International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 702-710 www.ijprajournal.com ISSN: 2249-7781

Hypolipidemic and Antioxidant Activity of Aerial Parts of Michelia Champaca Linn in Hyperlipidemic Rats

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Date of Submission: 25-09-2021 Date of Acceptance: 08-10-2021

ABSTRACT-
To evaluate hypolipidemic activity of the methanolic and aqueous extracts of aerial parts of the plant Michelia champaca Linn. And to evaluate the antioxidant activity of the methanolic and aqueous extract of aerial parts of the plant Michelia champaca Linn in hyperlipidemic rats.
Methods: High fat diet induced hyperlipidemia in rats.
Result: These result indicate that methanolic extract of M. champaca Linn shows statistically significant hypolipidemic activity compared to aqueous extract of M. champaca Linn
Conclusion: Theaerial parts of Michelia champaca Linn Possesses antioxidant effect in addition to hypolipidemic effect. Furthermore methanolic extract is more potent than aqueous extract. Antioxidant effect of extracts might be due to polyphenolic compounds and flavonoids of aerial parts of Michelia champaca Linn and thus this plant have a promising role in the treatment of hyperlipidemia and atherosclerosis
Keyword- Hyperlipidemia, Antioxidant, Atherogenic index, Michelia Champaca, High fat diet.

I. INTRODUCTION

Hyperlipidemia is a vital segment of heart and coronary syndromes, as well as atherosclerosis, causing approximately 17 million deaths worldwide each year. Endothelial is support system, the entry of low- density lipoproteins (LDL) through the intimae layer, causing oxidation and atherosclerotic destruction. [1] Hyperlipidemia is a dangerous complication that interferes with daily life. It is a medical condition during which any or all lipid characteristics and/or lipoproteins in the blood are elevated. Because these fatty substances circulate in the blood coupled to proteins, it's also known as hyperlipoproteinemia.

Hyperlipidemia is considered one of the major risk factors causing cardiovascular diseases (CVDs).CVDs accounts for one third of total deaths around the world, it is believed that CVDs will turn out to be the main cause of death and disability worldwide by the year 2020. [2, 3]

Hyperlipidemia is an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters and phospholipids and or plasma lipoproteins including very low-density lipoprotein and low-density lipoprotein, and reduced high-density lipoprotein levels. [4, 5] Hypercholesterolemia and hypertriglyceridemia are the main cause of atherosclerosis which is strongly related to ischemic heart disease (IHD). [6] There is a strong relation between IHD and the high mortality rate. Furthermore elevated plasma cholesterol levels cause more than four million deaths in a year. [7]

Atherosclerosis is a process of arteries hardening due to deposition of cholesterol in the arterial wall which causes narrowing of the arteries. Atherosclerosis and atherosclerosis-associated disorders like coronary, cerebrovascular and peripheral vascular diseases are accelerated by the presence of hyperlipidemia. [8]

The underlying cause of heart attacks, aneurysms, and peripheral vascular disease, atherosclerosis, is a leading cause of morbidity and mortality throughout. Lipid accumulation originates and disturbs the condition, which can be thought of as a variety of chronic inflammation. Hypercholesterolemia and hypertriglyceridemia are two separate risk factors that can accelerate the onset of atherosclerosis and the evolution of atherosclerotic lesions, either alone or in combination. The buildup of cells storing excess lipids within the artery wall is one of the first stages in the development of atherosclerosis. Moreover, increased intracellular formation of reactive oxygen species (ROS) has been shown to

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 702-710 www.ijprajournal.com ISSN: 2249-7781

play a key role in chronic inflammatory responses to atherosclerosis. Oxidative stress caused in aerobic organisms during physiological or physiopathological mitochondrial oxidative metabolism. [9]

Traditional medicine is widely practiced in poor nations, and the majority of the population relies on it for their main health care. Traditional medicines are derived largely from medicinal plants. Various plants are utilized in traditional medicine for the treatment of oxidation and HL, and various plants have yet to be properly researched. Plant products are widely utilized for the treatment of a variety of diseases around the world, especially when allopathic medicine fails or has significant adverse effects. Adverse effects of hypolipidemic medications include dizziness, elevated or irregular heart rate, constipation, low blood pressure and drowsiness. [10]

