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

ABSTRACT

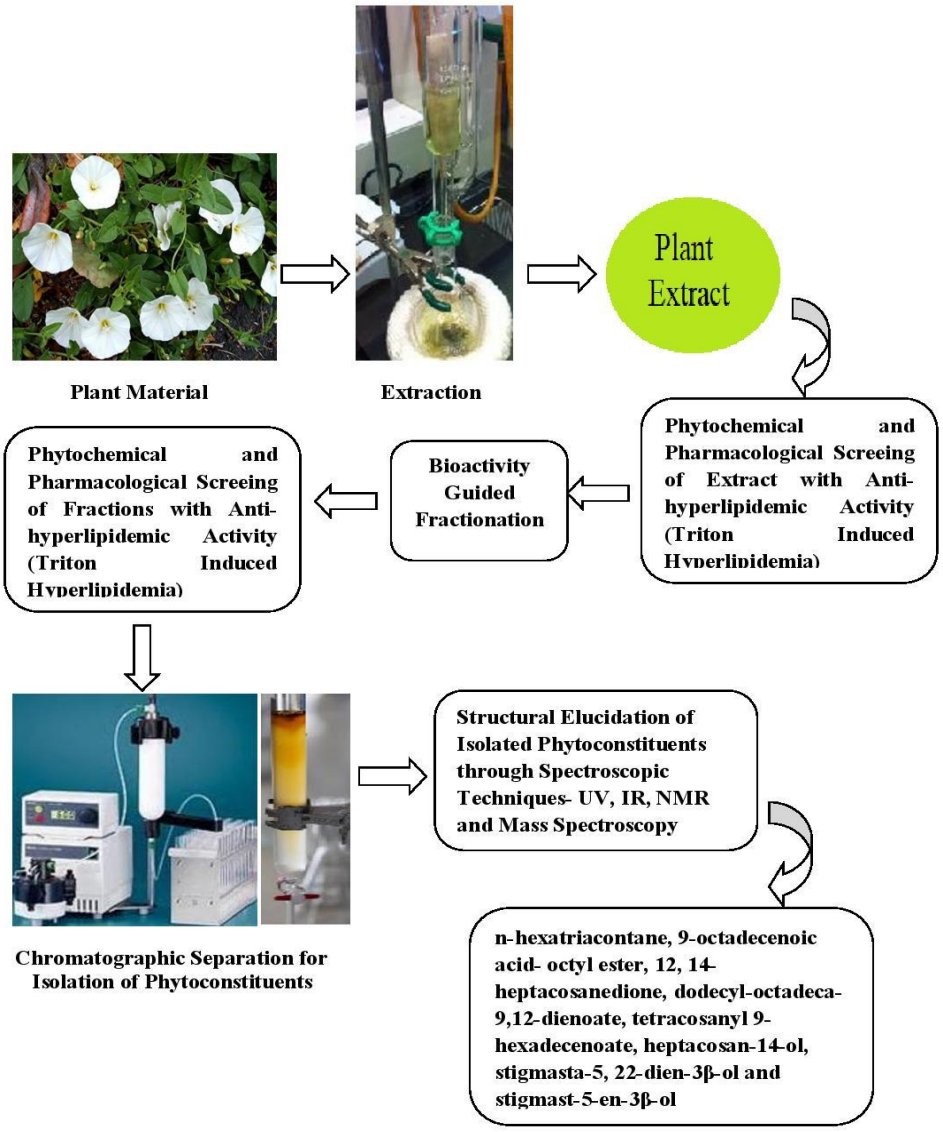
Coronary heart diseases are the clinical manifestation of atherosclerosis. Development of hyperlipidemia involves accumulation of lipid containing particles in the walls of coronary arteries. The present study was undertaken to explore the antihyperlipidemic effect of ethanolic extract of stems of *Convolvulus pluricaulis* Choisy. and its chloroform fraction in Triton induced hyperlipidemic rats. Flash chromatography was done for the most active fraction obtained from antihyperlipidemic bioactivity resulting in the isolation of n-hexatriacontane; 9-octadecenoic acid- octyl ester; 12, 14-heptacosanedione; dodecyl-octadeca-9,12-dienoate; tetracosanyl 9-hexadecenoate; heptacosan-14-ol; stigmasta-5, 22-dien-3β-ol and stigmast-5-en-3β-ol. The structure of the components has been established on the basis of spectral data analysis. Animals were administered with i.p. injection of Triton WR 1339 at dose of 400 mg/kg body weight. After 24 h. of Triton administration the ethanolic extract and its fraction were administered orally at doses of 200 and 400 mg/kg body weight in rats. The study dose dependently inhibited the total cholesterol ($p<0.05$), triglycerides ($p<0.01$), LDL level ($p<0.05$), and significantly increased HDL ($p<0.01$) level. The chloroform fraction was found to be more effective in restoring lipid profile changes in rats treated with Triton probably due to the presence of phytoconstituents. The present work characterized different isolated phytoconstituents from active fraction of *C. pluricaulis.* Treatment with extract and fraction showed

significant decreased in triglyceride. HDL is considered to be a beneficial lipoprotein as it has an inhibitory effect in the pathogenesis of atherosclerosis. Low level of HDL is associated with high risk of coronary artery disease. It may be concluded that the lowering of lipid level from active fraction is due to the presence of isolated components.

Keywords:

Convolvulus pluricaulis; Anti-hyperlipidemic bioactivity; Flash Chromatography; Isolated Phytoconstituents

Graphical abstract



INTRODUCTION

Hyperlipidemia is an elevation of lipids in the bloodstream. These lipids include cholesterol, cholesterol esters, phospholipids and triglycerides. They are transported in the blood as part of large molecules called lipoproteins. Hyperlipidemia is a general term,

it could be either high cholesterol in the blood (hypercholesterolemia), high triglycerides in the blood (hypertriglyceridemia) or it could be both.

