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Screening of Antihyperlipidemic Herbal Drugs for their ACAT (Acyl Coenzyme: A Cholesterol Acyltransferase) Inhibitory Activity

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Abstract: ***Hyperlipidemia (elevated level of LDL, TG, TC or VLDL) is a major cause of atherosclerosis and associated cardio vascular disease. Lipid metabolism is regulated by various enzymes, ACTA enzyme one of them. The aim of present study was screen out various antihyperlipidemic herbal drugs for their ACAT enzyme inhibitory activity. Liver microsomal preparation was prepared from high fat diet male albino Wistar rats. ACAT enzyme inhibitory activity was measured addition of four different concentration (1 µg/ml, 10 µg/ml, 30 µg/ml 100 µg/ml) of herbal drugs. Successive extractions of herbal drugs were done by petroleum ether, chloroform, ethyl acetate and methanol, IC_{50} value of each extract was determined. The extract with lowest IC_{50} value were also sub fractionated and their IC_{50} values were also determined. All the plant extracts showed inhibition of ACAT activity with IC_{50} range of (1.70-24.85 µg/ml). The ethyl acetate extract of curcuma longa rhizomes (10.47 µg/ml), and chloroform extract leaf of Carica papaya (12.07 µg/ml) and Camellia senesis (13.04 µg/ml) and ethyl acetate extract of Achyrenthes aspera leaf (4.27 µg/ml) showed lowest IC_{50} values. The IC_{50} values of ethyl acetate sub fraction of Curcuma longa and Achyrenthes aspera leaf were(4.58 µg/ml) and (2.44 µg/ml) chloroform sub fraction of Carica papaya leaf (4.27 µg/ml) and hexane sub fraction of Camellia senesis leaf showed (1.70 µg/ml) lowest IC_{50} value. From the present study, it could be concluded that Curcuma longa rhizomes, Carica papaya leaf, Camellia senesis leaf, and Achyrenthes aspera leaf showed potent ACAT inhibitory activity and their further subfraction showed improve in inhibitory activity on ACAT enzyme.***

Keywords: Hyperlipidemia, ACAT (Acyl coenzyme A: cholesterol acyltransferase), LDL (low density lipoprotein), TG (Triglyceride), TC (Total cholesterol) and VLDL (very low density lipoprotein)

1. Introduction

Today in most of the developed and developing country, hyperlipidemia and thereby atherosclerosis is the leading cause of Coronary heart disease (CHD). Epidemiologic data also suggest that hypercholesterolemia and perhaps coronary atherosclerosis itself are risk factors for ischemic cerebrovascular accident. According to data from 2009 to 2012, >100 million U.S. adults ≥20 years of age have total cholesterol levels ≥200 mg/dL; almost 31 million have levels ≥240 mg/ dL. Hyperlipidemia is a metabolic disorder, that specific characterized by alteration of lipid metabolism and lipoprotein profile due to increase concentration of TC (Total cholesterol), Cholesteryl ester (CE), and TG (Triglycerides), with concomitant decrease in HDL (High density lipoprotein) level in the blood circulation.[1] [2] [3]There are several drugs available in market for the treatment of hyperlipidemia like of HMG-CoA reductase inhibitors (statins), fibric acid derivative (fibrates),bile acid binding resins (Cholestipol and cholestyramine). The developments of HMG-CoA reductase inhibitors, i.e., statin significantly reduces the mortality of CHD patients by 20-35%, savings life of millions of people each year. On the other hand, even with the aid of statins, CHD related mortality remain high. Rhabdomyolysis and myopathy is similar for all lipid lowering drugs and range of 0.1-0.5% with monotherapy, increasing to 05-2.5% with combination therapy.[4] [5]There is still a needs to identify other compounds that would complements the action of statins and might further reduces CHD incidences and mortality. Cholesterol, the chief sterol found in vertebrates, exists both as a free sterol and as a component of cholesterol esters, which are synthesized by acyl-CoA:cholesterol acyltransferase (ACAT) enzymes. Because cholesterol ester synthesis by ACAT enzymes is involved in the synthesis and secretion of lipoproteins and in macrophage foam cell formation, there has been considerable interest in developing ACAT inhibitors to treat or prevent atherosclerosis.[6]

