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Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (*ex vivo*) by medicinal plants from western ghats

P. Vanitha Reddy, Lakshmi Sarkar and Asna Urooj

Department of Studies in Food Science and Nutrition,University of Mysore, Manasagangotri, Mysore-570006, Karnataka, India

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Abstract

Medicinal plants are being explored as natural inhibitors of HMG-CoA reductase (vital enzyme in cholesterol biosynthesis) as the drugs (statins), used in the management of hypercholesterolemia, are associated with side effects. In this study, medicinal plants: *Abrus precatorius* Linn. (AP), *Canthium parviflorum* Lam. (CP) *and Costus speciosus* (Koenig)Sm. (CS) were studied for phytochemical composition and the ability of methanol extract (300 µg) to inhibit HMG-CoA reductase activity, using HMG-CoA as a substrate. The assay is based on the stoichiometric formation of Coenzyme A during the reduction of microsomal HMG-CoA to mevalonate. Phytochemicals glutathione, tannins, alkaloids and vitamin C were at higher amount in AP, CP and was rich in flavonoids and α tocopherol and polyphenols, β-carotene, saponins and steroidal saponins presence was indicated in CS. The plants showed varying degree of inhibition of HMG-CoA reductase. The CoA released at the end of assay in the control (119.70 nM) and statins (94.46 nM) was higher than that released in systems, containing CS (72.97 nM) > CP (55.41 nM) >AP (50.23 nM) extracts. From the results, it can be concluded that all the three plants studied possess HMG-CoA reductase inhibitory activity, indicating scope for their utilization as cholesterol lowering agents. However, further studies are needed, before recommending them as safe therapeutic agents.

Key words: *Abrus precatorius* Linn. (AP), *Canthium parviflorum* Lam. (CP) *and Costus speciosus* (Koenig)Sm. (CS), cholesterol, HMG CoA reductase, Coenzyme A

1. Introduction

In current times, there is a great deal of interest in the use of herbal remedies for human ailments. The use of these remedies is based on the fact that these medicines contain a large amount of natural substances which alleviate illness, thus, promoting health. World Health Organization (WHO) reported that more than 80% of the world population depends on traditional medicines for primary healthcare and they utilize the plant extracts or bioactive components present in them (Levy and Brink, 2005).

Epidemiological studies have indicated that hypercholesterolemia confers high risk for atherosclerosis and occlusive related vascular disorders which still occurs increasingly and accounts for the majority of mortality worldwide (NIHCE, 2008). Elevated cholesterol levels are treated with a diet, consisting of low saturated fat, trans fat-free, low cholesterol foods, often followed by one of various hypolipidemic agents, such as statins, fibrates, cholesterol absorption inhibitors, nicotinic acid derivatives or bile acid sequestrants (Rudney *et al*., 1981). Since these cholesterol lowering drugs are associated with some side effects, medicinal plants are being explored as natural hypocholesterolemic agents (Reddy *et al.,* 2012).

The enzyme HMG-CoA reductase plays a vital role in the *de novo* cholesterol synthesis, by catalyzing the conversion of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to mevalonate, a rate limiting step. Inhibition of HMG-CoA reductase alters the synthesis of cholesterol, proving beneficial in hypercholesterolemic condition (Reddy *et al.,* 2012).

Several medicinal plants such as *Moringa oleifera* (Ghasi *et al.,* 2000), *Terminalia arjuna* (Gupta *et al.,* 2001), *Sushruta Samhita* (Shields and Moranvillie, 2005), *Trigonella foenum-graecum* (Sowmya aand Rajyalakshmi, 1999), *Alfaalfa* (Malinow *et al*., 1980) *etc*., have been extensively studied for their cholesterol lowering effects. Our team has explored the lipid lowering and antioxidant potential of *Moringa oleifera* and *Morus indica* using *in vitro*, *ex vivo* and animal models (Urooj and Reddy, 2010; Oinum *et al*., 2012; Arabshahi-Delouee and Urooj 2007; Reddy and Urooj, 2013). Western Ghats of Karnataka, South India is abundant in medicinal plants, having several health benefits. Three plants: *Abrus precatorius* (AP), *Canthium parviflorum* (CP) *and Costus speciosus* (CS) (Arabshahi-Delouee and Urooj., 2007) found in this region have been explored for their medicinal properties. *Abrus precatorius* has been studied for its anti-inflammatory (Kuo, 1995); anticancer (Sujit *et al., 2011*) and *Canthium parviflorum* is reported for its antioxidant capacity (Baskar *et al*., 2008), wound healing and as a diuretic (Mohidin *et al*., 2003) and antimicrobial agent (Peter *et al*.,2011) well documented for its antidiabetic (Srivastava, 2011) and hepatoprotective (Verma and Khosa, 2009) effects. However, there are no reports on the hypocholesterolemic potency of these plants.

