CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.7489222

Available online at: http://www.iajps.com *Research Article*

# PHYTOCHEMICALS & ANTI HYPERLIPIDAEMIC ACTIVITY OF METHANOLIC EXTRACT OF *TEPHROSIA PURPUREAON RATS*

**Ankit Gour*, Sonpal Singh, Jagdish Chandra Rathi**
NRI Institute of Pharmaceutical Sciences, Bhopal (M.P.)

**Article Received:** October 2022 **Accepted:** November 2022 **Published:** December 2022

**Abstract:**
*Natural products, particularly of plant origin, are the main quarry for discovering promising lead candidates and play an imperative role in the upcoming drug development programs. Ease of availability, low cost, and least side effects make plant-based preparations the main key player of all available therapies. Tephrosia purpurea is a wild herb belongs to the family fabaceae and commonly known as sharpunkha. This plant has also been used for the treatment of several gastrointestinal disorders and has ability to cure disorder related to bowel, kidney liver spleen. Its dried parts can be used effectively for the treatment of boils, bleeding piles, bronchitis etc. It also has diuretic property. Its roots decoction is useful in enlargement and damage of liver. The aim of this study is to examine anti-hyperlipidaemic effect of Tephrosia purpurea. The leaves of plant were collected and subjected to extraction by methanol as solvent. Further qualitative & quantitative estimation of phytochemical and antioxidant activity was also checked. The anti -hyperlipidaemic activity was analysed in rats by examining various biochemical parameters. The results showed that the yields were found to be 7.28% w/w of Tephrosia purpurea of petroleum ether extract, 12.54 % w/w of methanolic extract. The phytochemical screening revealed presence of alkaloid, flavonoid glycosides, tannins, steroid, phenol. Total phenol & flavonoid content was found to be 0.865 mg/100mg and 0.947 mg/100mg of dried extract respectively. The IC 50 value for Methanolic extract of Tephrosia purpurea was found to be 53.37 as compared to ascorbic acid with 17.68 IC 50value. All the treatment groups produced a significant decrease in serum TC, TG, HDL-C, and VLDL-C levels. In addition to the above, the serum LDL-C levels were significantly decreased by the Tephrosia purpurea extracts. The Tephrosia purpurea leaves extract significantly increased fecal cholesterol excretion on dose dependant manner. Thus, from the results it can be concluded that the methanolic extract of Tephrosia purpurea exhibit potent anti hyperlipedemic activity.*

***Keywords:*** *Tephrosia purpurea, Anti -Hyperlipidaemic, Medicinal plants, TG, HDL, Cholesterol*

**Corresponding author:**
**Ankit Gour,**
*ankitgourvds121@gmail.com*

QR code

*Please cite this article in press Ankit Gour et al,* ***Phytochemicals & Anti Hyperlipidaemic Activity Of Methanolic Extract Of Tephrosia Purpureaon Rats..,*** *Indo Am. J. P. Sci, 2022; 09(12).*

## INTRODUCTION:

Natural products, particularly of plant origin, are the main quarry for discovering promising lead candidates and play an imperative role in the upcoming drug development programs. Ease of availability, low cost, and least side effects make plant-based preparations the main key player of all available therapies, especially in rural areas. Most tests have demonstrated the benefits of medicinal plants containing Hypoglycemic properties in diabetes management. Medicinal plants like aloe, banaba, bitter melon, caper, cinnamon, cocoa, coffee, fenugreek, garlic, guava, gymnema, nettle, sage, soybean, green and black tea, turmeric, walnut, and yerba mate used for treating diabetes and its comorbidities and the mechanisms of natural products as antidiabetic agents, with attention to compounds of high interest such as fukugetin, palmatine, berberine, honokiol, amorfrutins, trigonelline, gymnemic acids, gurmarin, and phlorizin [1,2].

Nowadays, treatments of diseases including diabetes using medicinal plants are recommended because these plants contain various phytoconstituents such as flavonoids, terpenoids, saponins, carotenoids, alkaloids, and glycosides, which may possess antidiabetic activities. The combined action of biologically active compounds (i.e., polyphenols, carotenoids, lignans, coumarins, glucosinolates, etc.) leads to the potential beneficial properties of each plant matrix, and this can represent the first step for understanding their biological actions and beneficial activities [3].