It is important to thoroughly characterized cholesteryl ester formation in human liver. Two enzymes are thought to be responsible for the synthesis of plasma cholesteryl ester (CE), i.e., ACAT (Acyl Coenzyme A: cholesterol acyltransferase) and LCAT (Lecithin: cholesterol acyltransferase). Lecithin cholesterol acyltransferase (LCAT, EC 2.3.1.43), is the enzyme producing most plasma cholesterol esters and a key participant in the process of reverse cholesterol transport. LCAT is a glycoprotein that is secreted by the liver into the blood its activity is necessary for the formation of mature high density lipoprotein (HDL) and for remodelling of HDL lipoprotein particles[7] [8]. There are two isoforms, ACAT1 and ACAT2.Acyl coenzyme A: cholesterol acyltransferase (ACAT) is an intracellular enzyme that catalyzes the formation of cholesterol esters from cholesterol and log chain fatty acids. ACAT is an integral membrane protein located in the rough endoplasmic reticulum (ER) and ACAT activity is present in a variety of cells and tissues, including the adrenal, aorta, intestine and liver. The regulation of ACAT is a key step in cholesterol homeostasis.[9] [10]

Herbs chemical constitute a major part in all traditional medicines they fitted immediate personal need, are easily available, accessible and inexpensive. Now a days there has been a tremendous increase in use of plants based health productive in developing as well as developed countries

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resulting exponential growth of herbal products globally. There are several antihyperlipidemic drugs available in marked including statins, fibrates, and niacin. Along with various herbal drugs like *Achyrenthus aspera leaf, Camellia senesis leaf, Curcuma longa rhizomes,* and *Carica papaya leaf* possess antihyperlipidemic activity, but there mechanism is not still clear. The present study is intended for that the proposal drugs on acting ACAT enzyme inhibitory activity.

Achyrenthus aspera leaf (Amaranthaceae) is an important medicinal herb found as a weed throughout India. Though almost all of its parts are used in traditional system of medicines, seeds, roots, leaf, and shoots are important parts which are used medicinally. The plant possess various medicinal activity like hypoglycaemic, hypertension, hyperlipidemia, anti-diabetic, psoriasis, anti-inflammatory, hepatoprotective, immunomodulatore, analgesic and nephron protective. Chemical constitute reported as major Oleanolic acid glycosides A, B, C, and D. flavonoids, tannins, alkaloids saponin. Previous study reported that Alcoholic extract of *Achyrenthus aspera* at 100mg/kg dose lowered serum TC, TG, and total lipid by 60%, 33%and53%. The chronic administration of this drug at the same doses to normal rats for 30 days, lowered serum TC, PL, TG and TL by 56, 62, 68 and 67% respectively followed by significant reduction in the levels of hepatic lipids.[11][12][13]

Carica papaya leaf **(**Caricaceae) commonly known as papaya or papita. There seeds, fruits and leaf used as traditional medicine. Carica papaya possess Anti-diabetic, Hypolipidemic, hypoglycaemic, and increase RBC counts in human.It has anti-lipidemic and anti-cholestrolemic activities and as such could be used in the management of hypercholesterolemic.The study also demonstrated the hypolipidemic effects of papaya by reducing the levels of TC, TG, VLDL and LDL.[14][15]

Flavonoids reach herbal drugs like *Curcuma longa* rhizomes and *Camellia senesis* leaf possess anti-inflammatory, anti-oxidants, anti-neoplastic, anti-inflammatory activity positive effect on cardiovascular system. Reduce plasma cholesterol level. *Curcuma longa* rhizomes (Zingiberaceae) commonly known as Indian saffron, turmeric, haldi and its used as a spice for millennia. The chief compound is curcuminoid is known as (curcumin-I) contain 0.3-5.4%, demethoxycurcumin (curcumin-II), didemethoxycurcumin (curcumin-III) Curcumin and curcuminoid reported to be responsible for yellow colour in species. Volatile oil content ranges from 1 to 6.5% and composed of mono and sesquiterpenes such as alpha and bête pinene, alpha phelandrene, camphor, camphene, alpha beta turmerones. [16][17][18]