Author for correspondence: Professor Asna Urooj
Department of Studies in Food Science and Nutrition,University of Mysore, Manasagangotri, Mysore-570006, Karnataka, India
E-mail: asnau321@gmail.com
Tel.: +91-09448489334; Telefax: +91 821 2419632

Hence, these medicinal plants namely: *Abrus precatorius* (AP), *Canthium parviflorum* (CP) *and Costus speciosus* (CS) were studied for the HMG-CoA reductase inhibitory effect, using an *ex vivo* assay and compared with statins.

2. Materials and Methods

Three medicinal plants: *Abrus precatorius* Linn. (AP), *Canthium parviflorum* Lam. (CP) *and Costus speciosus* (Koenig)Sm (CS) were collected from the Western Ghats of Karnataka, identified by Dr. Janardhan, Department of Studies in Botany, University of Mysore and voucher specimen was retained in the laboratory for future.

2.1 Preparation of the samples

The leaves were washed and dried in a hot air oven at 60°C for 8-10 hrs. The dried samples were ground into a fine powder, sieved through a 60mm mesh and stored in air tight containers at 4ºC until further use.

2.2 Estimation of phytochemicals

The dehydrated sample was assayed for phytochemical components, using standard methods. Total phenols were extracted from a weighed portion (50-500 mg) of dried sample with 5 ml of 1.2M HCl in 50% aqueous methanol for 2 h and analyzed by Folin-Ciocalteu micro method. Results are expressed as µmol gallic acid equivalent g-1dry weight. Flavonoids were determined by a pharmacopoeia method, using Rutin as a reference compound, alkaloids by the method based on the reaction with bromocresol green (BCG), using Atropine as a standard, saponins by using vanillin and sulfuric acid reagents and Diosgenin as standard, tannins estimated by Folin-Ciocalteu micro method, α-tocopherol was extracted by direct saponification of dried sample and estimated based on formation of a red complex from reaction of αα bipyridyl with ferrous ion due to reduction of ferric ion by tocopherol . β-carotene was quantified by column chromatography, followed by measuring the absorbance of elute at 450 nm against standard β-carotene. Reduced glutathione was determined based on the development of a yellow compound due to reaction of 5,5-Dithio (bis) nitro benzoic acid (DTNB) with compounds containing sulphydryll groups (Reddy and Urooj, 2013).

2.3 Preparation of extract

Fifteen grams sample was extracted with 100 ml methanol, in a mechanical shaker for 6 hrs. The extracts were evaporated at 40ºC under reduced pressure to dryness in a rotary evaporator (Superfit, India). The extract was further dried and stored in air-tight container at 0ºC until used.

2.4 Preparation of microsomes

A healthy male adult rat was obtained from the Central animal house, Department of Zoology, University of Mysore, after availing clearance from the University Animal Ethics Committee of University of Mysore (No. MGZ/2620/2011-12; dated: 31.01.2012). The rat was fasted 24 hrs and sacrificed after 9:00 pm to obtain the active HMG-CoA reductase enzyme. The liver was immediately removed from the rat and placed in cold triethanolamine HCl buffer (0-4°C) at pH 7.4. The liver was thoroughly chilled and homogenized. The homogenate was centrifuged at 60,000 g for 60 min, the supernatant was separated and microsomal pellet was then rinsed with buffer and frozen at -20° C. The resuspended microsomes to be used for the assay were diluted with buffer to give a protein concentration of 5-10 mg/ml. The procedure described here is partially modified from that reported by Shapiro and Rodwell (1971).

