

THE AMELIORATIVE ACTIVITY OF ETHANOLIC EXTRACT OF *ANTHOCEPHALUS CADAMBA* STEM BARK ON HYPOLIPIDEMIC ACTIVITY IN RATS

Anil Kumar yadav^{1*}, Kuldeep Singh¹

¹ Shambhunath Institute of Pharmacy Jhalwa Prayagraj

Corresponding Author: Anil kumar Yadav. [E-mail- ii12anilvadav@gmail.com](mailto:ii12anilvadav@gmail.com),
deepharmaworld@gmail.com

Abstract

Dyslipidemia is a critical risk factor for cardiovascular diseases, leading to an increasing interest in natural compounds as potential hypolipidemic agents. *Anthocephalus cadamba* (Roxb.) Miq., known for its medicinal properties in traditional systems, has been understudied for its lipid-lowering effects. This study aimed to investigate the hypolipidemic activity of the ethanolic extract of *Anthocephalus cadamba* stem bark (EACSB) in hyperlipidemic rats. Male Wistar rats were divided into five groups: a normal control, a hyperlipidemic control induced by a high-fat diet, and three treatment groups receiving different doses of EACSB (100, 200, and 400 mg/kg body weight) orally for 60 days. Lipid profiles, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and very-low-density lipoprotein cholesterol (VLDL-C), were assessed. Liver histopathology was also examined to evaluate the safety of the extract. Treatment with Ethanolic extract of *Anthocephalus cadamba* stem bark significantly reduced TC, TG, LDL-C, and VLDL-C levels while increasing HDL-C in a dose-dependent manner compared to the hyperlipidemic control. The most pronounced effect was observed at the highest dose of 400 mg/kg. Histopathological analysis showed no adverse effects on liver tissues, indicating the safety of the extract. The ethanolic extract of *Anthocephalus cadamba* stem bark exhibits significant hypolipidemic activity in rats, highlighting its potential as a natural therapeutic agent for managing dyslipidemia. Further studies are warranted to isolate the active compounds responsible for the observed effects and to understand the underlying mechanisms.

Keywords: Hypolipidemic, *Anthocephalus cadamba*, Lipid profiles, herbal medicine, Ethanolic extract.

INTRODUCTION

Since days of year, home grown medicinal plants have been utilized against various diseases. The ancient man utilized plants as helpful and therapeutic substances that he could without much of a

stretch get. For each life, WHO have helpful properties, nature provided plant lavishness richly. Some plant huge characteristics have been distributed for quite a while, yet a significant number of them have yet not been explored. Along these lines, their applications should be explored and pharmacognostic and drug examinations completed to decide their therapeutic characteristics. Regardless of critical advances in the administration of engineered prescriptions for the treatment of diabetes mellitus, research is in progress on regular enemy of diabetic plant compounds.[1] Many hypoglycetic spices are known through fables, however the disclosure of creature tests that are almost like the obsessive course of diabetes in human individuals is because of their presentation in the contemporary arrangement of therapy.[2] World-wide, for their alleged hypoglycaemic activity in excess of 1200 plants have been utilised.[3] Plants keep on assuming a significant part in the therapy of diabetes, particularly in helpless nations where a great many people have little means, where the general wellbeing framework can't furnish the populace with essential clinical and drug mind and have no admittance to contemporary treatment. [4-5]

Hyperlipidemia

Expanded plasma lipid levels are to a great extent all out cholesterol. Inception and improvement of the atherosclerotic impasse are known to prompt high thickness lipoprotein triglyceride and low thickness lipoprotein just as diminishing high-thickness lipoprotein. Expanded danger of factors for the improvement of diabetes isn't simply auxiliary metabolic deregulation related with diabetes.[6] in case of diabetes causes high blood levels of fatty substances and helpless thickness, cholesterol and lipoproteins are key danger factors for cardiovascular infection early advancement, for example, arthrosclerosis, hypertension, heart thus on.[8] High lipid levels emerge from expanded intestinal retention or worked on endogenous blend, subsequently hyperlipidemias can be diminished in two distinctive manners; endogenous combination can be obstructed or ingestion diminished. In typical creatures without counterfeit suppers the two angles might be tested.[9-11] The etiology of arteriosclerosis with its twin impacts of apoplexy and encroachment is viewed as hyperlipidemia with a raised cholesterol, fatty oils conveying lipoproteins. The lipoprotein orders are six: chylomicron, nutrient chylomicron, VLDL (extremely low lipoprotein thickness), LDL (center lipo-protein thickness), LDL (low lipoprotein thickness) and HDL (low lipoprotein thickness) (high thickness lipoprotein).²⁶ HDL builds cholesterol freedom from fringe cells and works with the arrival of cholesterol to the liver. Consequently, it is attractive to raise HDL levels High VLDL and LDL levels empower arteriosclerosis, in actuality. Macrophages utilize a forager gadget to ingest LDL explicitly in the oxidized state in this way hostile to arteriocholesterol should