Camellia senesis leaf (Thecae) known as tea most common drink in the world. Prospective studies suggested that the polyphenolic flavonoids in tea may exert a protective effect against CHD. The reported beneficial effect of Green tea catechins and gallate esters to reduces intestinal cholesterol absorption and inhibit platelate aggregation. Other research suggested that tea increase the expression of the hepatic LDL-C receptore, a cell surfaces protein involved in control of plasma cholesterol, and increased the faecal excreation of bile acids and cholesterol.[19] [20]

2. Materials and Methods

2.1 Study Approval

The experiment protocol was approved by the Institutional Animal Ethics Committee, (IAEC) of K. B. Institute of Pharmaceutical Education & Research, Gandhinagar; protocol approval registration number: KBIPER/13/447.

2.2 Animals

Male albino rats of Wister strain were house at 25^0 c; 1 hrs. light dark cycles in cages with free access to normal pellet diet and water ad libitum and acclimatized to the surrounding one week prior to the experiment start.[12]

2.3 Authentification of herbal drugs

Authentification of herbal plants done by department of pharmacognosy, K. B. Institute of Pharmaceutical Education & Research, Gandhinagar.

2.4 Chemical and reagents

Sucrose, Tris buffer (pH 7.4), Iodoacetic acid, Lignocaine, Potassium hydrogen phosphate buffer (0.2 M, pH 7.4), Bovine serum albumin, Chloroform, methanol, petroleum ether, ethyl acetate, hexane, and Total cholesterol kit (span diagnostic).

2.5 Methods

Preparation of liver microsomes: Male Sprague Dawley rat was killed by decapitation and its liver removed to ice-cold 0.25 M sucrose solution. The liver was homogenized (after mincing with scissors) using homogenizer in 10 ml/g tissue wet weight if ice-cold 0.25 M sucrose solution. The crude homogenate was centrifuged for 20 min at 11,500 RPM and the pellet was discarded. The resulting supernatant was centrifuged at 13,500 RPM for 35 min after addition of $CaCl_2$ (0.1 ml per 1 ml of supernatant). The pellet was resuspended in 10 ml of 0.1M tris buffer, at pH 7.4 by homogenization, and it was stored at -20°C until use.[21][22]

2.6 ACAT assay

There are two enzymes present in the liver microsomes (hepatocytes of liver) preparation one LCAT and another is ACAT inhibition of LCAT enzyme by adding LCAT inhibitor 10µl iodoacetic acid dissolve in Tris-buffer isotonic saline (154 mmol/l Nacl, 110 mmol/l Tris,1mmol/l EDTA, pH 7.4) add into liver microsome preparation and inhibition of cholesterol esterase by adding 20 µl of lignocaine after addition of reagent 1 and incubate for 5 min at37^0c.[22][23]
The in vitro ACAT inhibition assay study carried out under below procedure:

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Table 1: ACAT enzyme inhibition assay procedure.

Sr. No	Reagents	Control	1 (µg/ml)	10 (µg/ml)	30 (µg/ml)	100 (µg/ml)
1.	Liver homogenate (LH)	50 µl	50 µl	50 µl	50 µl	50 µl
2.	Iodoacetic acid (LCAT inhibitor)	10 µl	10 µl	10 µl	10 µl	10 µl
3.	Reagent 1 (R1)	100 µl	100 µl	100 µl	100 µl	100 µl
4.	Lignocaine (cholesterol esterase inhibitor)*	20 µl	20 µl	20 µl	20 µl	20 µl
INCUBETED FOR 5 MIN AT 37^0 C						
5.	Reagent 2 (R2)	1 µl	1 µl	1 µl	1 µl	1 µl
6.	Herbal drugs	--	30 µl	30 µl	30 µl	30 µl
INCUBETED FOR 10 MIN AT 37^0 C						

Reagent 1: Cholesterol esterase, Cholesterol oxidase, Phenol, 4-amino antipyrine
Reagent 2: STD Cholesterol
Step 1:- LH + R1. (Absorbance = Total cholesterol in LH)
Step 2:- LH + R1 + R2. (Absorbance = Total cholesterol in LH + STD total cholesterol)
Step 3:- R1 + lignocaine (kept for 5 min.) + LH. (Absorbance = Free cholesterol in LH)
Step 4:- R1 + lignocaine (kept for 5 min.) + LH + R2. (Absorbance = Free cholesterol in LH + STD free cholesterol)
Step 5:- R1 + lignocaine (kept for 5 min.) + Compound+ LH + R2. (Absorbance = Free cholesterol in LH + STD free cholesterol)
*All steps repeated in presences of Iodoacetic acid as LCAT inhibitor than calculate it.