Elevated plasma lipid levels, mainly total cholesterol (TC), triglyerides (TG) and low density lipoproteins (LDL) along with decrease in high density lipoproteins (HDL) are known to cause hyperlipidemia which is core in initiation and progression of arteriosclerosis impasse. Therefore prime consideration in therapy for hyperlipidemia and arteriosclerosis is to enervate the elevated plasma level of TC, TG and LDL along with increase in HDL lipid levels.

Convolvulus pluricaulis Choisy. (Convolvulaceae) is commonly known as Shankhpushpi in Indian traditional medicine. The plant contains alkaloids- shankhapushpine, convolvuline and betaine. Fresh plant contains volatile oil and potassium chloride. It also contains a yellow neutral fat, an organic acid and saline substances. The plant is used as a vermifuge and, with oil for promoting growth of hair [1]. It is well known for its therapeutic effect on brain disorders in Ayurvedic system of medicine [2], anxiolytic activity [3], antiulcer activity [4], immunomodulatory activity [5], adaptogenic [6] and antioxidant properties [7]. Phytochemically the plant has been reported to contain aliphatic hydrocarbons, fatty acids and alkaloids [8].

MATERIAL AND METHODS

Plant Material:

The aerial parts of *Convolvulus pluricaulis* L. were collected from Bhopal (M.P.), India and were identified and authenticated by Dr. Zia ul Hassan, Assistant professor,

Department of Botany, Saifia College of Science & Education, Bhopal. A voucher specimen No.175/Bot/Saifia/2010 is deposited in the herbarium of botany department.

Extraction and Fractionation:

The dried drug was coarsely powdered and then exhaustively extracted with 90% ethanol in soxhlet apparatus. The ethanolic extract was freed of solvent under vacuum to get 94 g (8.2% yield) of dark greenish mass. The solvent free ethanolic extract was further dissolved and extracted with chloroform. The chloroform layer was separated and allowed to evaporate. Ethanolic fraction and chloroform fraction were thus obtained.

Phytochemical Profiling:

Qualitative chemical test were performed to assess the presence of various phytoconstituents. The preliminary phytochemical screening revealed the presence of tannins, flavanoids and alkaloids in ethanolic extract of *C. pluricaulis* while chloroform fraction revealed the presence of sterols.

Instrumentation:

Flash chromatography was done using a Buchi controller C-610 apparatus (NIPER, Mohali, India). The ultraviolet (UV) spectra were recorded on a spectrometer by Shimadzu UV 1700 model in MeOH/EtOH (VNS Institute of Pharmacy, Bhopal, MP, India). The infrared (IR) spectra were measured on a Jasco FT/IR-5300 spectrophotometer (RGTU, Bhopal, MP, India). The ^{1}H and ^{13}C spectra were recorded on a V 300 and BKS nuclear magnetic resonance (NMR) spectrometer (NIPER) using dimethyl sulfoxide (DMSO) and $CDCl_3$ as solvents. Mass spectra were recorded on a Scan AP spectrometer/JEOL JMS AX-500 spectrometer (RGTU, Bhopal, MP, India).

Screening for hypolipidemic activity:

Screening for hypolipidemic activity was carried out in Triton loaded albino rats of either sex, 6 - 8 weeks old and weighing 100-120 g were selected for the experiments.

Preparation of test material:

Ethanolic extract and chloroform fraction were suspended in distilled water plus polyoxyethylenesorbiton Mono-oleate (Tween 80).

Animal Model:

The Swiss albino rats were selected and housed in polypropylene cages maintained under controlled conditions. The animals were fed with pellet food and water *ad libitum*. The animals fasted for 12-14 h before experimentation but it was allowed free access to water. Rats of either sex, 6 - 8 weeks old and weighing 100-120 g, were taken for the experiments. The usage of animals were approved by the ethical committee of the Research Centre having following CPCSEA Reg. No.-778/03/c/CPCSEA.

Measurement of biochemical parameters:

Albino rats were divided into seven groups of six rats each. Group I served as vehicle control. Group II was kept as hyperlipidemic and administered with Triton only. Group II-VII were given i.p. injection of Triton WR 1339 at dose of 400 mg/kg body weight. After 24 h. of Triton administration, animals of Group III received atrovastatin at the oral dose of 50 mg/kg. Group IV and V were treated with ethanolic extracts of *C. pluricaulis* at the oral dose of 200mg/kg and 400mg/kg. Group VI and VII were treated with chloroform fraction, at the oral dose of 200mg/kg and 400mg/kg. The treatment was continued for 5 days with a view to see the effect on lipid profile [9].

The blood samples were withdrawn by ocular puncture and transferred directly into centrifuge tubes and allowed to clot at room temperature for 20-25 min and centrifuged

for 20 min at 3000 rpm. The supernatant clear serum thus obtained was transferred carefully with the help of micropipette into small test tubes for estimation. The serum concentration of total cholesterol, HDL and triglyceride were measured by standard procedure using auto- analyzer.

Statistical analysis:

Statistical evaluation of the data was done by Student't' test. (Graph PAD Instat software, Kyplot). A value of $p<0.05$ was considered to be significant.

Chromatographic Studies and Isolation of Compounds:

The solvent free ethanolic extract was dissolved and extracted with chloroform. It was dried, packed and chromatographed over silica gel column. The column was eluted with petroleum ether, chloroform and methanol successively in the order of increasing polarity to isolate phytoconstituents.

Flash chromatography (Buchi controller C-610) was done for the chloroform fraction. Elution of the column with Petroleum ether: $CHCl_3$ (1:1) [fraction 1–42] yielded colourless sticky mass, recrystallised from acetone. The yield was found to be 84 mg (0.004% yield). Thin layer chromatography (TLC) of the powdered sample was carried out using various solvent systems. The appropriate one found to be $CHCl_{3:}$ Petroleum ether (1: 1). This solvent system gave the best resolution. R_f value was found to be 0.54.