2.5 3-Hydroxy-3-methylglutaryl CoA reductase assay

For the assay of 3-hydroxy-3-methylglutaryl CoA reductase, the incubation mixtures contain 0.5-1.0 mg of microsomal protein, 150 nmoles of HMG- CoA, and 2imoles of NADPH. These components are added to 0.8 ml of 0.1 M triethanolamine-0.02 M EDTA buffer at pH 7.4 without dithiothreitol. The dithiothreitol (0.2 µmole) was added along with the microsomal preparation. The final incubation volume was 1 ml. contents in tubes were as follows: (a) All components, (b) all components except NADPH, (c) all components except NADPH with 300 µg of AP, CP and CS methanol extracts separately, and (d) all components except NADPH with statins (Frankel *et al*., 1973). After a series of steps, the Coenzyme A released was calculated using the following formula:

$$\text{n moles/min} = \frac{[\text{A reaction} - \text{A control}] \times 1.430}{0.0136 \times \text{time}}$$

The 1.43 is the dilution factor, and A is the absorbance at 412 nm. The difference in absorbance between the complete reaction and that of all components except NADPH represents the activity due to HMG-CoA reductase.

2.6 Statistical analysis

Each experiment was conducted in triplicates and data are expressed as Mean±SD.

3. Results

3.1 Phytochemical composition

Table 1 depicts the phytochemical composition of the three samples: AP, CP and CS. The glutathione (375±176.77 mM), tannins (1160±0.076 mg), alkaloids (1100.0±0.03 mg) and vitamin C (Fresh basis-0.443±0.011 g; dry basis-0.643±0.04 g) was highest in AP, the flavonoids (2.443±0.811 mg) and α-tocopherol (164.6±22.1 mg) content was high in CP, and CS was rich in polyphenols (3.211±0.121 g), β-carotene (1377.3±2.51 mg), saponins (0.282±0.04 mg)and steroidal saponins (0.238±0.038 mg) respectively.

3.2 3-Hydroxy-3-methylglutaryl CoA reductase assay

Liver microsomes were treated with methanol extracts (300 µg) of the three plants and their effect on the activity of 3-hydroxy-3-methyl glutaryl CoA reductase was measured based on the coenzyme A released during the reduction of 3- hydroxy-3-methylglutaryl CoA to mevalonate. The assay was run against a control and compared with the statins. The results indicate that all the three samples were highly effective (51-66 %) compared to statins (37 %) in inhibiting the HMG-CoA reductase in the liver microsomes (Figure 1).

The Coenzyme A released during the assay was higher in control (119.7 nmoles) than the microsomes treated with AP, CP, CS and statins (Figures 2 and 3) and among all, Abrus (AP) was more potent in inhibiting the enzyme activity (50.23 nmoles). Figure 2 shows the time course of activity of HMG CoA reductase for a period of 4 min. From the Figure, it can be observed that the rate of

release of Coenzyme A was constantly increasing from zero to 4th min, both in control and statins. The Co enzyme A released at the zero min. was low in statins (36.54 n moles) than the other samples and control. The rate of release of Co enzyme increased rapidly at 0.5th min in statins (57.14 n moles) and was higher than that of AP (47.52 n moles) and CP (49.62 n moles). At the end of the 4th minute, the Co enzyme A released in the presence of AP (50.23 n moles), CP (55.41 n moles) and CS (72.97 n moles) was lower than that of statins (94.46 n moles).

Figure 3 shows the total HMG Co A reductase activity as nmoles of Coenzyme A released at the end of 4th min in the following order: control (119.7 n moles) > statins (94.46 n moles) > CS (72.97 n moles) > CP (55.41n moles) > AP (50.23nmoles). No significant ($p<0.05$) difference was observed between the Co enzyme A released by AP and CP. The low amount of CoA released by three medicinal plants compared to statins is indicative of the inhibitory effect on HMG- CoA reductase.