2.7 Method for herbal drug extraction:[24] [25]

Curcuma longa rhizomes: Aliquots of 100 grams of powdered plant material were extracted successive extraction with solvents of increasing polarity, such as petroleum ether, chloroform, ethyl acetate, methanol and water by soxhlet apparatus. The extracts were filtered, concentrated to dryness and stored in refrigerator below 4^0C, until further use.

Carica papaya leaf:Five gram aliquots of powdered plant material were extracted separately with Solvents of increasing polarity, such as Petroleum ether (PE), Chloroform (CF), Ethyl acetate (EA), and methanol (ME) by hot maceration, under reflux. The extracts were filtered, concentrated to dryness and stored in refrigerator below 40C, until further use.

Camellia senesis leaf: Five gram aliquots of powdered plant material were extracted separately with Solvents of increasing polarity, such as Petroleum ether (PE), Chloroform (CF), Ethyl acetate (EA), and methanol (ME) by hot maceration, under reflux. The extracts were filtered, concentrated to dryness and stored in refrigerator below 40C, until further use.

Achyrenthes aspera leaf: Five gram aliquots of powdered plant material were extracted separately with Solvents of increasing polarity, such as Petroleum ether (PE), Chloroform (CF), Ethyl acetate (EA), and methanol (ME) by hot maceration, under reflux. The extracts were filtered, concentrated to dryness and stored in refrigerator below 40C, until further use.

2.8 Sub fractionation of herbal drugs:[24] [25]

Curcuma longa* rhizomes*: *Curcuma longa* ethyl acetate extract show lowest IC 50 value comparative other solvents. This extract was further sequentially extracted with n-hexane and ethyl acetate.

Carica papaya* leaf*: Chloroform extract of *Carica papaya* show lowest IC 50 value comparative other solvents. This extract was further sequentially extracted with n-hexane and chloroform.

Achyrenthes aspera* leaf*: *Achyrenthes aspera* ethyl acetate extract show lowest IC 50 value comparative other solvents. This extract was further sequentially extracted with n-hexane and ethyl acetate.

Camellia senesis* leaf*: Chloroform extract of *Camellia senesis* show strong ACAT enzyme show lowest IC 50 value comparative other solvents. This extract was further sequentially extracted with n-hexane and chloroform.

3. Results

Table 2: Effect of Curcuma longa rhizomes on cholesteryl ester formation:

Effect of Curcuma longa rhizome on cholesteryl ester formation (ACAT enzyme inhibitory activity)						
Sr. No	Herbal drugs Extraction	Control	Concentration of cholesteryl ester (µg/ml) at Following concentration of herbal drugs			
			1 µg/ml	10 µg/ml	30 µg/ml	100 µg/ml
1.	Petroleum ether	35.872±1.13	27.51 ±1.72*	23.55±0.23*	20.47 ± 1.07*	19.00±0.59*
2.	Chloroform	35.872±1.13	29.64± 1.72*	28.82± 1.18*	23.42 ± 1.71*	15.72± 0.28*
3.	Etyhlacetate	35.872±1.13	25.06± 0.28*	23.91± 0.59*	20.96 ± 0.71*	18.83± 0.86*
4.	Methanolic	35.872±1.13	32.10± 1.31	28.99± 1.36*	25.88 ± 0.99*	12.94± 0.99*
5.	Sub fraction of Hexane	35.872±1.13	26.53±2.52*	24.57±0.85*	16.54±2.69*	13.92±2.018*
6.	Subfraction of Chloroform	35.872±1.13	27.02±1.02*	23.91±1.31*	13.43±0.86*	8.35±2.60*

* indicate Values are Mean ± SEM. *P< 0.05 when compared with respective control group.

