}H_{50}O_2$).; 12, 14-heptacosanedione (Compound III)- +ve ion FABMS m/z: 408.7 [M]+ ($C_{27}H_{52}O_2$).; dodecyl-octadeca-9,12-dienoate (Compound IV)- + ve ion FABMS m/z: 448.8 [M]+ ($C_{30}H_{56}O_2$).; tetracosanyl

Table 1. *C. pluricaulis* ethanol extract and $CHCl_3$ fraction effect on the total cholesterol (TC) level in Triton WR- 1339 induced hyperlipidemia model

Groups	Level of total cholesterol after triton injection (mg/dl)	Level of total cholesterol after vehicle/drug administration (mg/dl)
Control Group	67.18 ± 1..20	68.55 ± 1.59
Hyperlipidemic (Triton)	69.51 ± 1.40	99.80 ± 1.26
Atorvastatin (5 0 mg/kg)	87.45 ± 0.56	62.23 ± 0.42 [a]
C. pluricaulis ethanol extract (200 mg/kg)	84.93 ± 1.51	65.73 ± 1.56 [b]
C. pluricaulis ethanol extract (400 mg/kg)	86.84 ± 1.92	57.84 ± 1.31 [b]
C. pluricaulis $CHCl_3$ fraction (200 mg/kg)	87.59 ± 1.15	62.34 ± 0.57 [b]
C. pluricaulis $CHCl_3$ fraction (400 mg/kg)	89.36 ± 1.30	55.42 ± 0.66 [b]

Total cholesterol concentration is determined by standard method. Values given as mean ± S.E.M for 6 animals in each group
[a]: p<0.01 [b]: p<0.05 compared to the Triton hyperlipidemic group

Table 2. *C. pluricaulis* ethanol extract and $CHCl_3$ fraction effect on the triglyceride (TG) level in Triton WR- 1339 induced hyperlipidemia model

Groups	Level of triglyceride after triton injection (mg/dl)	Level of triglyceride after vehicle/drug administration (mg/dl)
Control Group	52.36 ± 0.47	54.23 ± 0.34
Hyperlipidemic (Triton)	51.40 ± 1.56	88.74 ± 1.73
Atorvastatin (50 mg/kg)	66.59 ± 1.24	52.74 ± 1.52 [a]
C. pluricaulis ethanol extract (200 mg/kg)	58.47 ± 1.42	60.73 ± 1.01 [b]
C. pluricaulis ethanol extract (400 mg/kg)	58.89 ± 1.27	57.96 ± 1.12 [a]
C. pluricaulis $CHCl_3$ fraction (200 mg/kg)	58.61 ± 1.85	55.25 ± 1.29 [a]
C. pluricaulis $CHCl_3$ fraction (400 mg/kg)	59.94 ± 1.02	53.48 ± 1.70 [a]

Triglyceride concentration is determined by standard method. Values given as mean ± S.E.M for 6 animals in each group
a: p<0.01 b: p<0.05 compared to the Triton hyperlipidemic group

Table 3. *C. pluricaulis* ethanol extract and $CHCl_3$ fraction effect on the high density lipoprotein (HDL) level in Triton WR- 1339 induced hyperlipidemia model

Groups	Level of HDL after triton injection (mg/dl)	Level of HDL after vehicle/drug administration (mg/dl)
Control Group	35.36 ± 0.98	35.86 ± 0.34
Hyperlipidemic (Triton)	33.43 ± 0.87	34.02 ± 0.91
Atorvastatin (50 mg/kg)	46.24 ±0.43	57.96.± 0.61 [a]
C. pluricaulis ethanol extract (200 mg/kg)	45.66 ± 0.57	47.40 ± 0.71 [b]
C. pluricaulis ethanol extract (400 mg/kg)	44.27 ± 1.50	49.25 ± 1.54 [b]
C. pluricaulis $CHCl_3$ fraction (200 mg/kg)	48.41 ± 0.59	51.84 ± 0.42 [a]
C. pluricaulis $CHCl_3$ fraction (400 mg/kg)	46.38 ± 0.92	52.42 ± 0.96 [a]

