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A kinetic study comparing Terminalia arjuna L. and Bacopa monnieri L. for lowering total serum cholesterol- in vitro

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ABSTRACT

Every cell in the human body needs cholesterol to maintain the structure of its cellular membranes. All forms of cholesterol are a class of lipid molecules and are not dangerous within normal range. They are also crucial for the healthy functioning of the human body. However, the body makes the required amount of cholesterol, some people are by heredity prone to producing more cholesterol than required. Also, cholesterol levels increase with increase in age, high fatty diets, and unhealthy lifestyles. The risk for heart and cardio vascular illnesses has been linked to high cholesterol levels recently. Hence, an accurate, sensitive, exact, dependable, quick method is developed to detect the total cholesterol level using high-performance thin layer chromatography(HPTLC). Precoatedsilica gel 60 F254 glass plates served as the stationary phase in this technique, the samples are applied and developed utilizing a fully automatic CAMAG Pro sample applicator and developer module application. It has been demonstrated that this method is repeatable and can even be used for samples with intricate matrixes and showed encouraging results.

Keywords: High-performance thin layer chromatography (HPTLC), cholesterol and cardiovascular illnesses.

INTRODUCTION

All forms of cholesterol are a class of lipid molecules and are not dangerous within normal range. They are also crucial for the healthy functioning of the human body. Every cell in the body needs cholesterol to maintain the structure of its cellular membranes. It is also necessary to producesome type of hormones, vitamins etc. It is animportant part of the mammalian cell membrane structure and is necessary to preserve its fluidity and permeability. This fatty molecule, is practically present in all human body cells. It is naturally made in the human liver and is also present in various foods, such as eggs, various types of meats, milk and milk products like cheese, butter etc.

However, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol are the two different forms of cholesterol. Many individuals consider and refer to LDL cholesterol as the bad type of cholesterol. People perceive HDL to be the good type of cholesterol. Plaque accumulation in the arteries can result from having too much LDL cholesterol in the blood. When this happens, cardiovascular disease (CVD), such as heart attack and stroke, may result. Although the body makes the required amount of cholesterol, some people are genetically prone to producing more cholesterol than required. Also, cholesterol levels increase with age,high fattydiets, and unhealthy lifestyles.

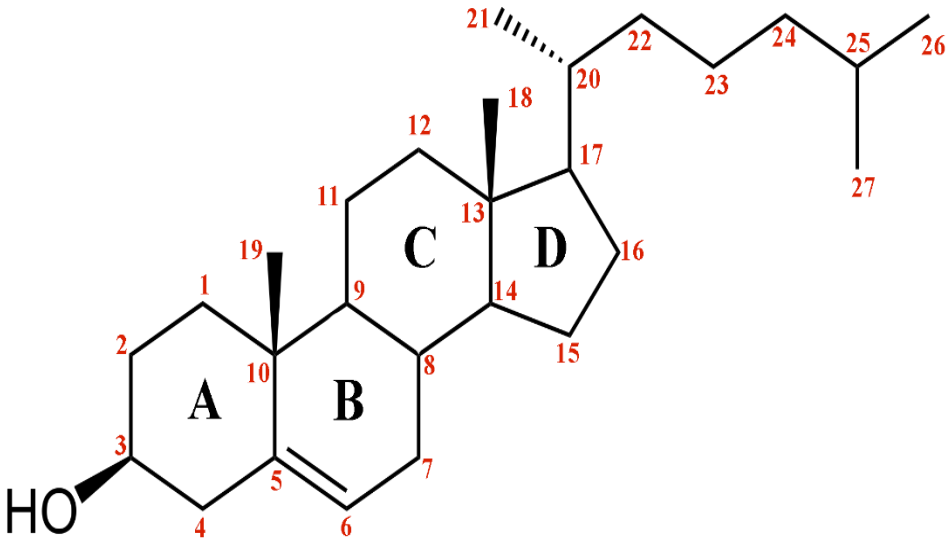

Fig.1 structure of cholesterol

The risk for heart and vascular illnesses has been linked to high cholesterol levels. Since the nineteenth century , there has been a growing interest in determining its concentration in foods due to its link to plasma cholesterol concentration and atherosclerosis. Therefore, even though the human body requires cholesterol to survive, too much of it can result in potentially dangerous cardiac conditions as well as other related illnesses like diabetes, strokes, etc. As a result, the measurement of cholesterol has grown significant in composition research on diverse food products.[1]