Table 1: Phytochemical composition of selected medicinal plants (g/100 g)

Phytochemicals	AP	CP	CS
Polyphenols	1.06 ± 0.06	1.279 ± 0.32	3.211 ± 0.121
Flavonoids*	1.325 ± 0.085	2.443 ± 0.811	1.174 ± 0.42
Glutathione**	375 ± 176.77	311.55 ± 25	285.6 ± 33
α- Tocopherol *	36.6 ± 3.05	164.6 ± 22.1	90.94 ± 3.29
Tannins *	1160 ± 0.076	605.04 ± 0.025	1088.0 ± 0.036
β- carotene ***	1260.0 ± 0.05	1060.0 ± 0.6	1377.3 ± 2.51
Alkaloids *	1100.0 ± 0.03	400.0 ± 0.06	120.0 ± 0.03
Total saponins	0.244 ± 0.01	0.200 ± 0.04	0.282 ± 0.04
Steroidal saponins	0.186 ± 0.028	0.148 ± 0.051	0.238 ± 0.038
Vitamin C (Fresh basis)	0.443 ± 0.011	0.141 ± 0.001	0.135 ± 0.008
Vitamin C (Dry basis)	0.643 ± 0.04	0.0228 ± 0	0.0237 ± 0.004

AP-Abrus precatorius; CP-Canthium parviflorum; CS-Costus speciosus; *mg/100 g, **mmol/100g : *** µg/100g dry basis: Values are mean of triplicates

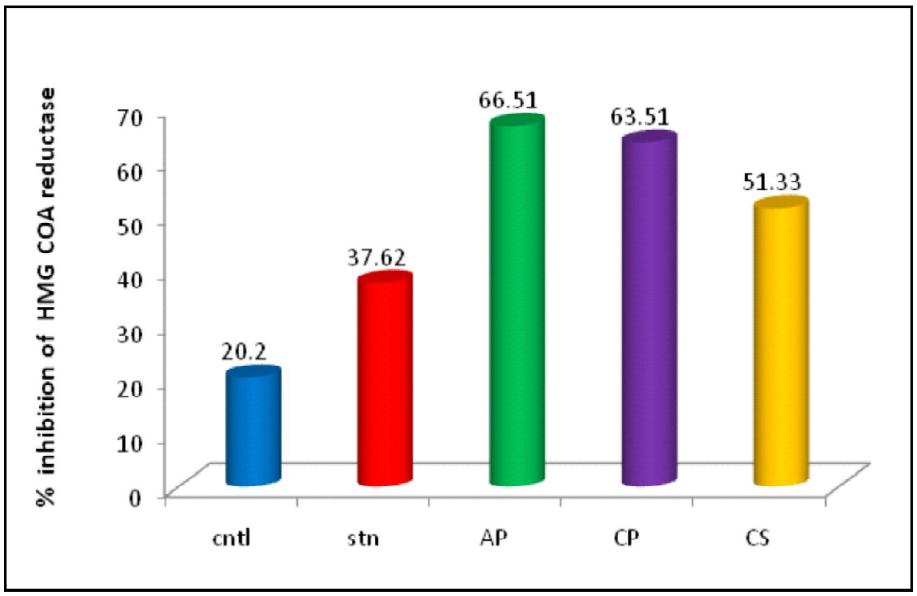

Figure 1: Percent inhibition of HMG CoA reductase by selected medicinal plants (300 µg) Ctrl-control; stns-statins; AP-*Abrus precatorius*; CP-*Canthium parviflorum;*CS-*Costus speciosus*