*Tephrosia purpurea* is a wild herb belongs to the family fabaceae and commonly known as sharpunkha. It is distributed among India, Australia, China, Sri Lanka up to 400 m to 1300 m altitude. As in Ayurveda system it is called as “Sarwa wran vishapah” which reveals that it has the ability to heal any type of wound. It is being used as a home remedy for healing wounds. Several ethno botanical articles revealed this plant as a folk medicine and is being used for the treatment of cuts and wounds in broad spectrum. It is one of the effective folk medicine for the treatment of inflammation as well as enlargement of liver and spleen. Because of this property it is also known as plihari or plihasathru where plihari denotes spleen [4].

This plant has also been used for the treatment of several gastrointestinal disorders and has ability to cure disorder related to bowel, kidney liver spleen. Its dried parts can be used effectively for the treatment of boils, bleeding piles, bronchitis etc. It also has diuretic property. Its roots decoction is useful in enlargement and damage of liver. It can be used as mouthwash and very helpful against gingivitis. Its roots are able to cure several skin disorders, can be used in elephantiasis, flatulence, asthma, anaemia, chronic fever. Moreover, roots and seeds of this herb can be used as insecticide as well as pesticide. Its roots being used as herbal fish poison by many hunters in Gunia. Its seeds oil has anthelmintic properties and also used in scabies and leukoderma. Leaves of this herb can be used in syphilis, gonorrhoea, pectoral diseases etc [5,-7]. So, This study deals with phytochemical & antihyperlipidemic activity of *Tephrosia purpurea* methanolic extract on rats

## MATERIAL AND METHODS:

### Collection of plant material

Organoleptic, morphological and microscopic properties could all be used to distinguish between crude medicines. *Tephrosia purpurea* was the chosen plant, and its leaves were located and collected from Bhopal based on their geographic accessibility. Selected plant material was collected, cleaned, dried in the shade, ground to a fairly coarse powder, and then kept in an airtight container for later use.

### Animals

Animal’s Albino rats (SD strain) weighing 150–200g of either sex were used in the present study. They were provided normal diet and tap water ad labium and were exposed to 12-h light and 12-h dark cycle. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

### Extraction

55.3 gram weight of powdered plant medicine was measured and placed in a soxhlet container. A drop from the thimble was placed on a piece of filter paper that did not have any oily spots, indicating that the plant material had been completely defatted. The plant material was defatted with petroleum ether (40°-60°C) for around 12 hours separately.

The defatted material was taken out of the Soxhlet device and allowed to air dry to get rid of any remaining petroleum ether. The medicine from the defatted plant was extracted using methanol as the solvent. For various solvents, the process was run for approximately varying lengths of time. The liquid extracts were collected in a tarred conical flask. The

solvent removed by distillation. Last traces of solvent being removed under vacuum. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated.

**Preliminary phytochemical screening**

To analyze the plant material in terms of its active ingredients, one must first perform a preliminary phytochemical screening. Leaves of *Tephrosia purpurea* extracts were put through routine phytochemical testing in order to identify the various components they contained [8,9].

**Estimation of total phenolic content**

The total phenolic content of dry extracts was performed with Folin-Ciocaltaeu assay. 1 ml of sample (1 mg/ml) was mixed with 1 ml of Folin Ciocalteu's phenol reagent. After 5 minutes, 10 ml of 7% sodium carbonate solution was added to the mixture followed by the addition of 13ml of demonized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 minutes at 23 $^0$ C, after which the absorbance was read at 760 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of Gallic acid equivalents (GAE)/100mg of dried sample [10].

