Original Article

Structural Elucidation of Isolated Phytochemicals from Selected Medicinal Plants with Anti-hyperlipidemic Activity and Development of Polyherbal Formulation with Densitometric Analysis of Isolated Phytoconstituents

Deepa Iyer[1,*], Umesh K Patil[2]

[1]Department of Pharmacognosy, Swami College of Pharmacy, Bhopal, Madhya Pradesh, INDIA.
[2]Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya (A Central University), Bhopal, Madhya Pradesh, INDIA.

ABSTRACT

Objectives: The present systematic research aimed to express the antihyperlipidemic effects of different types of herbs based on animal studies. The purpose of the present project is to perform the phytochemical investigation and exploration of hypolipidemic activity with the development and quantification of phytoconstituents of polyherbal hypolipidemic formulation employing *Apium graveolens, Salvadora persica, Carica papaya* and *Evolvulus alsinoides.* **Materials and Methods:** Flash chromatography technique was used for the isolation of phytoconstituents from the selected medicinal plants. Antihyperlipidemic study was done on triton induced model. Triton- WR 1339 (400 mg/kg b.w) i.p. injection were given to different animal groups. HPTLC densitometric method was established for the quantification of the phytoconstituents in polyherbal formulation. **Results:** The phytoconstituents so isolated were identified as 8, 8-Dimethyl-2H, 8H-pyrano [2, 3-f] chromen-2-one, 9- methoxyfuro [3, 2-g] chromen-7-one, 7- hydroxy-2H-chromen -2-one, Isothiocyanatomethylbenzene and 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro 1Hcyclopenta[α]phenanthren-3-ol through chromatographic separation techniques and their structures were elucidated by spectroscopic analysis. The formulation showed significant reduction in TC, TG, LDL and VLDL level ($p<0.01$) and significantly raised HDL level ($p<0.01$) compared to hyperlipidemic group. Furthermore, HPTLC method was established for the quantification of isolated phytoconstituents in polyherbal formulation through densitometric analysis. **Conclusion:** The present work also characterized active phytoconstituents from different parts of selected plants. The research work involved development of polyherbal hypolipidemic formulation with a view to reduce the serum levels of TC, TG, LDL and to increase the serum level of HDL cholesterol and to establish HPTLC densitometric method for quantification of its phytoconstituents.

Key words: Hyperlipidemia, Medicinal Plants, Flash Chromatography, Isolated Phytoconstituents, HPTLC Densitometric Analysis.

Submission Date: 19-07-2019;
Revision Date: 06-02-2020;
Accepted Date: 15-04-2020

DOI: 10.5530/ijper.54.2s.89

Correspondence:
Prof. Umesh K. Patil,
Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya (A Central University), Madhya Pradesh, 470003, INDIA.
Phone: +91-9425172165,
Email: umeshpatil29@gmail.com

INTRODUCTION

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.[1] High plasma lipid levels, mainly TC, TG and LDL along with decrease in HDL are known to cause hyperlipidemia which is core in initiation and progression of arteriosclerosis impasse.[2] Therefore, prime consideration in therapy for hyperlipidemia and arteriosclerosis is to enervate the

www.ijper.org

elevated plasma level of TC, TG and LDL along with increase in HDL lipid level.[3]

Phyto-chemists prepare extracts for biological screening methods using pharmacologically relevant assays and commence the process of fractionation, isolation and characterization of the active compound(s) through bioassay-guided fractionation.[4] In more recent history, various medicinal plants have been continue to be explored for the isolation and structural elucidation of pharmacological active phytoconstituents.[5,6]

Pharmacological screening techniques based on bioassay guided procedures have become very important protocol for the exploration of new bioactive phytoconstituents from plants so that the chemical profiling can be more accurate.[7,8]

Herbal drugs could overcome the adverse effects like cardiac arrhythmias, cardiac arrest, arrythmias, mental distress etc. of modern or allopathic drugs. The side effects caused by using modern drugs is still a matter of concern. Herbs could be potentially used to reduce the level of cholesterol inside the body with less or minimal side effects.[9]

The present research has been done to explore the potential of medicinal plants in reduction of hyperlipidemia. The study involved densitometric analysis by HPTLC of polyherbal formulation using active fractions of *Apium graveolens*, *Salvadora persica*, *Carica papaya* and *Evolvulus alsinoides*.

MATERIALS AND METHODS

Plant material

The plant materials were procured in the month of July 2010 from Bhopal (Madhya Pradesh), India. The *Apium graveolens* seeds, *Salvadora persica* stems, *Carica papaya* seeds and *Evolvulus alsinoides* aerial parts were collected, identified and authenticated by Assistant Professor Dr. Zia ul Hassan, Botany, Saifia College of Science and Education, Bhopal. A voucher specimen no. of 175/Bot/Safia/2010 has been deposited in the herbarium in the respective department.

Extraction and fractionation

900g of seeds of *A. graveolens*, 800g of stems of *S. Persica*, 750g of seeds of *C. papaya* and 850g of aerial parts of *E. alsinoides* were coarsely powdered and extracted in Soxhlet apparatus with 90% ethanol. The ethanolic extracts so obtained were freed of solvent under vacuum to get 92 g (10.22% yield) of dark greenish brown mass of *A. graveolens*, 74 g (9.25% yield) of dark brown mass of *S. persica*, 61 g (8.13% yield) of brown mass of *C. papaya* and 72 g (8.47% yield) of dark green mass of *E. alsinoides*.

Ethanol extracts and their fractions were tested for the presence of phytoconstituents by qualitative analysis. The phytochemical analysis of extracts revealed the presence of different phyto-constituents. The *Apium graveolens* ethanolic extract revealed the presence of alkaloids, glycosides, sterols, flavonoids, tannins, phenolic compounds, proteins, fats and oils. The *Salvadora persica* ethanolic extract revealed the presence of alkaloids, glycosides, sterols, tannins, phenolic compounds and proteins. The *Carica papaya* ethanolic extract revealed the presence of alkaloids, glycosides, tannins, proteins, fats and oils. The ethanolic extract of *Evolvulus alsinoides* revealed the presence of alkaloids, glycosides, sterols, flavonoids, tannins, proteins, fats and oils.

Instrumentation used

Buchi controller C-610 apparatus (NIPER, Mohali, India) was used to perform Flash Chromatography. Shimadzu UV 1700 model in MeOH/ EtOH (VNS Institute of Pharmacy, Bhopal, M.P., India) was used to record UV spectra. Jasco FT/IR-5300 spectrophotometer was used to measure IR spectra. The ^{1}H spectra and ^{13}C spectra were recorded on aV 300 and bks NMR spectrometer (NIPER, Mohali, India), using DMSO and $CDCl_3$ as solvents. Mass spectra were obtained from Scan AP spectrometer/JEOL JMS AX-500 spectrometer (RGTU, Bhopal, M.P., India).

Chromatographic isolation of compounds

The bioactivity guided fractionation resulted in the selection of active fraction that is responsible for significant antihyperlipidemic activity. Active fraction was further subjected to chromatographic studies with a view to isolate phytoconstituents. Isolation was done using Flash and Column chromatography.

Flash chromatography was done for the *A. graveolens* chloroform fraction. Elution of the column was done with n-hexane: $CHCl_3$ (25:75). TLC of the colorless crystalline powder so obtained was carried out using Petroleum ether: chloroform: methanol: acetic acid (10:2:1:1) solvent system. The yield of the sample was found to be 0.011% (compound I). The melting point of compound I was 117°C.

Further elution of the column with chloroform (fraction 11-16) yielded white needle shaped crystals. Again TLC of the powdered sample so obtained was carried out using Petroleum ether: chloroform: methanol: acetic acid (10:2:1:1). The yield of the sample was found to be 0.157% (compound II). The melting point of compound II was 146°C.

Elution of the flash column was done for the chloroform fraction of *S. persica* with Chloroform: methanol (110:90). This yielded a creamish yellow amorphous powder followed by recrystallisation from ethanol.

The powder was further eluted through the column with chloroform: methanol (60:40) which yielded creamish white crystals. TLC of the powdered sample was carried out using Chloroform: *n*-butanol: ethanol: acetic acid (2:4:1:1) solvent system. The yield of the sample was found to be 0.041% (compound III). The melting point of compound III was 228°C.

Elution of the flash column was done for the water-soluble fraction of *C. papaya* with Chloroform: methanol (120:80). This yielded a creamish yellow powder followed by recrystallisation from ethanol. The powder was further eluted through the column was done with $CHCl_3$: methanol (70:30). TLC of the powdered sample was carried out using chloroform: ethanol: acetic acid (18:6:1). The yield of the sample was found to be 0.089% (compound IV). The melting point of compound IV was 41°C.