HDL concentration is determined by standard method. Values given as mean ± S.E.M for 6 animals in each group
a: p<0.01 b: p<0.05 compared to the Triton hyperlipidemic group

Table 4. *C. pluricaulis* ethanol extract and $CHCl_3$ fraction effect on the low density lipoprotein (LDL) level in Triton WR- 1339 induced hyperlipidemia model

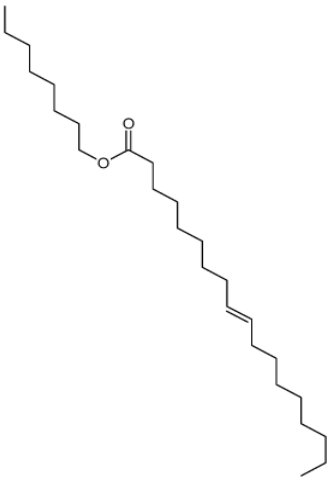
Groups	Level of LDL after triton injection (mg/dl)	Level of LDL after vehicle/drug administration (mg/dl)
Control Group	35.32 ± 0.27	34.75 ± 0.49
Hyperlipidemic (Triton)	35.78 ± 0.66	60.53 ± 0.74
Atorvastatin (50 mg/kg)	58.24 ± 0.42	37.24 ± 0.64 [a]
C. pluricaulis ethanol extract (200 mg/kg)	53.26 ± 0.74	46.73 ± 0.43 [b]
C. pluricaulis ethanol extract (400 mg/kg)	54.84 ± 1.20	43.21 ± 1.54 [b]
C. pluricaulis $CHCl_3$ fraction (200 mg/kg)	56.34 ± 1.32	44.34 ± 0.83 [b]
C. pluricaulis $CHCl_3$ fraction (400 mg/kg)	57.24 ± 1.15	41.45 ± 1.05 [b]

LDL concentration is determined by standard method. Values given as mean ± S.E.M for 6 animals in each group.
a: p<0.01 b: p<0.05 compared to the Triton hyperlipidemic group

9-hexadecenoate (Compound V)- +ve ion FABMS m/z: 591 [M]+ ($C_{40}H_{78}O_2$).; heptacosan-14-ol (Compound VI)- +ve ion FABMS m/z (rel. int.):396.7[M]+ ($C_{27}H_{56}O$).; stigmasta-5, 22-dien-3 beta -ol (Compound VII)- +ve ion FABMS m/z:412.7 [M]+ ($C_{29}H_{48}O$) and stigmast-5-en-3 beta-ol (Compound VIII)- +ve ion FABMS m/z :414.7[M]+ ($C_{29}H_{50}O$) (Figs. 1-8). Modern pharmacological studies have shown that these phytocompounds exhibit antioxidant, antimicrobial, anti-inflammatory, hypoglycaemic, anti-tumor and immune regulating effects. It is of great nutritional and medicinal value. Phytosterols are the plant based bioactive compounds. They exert a wide number of effects like antimicrobial, immunomodulatory, antidiabetic, antiobesity, anti-inflammatory etc. Phytosterols have a great potential as anticancer agents and phytosterol-rich diets may reduce the risk of cancer by 20% [10-13].

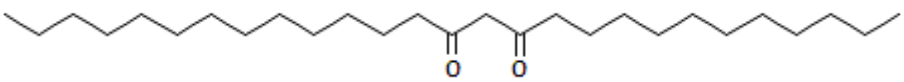
$CH_3(CH_2)_{34}CH_3$
n-hexatriacontane (Compound I)

Fig. 1.



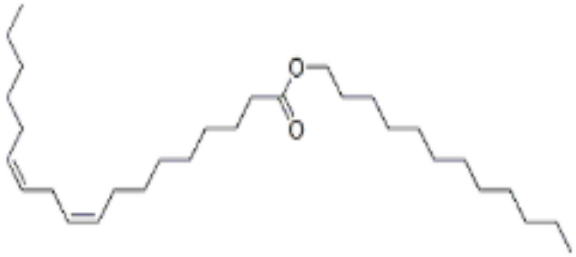
9-octadecenoic acid- octyl ester (Compound II)

Fig. 2.