Statins are the first-line treatment for primary hyperlipidemias with elevated LDL and CH levels, with or without elevated TG levels, as well as secondary hypercholesterolemia (diabetes, nephritic syndrome). However, all statins have a variety of adverse effects, such as gastrointestinal issues and headaches are minor. Rashes and sleep problems aren't prevalent. Although a rise in serum transaminase is possible, liver injury is uncommon. [11]

Champak (Michelia champaca L.) is a flowering plant native to India, Thailand, Myanmar, and Malaysia in South and Southeast Asia. Michelia champaca L. (Magnoliaceae) is a big medicinal plant that has been used for centuries to treat a variety of ailments, including inflammation. M. champaca L. leaf extract was found to be antioxidant, analgesic, and cytotoxic. [12] As a result, the current study aims to assess Michelia champaca L.'s hypolipidemic and antioxidant activity in hyperlipidemic rats.

II. MATERIAL AND METHODS:

Procurement, authentication, drying and storage of plant material.

Aerial parts (leaves, fruits, flowers, buds and stem) of Michelia champaca Linn, were collected in month of November 2020 from local area of Karad. The plant was authenticated by the botanist Dr. Girish Potdar

The aerial parts of Michelia champaca Linn were shade dried for week then immediately aerial parts were grinded using the grinder, the coarse powder was collected by passing through the sieve number 85.

- **Preparation of extracts** [13]

Different extract were prepared from aerial parts of Michelia champaca Linn by following methods.

- **Preparation of aqueous extract of Michelia champaca Linn**

Drug powder was taken in a 1000 ml conical flask and macerated with sufficient quantity of chloroform water for 7 days. During maceration, it was shaked occasionally. On 7^{th} day it was filtered and the filtrate was concentrated. The remaining solvent was evaporated by heating on a water bath (50^0c). The extract was kept in a vacuum desiccators to remove excess of moisture present in extract.

- **Preparation of methanolic extract of Michelia champaca Linn.**

The powder was extracted with methanol (95%) by soxhlet extraction method for 72hr. After completion of extraction the solvent was removed and obtained extract was kept in vacuum desiccators to remove excess of moisture present in extract.

YIELD VALUE

- **Percentage yield of methanolic extract of aerial parts of Michelia champaca L**.

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug}} \times 100$$

$$\frac{23\text{gm}}{290\text{gm}} \times 100$$

Percentage yield = 7.93 %

- **Percentage yield of aqueous extract of aerial parts of Michelia champaca L.**

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug}} \times 100$$

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$$= \frac{22gm}{250gm} \times 100$$

Percentage yield = 8.8 %

Phytochemical analysis of aqueous and methanolic extracts of Michelia champaca Linn

Chemical tests were carried out for different extract of Michelia champaca Linn. to identify the presence of various phytochemical constituents.

Experimental Animals

Healthy, adult male and female albino Wister Rats, weighing 250-300 gm were obtained from the animal house of Appasaheb Birnale College of Pharmacy.

Housing the Animals:

The animals were kept in a well ventilated room. The animal was placed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with food pellet and water ad libitum.

Drug Solutions:

The rosuvastatin solution was prepared in normal saline and administered orally as 10mg/kg.

The aqueous extract of aerial parts of Michelia champaca Linn was prepared in distilled water.

The methanolic extract of aerial parts of Michelia champaca Linn was prepared in normal saline.

Table no.1 List of drugs with dose and route of administration

Sr.No.	Drug used	Dose	Route of administration
1.	Rosuvastatin	10mg/kg	Oral
2.	Aqueous Extract of Michelia champaca L.	200mg/kg	Oral
3.	Methanolic extract of Michelia champacaL.	200mg/kg	Oral
4.	High fat diet	-	Oral

Dose selection.

In order to decide the dose of plant extract it is essential to go through the toxicity study of the extracts according to OECD guidelines. Hence for acute oral toxicity and LD50 determination OECD guidelines 425 (organization for economic cooperation and development) was allowed.