Flash chromatography was done for the chloroform fraction of *E. alsinoides*. Elution of the column was done with $CHCl_3$: Methanol (24:1) yielded colorless amorphous powder, recrystallised from methanol. TLC of the powdered sample was carried out using various solvent systems. The appropriate one found to be - Petroleum ether: $CHCl_3$: Methanol (1: 4: 1). The yield of the sample was found to be 0.190% (compound V). The melting point of compound V was 137°C.

Characterization of compounds

Isolated compounds obtained from different fractions were then analyzed using spectroscopic methods. The structure elucidation was done using spectroscopic methods such as UV, IR, NMR and Mass. λ_{max} was noted from scanned spectra using UV analysis. λ_{max} of isolated compound I was found to be 318, λ_{max} of compound II was found to be 312, λ_{max} of compound III was found to be 324, λ_{max} of compound IV was found to be 217 and λ_{max} of compound V was found to be 206.

IR study was done to know the actual position of bonds depending upon the nature of groups attached to the ring. Compound I showed 2864.09 (C-H Stretching), 1832.25 (C=O Stretching), 1101.28 (C-O Stretching), 813.90 (C-H Bending) vibrations. Compound II showed 2887.24 (C-H Stretching), 1785.96 (C=O Stretching), 1326.93 (α, β unsaturated lactone), 1145.64 (C-O Stretching), 835.12 (C-H Bending) vibrations. Compound III showed 3664.50 (O-H Stretching), 2659.65 (C-H Stretching), 1814.89 (C=O Stretching), 1602.74 (α, β unsaturation), 840.91 (C-H Bending) vibrations. Compound IV showed 3355.91 (N-H Stretching), 2864.00 (C-H Stretching), 1909.40 (N=C=S Stretching), 1247.86 (C-N Streching), 871.76 (C-H Bending) vibrations. Compound V showed 3407.98 (O-H Stretching), 2920.03 (CH_2 Stretching), 2854.45 (C-H Stretching), 1670.24 (C=C Stretching), 1053.06 (C-O Stretching) vibrations.

NMR spectrum of a substance gives very valuable information about its molecular structure in terms of chemical shifs (δ ppm) values.

Compound I showed following values ^{1}H-NMR spectrum: δ 7.61 (1H, d, H-3, J=9.48 Hz), 7.21 (1H, d, H-4, J=8.48 Hz), 6.88 (1H, d, H-5, J= 10.08 Hz), 6.72 (1H, d, H-6, J= 8.48 Hz), 6.23 (1H, d, H-9, J= 9.44 Hz), 5.74 (1H, d, H-10, J= 10.08 Hz), 1.47 (3H, s, CH_3), 7.27 (3H, s, CH_3). ^{13}C-NMR spectrum: δ 161.06 (C-2), 156.30 (C-3), 150.09 (C-4), 143.94(C-5), 130.76(C-6), 127.78(C-8), 114.99 (C-9), 113.54 (C-10), 112.59 (C-4a), 109.28(C-6a), 77.61 (C-10a), 77.37 (C-10b), 77.05 (8- CH_3), 76.73 (8- CH_3).

Compound II showed following values ^{1}H-NMR spectrum: δ 4.28 (3H, s, CH_3O), 6.29 (1H, d, H-6, J=9.8 Hz), 7.27 (1H, s, H-4), 7.13 (1H, s, H-3), 7.60 (1H, d, H-2, J=2.4 Hz), 8.17 (1H, s, H-5). ^{13}C-NMR spectrum: δ 161.26 (C-2), 93.82 (C-3), 144.79 (C-4), 112.63 (C-5), 106.37(C-6), 149.56 (C-7), 139.29 (C-9), 158.37 (C-9a), 152.69 (C-8a), 105.05 (C-3a), 77.02 (C-4a), 60.08 (9-OCH_3).

Compound III showed following values ^{1}H-NMR spectrum: δ 10.58 (1H, s, OH-7), 7.95 (1H, d, H-4, J=9.3 Hz), 7.55 (1H, d, H-5, J=8.7 Hz), 6.23 (1H, d, H-3, J=10.32 Hz), 6.74 (1H, s, H-8), 6.83 (1H, dd, H-6, J = 2.7 Hz). ^{13}C-NMR spectrum: δ 160.52 (C-2), 155.56 (C-6), 144.55 (C-4), 129.74 (C-8), 113.17 (C-3), 111.46 (C-5), 111.33(C-7), 102.23 (C-4a), 161.35 (C-8a).

Compound IV showed following values ^{1}H-NMR spectrum: δ 4.89 (1H, s, H-1), 3.49 (1H, s, H-2), 7.44 (1H, s, H-3), 7.41(1H, d, J=1.5 Hz, H-4), 7.39 (1H, d, J=2.1 Hz, H-5), 7.33 (2H, d, J=1.8 Hz). ^{13}C-NMR spectrum: δ134.56 (C-1), 128.69 (C-2), 128.04 (C-3), 126.98(C-4), 47.97(C-5), 39.78(C-6), 39.50 (C-7), 39.23 (C-8).

Compound V showed following values ^{1}H-NMR spectrum: δ 3.51 (1H, brs, H-3), 5.30 (1H, d, H-6, J=5.5 Hz), 0.67 (3H, brs, Me-18), 1.01(3H, brs, H-19), 0.97 (3H, d, J=6.5 Hz, Me-21), 0.82 (3H, t, J=6.2 Hz, Me-26), 0.85 (3H, d, J=6.0 Hz, Me-27), 0.86 (3H, d, J=6.0 Hz, Me-29). ^{13}C-NMR spectrum: δ37.33 (C-1), 31.63 (C-2), 71.8(C-3), 72.3(C-4), 141.7(C-5), 121.4(C-6), 31.6(C-7), 31.81(C-8), 49.57(C-9), 36.74(C-10), 20.9(C-11), 31.80(C-12), 40.4(C-13), 56.04(C-14), 24.19(C-15),

28.60(C-16), 50.1(C-17), 11.36(C-18), 19.30(C-19), 36.74(C-20), 18.15(C-21), 33.30(C-22), 25.73(C-23), 42.3(C-24), 30.5(C-25), 20.37(C-26), 19.30(C-27), 23.56(C-28), 11.5(C-29).

Mass spectroscopy helps us to know the molecular mass of a compound. Compound I showed following peaks: Positive ion FAB MS m/z: 229 $[M+H]^+$ ($C_{14}H_{12}O_3$), 212, 187, 160. Compound II showed following peaks: Positive ion FAB MS m/z: 215 $[M-H]^-$ ($C_{12}H_8O_4$), 201, 176, 148, 91, 67. Compound III showed following peaks: Positive ion FAB MS m/z: 161 $[M-H]^-$ ($C_9H_6O_3$), 77,89,105,117,133. Compound IV showed following peaks: Positive ion FAB MS m/z: 150 $[M+H]^+$ (C_8H_7NS), 132,115, 88,75,69. Compound V showed following peaks: Positive ion FAB MS m/z: 414$[M]^+$ ($C_{29}H_{50}O$), 397, 396, 381, 365, 339, 371, 255, 239, 213, 199, 159, 145, 119, 105.

Preparation of polyherbal formulation using active fractions

a. Formulation of Polyherbal tablets

Polyherbal tablets were prepared by direct compression method.

- Fractions obtained from selected plants were used for formulation of polyherbal tablets.
- Excipient like microcrystalline cellulose is used as directly compressible agent, magnesium stearate used as a lubricant and lactose used as a diluent.

b. Preparation and compression of mixed blend of active ingredients and excipients

All the ingredients were mixed in a mortar and pestle. The mixed blend of different fractions and excipients were compressed using a single punch tablet-punching machine to produce tablets weighing 500 mg. (Table 1)

Screening for hypolipidemic activity

Screening for hypolipidemic activity was carried out in male albino rats weighing 100-120 g. Tyloxapol (Triton WR 1339) was purchased from Sigma Chemicals, USA.

Preparation of test material

Ethanol extracts and different fractions were suspended in distilled water plus Polyoxyethylenesorbiton Monooleate (Tween 80). Triton was dissolved in normal saline to give a 7% solution.

Animal model

The albino rats were selected and housed in polypropylene cages maintained under controlled conditions. The animals were fed standard mice feed and acidified water *ad libitum*. Male albino rats of 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 for groups treated with ethanolic extracts, different fractions, isolated phytoconstituents and Polyherbal formulation

Acute toxicity studies of the ethanolic extracts of selected plants and different fractions were determined using albino rats. OECD Guideline No. 423 of CPCSEA was adopted for the toxicity studies.