bring down VLDL and increment HDL.[12,13]

Symptoms

Contract persons with normal hyperlipidemia may have cardiovascular signs of family combined hyperlipidemia within a couple of years, for example:[14]

- Chest pain (at a young age)
- Heart attack (at a young age)
- Cramping in the calves while walking
- Sores on the toes that don't heal properly
- Stroke symptoms, including trouble speaking, drooping on one side of the face, or weakness in the extremities.

Raw material collection

Develop natural product from the nearby market has been acquired. The plant flushed into faucet water and washed with refined water. The skin was isolated, dried in conceal, finely powdered, and presented to 30 hour extraction of Soxhlet apparatus utilizing mortar and pestle (1 kg separately). The concentrate was sifted and dried at 40°C and brought down under tension. For all after examinations this dried powder is considered as a parent.[15]

Preparation of Stem peel powder

Right away, the strip was taken out from the leafy foods into fine powder and protected in fine air bottles with refined water and shades. Contrasted with natural solvents in a funnel shaped jug, determined the measure of powder and afterward put away for 24 hours in a turning shaker at 190-220 rpm. Also, it was then separated and centrifuged utilizing the muslin material. The supernatant was gathered utilizing the dissolvable dissipating gadget for the last volume of 1 fourth of the first volume. The dissolvable was dissipated. For ensuing exploration it was kept at 40°C in hermetically sealed containers.

Preparation of Plant extracts (Ethanol extract) from stem bark of Anthocephalus cadamba

The cleaned test (approx. 50 gm) was then pressed in a soxhlet gadget and separated for 8 to 12

hours with 95% ethanol. The separated powder was disposed of after the extraction and the ethanol extricate was additionally disposed of for additional preparing. By refining the excess dissolvable in the concentrate and the concentrated concentrate was dried further at a temperature not surpassing 40 °C in a turning evaporator under diminished pressing factor. It was then gathered in Petri plate and put away in a cooler (extractive worth 10gm). The dense concentrates have been used to pre-look at changed plant mixtures like steroids, glycosides, flavonoids, tannins, carbs, and so forth

Animal

Adult Wistar albino rats weighing 150-200 grams. Animals were stored in (50cm x 30cm x 25cm) polypropylene cages, fed, and treated in accordance with CPCSEA (Reg. No. 324) in-house rules, and under the guidance of an Animal Ethics Committee, in accordance with the Indian National Legislation on Animal Care and Use. The animals were allowed to adapt to the room conditions as per the procedure .

The animal room and the polypropylene cages were cleaned thoroughly and regularly with suitable aeration, to keep the environment clean and airy.

- Left over food and excreta were removed daily.
- The temperature of the animal room was maintained at $26 \pm 2^{\circ}\text{C}$.
- Rats were fed daily with food pellets supplied by Poultry Research Station, Chennai and clear drinking water was provided ad libitum.
- The rats were handled gently and care was taken not to press the animals

Preparation of the hyperlipidemic diet The preparation of a high-fat diet is done by mixing 1% cholesterol, 1% cholesterin, and 1 mL of coconut oil.

Cholesterol fed diet hyperlipidemia

30 animals were divided in 5 groups (n=5). All groups contain 6 animals. Group I (Normal group) received a normal diet ad libitum. Group II (inducing agent) received a high-fat diet. The IV and V groups, after fifteen days, received a high-fat diet with the plant ethanolic extract for thirteen days. That dose was 100mg/kg and 250mg/kg p.o. Group III received the standard drug Rosuvastatin for fifteen days at a dose of 200mg/kg with a high-fat diet. After thirty days, blood was collected from reterobulbar, allowed to clot for 45 min, and separated the serum by centrifugation. Using commercially available kits, a lipid profile was done.