**Estimation of total flavonoid content**

10mg quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pippeted out and made up to 10ml with Methanol to get 100 mcg/ml Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 5, 10, 15, 20, and 25mcg/ml were prepared. To each of these 4ml water was added followed by 0.3ml of 5% sodium nitrite. After 5min 0.3ml of 10% Aluminium chloride solution and at the 6$^{th}$ minute 2ml of 1M Sodium hydroxide was added. The total volume was made up to 10ml with distilled water. A blank was prepared without addition of aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 510nm using UV-Visible spectrophotometer.

***In vitro* DPPH radical scavenging**

DPPH scavenging activity was measured by the spectrophotometer with slightly modification . Stock solution (6 mg in 100ml methanol) was prepared Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm [11].

**Drugs and extract**

All the extracts were suspended in distilled water using 1% w/v gum acacia. The reference drugs Atorvastatin suspended in distilled water using 1% carboxymethyl cellulose (CMC). The control group received 1% w/v gum acacia in distilled water and 1% CMC solution as vehicles.

**Acute oral toxicity study**

Adult Albino rats (SD strain) weighing 150–200g of either sex, fasted overnight and were used for acute toxicity study, as per the Organization for Economic Co-Operation and Development (OECD 423) guideline. Four groups of mice of both sexes were fasted overnight. The first control group mice received 0.5% carboxymethyl cellulose (CMC) suspension in distilled water while the other groups received methanolic extract of *Tephrosia purpurea* in 0.5% CMC at doses of 200, 600, and 2000 mg/kg. Animals were observed closely for first 4 hours, for any toxicity manifestation, like increased motor activity, salivation, convulsion, coma, and death. Subsequently observations were made at regular intervals for 24 h. The animals were under further investigation up to a period of 14 days and no mortality was reported within the study period.

**Diet-induced hyperlipidemia in rats**

The normal group received a standard chow diet and all other groups received a high-cholesterol diet consisting of normal chow diet 92%, cholesterol 2%, cholic acid 1%, and coconut oil 5% for 7 days. The reference drug (Atorvastatin 50 mg/kg) and extracts were administered once daily between 8:00 and 9:00 a.m. for 7 days. The daily food intakes were determined before treatments. On the last day, animals were deprived of food but not water. Blood samples were collected by retro orbital puncture technique under light anesthesia. The animals were sacrificed and liver tissues were collected and preserved at 40°C for further analysis [12].

**Table 1: Diet-induced Hyperlipidemia Model: Summary of Animal Groups and Treatments**

| S. No | Groups | Treatments |
|---|---|---|
| 1. | Normal | Vehicles (1 mL of 1% gum acacia and 1% CMC) |
| 2. | Hyperlipidimic control | High cholesterol diet |
| 3. | Treated with Standard (Atorvastatin) | High cholesterol diet + Atorvastatin (50mg/kg, p.o.) |
| 4. | Treated with MtTP 200mg/kg | High cholesterol diet + MtTP (200mg/kg, p.o.) |
| 5. | Treated with MtTP 400mg/kg | High cholesterol diet + MtTP (400mg/kg, p.o.) |

**Estimation of biochemical parameters**

**Lipid profile**

The serum lipid profile was determined on day 8 in the case of diet-induced hyperlipidemia. The total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) levels were estimated using commercially available kits (Erba; Transasia Bio-Medicals Ltd., Daman, India). Very low-density lipoprotein cholesterol (VLDL-C) was calculated as TG/5. LDL-cholesterol (LDL-C) levels were calculated using Friedewald's formula [ The atherogenic index was calculated using the formula: atherogenic index [13].

**Estimation of serum lipid profiles**

Estimation of lipid profiles were placed major role in obesity condition. Usually, in obese condition the levels of lipids were higher than normal. So that, to know the activity of plant extract lipid profiles was studied.

**Estimation of Cholesterol**

Ferric chloride-acetic acid (9.9 ml) reagent was added to 0.1 ml of serum for deproteinization. The contents were centrifuged at 3000 rpm for 15 min. 5 ml of the supernatant was taken and to this added 3 ml of concentrated Sulphuric acid and kept for 20 min at room temperature. The pink colour formed was read at 540 nm against a blank containing 5 ml of ferric chloride-acetic acid reagent. A set of standards were also performed in the similar manner [14].