Albino rats were divided into twelve groups of six rats each. Group 1 served as vehicle control. Triton WR 1339 at dose of 400 mg/kg body weight was injected to the animals of groups 2-16. After 24 hr. of Triton administration, animals of Group 3-16 were administered orally with respective test drugs. Group 1- Vehicle control group; Group 2- Hyperlipidemic group and administered with Triton (400 mg/kg) only; Group 3-Standard drug (Atrovastatin) treated rats at the oral dose of 80 mg/kg; Group 4- *A. graveolens* ethanolic extract at the oral dose of 200mg/kg; Group 5- Chloroform fraction of *A. graveolens* ethanolic extract at the oral dose of 200mg/kg; Group 6- *S. persica* ethanolic extract at the oral dose of 200mg/kg; Group 7- Chloroform fraction of *S. persica* ethanolic extract at the oral dose of 200mg/kg; Group 8- *C. papaya* ethanolic extract at the oral dose of 200mg/kg; Group 9- Water soluble fraction of *C. papaya* ethanolic extract at the oral dose of 200mg/kg; Group 10- *E. alsinoides* ethanolic extract at the oral dose of 200mg/kg; Group 11- Chloroform fraction of *E. alsinoides* ethanolic extract at the oral dose of 200mg/kg; Group 12- Phytoconstituent I at the oral dose of 50 mg/kg; Group 13- Phytoconstituent II at the oral dose of 50 mg/kg; Group 14- Phytoconstituent III at the oral dose of 50 mg/kg; Group 14- Phytoconstituent IV at the oral dose of 50 mg/kg; Group 15- Phytoconstituent V at the oral dose of 50 mg/kg; Group 16- Polyherbal formulation at the oral dose of 200 mg/kg.[10]

The lipid profile was studied after the 5 days of treatment. The serum concentration of total cholesterol, HDL and triglyceride were measured by standard procedure using auto- analyzer.[11]

Densitometric analysis of standard markers in polyherbal tablet

HPTLC finger printing profile: HPTLC study of polyherbal tablet formulation was carried out along with the different marker compounds corresponding to the active ingredients to ensure the presence of active ingredients in the formulation.

Reference Standards: All the reference standards used in the experiment were purchased from Sigma Aldrich, USA.

Apparatus

Spotting device: Spotting device was a Linomat V Automatic Sample Spotter (CAMAG, Muttenz, Switzerland)

Syringe: 100 µl (Hamilton, Bonaduz, Switzerland)

Thin layer chromatographic (TLC) chamber: Glass twin trough chamber (20 x 10 x 4 cm) (CAMAG)

Densitometer was a TLC Scanner 3 linked to Win Cats software (CAMAG)

HPTLC plates: 20 x 10cm, 0.2 mm thickness precoated with silica gel 60 F_{254} (Merck, Mumbai)

Preparation of standard solution

The stock solutions of isolated Phytoconstituent II, Phytoconstituent III and Phytoconstituent V were prepared in methanol. A stock solution of all the markers was prepared by dissolving 50 mg of accurately weighed markers in methanol and making up the volume to 50 ml of methanol. From these stock solutions, standard solutions of Phytoconstituent II, Phytoconstituent III and Phytoconstituent V at concentrations of (1-10 µg/ml) were prepared by transferring aliquots of stock solution to volumetric flasks and adjusting the final volume with methanol.

Preparation of sample solution

Accurately weighed 500 mg of polyherbal was dissolved in methanol. The solution was strained with the help of Whatman filter paper. The filterate obtained was evaporated to dryness and the residue obtained was reconstituted in 10 ml of methanol.

Calibration curve for Phytoconstituent II, Phytoconstituent III and Phytoconstituent V

10 µl of each of the standard solutions of Phytoconstituent II, Phytoconstituent III and Phytoconstituent V at concentrations of (1-10 µg/ml) were applied in triplicate on to an HPTLC plate. The plates were developed in a solvent system of toluene: ethyl acetate: methanol: glacial acetic acid (3:3:0.2:0.8) up to a distance of 9 cm. After development, the plate was dried in air and was scanned densitometrically at 232 nm using absorbance reflectance mode by CAMAG Scanner 2 and Wincats software III. λ_{max} of all three marker compounds fall in the range of 225-232 nm, hence 232 nm was chosen for scanning the three markers to record the peak.

Simultaneous quantification of Phytoconstituent II, Phytoconstituent III and Phytoconstituent V in polyherbal tablet

Sample solutions (10 µl) were applied in triplicate on a precoated HPTLC plate. The plate was developed in the same solvent system as above and scanned at 232 nm. The peak area and absorption spectrum was recorded. The amount of Phytoconstituent II, Phytoconstituent III and Phytoconstituent V were calculated using the respective calibration curve.

Validation of the method

Validation of the analytical procedure was followed as per ICH guidelines (CPMP/ICH/381/95; CPMP/ICH/281/95). The method was validated for precision and accuracy.

Statistical analysis

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

RESULTS

The preliminary phytochemical screening of extracts revealed the presence of various phyto-constituents. The ethanolic extract of *A. graveolens* revealed the presence of alkaloids, glycosides, sterols, flavonoids, tannins, phenolic compounds, proteins, fats and oils. The ethanolic extract of *S. persica* revealed the presence of alkaloids, glycosides, sterols, tannins, phenolic compounds and proteins. The ethanolic extract of *C. papaya* revealed the presence of alkaloids, glycosides, tannins, proteins, fats and oils. The ethanolic extract of *E. alsinoides* revealed the presence of alkaloids, glycosides, sterols, flavonoids, tannins, proteins, fats and oils.

Preliminary thin layer chromatography (TLC) was performed to select suitable solvent system on the basis of good resolution of ethanolic extracts. Pet. ether: chloroform: methanol: glacial acetic acid (10:2:1:1) solvent system was found suitable for *Apium graveolens* extract, Chloroform: n-butanol: ethanol: acetic acid (2:4:1:1) for *Salvadora persica* extract, Chloroform: ethanol: acetic acid (18:6:1) for *Carica papaya* extract and Pet. ether: chloroform: methanol (1:4:1) for *Evolvulus alsinoides* extract. Four spots were seen in *Apium graveolens* extract having R_f values of 0.72, 0.61, 0.53 and 0.45. Two spots were detected in *S. persica* extract having R_f values of 0.35 and 0.47. One spot was detected in *C. papaya* extract having R_f values of 0.65. Seven spots were seen in *E. alsinoides* extract having R_f values of 0.81, 0.76, 0.63, 0.51, 0.49, 0.42 and 0.37.

Flash chromatography was done for the *A. graveolens* chloroform fraction. Elution of the column was done with n-hexane: $CHCl_3$ (25:75). TLC of the colorless crystalline powder so obtained was carried out using Petroleum ether: chloroform: methanol: acetic acid (10:2:1:1) solvent system. The yield of the sample was found to be 0.011% (compound I). The melting point of compound I was 117°C.

Further elution of the column with chloroform (fraction 11-16) yielded white needle shaped crystals. Again TLC of the powdered sample so obtained was carried out using Petroleum ether: chloroform: methanol: acetic acid (10:2:1:1). The yield of the sample was found to be 0.157% (compound II). The melting point of compound II was 146°C.

Elution of the flash column was done for the chloroform fraction of *S. persica* with Chloroform: methanol (110:90). This yielded a creamish yellow amorphous powder followed by recrystallisation from ethanol.

The powder was further eluted through the column with chloroform: methanol (60:40) which yielded creamish white crystals. TLC of the powdered sample was carried out using Chloroform: *n*-butanol: ethanol: acetic acid (2:4:1:1) solvent system. The yield of the sample was found to be 0.041% (compound III). The melting point of compound III was 228°C.

Elution of the flash column was done for the water-soluble fraction of *C. papaya* with Chloroform: methanol (120:80). This yielded a creamish yellow powder followed by recrystallisation from ethanol. The powder was further eluted through the column was done with $CHCl_3$: methanol (70:30). TLC of the powdered sample was carried out using chloroform: ethanol: acetic acid (18:6:1). The yield of the sample was found to be 0.089% (compound IV). The melting point of compound IV was 41°C.

Flash chromatography was done for the chloroform fraction of *E. alsinoides*. Elution of the column was done with $CHCl_3$: Methanol (24:1) yielded colorless amorphous powder, recrystallised from methanol. TLC of the powdered sample was carried out using various solvent systems. The appropriate one found to be - Petroleum ether: $CHCl_3$: Methanol (1: 4: 1). The yield of the sample was found to be 0.190% (compound V). The melting point of compound V was 137°C.

Isolated compounds obtained from different fractions were then analyzed using spectroscopic methods. The structure elucidation was done using spectroscopic methods such as UV, IR, NMR and Mass. λ_{max} was measured using UV analysis. λ_{max} of isolated compound I- 318, λ_{max} of compound II - 312, λ_{max} of compound III- 324, λ_{max} of compound IV- 217 and λ_{max} of compound V- 206.