Collection of Blood

The control of cholesterol animals were anaesthetized by diethyl ether at the completion of the tests (days specified) and slaughtered on the 31st day by cervical dislocation, with the slaughter of the treatment groups. Fast blood samples were collected from rat with haematological and biochemical analytical conservatives in separate tubes.

Physico-Chemical Evaluation of Crude Extract

The rough concentrate was assessed for a few attributes truly and synthetically. The principle stage in recognizing and normalizing unrefined concentrate is actual appraisal. It assists with deciding the legitimacy of the rough drug for debasements. The principle stage is additionally taken to recognize substance segments and normalize unrefined medications.

Loss on drying

Loss of drying is a level of m/m of loss of mass. In a Petry dish it is definitely weighed to around 5-6g of medication powder and put away for 4-5 hours in a hot-air stove. In every model, the weight reduction was reported after the dessicator cooling. This procedure was proceeded until the weight was reliable.

$$\text{Loss on drying (\%)} = \frac{\text{loss in weight}}{W} \times 100$$

W= weight of the drugs in grams.

Total ash Values

Take around 2-3g of powdered concentrate appropriately gauged, pre-excited and made an appearance either platinum or silica dish. Dissipate on the lower part of the plate the powdered prescription. Bit by bit builds the warmth and doesn't outperform dull red warmth until it is sans carbon, cool and gauges. Except if this is conceivable, the carbon free debris will be taken out from boiling water, the waste will be gathered in an ash less channel paper, channels will be added to the waste and afterward vanishes the buildup and excites at low temperatures. Compute the extent of each air dried medication.

$$\% \text{ Total Ash} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

Water-insoluble ash

The ash was heated with ~ 30 mL diluted hydrochloric acid for 10-15 minutes and the insoluble material was taken in a sink. Washed, lit and weighted with hot water. With reference to air-dried medication, the proportion of acid-insoluble ash is determined.

Water-soluble ash

The entire debris was cooked with ~30 ml of water for 4-5 minutes. The insoluble material was accumulated in a pit. Washed, lit and weighted with heated water. Comparable to air dried med the extent of water-dissolvable not set in stone.

Preliminary Phytochemical Screening

A subjective synthetic test was trailed by the strategies used to distinguish different phytochemical substances, to give a wide idea of the sort of parts in the pelts.

Thin-layer Chromatography (TLC)

To recognize a specific number of compound parts supporting the synthetic test, the ethanol concentrate of the stem strip was presented to chromatographically assessment in meager layered structure. The procedural particulars are as per the following: By adding silica gel G slurry on glass plates, scientific TLC plates were delivered. The pre-arranged chromatographic plates, which are dried for 60 min noticeable all around and afterward warmed at 100°C for another 60 min were parted in liquids connected with TLC by drying the plates.

Preparation of Mobile Phase

Set up the top alongside a blender of 5% acidic corrosive and 95% ethyl acetic acid derivation. Inside the chamber, place TLC plate, which grants to move to the dissolvable. Permit dissolvable to vanish and afterward see the bright layout, the dissolvable moved and the distance between spots, to be assessed from this RF. 15 µl of ethanolic remove from the example utilized on one side of the fine layer plate, around 5 cm from the lines using capillary cylinder, for my subjective examination. The outcome is a solitary piece of test. The degree of the example volume was checked to a most extreme degree of 0.5 cm. The decision of dissolvable depends on two factors: (a) the idea of the substance to be

eliminated and (b) the material to be exposed to partition. The front of the dissolvable was demonstrated and the plate could be dried. On the chromatogram, the beautiful mixtures were apparent. Utilizing the representation specialist Ninhydrin specialist fumes, dull parts were distinguished. To qualitate the plate, the transitory conduct of the independently provided synthetic not really set in stone as a RF esteem.