Since cholesterol in (Fig. 1) is insoluble in water or aqueous medium, it is challenging to estimate its content using conventional techniques. Complex techniques, such as isotopic tracking and mass fragmentation, were developed for the qualitative detection of cholesterol and to investigate the cell's related metabolic pathways. Additionally, severalother techniques, such as colorimetric and spectrophotometric analysis & estimations, have been discovered to measure cholesterol concentrations. [2-9]Spectrophotometric analysis, fluorometric analysisand high-performance liquid chromatography(HPLC), among other techniques have also been used.[10, 11]Most of the above-mentioned techniques require the involvement of various chemicals and laborious laboratory procedures, and some of them are not appropriate for materials with complex matrixes.

The advantages of thin layer chromatography are utilised in the sophisticated and advanced process known as high-performance thin layer chromatography (HPTLC). The main benefits of HPTLC include its low analysis cost, fully automated analysis system, few sample requirements, and less sample preparation stages. It also makes it possible to analyse multiple samples at once while doing chromatography, derivatization, and detection. HPTLC is a vital tool for estimating diverse samples because it is an advanced analytical tool suitable for both qualitative and quantitative analysis of samples. One of the major features of this technique is its capacity to simultaneously determine both qualitatively and quantitatively for numerous samples with various contents, enabling comparisons between reference standards and test samples for easy identification of the primary component.

When using a TLC scanner and detecting the constituents' absorption and/or fluorescence at the right wavelength, HPTLC CAMAG Pro creates visible chromatograms, peaks, and data that can also be densitometrically evaluated and compared. [12,17,18]

Today's scientific community is particularly interested in lipoproteins because of their connection to atherosclerosis and other diseases. A study by Touchstone [13] compiles several studies that have been written about the analysis of cholesterol and its compounds by TLC. But many methods for determining cholesterol concentrations rely on mass spectrometry or chemical post-chromatographic derivatization.

Herbals nowadays have gained significant importance due to lesser side effects, additionallyarjuna bark lowers blood cholesterol levels and reduces blood vessel hardening by lowering fat build up in the arteries, hence preventing atherosclerosis. [14] In Ayurveda, Brahmi (Bacopa monnieri L.) is recognised as a superior mental tonic that improves human thinking, learning, memory, and cognitive function.[15] Brahmi is used to treat asthma, mental illnesses, as a nervine tonic and as a diuretic, etc. In addition to its use as a brain tonic, either alone or in combination with other herbs,reducing the major enzymes responsible to produce reactive oxygen species (ROS) also aids in lowering their scavenging activity. [16,17]

Cow urine (distilled and branded) has several qualities, one of which is bio enhancing. As a bioenhancer, cow urine distillate is more effective at boosting the effects of antibacterial, antifungal, and anticancer medications. [18-20]

The aim of the study was to develop and evaluate a simple HPTLC method for the measurement of pooled serum total cholesterol levels (discarded and non-infectious) before and after treatment with herbal filtrates at fixed time intervals. The analytical methodology stands out by preserving the correctnessof the analysis. The cholesterol concentration in the pooled serum sample (before & after treatment) was estimated to confirm the repeatability of this method.

MATERIALS & METHODS

Chemicals & reagents

The laboratory employed only analytical grade solvents and chemicals that were purchased from standardised vendors for the duration of the experiment. Pooled serum total cholesterol samples that had been discarded and were not infectious were used for the analysis, and methanol and n-hexane were used in the extraction process.