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Table 3: Effect of Carica papaya leaf on cholesteryl ester formation:

Effect of Carica papaya leaf on cholesteryl ester formation (ACAT enzyme inhibitory activity)						
Sr. No	Herbal drugs Extraction	Control	Concentration of cholesteryl ester (µg/ml) at Following concentration of herbal drugs			
			1 µg/ml	10 µg/ml	30 µg/ml	100 µg/ml
1.	Petroleum ether	35.872±1.13	31.61±2.12	30.46±1.98	19.65±0.75	13.75±0.71
2.	Chloroform	35.872±1.13	29.32±3.36*	26.20±2.27*	20.47±1.18*	13.92±2.14*
3.	Etyhlacetate	35.872±1.13	28.50±1.77*	27.68±1.07*	20.47±1.14*	17.85±2.05*
4.	Methanolic	35.872±1.13	35.21±2.57	35.05±1.27	23.25±2.68*	18.18±1.72*
5.	Sub fraction of Hexane	35.872±1.13	25.88±1.14*	21.62±1.23*	19.16±2.04*	12.28±0.75*
6.	Subfraction of Chloroform	35.872±1.13	18.50±1.61*	22.60±1.41*	20.80±1.27*	17.28±3.27*

* indicate Values are Mean ± SEM. *P< 0.05 when compared with respective control groups.

Table 4: Effect of Camellia senesis leaf on cholesteryl ester formation

Effect of Camellia senesis leaf on cholesteryl ester formation (ACAT enzyme inhibitory activity)						
Sr. No	Herbal drugs Extraction	Control	Concentration of cholesteryl ester (µg/ml) at Following concentration of herbal drugs			
			1 µg/ml	**10 µg/ml**	**30 µg/ml**	**100 µg/ml**
1.	Petroleum ether	35.872±1.13	28.33±0.81*	24.40±1.71*	21.294±0.71*	19.81±3.38*
2.	Chloroform	35.872±1.13	29.48±3.27	26.53±2.25*	18.01±1.66*	13.59±2.36*
3.	Ethyl acetate	35.872±1.13	35.54±0.59	33.25±1.63	22.27±1.27*	13.26±0.56*
4.	Methanolic	35.872±1.13	32.59±0.65	30.79±2.05	21.45±0.59*	19.32±3.22*
5.	Sub fraction of Hexane	35.872±1.13	22.93±0.99*	19.32±0.71*	16.54±0.86*	10.31±2.04*
6.	Subfraction of Chloroform	35.872±1.13	26.53±1.50*	18.50±1.66*	13.59±1.18*	8.02±1.14*

* indicate Values are Mean ± SEM. *P< 0.05 when compared with respective control group.

Table 5: Effect of Achyrenthes aspera leaf on cholesteryl ester formation

Effect of *Achyrenthes aspera* leaf on cholesteryl ester formation (ACAT enzyme inhibitory activity)						
Sr. No	Herbal drugs Extraction	Control	Concentration of cholesteryl ester (µg/ml) at Following concentration of herbal drugs			
			1 µg/ml	**10 µg/ml**	**30 µg/ml**	**100 µg/ml**
1.	Petroleum ether	35.872±1.13	28.17±1.84*	28.00+ 0.49*	19.32+1.73*	12.61±1.27*
2.	Chloroform	35.872±1.13	31.61±0.91	23.25±1.34	14.25±1.50*	11.79±1.50*
3.	Etyhlacetate	35.872±1.13	24.40±1.27*	22.93±3.27*	19.81±0.71*	14.74±1.02*
4.	Methanolic	35.872±1.13	32.10±2.12	32.92±0.85	27.84±3.03*	10.48±2.36*
5.	Sub fraction of Hexane	35.872±1.13	20.63±1.30*	17.19±1.02*	16.05±1.88*	13.26±1.022*
6.	Subfraction Chloroform	35.872±1.13	25.06±1.30*	16.70±3.0*	13.923±2.68*	10.97±0.59*

* indicate Values are Mean ± SEM. *P< 0.05 when compared with respective control group.