Physical Test Of Crude Drugs (Table 1.1)

Crude drugs	Physical Test			
	Nature	Colour	Odour	Taste
<i>Anthocephalus cadamba</i> Stem Extract	Coarse powder	Yellowish brown	Characteristic	Astringent

Extractive Values (Table 1.2)

Crude drugs	Alcohol % w/w	Aqueous % w/w
<i>Anthocephalus cadamba</i> Stem Extract	20.15	22.86

Loss on Drying And Foreign Organic Matter (Table 1.3)

Crude drugs	Loss on drying (% w/w)*	Foreign matter (% w/w)*
<i>Anthocephalus cadamba</i> Stem Extract	7.35	1.25

Total Ash, Acid Insoluble Ash And Water Soluble Ash Values (Table 1.4)

Crude drugs	Total ash value* % w/w	Water soluble ash* % w/w	Acid insoluble ash value* % w/w
<i>Anthocephalus cadamba Stem Extract</i>	7.5	4.5	1.25

Table 1.6: Effect of Anthocephalus cadamba Stem Extract Peel Extract on Serum Total cholesterol levels(mg/dl)

S.No	Groups	Total Cholesterol (mg/dl)
1.	Normal	95 ± 1.5
2.	High fat diet	120±2.5
3.	Rosuvastatin	98±2.7
4.	Peel extract (100mg/kg)	104±2.1
5..	Peel extract (250mg/kg)	102±1.6

All values were expressed as mean ± SEM and n=6

Fig 1. Effect of Anthocephalus cadamba Stem Extract Extract on Serum Total cholesterol evels

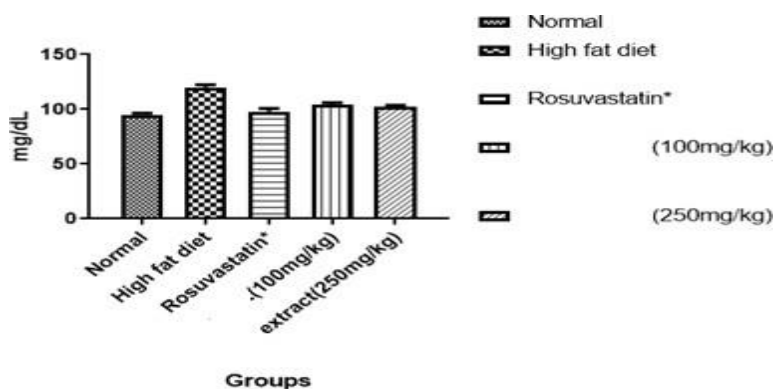


Table 1.7: Effect of Anthocephalus cadamba Stem Extract on Serum Triglycerides levels (mg/dl)

S.No	Groups	Triglycerides (mg/dl)
1.	Normal	87 ± 1.5
2.	High fat diet	118±1.5
3.	Rosuvastatin	86±1.5
4.	Stem extract (100mg/kg)	94±2.1
5.	Stem extract (250mg/kg)	88±1.6

All values were expressed as mean ± SEM and n=6

Fig 2. Effect of Anthocephalus cadamba Stem Extract on Serum Triglycerides levels

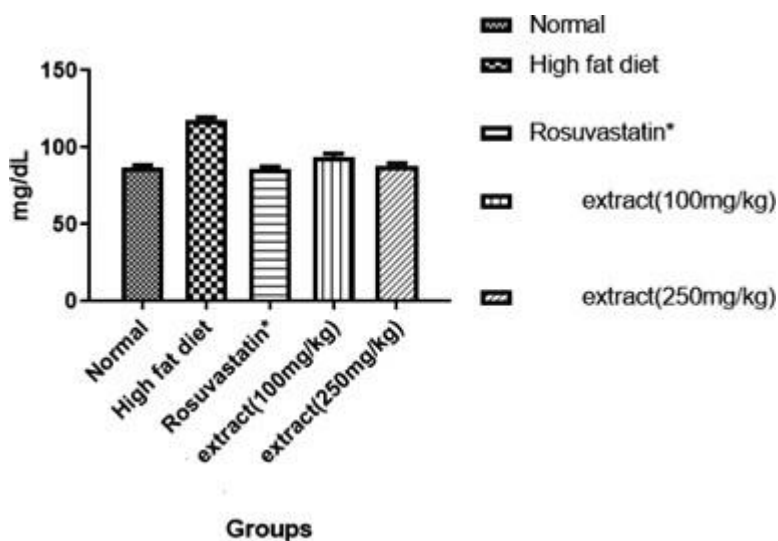


Table 1.8: Effect of Anthocephalus cadamba Stem Extract on Serum Phospholipids levels (mg/dl)