Further elution of the column with Petroleum ether: $CHCl_3$ (1:1) [fraction 43–60] gave colourless amorphous powder and recrystallised from acetone. The yield so obtained was 130 mg (0.005% yield). R_f value was found to be 0.78 from $CHCl_3$: Petroleum ether (1:1) TLC solvent system. Again elution of the column with Petroleum ether: $CHCl_3$ (1:1) [fraction 61–81] produced colourless amorphous powder which was

recrystallised from acetone, 142 mg (0.008% yield); R_f: 0.75 ($CHCl_3$: Petroleum ether, 1:1). Elution of the column with Petroleum ether: $CHCl_3$ (1:3) [fraction 82–110] afforded colourless amorphous powder, recrystallised from acetone, 175 mg (0.008% yield). R_f value was found to be 0.70 from $CHCl_3$: Petroleum ether (3:1) TLC solvent system.

Further elution of the column with Petroleum ether: $CHCl_3$ (1:3) [fraction 111–128] furnished colourless amorphous powder, recrystallised from acetone; 138 mg (0.006% yield); R_f: 0.61 ($CHCl_3$: Petroleum ether, 3:1). Again elution of the column with $CHCl_3$ (129–155) yielded a colourless amorphous powder which was further recrystallised from acetone: ethyl acetate (1:1), 203 mg (0.01% yield); R_f: 0.55 ($CHCl_3$).

Elution of the column with $CHCl_3$: Methanol (99:1) (156–185) yielded colourless amorphous powder and recrystallised from methanol, 240 mg (0.011% yield); R_f: 0.45 (Petroleum ether: $CHCl_3$: Methanol, 7:1:2) and Elution of the column with $CHCl_3$:Methanol (24:1) [fraction 186–201] yielded colourless amorphous powder of which was recrystallised from methanol; 215 mg (0.009% yield); R_f: 0.47 (Petroleum ether: $CHCl_3$: Methanol, 1:4:1).

Characterization of Compound I:

The UV spectra were recorded on spectrometer by Shimadzu UV 1700 model in EtOH. The IR spectra were measured on a Jasco FT/IR-5300 spectrophotometer. The ^{1}H spectra and ^{13}C spectra were recorded on aV 300 and bks NMR spectrometer, using Dimethyl

sulfoxide (DMSO) and $CDCl_3$ as solvents. Mass spectra were recorded on a Scan AP spectrometer/JEOL JMS AX- 500 spectrometer.

The compound has m.p.: 74–76 °C; UV λ_{max} : 245 nm; IR (KBr): 2924, 2852, 1461, 1378, 1020, 723 cm^{-1}; ^{1}HNMR (DMSO-): δ 1.53 (4H, m, 2 × CH_2), 1.29 (56H, brs, 28 × CH_2), 1.21 (8H, brs, 4 × CH_2), 0.84 (6H, brs, Me-1, Me-36); ^{13}C NMR (DMSO): δ 31.59 (CH_2), 28.54 (32 × CH_2), 22.13 (CH_2), 14.18 (Me-1, Me-36); Positive ion FAB MS *m/z*: 506.985 $[M]^+(C_{36}H_{74})$.

Characterization of Compound II:

The compound has m.p.: 60–62 °C; UV λ_{max}: 251 nm; IR (KBr): 2914, 2852, 1725, 1639, 1330, 1115, 781, 749, 703 cm^{-1}; ^{1}H NMR (DMSO): δ 5.34 (1H, m, H-9), 5.11 (1H, m, H-10), 3.65 (2H, brs, H_2-1′), 2.33 (2H, brs, H_2-2), 1.63 (4H, brs, H_2-8, H_2-11), 1.26 (34H, brs, (17 × CH_2), 0.87 (6H, brs, Me-18, Me-8′); ^{13}C NMR (DMSO): δ 173.12 (C-1), 129.14 (C-9), 115.40 (C-10), 63.28 (C-1′), 36.71 (CH_2), 34.89 (CH_2), 33.41 (CH_2), 30.93 (CH_2), 28.65 (CH_2), 25.11 (CH_2), 24.16 (CH_2), 23.47 (CH_2), 21.66 (CH_2), 20.30 (CH_2), 19.07 (CH_2), 13.24 (Me-18), 11.35 (Me-8); +ve ion FABMS *m/z* : 394.7 $[M]^+(C_{26}H_{50}O_2)$.

Characterization of Compound III:

The compound has m.p.: 70–72 °C; UV λ_{max} (MeOH): 248 nm; IR (KBr): 2921, 2853, 1736, 1638, 1463, 1377, 1260, 1080, 970, 804, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.37 (1H, m, H-9), 5.11 (1H, brs, H-10), 4.12 (2H, brs, H_2-1′), 2.04 (2H, brs, H_2-2), 1.69 (2H, brs, H_2-8), 1.59 (2H, brs, H_2-11), 1.25 (30H, brs, 15 × CH_2), 1.02 (6H, brs, 3 × CH_2), 0.87 (3H, t, J = 6.2 Hz, Me-18), 0.85 (3H, t, J = 6.2 Hz, Me-9′); ^{13}C NMR ($CDCl_3$): δ 173.11 (C-1), 123.62 (C-9), 121.28 (C-10), 63.17 (C-1′), 30.81 (CH_2), 28.57 (20 × CH_2), 13.37 (Me-18, Me-9′); +ve ion FABMS *m/z*: 408.7 $[M]^+(C_{27}H_{52}O_2)$.