**Estimation of Triglycerides**

Plasma (0.1 ml) was taken in a glass stoppered centrifuge tube and to this added 4 ml of isopropanol and 400 mg of alumina. The tubes were tightly capped and shaken vigorously for 10 min. The tubes were centrifuged at 3000 rpm for 15 min and 2 ml of the supernatant was pipette into clean, dry test tubes. To these added 0.6 ml of alcoholic KOH and kept at 70°C for 15 min. The tubes were cooled to room temperature. To this added 0.5 ml of acetyl acetone reagent, 1.0 ml of meta periodate reagent and incubated at 50°C for min. Standard was also run in the same fashion with triolein instead of plasma. The colour developed was read at 405 nm against the reagent blank [15].

**Estimation of HDL-Cholesterol (HDL-C)**

Plasma (0.5 ml) was taken in a centrifuge tube and to this added 0.25 ml of Phosphotungstic acid reagent and 0.25 ml of $MgCl_2$ and was centrifuged at 1500 x g for 30 min in a refrigerated centrifuge and the amount of cholesterol was determined in the supernatant.

**Estimation of VLDL and LDL cholesterol (VLDL-C and LDL-C)**

By using Freidwald formula the concentration of VLDL and LDL cholesterol in serum were calculated.

**Results & Discussion**

The yields were found to be 7.28% w/w of *Tephrosia purpurea* of petroleum ether extract, 12.54 % w/w of methanolic extract. The total phenolic Content in leaves (mg/100mg) in methanolic extract was found to be 0.865mg/100mg dried extract. Total flavonoids content (TFC) of methanolic extract of *Tephrosia purpurea* was calculated using calibration curve method. The Total flavonoid content in leaves (mg/100mg) in methanolic extract was found to be 0.947mg/100mg dried extract. The phytochemical screening revealed presence of alkaloid, flavonoid glycosides, tannins, steroid, phenol. The IC 50 value for Methanolic extract of *Tephrosia purpurea* was found to be 53.37 as compared to ascorbic acid with 17.68 IC 50value.

The acute toxicity study did not result in any mortality of treatment rats and no toxic effect was observed throughout the 14 days study period. Physical observation of the test article-treated rats throughout the study indicated that none of the them

showed signs of toxic effect such as changes on skin and fur, eyes and mucus membrane, behavior pattern, tremors, salivation, diarrhea, sleep and coma. No mortality was observed in any of the rats.

No significant difference in food intake among the different groups was observed for day 1 to day 7. There was a significant increase in the serum levels of TC, TG, LDL-C, VLDL-C, and HDL-C in the hyperlipidimic control group as compared with the normal control group. The *Tephrosia purpurea* leaves extract significantly increased fecal cholesterol excretion on dose dependant manner. The fecal bile acid excretion was significantly increased by all the treatment groups except atorvastatin.

All the treatment groups produced a significant decrease in serum TC, TG, HDL-C, and VLDL-C levels. In addition to the above, the serum LDL-C levels were significantly decreased by the *Tephrosia purpurea* extracts.

**Table 2: Extractive values obtained from *Tephrosia purpurea***

| S. No. | Extracts | Time of extraction (Hours) | Color of extract | % Yield |
|---|---|---|---|---|
| 1 | Petroleum ether | 12 | Brown | 7.28% |
| 2 | Methanolic | 12 | Bark brown | 12.54% |

**Table 3: Preliminary phytochemical screening *Tephrosia purpurea* extract**

| S.N. | Phytoconstituents | Test Name | Methaolic Extract |
|---|---|---|---|
| 1 | Alkaloids | Mayer's Test | Present |
| | | Dragendorff's Test | Present |
| 2 | Glycosides | Raymond's Test | Present |
| | | Killer Killani Test | Present |
| 3 | Carbohydrates | Molisch's Test | **Absent** |
| | | Fehling's Test | **Absent** |
| 4 | Tannins | Vanillin- HCl Test | Present |
| | | Gelatin Test | **Absent** |
| 5 | Flavonoids | Lead acetate | Present |
| | | Shinoda Test | Present |
| 6 | Resins | Color detection with ferric chloride | **Absent** |
| | | Turbidity Test | **Absent** |
| 7 | Steroids | Libermann- Bur chard Test | Present |
| | | Salkowski Reaction | Present |
| 8 | Proteins & Amino acids | Biuret Test | Present |
| | | Precipitation test | **Absent** |
| | | Ninhydrin Test | Present |
| 9. | Phenols | Ellagic Acid Test | Present |