Instrumentation and conditions

The stationary phase consisted of glass precoatedsilica gel 60 F254 plates from Merck (Germany) that were 20 cm by 10 cm in size. The samples were applied to the HPTLC plate and developed using a fully automatic CAMAG Pro sample applicator and developer module system under the flow of nitrogen gas and filtered air. The chromatogram was made using twin trough chambers (CAMAG) that were 20 cm × 10 cm in size. Densitometric analysis was carried out using the CAMAG TLC Scanner 4 and the vision CATS planar chromatography manager programme (version 3.1; CAMAG, Muttenz, Switzerland) was used. The plates were heated on a hot plate and then dried after derivatization with 5% copper sulphate solution.

Standard preparation

The cholesterol standard used from the Erba chem Transasia kit gave result of 200 mg/dL. Kinetic study was conducted utilising it as the reference standard for cholesterol.After adding herbal Arjuna filtrate (Terminalia arjuna L. branded bark powder) to the pooled serum samples in distilled water and cow urine, respectively, a chromatogram was developed.

Sample preparation

The discarded, non-infectious pooled serum sample was used and Brahmi (Bacopa monnieri L. branded leaf powder) herbal filtrate in distilled water and cow urine, respectively was added to the serum sample and aliquots were obtained from each tube stored in a hot water bath at 37^0 C. The proteins were precipitated with methanol at 2-, 4-, and 6-hour intervals, and then they were equilibrated with n-hexane. After five days of standing, the n-hexane layers were transferred to the vials for further examination, and the samples were stored at 4^0 Celsius.

Chromatographic conditions

On the HPTLC Pro (CAMAG) autoanalyzer, samples and standards were both prepared and evaluated. The mobile phase was improved for higher resolution before the quantitative analysis by employing the trial-and-error method. The mobile phase and the improved solvent system were then used for further analysis. The plates were labelled and the chambers were saturated with the mobile phase before adding the sample. A 20 cm x 10 cm CAMAG twin through chamber and 20 ml of the mobile phase—9.5:0.5 v/v of methanol and chloroform, respectively—were used to create the chromatogram. The application locations were 10 mm away from the plate's edges and bottom. Before usage, the Whatman filter paper No. 1 was wetted with the solvent solution. It was then put in the twin trough chamber for development so that the chamber could be saturated with mobile phase vapours for 20 minutes before to each run. The ascending chromatographic technique was used to develop the plate to a migration distance of 70 mm. After being derivatized with a 5% copper sulphate solution, the plates were dried on a hot plate.

The CAMAG TLC Scanner 4 was employed for detection and densitometric scanning, with a 540 nm absorption wavelength. The scanning speed employed was 100 mm/s. The Vision CATS planar chromatographic management programme (CAMAG, Muttenz, Switzerland, version 3.1) was used to oversee the entire procedure. A constant temperature of 37± 3°C was used for all laboratory activities.