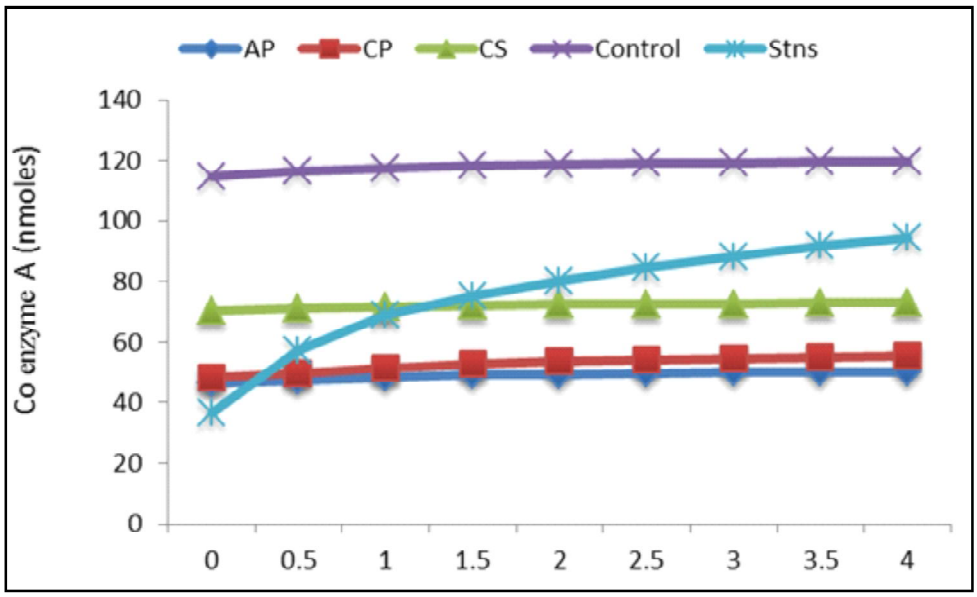

Figure 2: Effect of selected medicinal plants on the HMG COA reductase activity and release of CO enzyme A AP-*Abrus precatorius*; CP- *Canthium parviflorum;* CS-*Costus speciosus*

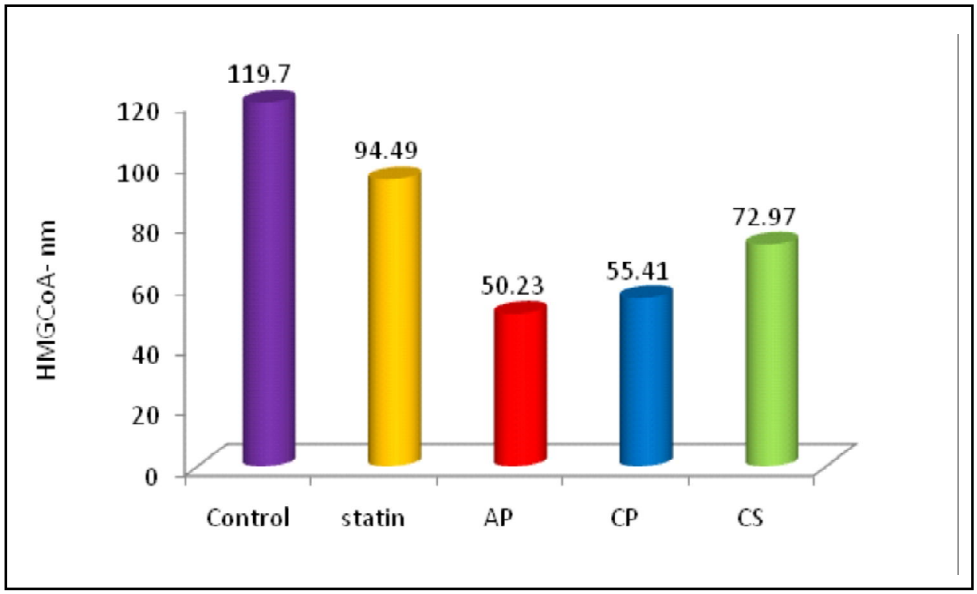

Figure 3: Total coenzyme A (n moles/mg protein) released during the reduction of HMG COA to Mevalonate, AP-*Abrus precatorius*; CP-*Canthium parviflorum;* CS-*Costus speciosus;* (p>0.05)

4. Discussion

Cholesterol is an insoluble lipid molecule that plays a critical role in the structure and function of membrane bi-layers. Membrane cholesterol contents that are either too high or too low are detrimental to cell function. When present in excess amounts in cells, cholesterol becomes toxic. Certainly, cholesterol-induced cytotoxicity represents a key initiating event leading to the development of atherosclerotic cardiovascular disease (Tabas, 2002).

Statins are the most commonly administered class of drugs to lower plasma LDL cholesterol. Their primary mechanism of action is to promote clearance of LDL particles from the plasma. Statins reduce the rate of intracellular cholesterol synthesis by inhibiting HMG-CoA reductase, the rate-limiting step in cholesterol biosynthesis (Brown and Goldstein, 1986).

The enzyme inhibitory effect correlates with the medicinal properties and phytochemical components such as phenolic compounds, alkaloids, saponins *etc*. Phytochemical analysis revealed the presence of alkaloids, glutathione, vitamin C and tannins in higher quantities in Abrus. The phytoconstituents, antioxidant and antiproliferative activities of various solvent extracts of Abrus has been reported (Umamaheswari, 2012). No data is reported for comparison of present observations.