Acute oral toxicity study of aqueous and methanolic extracts performed by Up and Down procedure (OECD guidelines)

Acute oral toxicity study was carried out according to the OECD 425 guidelines. six wister rats weighing between 250-300gm respectively were used. Animal were tested for limit doses 2000mg/kg. Animals were observed individually at least once during the first 30min. after dosing and periodically during the first 24hr. (with special attention given during the first 4hr.) Additionally observed for the changes in the skin, eyes, mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, and locomotor activity and behavior pattern. As per OECD guidelines the substance might be considered to have an LD50 value above 2000mg/kg body wt. As no any sign of toxicity was observed, therefore dose $1/10^{th}$ of above dose was taken as safe dose i.e. 200mg/kg body wt. of each extract(aqueous and methanolic)were selected for the present study.

Pharmacological Screening

200mg/kg doses of the aqueous and methanolic extracts were tested for their antihyperlipidemic effect using the following experimental models:

High fat induced Hyperlipidemia.

Experimental Design :

The male wistar rat weighing 250-300gm was used for the experimental study. The animal were divided into five groups of 6 animals each and fed orally.

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Table no. 3 High fat diet induced hyperlipidemia

Group	Treatment
I	Normal or Vehicle
II	High fat diet control
III	HCD+ Rosuvastatin (10mg/kg)
IV	HCD+ aqueous extract (200mg/kg)
V	HCD+ methanolic extract (200mg/kg)

Composition of high cholesterol diet cocktail.

In rats, Hyperlipidemia induced by daily feeding fresh prepared high fat diet. first normal feeding pellets grinded with grinder , prepare a fine powder to each 500 gm of pellet powder added 5 gm cholesterol, 2.5 gm cholic acid , 5 teaspoon wheat and gram powder, 15 gm amul butter, 10gm milk powder and finally add vegetable oil to the appearing of smoothing of mixture preparation. The animals were divided into five groups. Group first receives standard pellet diet. Second group were fed with high fat diet for 20 days to induce hyperlipidemia. On 10^{th} day standard drug and test extract treatment were given to animals respectively upto 20^{th} day. At the end of the experiment, the rats were anaesthetized with thiopentone sodium (40mg/kg) to collect the blood sample for the biochemical estimation. [14]

Biochemical parameter estimation for evaluation of hypolipidemic activity from model High fat diet

On the last day of experiment animal were sacrificed after blood collection and the following parameter were monitored.

Biochemical parameters

- Total cholesterol
- Triglycerides (TG)
- High density lipoprotein (HDL)
- Low density lipoprotein (LDL)
- Very low density lipoprotein (VLDL)
- Atherogenic index

Tissue Antioxidant parameters

- Melondialdehyde (MDA)
- Superoxide dismutase SOD
- Catalase (CAT)

Statistical Analysis:

Value as expressed as mean ± SEM; Statistical analysis was performed using Benferroni- test. *$p<0.05$ was taken as the criteria of significance.

III. RESULT

Table no.4 Preliminary phytochemical study of aqueous and methanolic extracts of aerial parts of Michelia champaca Linn.

Sr. No.	Chemical Test	Aqueous Extract	MethanolicExtract
1.	Alkaloid	-	-
2.	Saponin glycoside	-	-
3.	Cardiac glycoside	+	-
4.	Tannins and Phenolic compound	+	+
5.	Flavonoid	+	+
6.	Test for carbohydrates	+	+

Methanolic extract- Methanolic extract of aerial parts of Michelia champaca L. shows the presence of steroids, tannins and phenolic compounds, flavonoids, and carbohydrates.

Aqueous extract - Aqueous extract of aerial parts of Michelia champaca Linn Shows presence of cardiac glycoside,steroids, tannins and phenolic compounds, flavonoids, and carbohydrates.

Acute oral toxicity study.

LD_{50} value of aqueous and methanolic extract of Michelia champaca Linn is more than 2000mg/kg. Hence $1/10^{th}$ dose of 2000mg/kg was selected which is safe dose, with no mortality in studied subject.

High Fat Diet induced hyperlipidemia

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Table no. 5 Effect of methanolic and aqueous extract of aerial parts of Michelia champaca Linn on Lipid profile in High Fat diet induced hyperlipidemic rats.