**Table 4: Estimation of total phenol and flavonoids content of *Tephrosia purpurea***

| S. No. | Total phenol content | Total flavonoids content |
|---|---|---|
| | mg/ 100 mg of dried extract | |
| 1. | 0.865 | 0.947 |

**Results of antioxidant activity using DPPH method**

**Table 5: % Inhibition of ascorbic acid and methanolic extract of *Tephrosia purpurea***

| S. No. | Concentration (µg/ml) | % Inhibition | |
|---|---|---|---|
| | | Ascorbic acid | Methanolic extract of *Tephrosia purpurea* |
| 1 | 10 | 44.65 | 25.45 |
| 2 | 20 | 48.62 | 35.56 |
| 3 | 40 | 65.34 | 45.58 |
| 4 | 60 | 69.65 | 52.23 |
| 5 | 80 | 77.41 | 61.45 |
| 6 | 100 | 84.13 | 69.98 |
| **IC 50** | | **17.68** | **53.37** |

**Table 6: Effects of different treatments on food intake of diet-induced hyperlipidimic rats**

| Group (n = 6) | Daily food intake (g) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
| Normal | 20.63±0.44 | 21.57±0.23 | 20.75±0.21 | 21.92±0.44 | 21.15±0.40 | 20.78±0.45 | 20.92±0.55 |
| Hyperlipidimic control | 20.79±0.21 | 20.44±0.41 | 20.52±0.39 | 20.68±0.78 | 21.75±0.31 | 21.13±0.50 | 20.48±0.54 |
| Atorvastatin | 19.36±0.52 | 20.48±0.42 | 21.12±0.34 | 21.03±0.32 | 22.77±0.75 | 20.41±0.41 | 22.57±0.48 |
| Treated with MtTP 100mg/kg | 20.33±0.31 | 21.03±0.52 | 21.66±0.32 | 20.17±0.42 | 22.65±0.58 | 22.15±0.29 | 22.21±0.54 |
| Treated with MtTP 200mg/kg | 21.04±0.35 | 22.07±0.35 | 20.44±0.42 | 22.53±0.47 | 20.20±0.45 | 20.19±0.62 | 20.30±0.52 |

**Table 7: Effect of *Trichosanthes cucumerina* leaves extract on serum lipid profile of diet-induced hyperlipidemia in rats**

| Group (n = 6) | TC (mg/dL) | TG (mg/dL) | HDL-C (mg/dL) | LDL-C (mg/dL) | VLDL-C (mg/dL) |
|---|---|---|---|---|---|
| Normal | 138.79±1.47 | 109.46±4.54 | 70.11±1.32 | 61.43±2.45 | 29.09±0.83 |
| Hyperlipidemic control | 337.23±6.11* | 312.80±12.57* | 112.55±3.54* | 171.91±9.58 | 69.76±2.52* |
| Atorvastatin | 171.00±6.37** | 171.99±1.82** | 75.88±2.47 | 71.53±6.48** | 41.60±0.39** |
| Treated with MtTP 100mg/kg | 251.26±3.21** | 118.42±2.11** | 75.52±2.41 | 162.08±3.61 | 30.68±0.42** |
| Treated with MtTP 200mg/kg | 208.56±8.91** | 131.51±5.43** | 75.99±2.31† | 117.46±7.73** | 27.10±1.09** |