Oral administration of *Costu signeus* leaf powder (500 mg/kg bw) and *Canthium parviflorum* ethanol extract at different concentrations produced a significant hypoglycaemic effect in alloxan and STZ- induced diabetic rats. The concurrent effect of *Costus and Canthium* on lipid metabolism was significant in diabetic rats, this was evidenced by the reductions in serum cholesterol, triglycerides and lipid peroxides (Devi and Urooj, 2008; Purushoth *et al.,* 2012). However, the mechanism involved in the cholesterol lowering by *Costus* and *Canthium* leaves has not been reported.

The inhibition of Coenzyme A reductase has greater significance in cholesterol metabolism and, thus, has various health benefits. The synthetic drug statin along with inhibiting the HMG-CoA also blocks Coenzyme Q required for energy production and, thus, impairing cardiac and liver function (Rudney *et al*., 1981). Several clinical studies have reported that the patients with/without coronary heart disease (CHD), when treated with HMG-CoA reductase inhibitors and other lipid-lowering drugs like fibrates to lower plasma cholesterol levels, a reduction in cardiovascular morbidity and mortality was also seen (Scandinavian, 1994; Shepherd *et al.,* 1995; Stael *et al.,* 1998). However, most commercial HMG-CoA reductase-inhibitors are associated with adverse effects, including the induction of cutaneous vasodilation, rashes, gastrointestinal discomfort, hyperuricemia, hyperglycemia and hepatic dysfunction (Reddy *et al.,* 2012). Hence, the exploitation of natural remedies in inhibiting the HMG-CoA reductase becomes very essential.

There are many reports, on the hypocholesterolemic potency of herbal drugs. Epidemiologic studies have shown a correlation between an increased consumption of phenolic antioxidants and a reduced risk of CVD which is supported by a study where the HMG CoA reductase was inhibited by *Moringa oleifera* polyphenols at different concentrations (Reddy and Urooj, 2012). and supplementation of *Moringa* leaf polyphenol extract resulted in a significant decrease in plasma cholesterol in rats fed with high fat and cholesterol diet.

3-Hydroxy- 3-methylglutaryl Coenzyme A reductase, the enzyme that synthesizes mevalonate, appears to be regulated through a multivalent feedback mechanism. Full suppression of the reductase requires the presence of at least two regulators: cholesterol, which is normally derived exogenously from plasma low density lipoprotein (LDL) and a non-sterol product, which is normally synthesized endogenously from mevalonate (Brown and Goldstein, 1980). In cultured mammalian cells such as human fibroblasts, the activity of HMG CoA reductase, and hence, the formation of mevalonate is controlled through a feedback mechanism mediated by cholesterol that enters cells bound to a plasma lipoprotein, low density lipoprotein (LDL). In the absence of plasma LDL, cells in culture synthesize their own cholesterol, maintaining high levels of HMG CoA reductase. This effect was demonstrated by adding LDL to the culture medium. The LDL-derived cholesterol reduced the activity of HMG CoA reductase, thereby turning off the cell's cholesterol synthesis (Brown *et al*., 1973; Brown and Goldstein, 1979). In the present study, the plant extracts and statins might have acted in a similar manner as LDL in inhibiting HMG CoA reductase, thus, preventing formation of mevalonate.

Although several medicinal plants have been reported to possess cholesterol lowering properties, data are reported on the cholesterol lowering mechanism (Mohamed *et al.,* 2009; Adaramoye *et al.,* 2008; Osorio-Esquivel *et al.,* 2012). The major metabolic pathway for reducing cholesterol is *via* conversion to bile acids or preventing the cholesterol synthesis by inhibiting the HMG CoA reductase enzyme. To establish the cholesterol lowering mechanism of the medicinal plants studied, we are also exploring its bile acid binding capacity using *in vitro* model system. Such preclinical studies will help in proper selection of medicinal plants for promoting their therapeutic utility.

5. Conclusion

The finding of the present study lends support to the reported data on the hypocholesterolemic role of *Abrus, Canthium and Costus* speciocus by inhibiting the activity of HMG CoA reductase, a key enzyme in cholesterol biosynthesis. Abrus, in particular exhibited maximum inhibition indicating scope for its utilization. Studies on the isolation and characterization of pure extracts of these medicinal plants can result in developing a neutraceutical for use in functional food formulations in the management of hypercholesterolemia.

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Conflict of interest

We declare that we have no conflict of interest.

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