Sr. No.	Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
1.	Normal	75.74 ±1.48	67.73 ±1.62	37.04 ±0.86	13.74 ±1.01	49.38 ±1.88
2.	Control (High fat diet)	174.5 ±3.78# (↑130.39)	154.2 ±0.97# (↑127.66)	18.04 ±0.84# (↓ 51.29)	30.06 ±2.22# (↑118.77)	128.6 ±1.78# (↑160.42)
3.	High fat diet + Rosuvastatin (10mg/kg)	92.68 ±3.44**** (↓ 46.88)	95.01 ±0.97**** (↓38.38)	31.59 ±0.59**** (↑ 75.11)	18.90 ±0.40**** (↓ 37.12)	70.51 ±6.27**** (↓45.17)
4.	High fat diet + Methanolic extract 200mg	127.5 ±3.97**** (↓26.93)	139.3 ±1.92**** (↓9.66)	27.32 ±1.13**** (↑ 51.44)	20.07 ±1.49*** (↓33.23)	98.67 ±6.23*** (↓23.27)
5.	High fat diet + Aqueous extract 200mg	143.2 ±3.30**** (↓ 17.93)	145.2 ±2.49**** (↓5.83)	23.64 ±2.11**** (↑31.04)	23.63** ±2.07 (↓ 21.04)	110.8** ±2.54 (↓ 13.85)

Values are expressed as mean±SD (n=6), Statistical analysis was performed using Benferroni test.*p<0.05 was taken as the criterion of significance. *p<0.05 when compared to HFD control. #p<0.05 when compared to Normal (untreated control).Value in brackets indicates %increase or decrease.

Table no.6 Effect of Methanolic and aqueous extract of aerial parts of Michelia champaca L. on Atherogenic index

Sr. No.	Groups	Atherogenic Index
1.	Normal	1.04 ±0.55
2.	Control (High fat diet)	8.97±0.48 ↑762.5
3.	HFD+ Rosuvastatin	1.93±0.96 ↓78.48
4.		3.66±0.59 ↓59.19
5.	HFD + Methanolic extract 200mg	5.05±0.23 ↓43.70

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	HFD + Aqueous extract 200mg	

Change in the serum TC, TG, LDL, and HDL-C levels: It was observed that feed rats with HFD significantly increased the TC, TG, and LDL- C level in serum ($p<0.05$) as compare to rats on normal diet. The rise in TC level was 130.39% and rise in TG was 127.66%.

However dramatic decrease of serum TC and TG levels were observed in all test extracts and rosuvastatin standard group compare to those of HFD control. The serum TC level of rosuvastatin ME200 and AE200 mg/kg of test extracts dosing group were decreased by 46.88 %, 26.93%, 17.93% compared to HFD control rat respectively. Serum TG level of Rosuvastatin ME200, AE200 mg/kg of test extracts dosing group were decreased by 38.38%, 9.66%, 5.83 % compared to HFD control rat respectively.

A significant increase of serum LDL-C level i.e. 160.42% was detected in HFD group compared to that of animal on normal diet. However dramatic decrease of serum LDL-C levels were observed in group treated with test extract and Rosuvastatin. The serum LDL-C levels on Rosuvastatin ,ME 200,AE 200 mg/kg of MC extracts dosing group were decreased by ,45.17%, 23.27%, 13.85% compared to HFD control respectively.

A significant decrease of serum HDL- C level i.e. 51.29% was detected in HFD group compared to that of animals on normal diet. However there was marked increase in serum HDL-C level in all test extract and Rosuvastatin dosing groups HDL-C level of rosuvastatin ME 200, AE 200 mg/kg of test extract treated groups were increased by 75.11%, 51.44%, 31.04% compared to HFD group respectively.

These result indicate that methanolic extract of M. champaca Linn shows statistically significant hypolipidemic activity compared to aqueous extract of M. champaca Linn

Table no. 7 Effect of Methanolic and aqueous extract of aerial parts of Michelia champaca Linn on Oxidative stress marker in High fat diet induced hyperlipidemic rats.