IR study was done to know the actual position of bonds depending upon the nature of groups attached to the ring. Compound I showed 2864.09 (C-H Stretching), 1832.25 (C=O Stretching), 1101.28 (C-O Stretching), 813.90 (C-H Bending) vibrations. Compound II showed 2887.24 (C-H Stretching), 1785.96 (C=O Stretching), 1326.93 (α, β unsaturated lactone), 1145.64 (C-O Stretching), 835.12 (C-H Bending) vibrations. Compound III showed 3664.50 (O-H Stretching), 2659.65 (C-H Stretching), 1814.89 (C=O Stretching), 1602.74 (α, β unsaturation), 840.91 (C-H Bending) vibrations. Compound IV showed 3355.91 (N-H Stretching), 2864.00 (C-H Stretching), 1909.40 (N=C=S Stretching), 1247.86 (C-N Stretching), 871.76 (C-H Bending) vibrations. Compound V showed 3407.98 (O-H Stretching), 2920.03 (CH_2 Stretching), 2854.45 (C-H Stretching), 1670.24 (C=C Stretching), 1053.06 (C-O Stretching) vibrations.

NMR spectrum of a substance gives very valuable information about its molecular structure in terms of chemical shifts (δ ppm) values.

Compound I showed following values ^{1}H-NMR spectrum: δ 7.61 (1H, d, H-3, J=9.48 Hz), 7.21 (1H, d, H-4, J=8.48 Hz), 6.88 (1H, d, H-5, J= 10.08 Hz), 6.72 (1H, d, H-6, J= 8.48 Hz), 6.23 (1H, d, H-9, J= 9.44 Hz), 5.74 (1H, d, H-10, J= 10.08 Hz), 1.47 (3H, s, CH_3), 7.27 (3H, s, CH_3). ^{13}C-NMR spectrum: δ 161.06 (C-2), 156.30 (C-3), 150.09 (C-4), 143.94(C-5), 130.76(C-6), 127.78(C-8), 114.99 (C-9), 113.54 (C-10), 112.59 (C-4a), 109.28(C-6a), 77.61 (C-10a), 77.37 (C-10b), 77.05 (8- CH_3), 76.73 (8- CH_3).

Compound II showed following values ^{1}H-NMR spectrum: δ 4.28 (3H, s, CH_3O), 6.29 (1H, d, H-6, J=9.8 Hz), 7.27 (1H, s, H-4), 7.13 (1H, s, H-3), 7.60 (1H, d, H-2, J=2.4 Hz), 8.17 (1H, s, H-5). ^{13}C-NMR spectrum: δ 161.26 (C-2), 93.82 (C-3), 144.79 (C-4), 112.63 (C-5), 106.37(C-6), 149.56 (C-7), 139.29 (C-9), 158.37 (C-9a), 152.69 (C-8a), 105.05 (C-3a), 77.02 (C-4a), 60.08 (9-OCH_3).

Compound III showed following values ^{1}H-NMR spectrum: δ 10.58 (1H, s, OH-7), 7.95 (1H, d, H-4, J=9.3 Hz), 7.55 (1H, d, H-5, J=8.7 Hz), 6.23 (1H, d, H-3, J=10.32 Hz), 6.74 (1H, s, H-8), 6.83 (1H, dd, H-6, J = 2.7 Hz). ^{13}C-NMR spectrum: δ 160.52 (C-2), 155.56 (C-6), 144.55 (C-4), 129.74 (C-8), 113.17 (C-3), 111.46 (C-5), 111.33(C-7), 102.23 (C-4a), 161.35 (C-8a).

Compound IV showed following values ^{1}H-NMR spectrum: δ 4.89 (1H, s, H-1), 3.49 (1H, s, H-2), 7.44 (1H, s, H-3), 7.41(1H, d, J=1.5 Hz, H-4), 7.39 (1H,

d, J=2.1 Hz, H-5), 7.33 (2H, d, J=1.8 Hz). ^{13}C-NMR spectrum: δ134.56 (C-1), 128.69 (C-2), 128.04 (C-3), 126.98(C-4), 47.97(C-5), 39.78(C-6), 39.50 (C-7), 39.23 (C-8).

Compound V showed following values ^{1}H-NMR spectrum: δ 3.51 (1H, brs, H-3), 5.30 (1H, d, H-6, J=5.5 Hz), 0.67 (3H, brs, Me-18), 1.01(3H, brs, H-19), 0.97 (3H, d, J=6.5 Hz, Me-21), 0.82 (3H, t, J=6.2 Hz, Me-26), 0.85 (3H, d, J=6.0 Hz, Me-27), 0.86 (3H, d, J=6.0 Hz, Me-29). ^{13}C-NMR spectrum: δ37.33 (C-1), 31.63 (C-2), 71.8(C-3), 72.3(C-4), 141.7(C-5), 121.4(C-6), 31.6(C-7), 31.81(C-8), 49.57(C-9), 36.74(C-10), 20.9(C-11), 31.80(C-12), 40.4(C-13), 56.04(C-14), 24.19(C-15), 28.60(C-16), 50.1(C-17), 11.36(C-18), 19.30(C-19), 36.74(C-20), 18.15(C-21), 33.30(C-22), 25.73(C-23), 42.3(C-24), 30.5(C-25), 20.37(C-26), 19.30(C-27), 23.56(C-28), 11.5(C-29).

Mass spectroscopy helps us to know the molecular mass of a compound. Compound I showed following peaks: Positive ion FAB MS m/z: 229 $[M+H]^+$ ($C_{14}H_{12}O_3$), 212, 187, 160. Compound II showed following peaks: Positive ion FAB MS m/z: 215 $[M-H]^-$ ($C_{12}H_8O_4$), 201, 176, 148, 91, 67. Compound III showed following peaks: Positive ion FAB MS m/z: 161 $[M-H]^-$ ($C_9H_6O_3$), 77,89,105,117,133. Compound IV showed following peaks: Positive ion FAB MS m/z: 150 $[M+H]^+$ (C_8H_7NS), 132,115, 88, 75, 69. Compound V showed following peaks: Positive ion FAB MS m/z: 414$[M]^+$ ($C_{29}H_{50}O$), 397, 396, 381, 365, 339, 371, 255, 239, 213, 199, 159, 145, 119, 105.

The phytoconstituents so isolated were identified as 8, 8-Dimethyl-2H, 8H-pyrano [2, 3-f] chromen-2-one(Phyoconstituent I), 9- methoxyfuro [3,2-g] chromen-7-one (Phyoconstituent II), 7-hydroxy-2H-chromen -2-one (Phyoconstituent III), Isothiocyanatomethylbenzene (Phyoconstituent IV), 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro -1Hcyclopenta[α]phenanthren-3-ol and 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro 1Hcyclopenta[α]phenanthren-3-ol (Phyoconstituent V) [Figure 1-5].

Pharmacological screening of ethanolic extracts, different fractions and isolated phytoconstituents were performed on triton- induced hyperlipidemic rats to confirm their antihyperlipidemic potential. *A. graveolens* ethanolic extract and chloroform fraction significantly decreased total cholesterol level ($p<0.01$ and $p<0.001$) at a dose of 200mg/kg in serum compared to hyperlipidemic group. On the other hand, ethanolic extract and chloroform fraction of *S. persica*, significantly reduced total cholesterol level ($p<0.01$) at a dose of 200mg/kg in serum compared to hyperlipidemic group. *C. papaya* ethanolic extract and water soluble fraction significantly decreased total cholesterol level ($p<0.05$ and $p<0.01$) at a dose of 200mg/kg in serum compared to hyperlipidemic group. On the other hand, ethanolic extract and chloroform fraction of *E. alsinoides*, significantly reduced total cholesterol level ($p<0.01$ and $p<0.001$) at a dose of 200mg/kg in serum compared to hyperlipidemic group. Phytoconstituents I, II, III, IV and V showed significant reduction in total cholesterol level ($p<0.05$, $p<0.01$, $p<0.01$, $p<0.05$, $p<0.01$) compared to hyperlipidemic group (Table 2).

A. graveolens ethanolic extarct and chloroform fraction significantly decreased triglyceride level ($p<0.01$ and $p<0.001$) in serum at doses of 200mg/kg to hyperlipidemic group. On the other hand, ethanolic exatrct and chloroform fraction of *S. persica*, significantly reduced triglyceride level ($p<0.05$ and $p<0.01$) in serum at doses of 200mg/kg compared to hyperlipidemic group. *C. papaya* ethanolic extract and water soluble fraction significantly decreased triglyceride level ($p<0.05$) in serum at doses of 200mg/kg compared to hyperlipidemic group. On the other hand, ethanolic extract and chloroform fraction of *E. alsinoides*, significantly reduced triglyceride level ($p<0.01$ and $p<0.001$) in serum at doses of 200mg/kg compared to hyperlipidemic group. Phytoconstituents I, II, III, IV and V showed significant reduction in triglyceride level ($p<0.01$, $p<0.001$, $p<0.001$, $p<0.05$, $p<0.001$) compared to hyperlipidemic group (Table 3).