Characterization of Compound IV:

The compound has m.p.: 76–78 °C; UV λ_{max}: 242 nm; IR (KBr): 2918, 2850, 1735, 1638, 1463, 1379, 719 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.29 (2H, m, H-10, H-12), 5.13 (2H, m, H-9, H-13), 3.63 (2H, m, H_2-1′), 2.28 (2H, m, H_2-11), 2.04 (2H, brs, H_2-2), 1.68 (2H, brs, H_2-8), 1.59 (2H, brs, H_2-14), 1.25 (30H, brs, 15 × CH_2), 1.00 (4H, m, 3 × CH_2), 0.87 (3H, t, J = 6.3 Hz, Me-18), 0.85 (3H, t, J = 6 Hz, Me-12′); ^{13}C NMR ($CDCl_3$): δ 177.11 (C-1), 131.88 (C-12), 128.81 (C-13), 124.43 (C-9), 119.08 (C-10), 63.08 (C-1′), 39.7 (C-11), 37.29 (C-8), 32.79 (C-14), 31.91 (CH_2), 31.42 (CH_2), 29.69 (12 × CH_2), 26.39 (CH_2), 25.72 (CH_2), 24.81 (CH_2), 24.45 (CH_2), 22.68 (CH_2), 21.07 (CH_2), 19.72 (Me-18), 14.11 (Me-12′); +ve ion FABMS *m/z*: 448.8 $[M]^+(C_{30}H_{56}O_2)$.

Characterization of Compound V:

The compound has m.p.: 85–87 °C; UV λ_{max}: 241 nm; IR (KBr): 2919, 2049, 1728, 1636, 1465, 1282, 1166, 723 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.35 (1H, m, H-9), 5.11 (1H, m, H-10), 3.66 (2H, brs, H_2-1′), 2.41 (1H, d, J = 7.2 Hz, H_2-2a), 2.13 (1H, d, J = 7.2 Hz, H_2-2b), 1.68 (2H, brs, H_2-8), 1.60 (2H, brs, H_2-11), 1.41 (2H, m, CH_2), 1.37 (2H, brs, CH_2), 1.33 (2H, brs, CH_2), 1.25 (56H, brs, 28 × CH_2), 0.87 (3H, t, J = 6.9 Hz, Me-16), 0.085 (3H, t, J = 6.9 Hz, Me-24′); ^{13}C NMR ($CDCl_3$): δ 173.15 (C-1), 124.38 (C-9), 119.08 (C-10), 61.18 (C-1′), 39.72 (CH_2), 37.37 (CH_2), 37.11 (CH_2), 36.64 (CH_2), 34.39 (CH_2), 33.44 (CH_2), 30.13 (CH_2), 29.70 (18 × CH_2), 27.11 (CH_2), 26.71 (CH_2), 25.02 (CH_2), 24.46 (CH_2), 22.69 (CH_2), 21.03 (Me-16), 14.11 (Me-24′); +ve ion FABMS *m/z*: 591 $[M]^+(C_{40}H_{78}O_2)$.

Characterization of Compound VI:

The compound has m.p.: 110–112 °C; UV λ_{max}: 241 nm; IR (KBr): 3425, 2935, 2850, 1465, 1380, 1059 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 4.13 (1H, brs, $w_{1/2}$ = 15.6 Hz, H-14α), 2.50 (2H, brs, CH_2), 2.19 (2H, brs, CH_2), 1.52 (2H, brs, CH_2), 1.27 (42H, brs, 21 × CH_2), 0.88 (6H, brs, Me-1, Me-27); ^{13}C NMR (DMSO): δ 73.11 (C-14), 33.32 (CH_2), 30.75 (CH_2), 28.43 (20 × CH_2), 24.06 (CH_2), 21.48 (CH_2), 13.23 (Me-1, Me-27); +ve ion FABMS *m*/*z*: 396.7 $[M]^+(C_{27}H_{56}O)$.

Characterization of Compound VII:

The compound has m.p.: 168–170 °C; UV λ_{max}: 215 nm; IR (KBr): 3419, 2922, 2852, 1636, 1463, 1378, 1164, 965 cm^{-1}; ^{1}H NMR (DMSO): δ 5.16 (1H, brs, H-6), 5.14 (1H, m, H-22), 5.07 (1H, m, H-23), 1.12 (3H, brs, Me-19), 0.97 (3H, d, *J* = 7.8 Hz, Me-21), 0.88 (3H, d, *J* = 6.5 Hz, Me-26), 0.85 (3H, d, *J* = 6.3 Hz, Me-27), 0.81 (3H, d, J = 6.0 Hz, Me-29), 0.68 (3H, brs, Me-18); ^{13}C NMR (DMSO): δ 37.33 (C-1), 31.09 (C-2), 69.74 (C-3), 41.92 (C-4), 141.08 (C-5), 119.83 (C-6), 33.23 (C-7), 34.97 (C-8), 50.18 (C-9), 36.68 (C-10), 21.09 (C-11), 39.78 (C-12), 41.92 (C-13), 55.98 (C-14), 24.34 (C-15), 27.29 (C-16), 55.33 (C-17), 11.48 (C-18), 20.31 (C-19), 35.09 (C-20), 18.65 (C-21), 137.38 (C-22), 128.69 (C-23), 45.08 (C-24), 28.52 (C-25), 25.73 (C-26), 18.69 (C-27), 23.50 (C-28), 18.71 (C-29); +ve ion FABMS *m*/*z*: 412.7 $[M]^+(C_{29}H_{48}O)$.