S.No	Groups	Phospholipids (mg/dl)
1.	Normal	80 ± 1.5
2.	High fat diet	119±1.5
3.	Rosuvastatin	90±1.5
4.	Stem extract (100mg/kg)	95±1.5
5.	Stem extract (250mg/kg)	91±1.5

All values were expressed as mean ± SEM and n=6

Fig 4. Effect of Anthocephalus cadamba Stem Extract on Serum Phospholipids levels

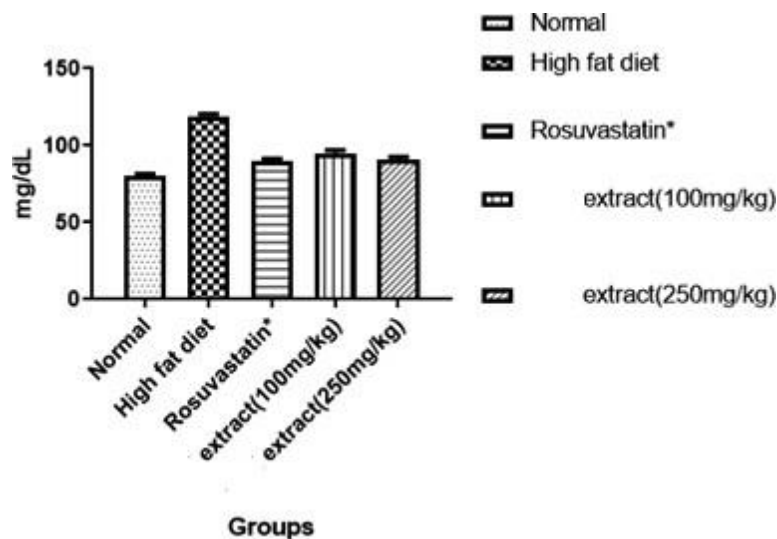


Table 1.9: Effect of Anthocephalus cadamba Stem Extract on Serum LDL levels (mg/dl)

S.No	Groups	LDL (mg/dl)
1.	Normal	50 ± 1.5
2.	High fat diet	110±1.5
3.	Rosuvastatin	55±1.5
4.	Stem-extract(100mg/kg)	59±1.5
5.	Stem-extract(250mg/kg) 56	± 1.5

All values were expressed as mean ± SEM and n=6

Fig 4. Effect of Anthocephalus cadamba Stem Extract on Serum LDL levels

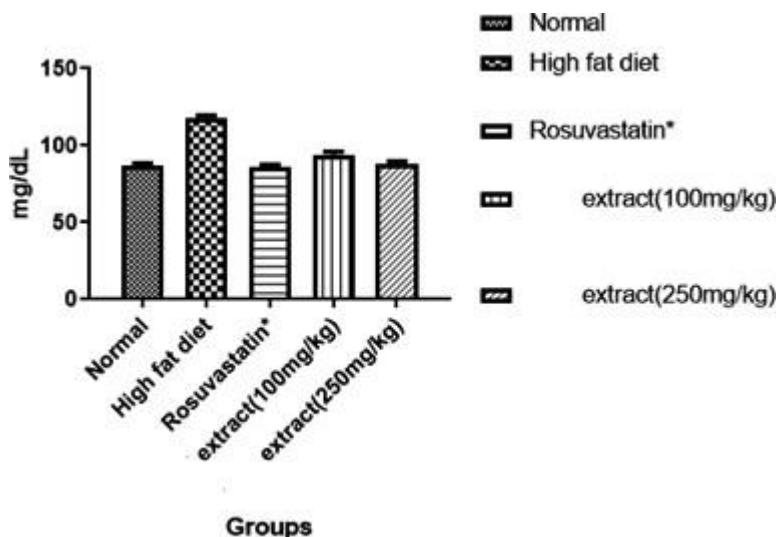


Table 1.10: Effect of Anthocephalus cadamba Stem Extract on Serum VLDL levels (mg/dl)

S.No	Groups	VLDL (mg/dl)
1.	Normal	28.5 ± 1.5
2.	High fat diet	60±1.5
ss3.	Rosuvastatin	24.5±1.5
4.	Peel extract (100mg//kg)	27.35±1.5
5.	Peel extract (250mg//kg)	25.21±1.5