A. graveolens ethanolic extract and chloroform fraction significantly increased HDL level ($p<0.05$ and $p<0.001$) in serum at doses of 200mg/kg to hyperlipidemic group. On the other hand, ethanolic extract and chloroform fraction of *S. persica*, significantly raised HDL level ($p<0.05$ and $p<0.01$) in serum at doses of 200mg/kg compared to hyperlipidemic group. *C. papaya* ethanolic extract and water soluble fraction significantly increased HDL level ($p<0.05$) in serum at doses of 200mg/kg compared to hyperlipidemic group. On the other hand, ethanolic extract and chloroform fraction of *E. alsinoides*, significantly raised HDL level ($p<0.05$ and $p<0.001$) in serum at doses of 200mg/kg compared to hyperlipidemic group. Phytoconstituents I, II, III, IV and V showed significant increase in HDL level ($p<0.01$, $p<0.001$, $p<0.01$, $p<0.05$, $p<0.001$) compared to hyperlipidemic group (Table 4).

A. graveolens ethanolic extract and chloroform fraction significantly decreased LDL level ($p<0.01$) in serum at doses of 200mg/kg to hyperlipidemic group. On the other hand, ethanolic extract and chloroform

fraction of *S. persica*, significantly reduced LDL level ($p<0.05$) in serum at doses of 200mg/kg compared to hyperlipidemic group. *C. papaya* ethanolic extract and water soluble fraction significantly decreased LDL level ($p<0.05$) in serum at doses of 200mg/kg compared to hyperlipidemic group. On the other hand, ethanoilc extract and chloroform fraction of *E. alsinoides*, significantly reduced LDL level ($p<0.05$ and $p<0.01$) in serum at doses of 200mg/kg compared to hyperlipidemic group. Phytoconstituents I, II, III, IV and V showed significant reduction in LDL level ($p<0.05$, $p<0.01$, $p<0.01$, $p<0.05$, $p<0.01$) compared to hyperlipidemic group.

A. graveolens ethanolic extract and chloroform fraction significantly decreased VLDL level ($p<0.01$ and $p<0.001$) in serum at doses of 200mg/kg to hyperlipidemic group. On the other hand, ethanolic extract and chloroform fraction of *S. persica*, significantly reduced VLDL level ($p<0.05$ and $p<0.01$) in serum at doses of 200mg/kg compared to hyperlipidemic group. *C. papaya* ethanolic extract and water soluble fraction significantly decreased VLDL level ($p<0.05$) in serum at doses of 200mg/kg compared to hyperlipidemic group. On the other hand, ethanolic extract and chloroform fraction of *E. alsinoides*, significantly reduced VLDL level ($p<0.01$) in serum at doses of 200mg/kg compared to hyperlipidemic group. Phytoconstituents I, II, III, IV and V showed significant reduction in VLDL level ($p<0.01$, $p<0.001$, $p<0.001$, $p<0.05$, $p<0.001$) compared to hyperlipidemic group.

Fractions obtained from selected plants were used for formulation of polyherbal tablets. Polyherbal tablets were prepared by direct compression method. Pharmacological screening of polyherbal tablet formulation was done. The formulation showed significant reduction in total cholesterol and triglyceride ($p<0.01$), LDL and VLDL level ($p<0.05$) compared to hyperlipidemic group. On the other hand the formulation significantly raised HDL level ($p<0.05$) compared to hyperlipidemic group (Table 2-4).

HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of active constituents in medicinal plants. HPTLC study of polyherbal tablet formulation was carried out along with the different marker compounds corresponding to the active ingredients to ensure the presence of active ingredients in the formulation. TLC densitometric methods were developed using High performance thin layer chromatography for the quantification of the three marker compounds, 9-methoxyfuro [3, 2-g] chromen-7-one (Phyoconstituent II), 7- hydroxy-2H-chromen -2-one (Phytoconstituent III) and 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethy l2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1Hcyclopenta[α]phenanthren-3-ol (Phytoconstituent V) from the polyherbal tablet formulation. Solvent systems were optimized to achieve best resolution of the marker compounds from the other components of the sample. Of the various solvent systems tried, the one containing toluene: ethyl acetate: methanol: formic acid (3: 3: 0.2: 0.8) gave best resolution of, Phytoconstituent II (R_f=0.6), Phytoconstituent III (R_f=0.35) and Phytoconstituent V (R_f=0.47) in the presence of the other compounds in the polyherbal tablet sample and enabled the quantification of the marker compounds (Figure 6 and 7). The methods were validated in terms of precision and accuracy. The relationship between the concentration of standard solutions and the peak response was linear within a correlation coefficient of 0.998 for Phyoconstituent III, 0.996 for Phyoconstituent V and 0.999 for Phyoconstituent II. Precision studies were done as intra-day variability and inter-day variability. The RSD values during intra-day precision were found to be 0.804, 1.958 and 1.650 respectively. The RSD values during inter-day precision were found to be were 1.221, 1.506 and 1.388 respectively for Phytoconstituent II. The RSD values during intra-day precision were found to be 0.217, 0.753 and 1.320 respectively. The RSD values during inter-day precision were found to be 0.804, 0.634 and 0.520 respectively for Phytoconstituent III. The RSD values during intra-day precision were found to be 0.528, 1.376 and 1.469 respectively. The RSD values during inter-day precision were found to be 1.277, 1.307 and 1.274 respectively for Phytoconstituent V. The average percent recovery at three different levels was found. The recoveries were found between 99.48 and 99.68% for Phytoconstituent II. The recoveries were found between 99.29 and 99.38% for Phytoconstituent III. The recoveries were found between 99.34 and 99.43% for Phytoconstituent V. The results indicated that all three phytoconstituents are easily detected in sample analyzed. The polyherbal formulation found to contain of 8.66 ± 1.547 % w/w of Phytoconstituent II, 0.381±0.042 % w/w of Phytoconstituent III, 0.524

Table 1: Formulation composition of polyherbal tablets using active fractions.

Ingredients	Qty. taken (%)
Active fraction of *A. graveolens*	20
Active fraction of *S. persica*	20
Active fraction of *C. papaya*	20
Microcrystalline cellulose	10
Magnesium stearate	05
Lactose	25

± 0.073% w/w of Phytoconstituent V (Table 5). The method was found to be suitable for the quantification of these marker compounds in polyherbal formulation. From the present study it was concluded that the herbal drugs can be potentially used to control the state of hyperlipidemia. The present work also characterized active phytoconstituents from different parts of selected plants. The study involved development of polyherbal hypolipidemic formulation employing active fractions of *Apium graveolens*, *Salvadora persica*, *Carica papaya* and *Evolvulus alsinoides* with a view to not only reduce the serum levels of TC, triglycerides TG and LDL cholesterol but also increase the serum level of beneficial HDL cholesterol.

DISCUSSION

Research on herbal medicines is gaining ground and the demand to use natural products in the treatment of various disorders is increasing worldwide.[12] Investigations on herbal products might lead to the development of alternative drugs and strategies. Such alternative strategies are required for the effective management of hyperlipidemic disorders. 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.[13] Moreover alteration in cholesterol metabolism has been associated with the etiology of most human diseases. It is widely reported that hypercholesterolemia occasioned by a defect in cholesterol transportation, biosynthesis or catabolism is a risk factor in coronary heart disease and atherosclerosis.[14]

Recently, a number of clinical studies suggest that the increased risk of coronary heart disease is associated with a high serum concentration of TC, LDL-C and triglyceride. The abnormally high concentration of serum lipids is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots. On the other hand, low serum concentration of HDL-C is also responsible for coronary heart disease.[15] Preclinical observations demonstrate that hyperlipidemia promotes accumulation of OXLDL in the arterial wall, promoting endothelial dysfunction and development of atherosclerosis and congestive heart diseases.[16] LDL play a very important role in arteriosclerosis and that hypercholesterolemia is associated with a defect relating to the lack of LDL receptors. The decrease of cholesterol and LDL levels achieved by administration

Table 2: Effect of ethanolic extracts, fractions, isolated phytoconstituents and polyherbal formulation on total cholesterol level (mg/dl) in triton induced hyperlipidemic model.