Sr.No.	Group	MDA nmol/mg of proteins	SOD U/mg of proteins	CAT µmoles/mg of proteins
1.	Normal	87.23 ±0.88	48.58 ±1.34	26.51 ±0.83
2.	Control (High fat diet)	223.7 ±1.33# (↑155.67)	23.26 ±0.86# (↓ 52.12)	14.87 ±1.11# (↓ 43.90)
3.	High fat diet +	118 ±0.75**** (↓47.25)	47.63 ±1.03**** (↑ 104.77)	24.36 ±1.36**** (↑ 63.81)
4.	Rosuvastatin	141.6 ±1.09** (↓ 36.70)	39.88 ±0.91** (↑ 71.45)	19.17 ±0.45** (↑ 28.91)
5.	High fat diet	192.2	28.27	17.86

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	+ methanolic extract (200mg) **High fat diet + aqueous extract (200mg)**	±1.74** (↓ 14.08)	±0.74** (↑ 21.53)	±0.36* (↑ 20.10)

Values are expressed as mean ± SD (n=6), Statistical analysis was performed using Benferroni test.*p<0.05 was taken as the criterion of significance. *p<0.05 when compared to HFD control.≠p<0.05 when compared to Normal (untreated control).Value in brackets indicate (%increase or decrease.)

MDA level (end product of lipid peroxidation) in liver showed a significant increase, whereas SOD and CAT level were showed a significant decrease in HFD fed wister rats. MDA level increased by 155.67% where as SOD and CAT decreased by 52.12% and 43.90 % compared to animals on normal diet only respectively.

However dramatic decrease in MDA content and marked increase in SOD and CAT levels were seen in all test extracts and Rosuvastatin Dosing groups. MDA level decreased in Rosuvastatin, ME 200, AE 200mg/kg of test extracts by 47.25, 36.70, 14.08 % compared to HFD group respectively. Whereas SOD level were increased in Rosuvastatin, ME 200, AE 200mg/kg of test extracts by 104.77, 71.45, 21.53 % compared to HFD group respectively. The significant increase in CAT level was observed by 63.81, 28.91, and 20.10 % respectively.

IV. DISCUSSION

Hyperlipidemia is modifiable risk factor for atherosclerosis and related cardiovascular diseases, including coronary heart disease, cerebral stroke, myocardial infraction and renal renal failure are becoming a major health problem in the world recently. [15]

Atherosclerosis, are the underlying cause of heart attack, stroke and peripheral vascular disease, in a main cause of morbidity and mortality worldwide. The disease can generally be viewed as a form of chronic inflammation that is induced and perturbed by lipid accumulation. Hypercholesterolemia and hypertriglyceridemia are independent risk factors that alone or together can accelerate the development of the atherosclerosis and progression of atherosclerotic lesions. One of the initial events in the development of atherosclerosis is the accumulation of cells containing excess lipids within the arterial wall. In addition, is has been demonstated that increased intracellular generation of reactive oxygen species (ROS) plays an important role in chronic inflammatory responses to atherosclerosis. [16]

Because of today's increased demand for herbal agents that have been regarded as relative safe in use, numerous types of herbal extract were tested in various in vivo and in vitro systems. The control of the excess of oxidative molecules includes especially exogenous intake of antioxidant molecules, which are largely found in plants. Michelia champaca L. flower is reported for the antioxidant activity. Phytochemical constituents of Michelia champaca L. flowers are tannins, polyphenols, flavonoids etc. Polyphenols is associated with anti- platelet aggregation and inhibition of vascular smooth muscle cell proliferation, all these effects might interfere with atherosclerotic plaque development and stability.

Hence in the present study, we evaluated the hypolipidemiac and antioxidant effect of aerial parts of Michelia champaca Linn aqueous and methanolic extracts in High fat diet induced hyperlipidemia in rats

Phytochemical Analysis

The phytochemical active constituents of M. champaca L. were qualitatively analysed for aqueous and methanolic extracts and the

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phytochemicals present in aqueous extract are cardiac glycoside, steroids, tannins, phenolic compounds, flavonoid and carbohydrate while in methanolic extract the phytochemicals found are steroids, tannins, phenolic compounds, flavonoid and carbohydrate.