Characterization of Compound VIII:

The compound has m.p: 137–138 °C; UV λ_{max}: 210 nm; IR (KBr): 3461, 2955, 2845, 1640, 1475, 1365, 1210, 1108 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.30 (d, *J* = 5.5 Hz, H-6), 3.51 (1H, brs, $w_{1/2}$ = 16.5 Hz, H-3α), 1.01 (3H, brs, Me-19), 0.97 (3 H, d, *J* = 6.5 Hz, Me-21), 0.86 (3 H, d, *J* = 6.0 Hz, Me-29), 0.85 (3 H, d, *J* = 6.0 Hz, Me-27), 0.82 (3H, t, *J* = 6.2 Hz, Me-26), 0.67 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): 37.33 (C-1), 31.63 (C-

2), 69.51 (C-3), 41.98 (C-4), 141.17 (C-5), 119.94 (C-6), 31.15 (C-7), 31.81 (C-8), 49.57 (C-9), 36.74 (C-10), 21.66 (C-11), 39.80 (C-12), 41.98 (C-13), 56.04 (C-14), 24.19 (C-15), 28.60 (C-16), 55.41 (C-17), 11.36 (C-18), 19.30 (C-19), 36.74 (C-20), 18.75 (C-21), 33.30 (C-22), 25.73 (C-23), 45.14 (C-24), 29.15 (C-25), 20.37 (C-26), 19.30 (C-27), 23.56 (C-28), 11.03 (C-29); +ve ion FAB MS *m/z* : 414.7 $[M]^+(C_{29}H_{50}O)$.

RESULTS AND DISCUSSION:

The rats treated with triton showed increase in serum cholesterol level as compared to initial level. Ethanolic extract and its chloroform fraction at dose of 200mg/kg decreased serum level of total cholesterol by 33.62 % ($p<0.05$) and 37.14 % ($p<0.05$). On the other hand ethanolic extract and its chloroform fraction at dose of 400mg/kg decreased serum level of total cholesterol by 35.09 % ($p<0.05$) and 39.15% ($p<0.05$) respectively (Table-1). Ethanolic extract and its chloroform fraction at dose of 200mg/kg decreased serum level of triglyceride level by 36.44 % ($p<0.05$) and 41.80% ($p<0.01$) respectively. On the other hand ethanolic extract and its chloroform fraction at dose of 400mg/kg decreased serum level of triglyceride level by 45.80% ($p<0.01$) and 51.23% ($p<0.01$) respectively (Table-2). Ethanolic extract and chloroform fraction at dose of 200mg/kg increased the serum HDL cholesterol level by 9.41% ($p<0.05$) and 11.66% ($p<0.05$). On the other hand ethanolic extract and fraction at dose of 400mg/kg increased the serum HDL cholesterol level by 12.57 % ($p<0.01$) and 14.79% ($p<0.01$) (Table-3). The reduction in LDL cholesterol level by ethanolic extract and chloroform fraction at dose of 200mg/kg were found to be 68.41% ($p<0.05$) and 70.64% ($p<0.05$). On the other hand ethanolic extract and its chloroform fraction at dose of 400mg/kg decreased LDL cholesterol by 72.90 % ($p<0.05$) and 76.88% ($p<0.05$) (Table-4).

Flash chromatography was done for the most active chloroform fraction based on bioactivity resulting in the isolation of phytoconstituents. The structure of phytocompounds obtained from chloroform fraction of *C. pluricaulis*, were elucidated as n-hexatriacontane (Compound I); 9-octadecenoic acid - octyl ester (Compound II); 12, 14-heptacosanedione (Compound III); dodecyl-octadeca-9,12-dienoate (Compound IV); tetracosanyl 9-hexadecenoate (Compound V); heptacosan-14-ol (Compound VI); stigmasta-5, 22-dien-3β-ol (Compound VII) and stigmast-5-en-3β-ol (Compound VIII) (Figures 1-8). Modern pharmacological studies have shown that these phytocompounds has anti-inflammatory, anti microbial, antioxidative, hypoglycaemic, anti-tumour, and immune regulating effects and is of great nutritional and medicinal value. Phytosterols are bioactive compounds found in foods of plant origin. They have been proposed to exert a wide number of effects like anti-obesity, anti-diabetic, anti-microbial, anti-inflammatory, and immunomodulatory effects. Also, anticancer effects have been strongly suggested, as phytosterol-rich diets may reduce the risk of cancer by 20% [10-13].

The compound I has m.p.: 74–76 °C; UV λ_{max} : 245 nm; IR (KBr): 2924, 2852, 1461, 1378, 1020, 723 cm^{-1}; ^{1}HNMR (DMSO-): δ 1.53 (4H, m, 2 × CH_2), 1.29 (56H, brs, 28 × CH_2), 1.21 (8H, brs, 4 × CH_2), 0.84 (6H, brs, Me-1, Me-36); ^{13}C NMR (DMSO): δ 31.59 (CH_2), 28.54 (32 × CH_2), 22.13 (CH_2), 14.18 (Me-1, Me-36); Positive ion FAB MS *m/z*: 506.985 $[M]^+$($C_{36}H_{74}$). The compound II has m.p.: 60–62 °C; UV λ_{max}: 251 nm; IR (KBr): 2914, 2852, 1725, 1639, 1330, 1115, 781, 749, 703 cm^{-1}; ^{1}H NMR (DMSO): δ 5.34 (1H, m, H-9), 5.11 (1H, m, H-10), 3.65 (2H, brs, H_2-1′), 2.33 (2H, brs, H_2-2), 1.63 (4H, brs, H_2-8, H_2-11), 1.26 (34H, brs, (17 × CH_2), 0.87 (6H, brs, Me-18, Me-