Group	Initial	Total cholesterol level after triton administration	Total cholesterol level after the vehicle or drug treatment
Control	63.30 ± 0.24	65.19 ± 0.35	66.54 ± 0.68
Hyperlipidemic Group	66.69 ± 0.16	87.50 ± 0.28	99.83 ± 0.22
Standard Group (Atrovastatin) (80 mg/kg)	60.64 ± 1.74	86.57 ± 1.58	60.19 ± 1.02 [a]
A. graveolens ethanolic extract (200 mg/kg)	65.12 ± 0.33	83.96 ± 0.25	67.22 ± 0.76 [b]
A. graveolens $CHCl_3$ fraction (200 mg/kg)	63.21 ± 0.11	85.43 ± 0.16	65.32 ± 0.25[a]
S. persica ethanolic extract (200 mg/kg)	67.85 ± 1.42	88.94 ± 1.39	73.04 ± 1.51[b]
S.persica $CHCl_3$ fraction (200 mg/kg)	65.49 ± 0.20	87.28 ± 0.44	71.96 ± 0.32 [b]
C. papaya ethanolic extract (200 mg/ kg)	65.02 ± 1.36	86.66 ± 1.52	77.84 ± 1.54 [c]
C. Papaya water soluble fraction (200 mg/ kg)	64.97 ± 1.71	89.85 ± 1.91	74.16 ± 1.35 [b]
E. alsinoides ethanolic extract (200 mg/kg)	68.26 ± 0.10	87.60 ± 0.12	69.34 ± 0.58 [b]
E. alsinoides $CHCl_3$ fraction (200mg/kg)	62.54 ± 1.63	89.39 ± 1.36	66.27 ± 1.43 [a]
Phytoconstituent I (50 mg/kg)	60.52 ± 0.50	87.37 ± 0.90	65.36 ± 0.78[c]
Phytoconstituent II (50 mg/kg)	67.18 ± 1.03	88.65 ± 0.20	62.41± 0.37 [b]
Phytoconstituent III (50 mg/kg)	63.45 ± 0.66	86.92 ± 0.31	63.27 ± 1.43 [b]
Phytoconstituent IV (50 mg/kg)	61.25 ± 0.70	88.73 ± 1.09	67.58 ± 0.69[c]
Phytoconstituent V(50 mg/kg)	68.44 ± 0.48	87.45 ± 0.42	62.41 ± 0.78[b]
Polyherbal Formulation(200 mg/kg)	64.97 ± 0.88	84.82 ± 1.07	66.35 ± 0.96[b]

Total cholesterol concentrations are estimated by standard method. Values are expressed for six animals in each group as mean ± S.E.M.
a: p<0.001 b: p<0.01 c: p<0.05 compared with hyperlipidemic group

Table 3: Effect of ethanolic extracts, fractions, isolated phytoconstituents and polyherbal formulation on triglyceride level (mg/dl) in triton induced hyperlipidemic model.

Group	Initial	Triglyceride level after the administration of triton	Triglyceride level after the vehicle/drug treatment
Control	49.53 ± 0.48	49.25 ± 0.65	49.42 ± 0.70
Hyperlipidemic Group	48.85 ± 1.04	69.70 ± 0.58	86.77 ± 0.73
Standard Group (Atrovastatin) (80 mg/kg)	49.26 ± 1.70	64.58 ± 1.30	50.72 ± 1.52 [a]
A. graveolens ethanolic extract(200mg/kg)	45.47 ± 1.22	68.54 ± 0.72	57.13 ± 0.15 [b]
A. graveolens $CHCl_3$ fraction (200mg/kg)	45.76 ± 1.40	65.60 ± 1.85	54.26 ± 1.39 [a]
S.persica ethanolic extract (200mg/kg)	48.93 ± 0.46	67.89 ± 0.56	60.16 ± 0.23 [c]
S.persica $CHCl_3$ fraction (200mg/kg)	46.38 ± 0.55	66.11 ± 0.73	57.40 ± 0. 67 [b]
C. papaya ethanolic extract (200mg/ kg)	48.03 ± 1.63	65.77 ± 1.90	62. 39 ± 1.85 [c]
C. Papaya water soluble fraction (200mg/ kg)	43.22 ± 1.08	67.52 ± 1.21	58.45 ± 1.96 [c]
E. alsinoides ethanolic extract (200mg/kg)	45.51 ± 0.19	68. 18 ± 0.84	59.26 ± 1.10 [b]
E. alsinoides $CHCl_3$ fraction (200mg/kg)	49.68 ± 1.10	63. 87 ±1.02	55.53 ± 0.96[a]
Phytoconstituent I (50 mg/kg)	47.46 ± 1.50	66.93 ± 1.81	57.84 ± 0.77[b]
Phytoconstituent II (50 mg/kg)	49.12 ± 0.26	66.89± 0.55	53. 46 ± 0.61[a]
Phytoconstituent III (50 mg/kg)	42.41 ± 0.11	65.36 ± 1.02	55.27 ± 0.38 [a]
Phytoconstituent IV (50 mg/kg)	46.92 ± 0.23	67. 68 ± 0.57	59.11 ± 0.23[b]
Phytoconstituent V(50 mg/kg)	48.33 ± 0.92	64. 53 ±1.14	52.33 ± 1.04[a]
Polyherbal ormulation(200mg/kg)	46.18 ± 0.50	67.28 ± 0.33	60.45 ± 0.19 [b]

Triglyceride concentrations are estimated by standard method. Values are expressed as mean ± S.E.M for six animals in each group.
a: p<0.001 b: p<0.01 c: p<0.05 compared with hyperlipidemic group

Table 4: Effect of ethanolic extracts, fractions, isolated phytoconstituents and polyherbal formulation on HDL level (mg/dl) in triton induced hyperlipidemic model.

Group	Initial	HDL level after the administration of triton	HDL level after the vehicle/ drug treatment
Control	32.34 ± 1.36	32.36 ± 1.11	32.86 ± 1.34
Hyperlipidemic Group	32.22 ± 0.58	42.53 ± 0.24	43.02 ± 0.49
Standard Group (Atrovastatin) (80 mg/kg)	33.45 ± 1.63	44.43 ± 1.43	52.96 ± 1.01 [a]
A. graveolens ethanolic extract (200mg/kg)	32.33 ± 1.10	41.63 ± 1.08	46.22 ± 1.11 [c]
A. graveolens $CHCl_3$ fraction (200mg/kg)	33.30 ± 1.74	46.26 ± 1.19	49.83 ±1.42 [a]
S. persica ethanolic extract (200mg/kg)	34.88 ± 0.94	41.13 ± 0.71	44.96 ± 0.68 [c]
S. persica $CHCl_3$ fraction (200mg/kg)	35.20 ± 0.12	44. 99 ± 0.36	47. 34 ± 0.51 [b]
C. papaya ethanolic extract (200mg/ kg)	36. 17 ± 1.27	40.87 ± 1.44	43.55 ± 1.09 [c]
C. Papaya water soluble fraction (200mg/ kg)	32.48 ± 1.24	41.32 ± 1.39	44. 06 ±1.57 [c]
E. alsinoides ethanolic extract (200mg/kg)	34.29 ± 0.77	40.15 ± 0.24	45.66 ± 0.25 [c]
E. alsinoides $CHCl_3$ fraction (200mg/kg)	36.34 ± 0.89	45.81 ± 0.41	48.73 ± 0.63 [a]
Phytoconstituent I (50 mg/kg)	33.68 ± 1.72	44.35 ± 1.92	49.44 ± 1.88[b]
Phytoconstituent II (50 mg/kg)	34.15 ± 1.02	43. 88 ± 0.19	43. 25 ± 0.61 [a]
Phytoconstituent III (50 mg/kg)	34. 28 ± 0.37	41.66 ± 0.83	44.62 ± 0.14 [b]
Phytoconstituent IV (50 mg/kg)	32.33 ± 0.24	42.20 ± 0.96	49. 04 ±0.73 [c]
Phytoconstituent V(50 mg/kg)	33.51 ± 0.87	42.33 ± 0.28	44.18 ± 0.36 [a]
Polyherbal Formulation (200mg/kg)	34.23 ± 1.17	41.32 ± 1.24	50.29 ± 1.40 [c]

HDL concentrations are estimated by standard method. Values are expressed as mean ± S.E.M for six animals in each group.
[a]: $p<0.001$ [b]: $p<0.01$ [c]: $p<0.05$ compared with hyperlipidemic group

Table 5: Phytoconstituents content in polyherbal formulation.

Phytoconstituent	Content (%w/w)
II	8.66 ± 1.547
III	0.381 ± 0.042
V	0.524 ± 0.073

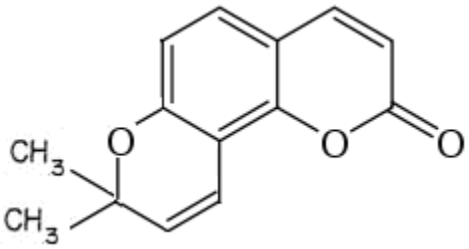


Figure 1: Phytoconstituent I.