Table 6: Phytochemical analysis of herbal drugs:[25]

1. Identification of ethyl acetate sub fraction of *Curcuma longa* rhizomes:		
Chemical test	**Observation**	**Inferences**
Liebermann burchard reaction	Green colour	Saponin, and steroids
Salkowaski test	Red colour	Terpenoids
1. Identification of chloroform sub fraction of *Carica papaya* leaf:		
Dragendroaf test	Orange precipitates	Alkaloids
Salkowaski test	Red colour	Terpenoids
2. Identification of chloroform sub fraction of *Camellia senesis* leaf:		
Dragendroaf test	Orange precipitates	Alkaloids
Salkowaski test	Red colour	Terpenoids
Foam test	Foam was observed	Saponin glycoside.
3. Identification of ethyl acetate sub fraction of *Achyrenthes aspera leaf*:		
Liebermann burchard reaction	Green colour	Saponin, and steroids
Foam test	Foam was observed	Saponin glycoside.

Table 7: IC_{50} values of herbal drugs.

Extraction of herbal drugs					
Sr. No.	Solvents used for extraction.	IC_{50} Values of *Curcuma longa* rhizomes	IC_{50} Values of *Carica papaya* leaf	IC_{50} Values of *Camellia senesis* leaf	IC_{50} Values of *Achyrenthes aspera* leaf
1.	Petroleum ether	15.72 µg/ml	20.20 µg/ml	20.07 µg/ml	19.01 µg/ml
2.	Chloroform	24.63 µg/ml	12.70 µg/ml	13.04 µg/ml	07.64 µg/ml
3.	Ethyl acetate	10.47 µg/ml	20.08 µg/ml	27.13 µg/ml	04.27 µg/ml
4.	Methanolic	24.85 µg/ml	17.52 µg/ml	23.59 µg/ml	28.83 µg/ml
Sub-fraction of herbal drug extraction by their respective solvent					
5.	N-hexane	06.12 µg/ml	08.85 µg/ml	01.70 µg/ml	03.35 µg/ml
6.	Ethyl acetate	04.58 µg/ml	--	--	02.44 µg/ml
7.	Chloroform	--	04.27 µg/ml	02.98 µg/ml	--

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4. Discussion

Hyperlipidemia include either a low high density lipoprotein cholesterol value or elevations in atherogenic lipoprotein particles, including cholesterol, cholesteryl esters and triglycerides. The increased LDL, TG or VLDL levels can be clinically controlled by reduced absorption of cholesterol from GIT, decreased synthesis in liver or increased deposition in the adipose tissue. Absorption of lipids from GIT can be reduced by using bile acid sequesterants such as Cholestipol also Ezetimibe by blocks the Niemann Pick channel which prevents absorption of cholesterol in enterocyte. The cholesterol which is entering into enterocyte is converted to cholesterylester with the help of Acyl-coenzyme A: cholesterol acyltransferase (ACAT) enzyme. This step is necessary for absorbed cholesterol to reach lymphatic circulation.Acyl-coenzyme A: cholesterol acyltransferase (ACAT) is an integral membrane protein located in the rough endoplasmic reticulum(ER) that catalyses are action in which a fatty acyl-CoA is esterified to a cholesterol molecule.[7] [9]

Various herbals have been reported to possess antihyperlipidemic activity. However, their mechanism of action remains obscure. In the present study we made an attempt to screen some of these plants for their inhibitory effect on ACAT enzyme. We selected plants *Curcuma longa rhizome, Carica papaya leaf, Achyrenthes aspera leaf, and Camellia senesis* leaf. The extract of dry powder of *curcuma longa rhizome, Carica papaya leaf, camellia senesis leaf, Achyrenthes aspera* leaf were prepared by successive extraction with petroleum ether, chloroform, Ethyl acetate and methanol. [25]