Flavonoids were reported to enhance HDL and cause reduction in LDL and VLDL levels in hypercholesteremic rats. [17] Flavonoids and phenols found in the aqueous and methanolic extracts of Michelia champaca L. plant could therefore be considered favorable in rising HDL and decreasing LDL and VLDL in extract treated rats.

Serum TC, TG, LDL-C

HFD-fed hyperlipidemic rat model has earlier been reported as an ideal in vivo model for testing antihyperlipidemic drugs. Havel and Rapaport reported that enriched fatty diets cause elevation of plasma TC and LDL cholesterol. High levels of TC and most importantly LDL cholesterol are predictors of atherosclerosis.

However the treatment of hyperlipidemic rats with methanolic) and aqueous extract of Michelia champaca L. at a dose 200mg/kg of body wt. and rosuvastatin std.(0.0001) along with cholesterol diet shown significantly decreases ($P<0.0001$) in serum TG,TC, and HDL-C level as compared to hyperlipidemic control rats. Bainton et al. showed that triglycerides are directly or indirectly related to coronary heart diseases. In the present study, treatment with test extract markedly decreased serum TG, TC, and LDL cholesterol, which indicates these test extracts may be use in treating coronary heart diseases.

Serum HDL- C And Atherogenic Index

There was marked decrease in the level of good cholesterol carrier, HDL-C in the animal treated with HCD by 51.29% as compared with normal group. Elevated level of blood HDL-C is considered as have cardioprotective effect. Treatment with methanolic extract($P<0.001$) of M. champaca L. extract (200mg/Kg) and Rosuvastatin ($P<0.0001$) showed significant increase in the level of HDL-C as compared with HCD control group ,where as group treated with 200mg/kg of aqueous extract of M. champaca L. shown non significant elevation in HDL-C. Atherogenic index which is most important indicator of CHD at high cholesterol level (M.champaca L. groups showed marked reduction in Atherogenic index.

The percentage of protection against the hyperlipidemia in the methanolic and aqueous extract treated group was 59.19%, 43.70%. whereas the standard group protection is 78.48%, which further confirms the significant protective effect of the plant extract against hyperlipidemia.

Antioxidant study

This study demonstrated that the elevated concentration of MDA, and end product of polyunsaturated fatty acid peroxidation, had present in higher lipid group. This suggest that ROS may already have exerted their cytotoxic effects in this early clinical stage of the disease. MDA levels continued to rise over the course of the disease, indicating overproduction of free radicals and leading to lipid peroxidation and cell oxidative injury, which is considered by some authors to be related to the development of hyperlipidemia complications. [18] Our result demonstrated that enhanced lipid peroxidation and decreased antioxidant enzyme activity represent early event in the development of hyperlipidemia in contrl group. [16]

The enzymatic antioxidant such as SOD, CAT can scavage reactive oxygen species and free radicals or stop their formation. [19] The oxidative balance in the body is regulated by endogenous and exogenous mechanisms, in which the excess of free radicals is related to many diseases. The present study showed that Rosuvastatin ($P< 0.0001$) and aqueous and methanolic ($P<0.001$) test extracts of plant significantly increase the SOD, catalase and decreased MDA contents ($P<0.001$). The present study demonstrate that the Rosuvastatin as well M. champaca test extracts given orally, were able to target the cardiac tissues, and found to decrease lipid peroxides contents and elevates the antioxidant enzyme levels, apart from improvement in lipid metabolism, in high fat fed wistar rats. These result present initial evidence that test extracts may be useful for the treatment of hyperlipidemia by enhancing the antioxidant defense mechanism. [20]

V. CONCLUSION

From the results obtained in this study, it is concluded that aerial parts of Michelia champaca Linn Possesses antioxidant effect in addition to hypolipidemic effect. Furthermore methanolic extract is more potent than aqueous extract. Antioxidant effect of extracts might be due to polyphenolic compounds and flavonoids of aerial parts of Michelia champaca Linn and thus this

plant have a promising role in the treatment of hyperlipidemia and atherosclerosis. Further studies on the activity guided isolation of the extract of this plant may yield valuable therapeutic compounds which may be useful for developing powerful hypolipidemic or antioxidant drug.

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