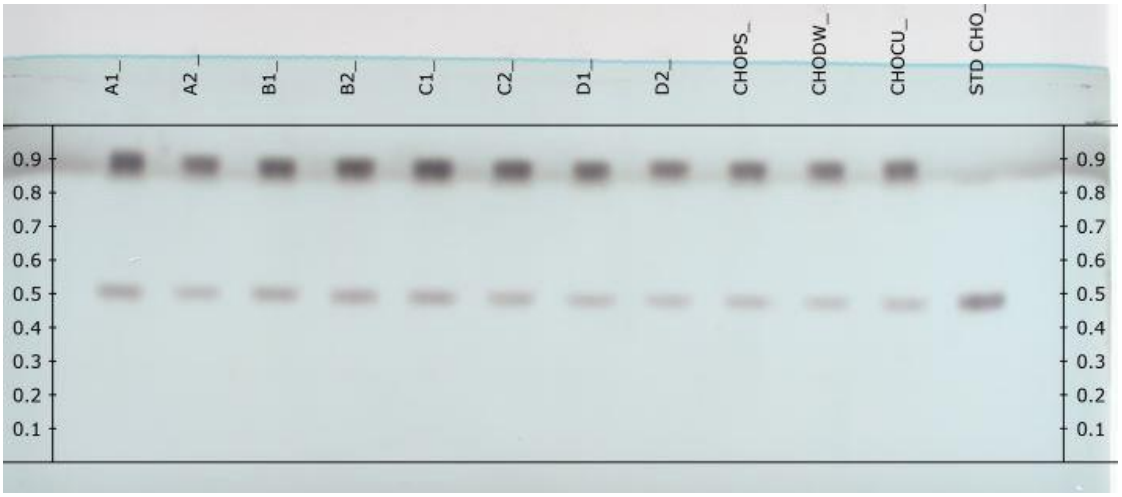

Fig 2. HPTLC plate image

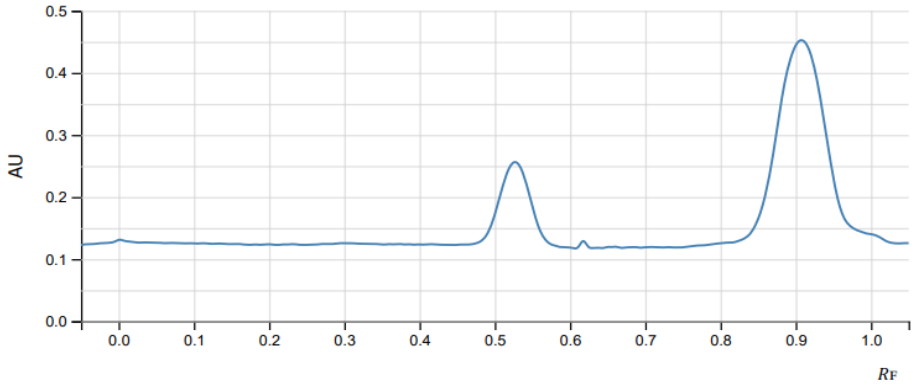

Fig 3. (for sample A1)

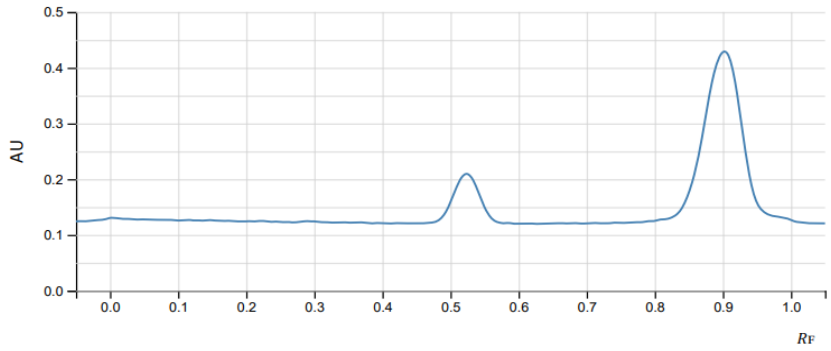

Fig 4.(for sample A2)

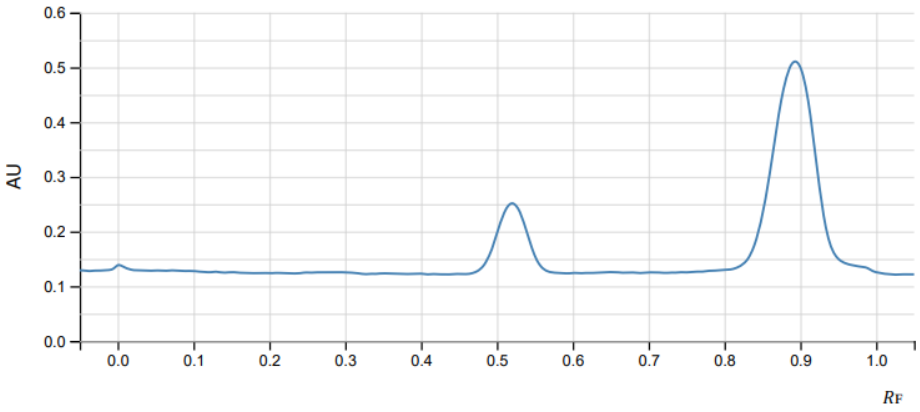

Fig 5. (for sample B1)