Fig 5. Effect of Anthocephalus cadamba Stem Extract on Serum VLDL levels

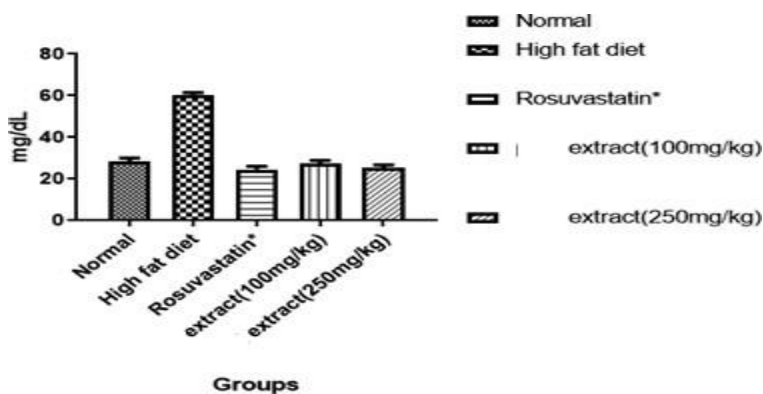


Table 1.11: Effect of Anthocephalus cadamba Stem Extract on Serum HDL levels (mg/dl)

S.No	Groups	HDL (mg/dl)
1.	Normal	40.5 ± 1.5
2.	Cholesterol	28.5±1.5
3.	Rosuvastatin	30±1.5
4.	Stem extract (100mg/kg)	27.5±1.5
5.	Stem extract (250mg/kg)	28±1.5

Fig 5. Effect of Anthocephalus cadamba Stem Extract on Serum HDL levels

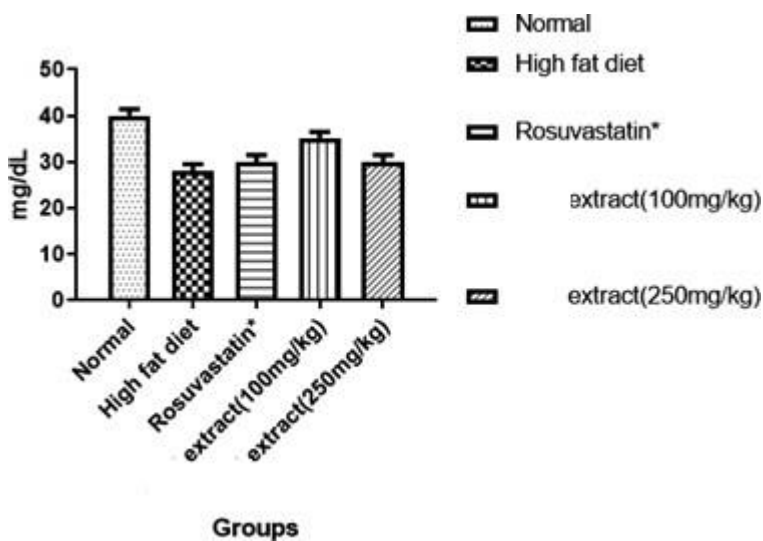


Table 2: RF values of six different constituents using TLC method

Constituents	Distance travelled	
	First test	Second test
Carbohydrates	0.07	0.08

Proteins	0.05	0.07
Tannins	0.06	0.05
Saponins	0.05	0.06
Alkaloids	0.41	0.45
Flavonoids	0.02	0.03

Conclusion

In this exploration, *Anthocephalus cadamba* Stem was obtained from the neighborhood market. It was cleaned with faucet water and afterward washed with deionized water prior to being dried. Plants and crude medications were assessed by botanists, who then, at that point squashed them to a coarse powder that was reliable in size. The presence of various phytoconstituents makes the fruit peel useful for extracting different additives and has a potential of providing useful food additives of human use. In the present study, we have found that most of the active phytochemical's constituents were present in the ethanolic extract of *Anthocephalus cadamba* Stem such as alkaloids, tannins, proteins, and carbohydrates using TLC method. This investigation exhibits that strip concentrates might be utilized as a wellspring of valuable designs in the advancement of new chemotherapeutic drugs to treat hypolipidemic messes in medical care. End To finish everything off, results are a rich wellspring of sugars and minerals just as filaments, which might be utilized to safeguard food and for remedial purposes, including hypolipidemic movement. As per the prior information, the *Anthocephalus cadamba* Stem may have antihyperlipidemic properties.

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Conflict of Interest

The authors declare no conflict of interest.

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