The ACAT enzyme inhibition by these extracts was carried out in in vitro using rat liver as the source of ACAT enzyme. The rat liver microsomes are rich in ACAT enzyme and therefor act as its source. Formation of cholesteryl ester from cholesterol in presences of rat liver microsomes was measured using total cholesterol kit. [21]This formation of cholesteryl ester and subsequent measurement in presences of different extracts of *Curcuma longa rhizomes, leaf of Carica papaya, Camellia senesis and Achyrenthes aspera* in four different concentration (1, 10, 30, 100). The inhibition of ACAT enzyme reflected by reduced formation of cholesteryl ester. IC_{50} value of each extract was determined. The extract with lowest IC_{50} value were further subfractionted and their IC_{50} values were also determined.[22] [23]

Curcuma longa rhizomes well known as turmeric has been reported to have antihyperlipidemic and antiatherosclerosis activity. The mechanisms of action however remain unclear. Ether extract of *Curcuma longa rhizome* in low dose (1.6-3.2 mg/kg) decrease in cholesterol and triglyceride levels. Turmeric extract effect on cholesterol level may be due to decrease cholesterol uptake in the intestines and increased conversion of cholesterol to bile acid in liver. The Ethanolic extract of curcuma longa rhizome (300mg/kg) orally in triton induced hyperlipidemia produced elevated serum HDL and serum total cholesterol, and significantly reduction in ratio of total cholesterol / phospholipid.[29] [30]

Extraction of herbal drugs carried out in successive method using solvents of increasing polarity like petroleum ether, chloroform, ethyl acetate and Methanol. Then its IC_{50} values determined. The extract with lowest IC_{50} value were further sub fractionated by respective solvents and their IC_{50} values were also determine. All the extracts of *Curcuma longa rhizomes* showed inhibition of ACAT activity with respective extracts petroleum ether, chloroform, ethyl acetate and Methanol showed IC_{50} values were 15.72 µg/ml, 24.63 µg/ml, 10.47 µg/ml, and 24.85 µg/ml. Significant change in cholesteryl ester formation each dose compare to control and also showed dose dependent fashion increase the dose of herbal drug concentration increase the percentage inhibition of ACAT activity. Ethyl acetate extract showed lowest IC_{50} value 10.47 µg/ml this extract was further sub fractionated by successive extraction with hexane and ethyl acetate solvents. Both subfraction hexane and ethyl acetate showed improve ACAT inhibitory activity with an IC_{50} values were 6.12 µg/ml and 4.58 µg/ml. The qualitative chemical test of ethyl acetate subfraction of *Curcuma longa* rhizomes showed presences of terpenoids and steroids.

Carica papaya leaf well known as the Papita (Papaya) and it reported as antihyperlipidemic and anti-atherosclerotic activity. Ether and water soluble fraction of *Carica papaya* leaf extract reduce the TC, TG, and increased HDL level.[14] [15] [31]

Extraction of *Carica papaya* leaf carried out in successive method using solvents of increasing polarity like petroleum ether, chloroform, ethyl acetate and Methanol. Then its IC_{50} values determined. The extract with lowest IC_{50} value were further sub fractionated by respective solvents and their IC_{50} values were also determine. All the extracts of *Carica papaya leaf* showed inhibition of ACAT activity with respective extracts petroleum ether, chloroform, ethyl acetate and Methanol showed IC_{50} values were 20.20 µg/ml, 12.70 µg/ml, 20.08 µg/ml, and 17.52 µg/ml. Significant change in cholesteryl ester formation each dose compare to control and also showed dose dependent fashion increase the dose of herbal drug concentration increase the percentage inhibition of ACAT activity. The chloroform extract of Carica papaya leaf extract show more potent ACAT inhibitory activity compare other three extract. This extract was further successive subfractionnation with hexane and chloroform. Both subfraction of *Carica papaya leaf* extract by hexane, and chloroform solvents. We found both sub fractionation of chloroform extract of *Carica papaya* leaf possess strong inhibition of ACAT enzyme with respective IC_{50} values 8.85 µg/ml, and 4..27 µg/ml. The qualitative chemical test of ethyl acetate subfraction of *Carica papaya leaf* showed presences of terpenoids and steroids.