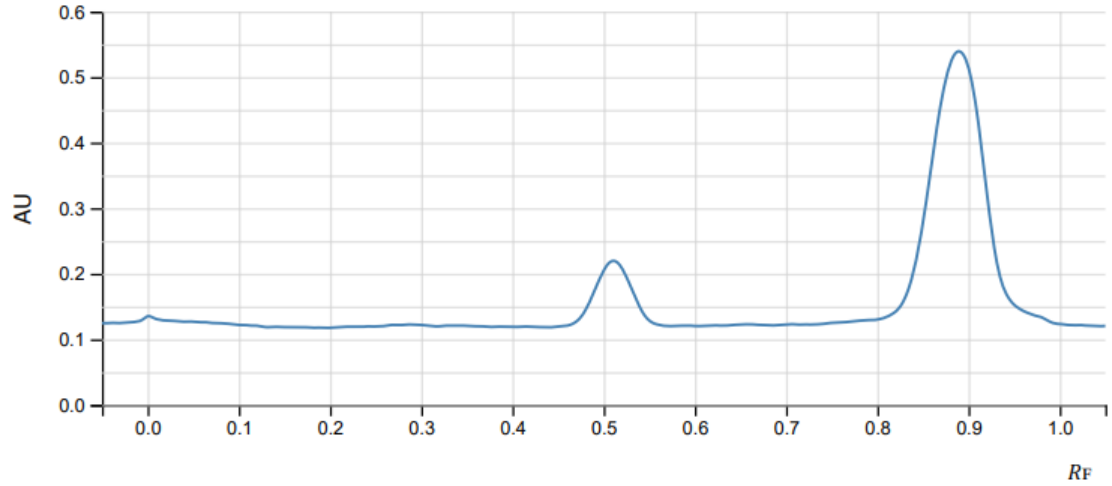

Fig 6. (for sample B2)