8′); ^{13}C NMR (DMSO): δ 173.12 (C-1), 129.14 (C-9), 115.40 (C-10), 63.28 (C-1′), 36.71 (CH_2), 34.89 (CH_2), 33.41 (CH_2), 30.93 (CH_2), 28.65 (CH_2), 25.11 (CH_2), 24.16 (CH_2), 23.47 (CH_2), 21.66 (CH_2), 20.30 (CH_2), 19.07 (CH_2), 13.24 (Me-18), 11.35 (Me-8); +ve ion FABMS *m/z* : 394.7 $[M]^+(C_{26}H_{50}O_2)$. The compound III has m.p.: 70–72 °C; UV λ_{max} (MeOH): 248 nm; IR (KBr): 2921, 2853, 1736, 1638, 1463, 1377, 1260, 1080, 970, 804, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.37 (1H, m, H-9), 5.11 (1H, brs, H-10), 4.12 (2H, brs, H_2-1′), 2.04 (2H, brs, H_2-2), 1.69 (2H, brs, H_2-8), 1.59 (2H, brs, H_2-11), 1.25 (30H, brs, 15 × CH_2), 1.02 (6H, brs, 3 × CH_2), 0.87 (3H, t, *J* = 6.2 Hz, Me-18), 0.85 (3H, t, *J* = 6.2 Hz, Me-9′); ^{13}C NMR ($CDCl_3$): δ 173.11 (C-1), 123.62 (C-9), 121.28 (C-10), 63.17 (C-1′), 30.81 (CH_2), 28.57 (20 × CH_2), 13.37 (Me-18, Me-9′); +ve ion FABMS *m/z*: 408.7 $[M]^+(C_{27}H_{52}O_2)$. The compound IV has m.p.: 76–78 °C; UV λ_{max}: 242 nm; IR (KBr): 2918, 2850, 1735, 1638, 1463, 1379, 719 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.29 (2H, m, H-10, H-12), 5.13 (2H, m, H-9, H-13), 3.63 (2H, m, H_2-1′), 2.28 (2H, m, H_2-11), 2.04 (2H, brs, H_2-2), 1.68 (2H, brs, H_2-8), 1.59 (2H, brs, H_2-14), 1.25 (30H, brs, 15 × CH_2), 1.00 (4H, m, 3 × CH_2), 0.87 (3H, t, *J* = 6.3 Hz, Me-18), 0.85 (3H, t, *J* = 6 Hz, Me-12′); ^{13}C NMR ($CDCl_3$): δ 177.11 (C-1), 131.88 (C-12), 128.81 (C-13), 124.43 (C-9), 119.08 (C-10), 63.08 (C-1′), 39.7 (C-11), 37.29 (C-8), 32.79 (C-14), 31.91 (CH_2), 31.42 (CH_2), 29.69 (12 × CH_2), 26.39 (CH_2), 25.72 (CH_2), 24.81 (CH_2), 24.45 (CH_2), 22.68 (CH_2), 21.07 (CH_2), 19.72 (Me-18), 14.11 (Me-12′); +ve ion FABMS *m/z*: 448.8 $[M]^+(C_{30}H_{56}O_2)$. The compound V has m.p.: 85–87 °C; UV λ_{max}: 241 nm; IR (KBr): 2919, 2049, 1728, 1636, 1465, 1282, 1166, 723 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.35 (1H, m, H-9), 5.11 (1H, m, H-10), 3.66 (2H, brs, H_2-1′), 2.41 (1H, d, *J* = 7.2 Hz, H_2-2a), 2.13 (1H, d, *J* = 7.2 Hz, H_2-2b), 1.68 (2H, brs, H_2-8), 1.60 (2H, brs,

H_2-11), 1.41 (2H, m, CH_2), 1.37 (2H, brs, CH_2), 1.33 (2H, brs, CH_2), 1.25 (56H, brs, 28 × CH_2), 0.87 (3H, t, J = 6.9 Hz, Me-16), 0.085 (3H, t, J = 6.9 Hz, Me-24′); ^{13}C NMR ($CDCl_3$): δc 173.15 (C-1), 124.38 (C-9), 119.08 (C-10), 61.18 (C-1′), 39.72 (CH_2), 37.37 (CH_2), 37.11 (CH_2), 36.64 (CH_2), 34.39 (CH_2), 33.44 (CH_2), 30.13 (CH_2), 29.70 (18 × CH_2), 27.11 (CH_2), 26.71 (CH_2), 25.02 (CH_2), 24.46 (CH_2), 22.69 (CH_2), 21.03 (Me-16), 14.11 (Me-24′); +ve ion FABMS m/z: 591 $[M]^+$($C_{40}H_{78}O_2$). The compound VI has m.p.: 110–112 °C; UV λ_{max}: 241 nm; IR (KBr): 3425, 2935, 2850, 1465, 1380, 1059 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 4.13 (1H, brs, $w_{1/2}$ = 15.6 Hz, H-14α), 2.50 (2H, brs, CH_2), 2.19 (2H, brs, CH_2), 1.52 (2H, brs, CH_2), 1.27 (42H, brs, 21 × CH_2), 0.88 (6H, brs, Me-1, Me-27); ^{13}C NMR (DMSO): δ 73.11 (C-14), 33.32 (CH_2), 30.75 (CH_2), 28.43 (20 × CH_2), 24.06 (CH_2), 21.48 (CH_2), 13.23 (Me-1, Me-27); +ve ion FABMS m/z (*rel. int.*): 396.7 $[M]^+$(C_{27} $H_{56}O$). The compound VII has m.p.: 168–170 °C; UV λ_{max}: 215 nm; IR (KBr): 3419, 2922, 2852, 1636, 1463, 1378, 1164, 965 cm^{-1}; ^{1}H NMR (DMSO): δ 5.16 (1H, brs, H-6), 5.14 (1H, m, H-22), 5.07 (1H, m, H-23), 1.12 (3H, brs, Me-19), 0.97 (3H, d, J = 7.8 Hz, Me-21), 0.88 (3H, d, J = 6.5 Hz, Me-26), 0.85 (3H, d, J = 6.3 Hz, Me-27), 0.81 (3H, d, J = 6.0 Hz, Me-29), 0.68 (3H, brs, Me-18); ^{13}C NMR (DMSO-d_6): δ 37.33 (C-1), 31.09 (C-2), 69.74 (C-3), 41.92 (C-4), 141.08 (C-5), 119.83 (C-6), 33.23 (C-7), 34.97 (C-8), 50.18 (C-9), 36.68 (C-10), 21.09 (C-11), 39.78 (C-12), 41.92 (C-13), 55.98 (C-14), 24.34 (C-15), 27.29 (C-16), 55.33 (C-17), 11.48 (C-18), 20.31 (C-19), 35.09 (C-20), 18.65 (C-21), 137.38 (C-22), 128.69 (C-23), 45.08 (C-24), 28.52 (C-25), 25.73 (C-26), 18.69 (C-27), 23.50 (C-28), 18.71 (C-29); +ve ion FABMS m/z: 412.7 $[M]^+$($C_{29}H_{48}O$). The compound VIII has m.p: 137–138 °C; UV λ_{max}: 210 nm; IR (KBr): 3461, 2955, 2845, 1640, 1475, 1365, 1210, 1108 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.30