IUPAC name: 8, 8-Dimethyl-2H, 8H-pyrano [2, 3-f] chromen-2-one.

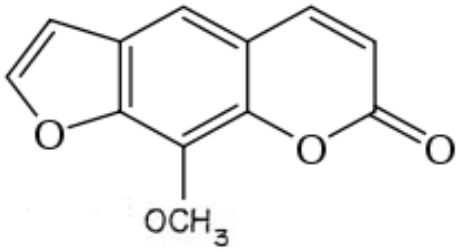


Figure 2: Phytoconstituent II.

IUPAC Name: 9- methoxyfuro [3, 2-g] chromen-7-one.

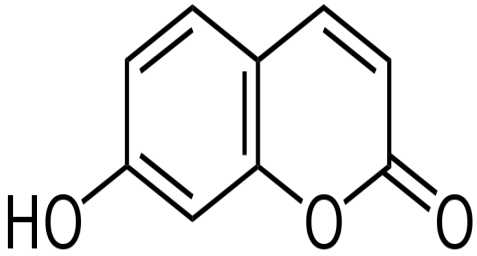


Figure 3: Phytoconstituent III.

IUPAC Name: 7- hydroxy-2H-chromen -2-one.

of test samples, demonstrates a possible protection against hypercholesterolemia and the harm this condition brings about.[17]

It is also known that HDL-cholesterol levels have a protective role in coronary artery disease. Similarly, increased level of serum LDL-cholesterol results in increased risk for the development of atherosclerosis. The increased level of HDL-cholesterol and decreased cholesterol level along with its LDL fraction which is evident from the results could be due to an increased cholesterol excretion and decreased cholesterol absorption through gastrointestinal tract.

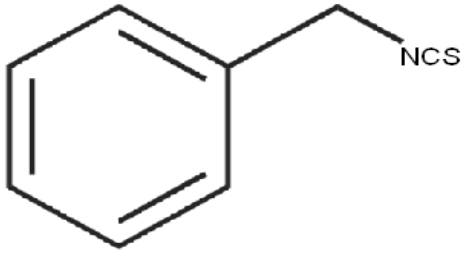


Figure 4: Phytoconstituent IV.

IUPAC Name: Isothiocyanatomethylbenzene.

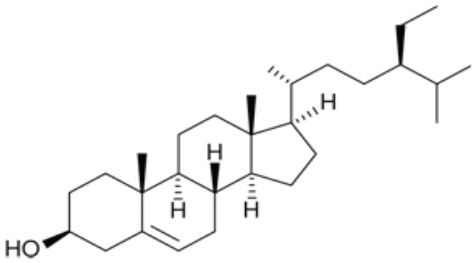


Figure 5: Phytoconstituent V.

IUPAC Name: 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl 2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[α] phenanthren-3-ol.

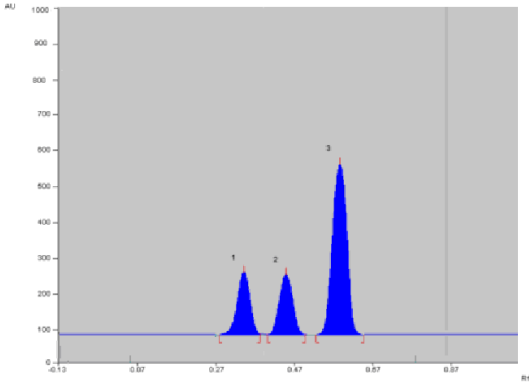


Figure 6: Densitogram of standard Umbelliferone, β- sitosterol and Methoxsalen.

Peak 3: Phytoconstituent II (R_f =0.6); Peak 1: Phytoconstituent III (R_f =0.35); Peak 2: Phytoconstituent V (R_f =0.47)

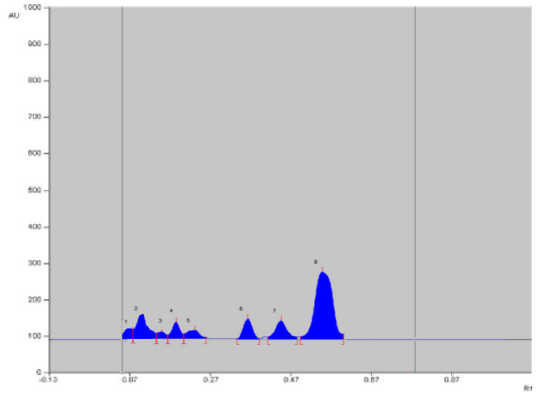


Figure 7: Densitogram of Polyherbal formulation.

Peak 8: Phytoconstituent II (R_f =0.6); Peak 6: Phytoconstituent III (R_f =0.35); Peak 7: Phytoconstituent V (R_f =0.47)

Phytosterols like beta-sitosterol lower cholesterol levels by competing with cholesterol for absorption in the intestine. Having a similar structure to cholesterol, phytosterols compete with cholesterol of dietary and biliary origin for incorporation into micelles in the gastrointestinal tract. Cholesterol displaced from the micelles is not absorbed and is destined for fecal excretion.

Increased triglyceride levels are a risk factor for cardiovascular disease. Beyond LDL cholesterol lowering, growing evidence suggest that phytosterols reduce triglyceride levels as well. The proposed mechanism behind the triglyceride lowering effect of phytosterols is due to a reduction in triglyceride rich VLDL particle produced by the liver. It is believed that the triglyceride lowering effects of phytosterols are more pronounced in individuals with elevated triglycerides. Beta-sitosterol present in *Evolvulus alsinoides* is a plant sterol which is structurally similar to cholesterol that acts in the intestine to lower cholesterol absorption. Because it has very low systemic absorption it proves beneficial to lower the cholesterol level in the body. Increasing the intake of phytosterols may help to reduce coronary heart disease with minimum risk.

The benzopyrans are a group of compounds whose members include coumarins and flavonoids.[18] Coumarins act by augmenting activity of LCAT, which regulates blood lipids.[19] 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. The possible mechanism of lipid lowering activity is may be due to enhancement of the activity of LCAT and inhibition of the action of hepatic TG- lipase on HDL. The presence of benzopyrans in *Apium graveolens* and *Salvadora persica* indicates that these derivatives act by augmenting Lecithin Acyl Transferase activity that further regulates the level of blood lipids. The hypocholesterolemic effect of *Apium graveolens* seeds and stems of *Salvadora persica* appears by enhancing the activity of Lecithin Acyl Transferase, as a result of which the action of hepatic TG- lipase on HDL was inhibited.

Sulphur containing compounds have an inhibitory effect upon the key enzymes involved in cholesterol biosynthesis, such as HMG-CoA reductase.

Hypocholesterolemic effect of papaya seeds is exerted by decrease in hepatic cholesterogenesis, where as the triacylglycerol lowering effect appears to be due to inhibition of fatty acid synthesis, by mallic enzymes, fatty acid synthatase and glucose-6-phosphate dehydrogenase. Bile acids are typically stored in fluid form in our gall bladder and when we eat a fat-containing meal, they get released into the intestine where they help ready the fat for interaction with enzymes to get absorbed in the body. The isothiocyanate glycosides present in *Carica papaya* seeds may bind together with some of the bile acids in the intestine and pass out of our body in a bowel movement rather than getting absorbed. When this happens, our liver needs to replace the lost bile acids by drawing upon our existing supply of cholesterol and as a result our cholesterol level drops down.

HPTLC fingerprinting helps in identification of chemical components even if the amount and concentration of the constituent is low. HPTLC method was established for the quantification of the isolated phytoconstituents in polyherbal formulation.

CONCLUSION

The present work characterized active phytoconstituent exhibiting anti-hyperlipidemic potential from selected medicinal plants. Coronary heart diseases are the clinical manifestation of atherosclerosis. Development of hyperlipidemia involves accumulation of lipid containing particles in the walls of coronary arteries. Treatment with different extracts, different fractions and polyherbal formulation 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. In the present investigation, treatment with ethanol extract and isolated phytoconstituent significantly decreased TC and TG level in serum while HDL level in serum was significantly increased. The active ingredients present in these medicinal plants may recover the disorders in lipid metabolism noted in hyperlipidemic state. HPTLC method was established for the quantification of the isolated phytoconstituents in polyherbal formulation.

The proposed method was found to be suitable for estimation of these markers in polyherbal formulation as it is proved to be precise, reproducible, reliable, accurate and robust. The present investigation may be quite useful as these medicinal plants are highly valued as traditional system of medicine.

ACKNOWLEDGEMENT

The author is thankful to the Research Institute for the support and providing facilities to carry out the research work.