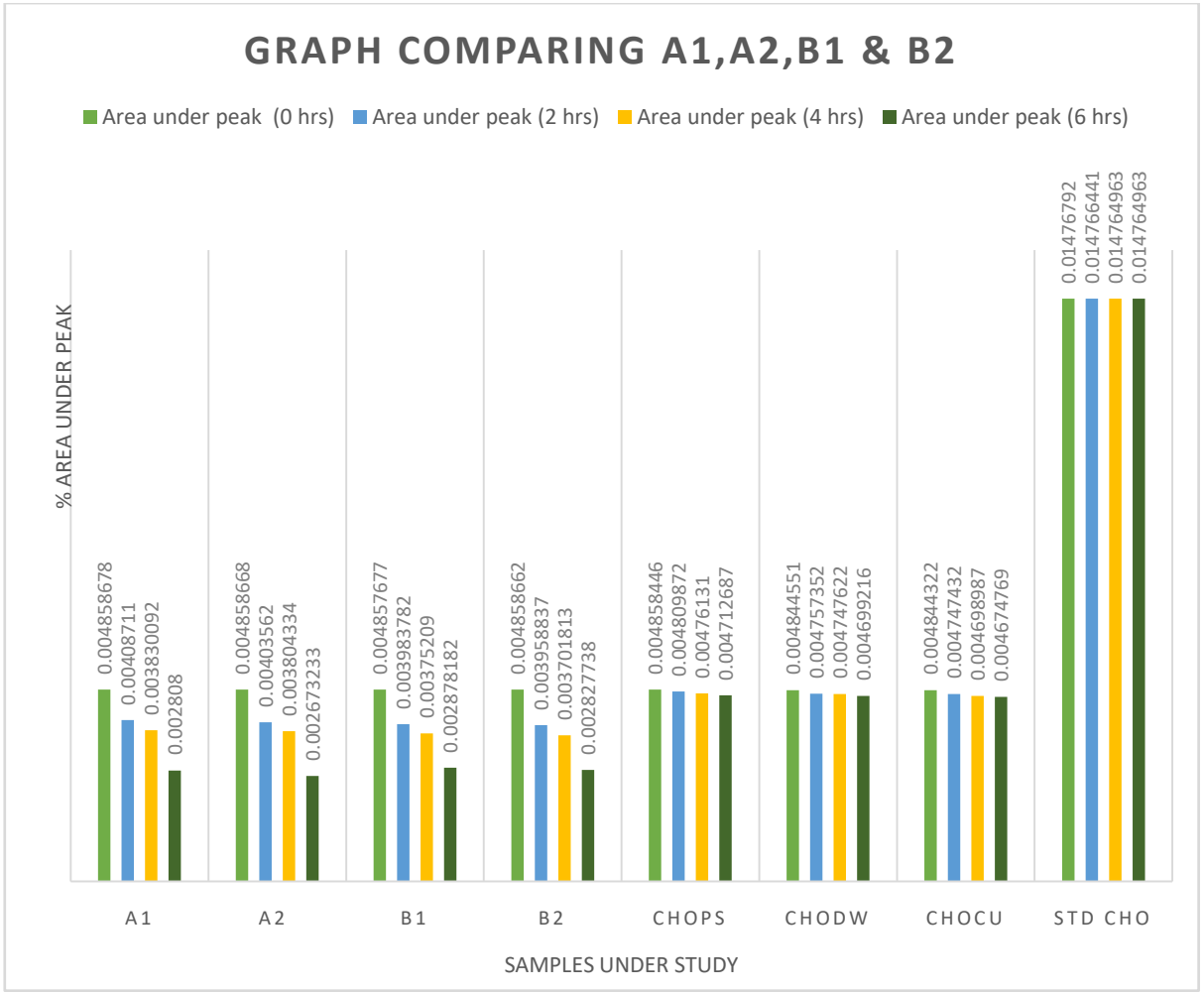

Fig 7. (Comparison between samples A1, A2, B1 & B2)

A1: Terminalia arjuna (ref std.) In distilled water
A2: Terminalia arjuna (ref std.) In cow urine
B1: Bacopa monnieri in distilled water
B2: Bacopa monnieri in cow urine
CHOPS: pooled serum sample discarded and non-infectious
CHODW: pooled serum sample in distilled water
CHOCU: pooled serum sample in cow urine
STD CHO: cholesterol standard from kit.

DISCUSSION

The root cause of coronary heart disease and myocardial ischemia has been connected to hypercholesterolemia and the following atherosclerosis. Since lowering cholesterol levels may reduce the risk of CVD[21], great efforts have been made to achieve this goal. The reduction in total cholesterol in a pooled serum sample that was discarded and non-infectious was utilised in the current investigation to assess the effects of Brahmi leaf powder (Bacopa monnieri L. branded), herbal filtrate in distilled water, and cow's urine on hyperlipaemia in- vitro. As a reference standard, distilled water, and cow's urine filtrates of Arjuna bark powder (Terminalia arjuna L. branded) were used. In the current study, test samples had significantly lower total cholesterol than the controls (CHOPS, CHODW, CHOCU & STD CHO) and demonstrated encouraging outcomes after 2, 4, and 6 hours of study.

A study found that taking T. Arjuna for a month improved the body's total lipid profile.[22] Triglycerides, LDL cholesterol, and total cholesterol were all reduced by 15%, 11%, and 16%, respectively, when statins and arjuna bark powder were combined for three months.[23]

For the determination of the total cholesterol content in serum samples, an accurate, and exact approach based on high performance thin layer chromatography was devised. Over the course of a six-hour kinetic investigation, the method's precision, accuracy, and specificity were confirmed. It has several advantages over traditional colorimetric analysis and other analytical processes, it is rapid and simple, requires a smaller sample size, and does not involve complicated chemical interactions, making it appropriate for regular laboratory analysis. By applying multiple samples to a single plate and allowing them to run, many samples can be applied and tested at any given moment. Due to this, the standardised and established HPTLC method is both accurate and reproducible and requires fewer individual sample volumes to determine total cholesterol concentration kinetically.[24,25,26]

CONCLUSION

As can be seen from the chromatogram above and the graph, the pooled serum sample for B1 and B2 had much lower total cholesterol levels when compared with the controls, which is an encouraging indicator. Therefore, Bacopa monnieri L. can be thought of as a viable therapeutic substitute alone or in combination forhyperlipemia patients, although more clinical research is needed.

Conflicts of interest: None.

Source of funding: None.

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