12, 14-heptacosanedione (Compound III)

Fig. 3.



Dodecyl-octadeca-9, 12-dienoate (Compound IV)

Fig. 4.

$CH_3(CH_2)_5CH = CH(CH_2)_7\text{-}CO\text{-}O\text{-}(CH_2)_{23}CH_3$

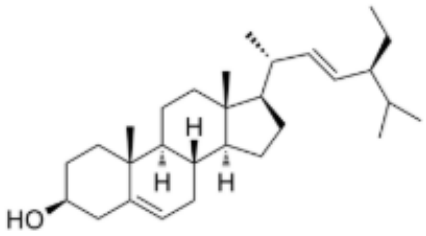
Tetracosanyl 9-hexadecenoate (Compound V)

Fig. 5.

$CH_3(CH_2)_{12}\text{-}CHOH\text{-}(CH_2)_{12}\text{-}CH_3$

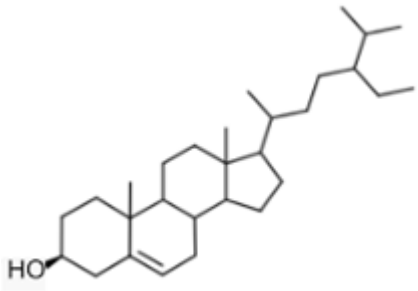
Heptacosan-14-ol (Compound VI)

Fig. 6.



Stigmasta-5, 22-dien-3 beta-ol (Compound VII)

Fig. 7.



Stigmast-5-en-3 beta-ol (Compound VIII)

Fig. 8.

Triton acts as surface active agent that obstructs the lipoproteins uptake from the circulation by extra-hepatic tissues through suppressing the action of lipases thus, resulting in raised lipid concentration [14]. The conceivable mode of action behind this is may be the enhanced action of lecithin-acyl transferase (LCAT) and decreased activity of hepatic TG lipase on HDL [15]. LCAT performs an important role in the fusion of HDL and free cholesterol, further transporting it back to VLDL and LDL [16-17].

Lack of LDL receptors is a crucial factor in hypercholesterolemia. By administration of the test samples, the reduction in the cholesterol and LDL levels demonstrates a possible protection against hypercholesterolemia [18, 19]. The ethanol extract, chloroform fraction and the active phytocomponents reduced the level of triglycerides, total cholesterol, LDL and increased significantly the HDL level. The accumulation of oxidatively modified low density lipoproteins in the arterial wall causes endothelial dysfunction leading to the development of congestive heart diseases and atherosclerosis [20, 21].

Treatment with *C. pluricaulis* ethanol extract and chloroform fraction showed inhibitory effects both on triglyceride and total cholesterol level after triton injection. The dose of 400 mg/kg chloroform fraction showed the maximum inhibitory action on the increase of serum TG and TC level. The drug extract and fraction also showed protective action by raising the HDL level.

Convolvulus pluricaulis is a traditional medicinal plant. It exhibits neuropharmacological actions like anxiolytic, anticonvulsant, sedative, antidepressant, nootropic, antistress and tranquilizing activities. It also exerts antiulcer, febrifuge, anti-microbial, insecticidal, anti-inflammatory, pain relieving, diuretic, hypoglycemic properties. It's reported pharmacological actions highlights its therapeutic potential in central nervous system disorders in Ayurveda.

4. CONCLUSION

The work involved the separation or isolation of active phytocomponents from the chloroform fraction of *C. pluricaulis* and could be responsible for lipid lowering action. Different types of hyperlipidemia can be effectively treated using various herbal remedies. They are not only

responsible to inhibit the raise of serum TG, TC and LDL level but also have a potential to elevate the beneficial HDL cholesterol level in the blood.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical committee of the Research Centre with CPCSEA Reg. No.-778/03/c/CPCSEA had approved the usage of animals for the experiment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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