(d, *J* = 5.5 Hz, H-6), 3.51 (1H, brs, $w_{1/2}$ = 16.5 Hz, H-3α), 1.01 (3H, brs, Me-19), 0.97 (3 H, d, *J* = 6.5 Hz, Me-21), 0.86 (3 H, d, *J* = 6.0 Hz, Me-29), 0.85 (3 H, d, *J* = 6.0 Hz, Me-27), 0.82 (3H, t, *J* = 6.2 Hz, Me-26), 0.67 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): 37.33 (C-1), 31.63 (C-2), 69.51 (C-3), 41.98 (C-4), 141.17 (C-5), 119.94 (C-6), 31.15 (C-7), 31.81 (C-8), 49.57 (C-9), 36.74 (C-10), 21.66 (C-11), 39.80 (C-12), 41.98 (C-13), 56.04 (C-14), 24.19 (C-15), 28.60 (C-16), 55.41 (C-17), 11.36 (C-18), 19.30 (C-19), 36.74 (C-20), 18.75 (C-21), 33.30 (C-22), 25.73 (C-23), 45.14 (C-24), 29.15 (C-25), 20.37 (C-26), 19.30 (C-27), 23.56 (C-28), 11.03 (C-29); +ve ion FAB MS *m/z* : 414.7$[M]^+$($C_{29}H_{50}O$).

Triton WR – 1339 acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extrahepatic tissues, resulting into increased blood lipid concentration[14]. The possible mechanism of activity may be due to enhancement of the activity of lecithin acyl transferase (LCAT) and inhibition of the action of hepatic TG- lipase on HDL[15]. LCAT plays a key role in the incorporation of free cholesterol into HDL and transferring it back to VLDL and LDL which are taken back later in liver cells [16, 17].

It is well known that LDL plays an important role in arteriosclerosis and that hypercholesterolemia is associated with a defect relating to the lack of LDL receptors [12]. The decrease of cholesterol and LDL levels achieved by administration of test samples, demonstrates a possible protection against hypercholesterolemia and the harm this condition brings about [18, 19]. The extract, fraction and the active phytoconstituent, inhibited the total cholesterol; triglycerides, low density lipoproteins level (LDL), and significantly increased high density lipoprotein level (HDL). Preclinical observations demonstrate that hyperlipidemia promotes accumulation of oxidatively modified low

density lipoproteins in the arterial wall, promoting endothelial dysfunction and development of atherosclerosis and congestive heart diseases [20,21].

The acute treatment with *C. pluricaulis* ethanolic extract and its chloroform fraction caused inhibitory effects both on total cholesterol (TC) and triglyceride level (TG) after Triton administration. The maximum inhibitory effect on serum TG and TC level was observed with 400 mg/kg chloroform fraction. The drug and its fraction showed protective action as it slightly increased the HDL cholesterol level.

Convolvulus pluricaulis has been widely screened for its various pharmacological activities. It has relatively well documented neuropharmacological actions such as nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, transquilising and sedative activities which justify its use in CNS diseases in the Ayurvedic system of medicine. It has antiulcer, antimicrobial, antipyretic, anti-inflammatory, analgesic, diuretic, antidiabetic and insecticidal properties. The various reported pharmacological activities of the plant highlight its therapeutic potential and limitations in our knowledge of its claimed traditional Indian usage.

CONCLUSION:

The present work characterized an isolated component from active fraction of *C. pluricaulis*. It may be concluded that the lowering of lipid level from active fraction is due to the presence of isolated components. Complete treatment of various types of hyperlipidemia is possible by herbal drugs. Various herbal remedies including one or more herbal drugs not only decrease the serum levels of TC, TG and LDL cholesterol but also increase the serum level of beneficial HDL cholesterol.

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DECLARATION:

No conflicts of interests.

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Effect of ethanolic extract and $CHCl_3$ fraction of *Convolvulus pluricaulis* on total cholesterol level (mg/dl) in Triton induced hyperlipidemic model (Table-1)

Group	Total cholesterol level after the administration of triton	Total cholesterol level after the vehicle/drug treatment
Control	66.19 ± 0.40	67.54±0.58
Hyperlipidemic	68.50 ± 0.39	98.83 ±0.25
Atrovastatin (50mg/kg)	87.45 ± 1.58	62.23 ±1.02 [a]
C. pluricaulis ethanolic extract (200mg/kg)	84.92± 0.52	65.72±.0.54[b]
C. pluricaulis ethanolic extract (400mg/kg)	86.83± 0.91	57.83±0.35[b]
C. pluricaulis $CHCl_3$ fraction (200mg/kg)	87.58± 1.12	62.32±0.58[b]
C. pluricaulis $CHCl_3$ fraction (400mg/kg)	89.37±1.28	55.41±0.64[b]

Total cholesterol concentrations are estimated by standard method. Values are expressed as mean ± S.E.M for six animals in each group.
[a]: $p<0.01$ [b] : $p<0.05$ compared to hyperlipidemic group