**Table 8: Effect of *Tephrosia purpurea* extract on fecal cholesterol and bile acid excretion in diet-induced hyperlipidimic rats**

| Group | Fecal cholesterol (mg/g of fecal matter) | Fecal bile acid‡ (mg/g of fecal matter) |
|---|---|---|
| Normal | 2.07±0.09 | 1.29±0.08 |
| Hyperlipidimic control | 4.21±0.07* | 1.12±0.05 |
| Atorvastatin | 3.77±0.07 | 2.56±0.06 |
| Treated with EtTC 200mg/kg | 5.74±0.13** | 4.87±0.04** |
| Treated with EtTC 300mg/kg | 9.54±0.13** | 2.87±0.10** |

## CONCLUSION:

In the present study, we have selected the *Tephrosia purpurea* for evaluating antihyperlipidemic activity since the phytochemical constituents are found to be in excellent quantity. In our study high fat diet induced hyperlipidemia in rats model was used. From the results, it is evident that methanolic extracts *Tephrosia purpurea* leaves can effectively decrease plasma cholesterol, triglyceride, LDL, and VLDL and increase plasma HDL levels. In addition, the methanolic extract of *Tephrosia purpurea* leaves has shown significant antioxidant activity. By the virtue of its antioxidant activity, *Tephrosia purpurea* leaves show antihyperlipidemic activity.

## REFERENCES:


1. Wadkar, K.; Magdum, C.; Patil, S.; Naikwade, N. Antidiabetic potential and Indian medicinal plants. J. Herb. Med. Toxicol 2008, 2, 45–50. [Google Scholar]
2. Asija R, Singh CH, Hemlata A. A comprehensive review on Antihyperlipidemic activity of various medicinal plants. Int J Curr Pharm Rev Res. 2016;7(6):407-15.
3. Koriem KM. Antihyperlipidemic activity of the medicinal plants among Kadazan and Dusun communities in Sabah, Malaysia: a review. Asian Pacific Journal of Tropical Biomedicine. 2014 Oct 1;4(10):768-79.
4. Rao AS, Yadav SS, Singh P, Nandal A, Singh N, Ganaie SA, Yadav N, Kumar R, Bhandoria MS, Bansal P. A comprehensive review on ethnomedicine, phytochemistry, pharmacology, and toxicity of Tephrosia purpurea (L.) Pers. Phytotherapy Research. 2020 Aug;34(8):1902-25.
5. Akanksha B, Avijit M, Chakraborthy GS, Seema G. Phytopharmacological uses of Tephrosia Purpurea—A review. Pharmacophore. 2014;5(4):658-65.
6. Dalwadi PP, Patel JL, Patani PV. Tephrosia purpurea Linn (Sharpunkha, Wild Indigo): a review on phytochemistry and pharmacological studies. Indian Journal of Pharmaceutical and Biological Research. 2014 Jan 1;2(1):108.
7. Babu N, Singh A, Singh R. A review on therapeutic potential and phytochemistry of Tephrosia purpurea. Bulletin of Pure & Applied Sciences-Botany. 2017 Jul 1(2).
8. Kokate CK. Ed. Practical Pharmacognosy, 4th Edn., *Vallabh Prakashan*: 1994; 112:120.
9. Harborne JB. Phytochemical methods A guide to modern techniques of plant analysis. Third ed. Chapman and Hall. 1998; 204-8.
10. Mishra AG, Singh R, Meha P, Parkhe G. Determination of total phenolic, flavonoid content, antioxidant and antimicrobial activity of Gloriosa superb seed extract. Asian J Pharm Educ Res. 2017;6(2):12-7.
11. Parkhe G, Jain P. Study of antioxidant potential of hydroalcoholic extract of Anethum graveolens. Career. Int J Sci Technol. 2018;1(2):39-45.
12. Blank B, Pfeiffer FR, Greenberg CM, Kerwin JF (1963): Thyromimetics.II. The synthesis and hypocholesterolemic activity of some betadimethylaminoethyl esters of iodinated thyroalkanoic acids. J Med Chem 6: 560–563.
13. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry. 1972 Jun 1;18(6):499-502.
14. Zlatkis A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. The Journal of laboratory and clinical medicine. 1953 Mar 1;41(3):486-92.
15. Foster LB, Dunn RT. Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. Clinical chemistry. 1973 Mar 1;19(3):338-40.