CONFLICT OF INTEREST

We wish to assure that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

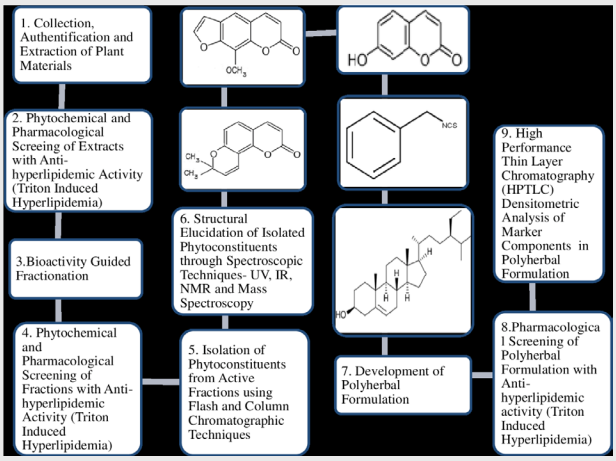
ABBREVIATIONS

HPTLC: High Performance Thin Layer Chromatography; **i.p.:** Intraperitoneal; **mg/kg:** Milligram/Kilogram; **TC:** Total Cholesterol; **TG:** Triglycerides; **LDL:** Low Density Lipoprotein; **VLDL:** Very Low Density Lipoprotein; **HDL:** High Density Lipoprotein; **UV:** Ultraviolet; MeOH/EtOH: Methanol/ Ethanol; **IR:** Infra-Red; **NMR:** Nuclear Magnetic Resonance; **DMSO:** Dimethyl sulfoxide; **$CDCl_3$:** Deuterated chloroform; **$CHCl_3$:** Chloroform; **TLC:** Thin Layer Chromatography; **CPCSEA:** Committee for the Purpose of Control And Supervision of Experiments on Animals; **OECD:** Organisation for Economic Co-operation and Development; **ICH:** International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; **CPMP:** Committee for Proprietary Medicinal Products; **RSD:** Relative Standard Deviation; **OXLDL:** Oxidized Low Density Lipoprotein; **LCAT:** Lecithin-Cholesterol Acyltransferase; **HMG-CoA:** 3-hydroxy-3-methylglutaryl-Coenzyme A.

REFERENCES

1. Barter PJ, Brewer HB, Chapman MJ, Hennekans CH, Rader DJ, Tall AR. Cholesteryl ester transfer protein: A novel target for raising HDL and inhibiting atherosclerosis. Arterioscler Thromb Vasc Biol. 2006;23(2):160-7.
2. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med. 1995;333(5):276-82.
3. Ahmed I, Lakhani MS, Gillet M, John A, Raza H. Hypotriglyceridemic and hypocholesterolemic effects of anti-diabetic Momordica charantia fruit extract in streptozotocin-induced diabetic rats. Diabetes Res Clin Pract. 2001;51(3):155-161.
4. Alder R, Lookinland S, Berry JA, Williams M. A systematic review of the effectiveness of garlic as an anti-hyperlipidemic agent. J Am Acad Nurse Pract. 2003;15(3):120-9.
5. Ansari SH. Essential of Pharmacognosy: Isolation, purification and screening of plant. India: Birla Publication Pvt. Ltd. 2007.
6. Asolkar LV, Kakkar KK, Chakre OJ. Second Supplement to Glossary of Indian Medicinal Plants with Active Constituents. India: NISCAIR. 1992.
7. Bruneton J. Pharmacognosy, Phytochemistry, Medicinal Plants. Hampshire, UK, Intercept Ltd. 1999.
8. Chatterjee A. Treatise of Indian Medicinal Plants. India: Council for Scientific and Industrial Research. 1990.
9. Bungorn S, Varima W, Pisamai L, Jamsai S, Durit J. Diuretic effects of selected Thai indegenous medicinal plants in rats. J Ethnopharmacol. 2001;75(2-3):185-90.
10. Patil UK, Dixit VK. Hypolipidemic activity of Cassia tora seeds. J Ethnopharmacol. 2004;90(2-3):249-52.
11. Friedman M, Bayer SO. The mechanism underlying hypocholesterolemia induced by Triton WR 1339. Am J Physiol. 1957;190(3):439-45.
12. Sofrata A, Claesson R, Lingstromn P, Gustafsson A. Strong antibacterial effect of miswak against oral microorganisms associated with periodontitis and caries. J Periodontol. 2008;79(8):1474-9.
13. Ghule BV, Ghante MH, Saoji AN, Yeole PG. Hypolipidemic and antihyperlipidemic effects of *Lagenaria siceraria* fruit extracts. Ind J Exp Biol. 2006;44(11):905-9.
14. Singh B, Solanki R, Bhatt V. Effects of antioxidant vitamins along with atorvastatin and atorvastatin–niacin combination on diet-induced hypercholesterolemia in rats. Int J Physiol Pathophysiol Pharmacol. 2010;2(1):57-63.
15. Parab RS, Mengi SA. Hypolipidemic activity of *Acorus calamus* in rats. Fitoterapia. 2002;73(6):451-5.
16. Aikawa M, Libby P. The vulnerable atherosclerotic plaque: Pathogenesis and therapeutic approach. Cardiovas Pathol. 2004;13(3):125-38.
17. Takahashi Y, Zhu H, Yoshimoto T. Essential roles of lipoxygenases in LDL oxidation and development of atherosclerosis. Antioxi Red Sig. 2005;7(3-4):425-31.
18. Kulkarni MV, Kulkarni GM, Lin CH, Sun CM. Recent advances in coumarins and 1-azacoumarins as versatile biodynamic agents. Curr Med Chem. 2006;13(23):2795-818.
19. Toyama DO, Diz FEB, Cavada BS, Rocha BA, Oliveira SC, Cotrim CA, *et al*. Umbelliferone induces changes in the structure and pharmacological activities of Bn IV, a phospholipase A(2) isoform isolated from *Bothrops neuwiedi*. Toxicon. 2011;57(6):851-60.

PICTORIAL ABSTRACT



SUMMARY

- The purpose of the present project is to perform the phytochemical investigation and exploration of hypolipidemic activity with the development and quantification of phytoconstituents of polyherbal hypolipidemic formulation employing *A. graveolens, S. persica, C. papaya* and *E. alsinoides*. The present work also characterized active phytoconstituents from different parts of selected plants. Flash chromatography technique was used for the isolation of phytoconstituents from the selected medicinal plants. Antihyperlipidemic study was done on triton induced model. Triton- WR 1339 (400 mg/kg b.w) i.p. injection were given to different animal groups. HPTLC densitometric method was established for the quantification of the phytoconstituents in polyherbal formulation.
- The phytoconstituents so isolated were identified as 8, 8-Dimethyl-2H, 8H-pyrano [2, 3-f] chromen-2-one, 9- methoxyfuro [3, 2-g] chromen-7-one, 7- hydroxy-2H-chromen -2-one, Isothiocyanatomethylbenzene and 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl2,3,4,7,8,9,11,12,14,15,16,17 dodecahydro -1Hcyclopenta[α]phenanthren-3-ol through chromatographic separation techniques and their structures were elucidated by spectroscopic analysis. The research work involved development of polyherbal hypolipidemic formulation with a view to reduce the serum levels of TC, TG and LDL and to increase the serum level of HDL cholesterol and to establish HPTLC densitometric method for quantification of its phytoconstituents. The formulation showed significant reduction in TC, TG, LDL and VLDL level ($p<0.01$) and significantly raised HDL level ($p<0.01$) compared to hyperlipidemic group. HPTLC method was also established for the quantification of isolated phytoconstituents in polyherbal formulation through densitometric analysis.

About Authors

Dr. Deepa Iyer has 14 years of experience in teaching and research. Her area of specialization is Natural Products Screening and Herbal Drug Research. She has been awarded "Fellowship for Training of Young Scientist" in 29th M.P. Young Scientist Congress, MPCOST, Bhopal. She has more than 30 research publications in reputed National and International Journals.

Umesh K. Patil is presently working as Professor of Pharmacognosy at Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya (A Central University), Sagar. His research interest includes Herbal Drug Technology, Ethnopharmacology and Natural Products. He has more than 22 years of experience in teaching and research. He is recipient of 7 prestigious awards given in the field of HMPs and remained BOYSCAST fellow of DST, Govt of India.

Cite this article: Iyer D, Patil UK. Structural Elucidation of Isolated Phytochemicals from Selected Medicinal Plants with Anti-hyperlipidemic Activity and Development of Polyherbal Formulation with Densitometric Analysis of Isolated Phytoconstituents. Indian J of Pharmaceutical Education and Research. 2020;54(2s):s316-s329.