Effect of ethanolic extract and $CHCl_3$ fraction of *Convolvulus pluricaulis*

on triglyceride level (mg/dl) in Triton induced hyperlipidemic model (Table-2)

Group	Triglyceride level after the administration of triton	Triglyceride level after the vehicle /drug treatment
Control	52.36 ± 1.45	54.23±1.32
Hyperlipidemic	51.40 ± 1.58	88.73 ±1.72
Atrovastatin (50mg/kg)	66.58 ± 2.26	52.72±2.52 [a]
C. pluricaulis ethanolic extract (200mg/kg)	58.48±2.42	60.72±.1.41[b]
C. pluricaulis ethanolic extract (400mg/kg)	58.85±2.27	57.95±1.32[a]
C. pluricaulis $CHCl_3$ fraction (200mg/kg)	58.60± 2.85	55.26±1.39[a]
C. pluricaulis $CHCl_3$ fraction (400mg/kg)	59.93±2.02	53.44±1.96[a]

Triglyceride concentrations are estimated by standard method. Values are expressed as mean ± S.E.M. for six animals in each group.
[a]: $p<0.01$ [b] : $p<0.05$ compared to hyperlipidemic group

Effect of ethanolic extract and $CHCl_3$ fraction of *Convolvulus pluricaulis*

on HDL level (mg/dl) in Triton induced hyperlipidemic model (Table-3)

Group	HDL level after the administration of triton	HDL level after the vehicle/drug treatment
Control	34.36 ± 1.11	35.86±1.34
Hyperlipidemic	33.43 ± 1.24	34.02 ±1.49
Atrovastatin (50mg/kg)	46.24 ± 1.43	57.96.± 1.01 [a]
C. pluricaulis ethanolic extract (200mg/kg)	45.66±1.57	47.40±.1.71[b]
C. pluricaulis ethanolic extract (400mg/kg)	44.27±1.49	49.35±1.52[b]
C. pluricaulis $CHCl_3$ fraction (200mg/kg)	48.46± 2.09	51.83±1.42[a]
C. pluricaulis $CHCl_3$ fraction (400mg/kg)	46.35± 1.92	52.44± 1.96[a]

HDL concentrations are estimated by standard method. Values are expressed as mean ± S.E.M. for six animals in each group.

[a]: $p<0.01$ [b] : $p<0.05$ compared to hyperlipidemic group

Effect of ethanolic extract and $CHCl_3$ fraction of *Convolvulus pluricaulis* on LDL level (mg/dl) in Triton induced hyperlipidemic model (Table-4)

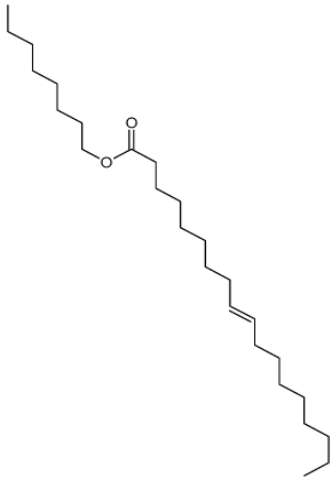
Group	LDL level after the administration of triton	LDL level after the vehicle/drug treatment
Control	35.32 ± 1.27	34.75±1.49
Hyperlipidemic	35.88 ± 1.66	60.73 ±1.74
Atrovastatin (50mg/kg)	58.22 ± 1.42	37.23± 1.20 [a]
C. pluricaulis ethanolic extract (200mg/kg)	53.26±1.74	46.73±.1.43[b]
C. pluricaulis ethanolic extract (400mg/kg)	54.84±1.60	43.38±1.58[b]
C. pluricaulis $CHCl_3$ fraction (200mg/kg)	56.33± 2.32	44.35±1.63[b]
C. pluricaulis $CHCl_3$ fraction (400mg/kg)	57.23±2.15	41.44±2.05[b]

LDL concentrations are estimated by standard method. Values are expressed as mean ± S.E.M. for six animals in each group.
[a]: $p<0.01$ [b] : $p<0.05$ compared to hyperlipidemic group

$CH_3(CH_2)_{34}CH_3$

n-hexatriacontane (Compound I)

Figure- 1

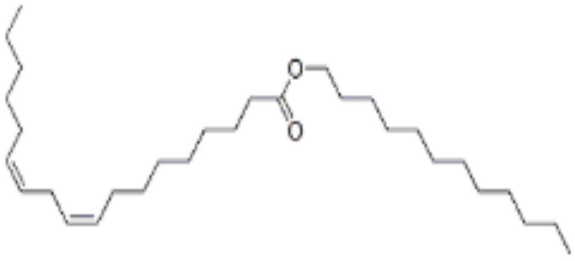


9-octadecenoic acid- octyl ester (Compound II)

Figure- 2

12, 14-heptacosanedione (Compound III)

Figure- 3



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

Figure- 4

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

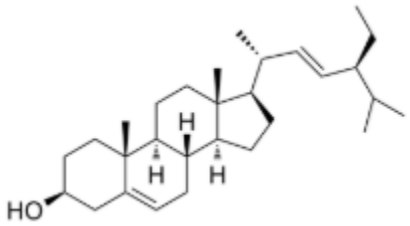
Tetracosanyl 9-hexadecenoate (Compound V)

Figure- 5

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

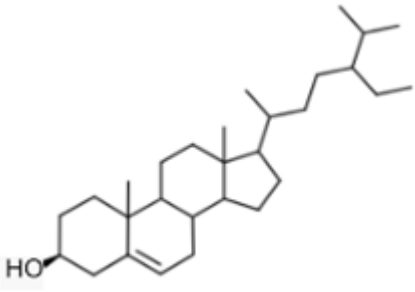
Heptacosan-14-ol (Compound VI)

Figure- 6



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

Figure- 7



Stigmast-5-en-3β-ol (Compound VIII)

Figure- 8