Camellia senesis leaf previous study reported that intestinal ACAT plays a key role in the intestinal absorption of cholesterol by esterifying cholesterol before its absorption. It has been shown that tea catechins decrease micelle solubility and intestinal absorption of cholesterol in rats. They hypothesized that GTE (green tea extract) may interfere with the absorption of cholesterol by inhibiting the

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ACAT activity. However, dietary GTE had no influence on the intestinal ACAT activity, suggesting that GTE increases the faecal output of cholesterol mainly as a result of its binding capacity and acceleration of cholesterol excretion. [20] [28]

Camellia senesis leaf powder extract with respective solvent petroleum ether, chloroform, ethyl acetate and methanol showed strong ACAT enzyme inhibition in all extracts with respective IC 50 values are 20.07 µg/ml, 13.04 µg/ml, 27.13 µg/ml, and 23.59 µg/ml. All the extracts showed significantly effective ACAT inhibitory activity compare to normal group. And also showed dose dependent fashion inhibition of ACAT inhibitory activity. Increase the dose of herbal drug increase the percentage inhibition of ACAT activity. The chloroform extract of *Camellia senesis* leaf showed lowest IC_{50} value13.04µg/ml as compare to other three extracts. This extract was further successive subfractionnation with hexane and chloroform. Both subfraction of chloroform extract by hexane, and chloroform solvents we found strong inhibition of ACAT with respective IC_{50} values 1.70 µg/ml, and 2.98 µg/ml. Hexane sub fraction of camellia senesis powder showed more potent than chloroform sub fraction extract.

The *Achyrenthes aspera* commonly known as apamarg, aghedo and it's used as hyperlipidemic and antiatherosclerotic activity were reported. The alcoholic extract of the plant *Achyranthus aspera* at100mg/kg dose has been reported to lower total serum cholesterol (TC) and phospholipid (PL), triglyceride (TG) and total lipids (TL) levels by 60, 51, 33 and 53percent, respectively by rapid excretion of bile acids causing low absorption of cholesterol and immense potential to lower serum lipid levels.[13][26][27]

Extraction of *Achyrenthes aspera leaf* carried out in successive method using solvents of increasing polarity like petroleum ether, chloroform, ethyl acetate and Methanol. Then its IC_{50} values determined. The extract with lowest IC_{50} value were further sub fractionated by respective solvents and their IC_{50} values were also determine. All the extracts of *Achyrenthes aspera leaf* showed inhibition of ACAT activity with respective extracts petroleum ether, chloroform, ethyl acetate and Methanol showed IC_{50} values were 19.01µg/ml, 7.64µg/ml, 4.27µg/ml, and 28.83µg/ml. Significant change in cholesteryl ester formation each dose compare to control and also showed dose dependent fashion increase the dose of herbal drug concentration increase the percentage inhibition of ACAT activity. Ethyl acetate extract of *Achyrenthes aspera leaf* showed lowest IC_{50} value 4.27 this extract was further sub fractionated by hexane and ethyl acetate solvents. Both sub fraction of ethyl acetate by hexane and ethyl acetate extracts showed potent ACAT inhibitory activity with respective IC_{50} values were 3.35 and 2.44. The ethyl acetate sub fraction of *Achyrenthes aspera leaf* showed potent ACAT inhibitory activity compare to hexane subfraction. The qualitative chemical test of ethyl acetate sub fraction showed presences of steroids, triterpene and saponin glycoside.

5. Conclusion

From the present study it can be concluded that the *Carica papaya leaf, camellia senesis leaf, curcuma longa rhizomes, and Achyrenthes aspera leaf* showed potent inhibitory activity on ACAT enzyme. And their further subfraction showed improve in inhibitory activity on ACAT enzyme. Results demonstrate that the plants *Curcuma longa rhizomes, Leaf of Carica papaya, Camellia senesis, and Achyrenthes aspera* possess ACAT inhibitors therefor support the traditional use of this plants for the treatment of hyperlipidemia and atherosclerosis.

6. Future Scope

Further, study would be required to isolate the active constituents from the above plants which are responsible for ACAT inhibitory activity. Future studies on in-vivo can be done for confirmation of the effectiveness of this *Curcuma longa rhizomes, and leaf of Carica papaya, Camellia senesis and Achyrenthes aspera* plants. Also future investigation on the isolation and characterization of the antihyperlipidemic chemical constituents is however required also purification of chemical constituents need for better activity.

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