



## Effect of saponin from *Tithonia diversifolia* leaf on the lipid profile of normal albino rats

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### Abstract

The study evaluated the effect of Saponins from *Tithonia diversifolia* leaf on lipid profile of normal rats. 35 rats were divided into six groups of five animals each. Group 1 served as control while groups 2-6 were administered 20, 40, 60, 80 and 100 mg/kg of Saponins from *Tithonia diversifolia* leaf for 21 days. Results indicated that administration of Saponins significantly reduced ( $p < 0.05$ ). Triglyceride (TG), Total Cholesterol (TC) and Low Density Lipoprotein (LDL) and increased the level of High Density Lipoprotein (HDL). These results suggest that Saponins from *T. diversifolia* leaf can prevent serum TG and cholesterol which are risk factors of coronary heart disease. Further studies at the molecular level are needed to understand the mechanism.

**Keywords:** *Tithonia diversifolia*, Lipid profile, Saponin, Thin Layer Chromatography (TLC), Atherosclerosis

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### 1. Introduction

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases (Grundy, 1987; and Elekofehinti et al., 2012), stroke, atherosclerosis and hyperlipidemia are the primary cause of death (Davey, 1993; and Elekofehiti, 2012). It has been proven to be a contributory cause of atherosclerosis in patients with diabetes (Pearl et al., 1987; Bierman, 1992; and Keaney and Localzo, 1999). Excessive accumulation of fatty acids leads to an accumulation of triglyceride in many tissues, particularly in the fat tissue, inducing lipolysis. The important role of serum lipid in atherosclerosis

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and coronary disease has been proved by many scientists. Growing evidence has demonstrated the cardiovascular disease risk to be positively associated with TC and TG, and inversely associated with High Density Lipoprotein (HDL) (Cooney et al., 2009). A lipid profile contains information about several different kinds of lipid that normally circulate in the blood. Values are numerical, but in order to simplify explanation, ranges of numerical values are often placed into categories such as 'low risk,' or 'high risk. The study was carried out to investigate the effect of Saponins from *Tithonia diversifolia* on serum lipid of normal rats. The reduction in triglyceride level by Saponins may be due to delay intestinal absorption of Triacylglycerol (TAG) by inhibiting pancreatic lipase activity (Han et al., 2000, and Francis et al., 2001).

Saponin is a class of chemical compounds, one of many secondary metabolites found in natural sources (Adanlawo and Akanji 2003; and Elekofehinti et al., 2012).

*T. diversifolia* (Hemsl.) A. Gray, Asteraceae (Compositae), commonly called Marigold, wild sunflower or Mexican sunflower, is a perennial noxious weed of field crops, waste land and road sides. It is a prolific shrub, perennial and erect, native to Mexico and Central America, and introduced in Africa, Australia, Asia and South America (Duke, 1982). It is widely cultivated as an ornamental shrub and for its medicinal value in different regions where it is commonly known as Mexican sunflower or tree marigold, as well as "nitobegiku" (Hui et al., 2009). An infusion made from *T. diversifolia* leaves and buds is used as a medicine for constipation, stomach pains, indigestion, some throat and liver pains (Patel et al., 2011; Patel et al., 2012; John-Dewole and Oni 2013; and Lifongo et al., 2014).

The primary aim of this research is to estimate the effect of varying concentration of Saponin on the lipid profile of a normal rat.

## 2. Materials and methods

### 2.1. Plant materials

The leaves of *T. diversifolia* were collected from a farm in Arigidi Akoko in Ondo State. They were identified and authenticated by Dr. Obembe Olutayo Ademola of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Nigeria. The leaves were air dried and grounded into a powdery fine texture and stored at room temperature in air tight Polythene bag prior to use.

### 2.2. Extraction and isolation of saponins from *T. diversifolia* leaf

Saponins were extracted as described by Elekofehinti et al. (2013). One hundred grams (100 g) of ground leaves was extracted with 2000 ml of methanol for 48 h using Soxhlet Extraction method. The methanolic extract was concentrated using a rotary evaporator and partitioned with hexane and water (1:2, v/v). After a thorough shaking, the mixture was allowed to stand overnight and the water layer was concentrated and partitioned between ethyl acetate and n-butanol (1:3, v/v). The butanol fraction was concentrated to obtain crude Saponin fraction. The crude Saponin fraction was spotted onto pre-coated Silica Gel TLC plate (Merck, Kleselgel 60F-254). The plates were developed with n-Butanol: Acetic Acid: Water (60:10:30 v/v/v). The spots on the chromatograms which were due to Saponins were identified by spraying with Lieberman-Burchard reagent (Methanol: Sulphuric acid: Acetic acid (50:5:5 v/v/v)). Concentrated crude Saponin extract was further purified using column chromatography. The crude saponins extract was applied to a silica gel column of (60-120 mesh). The impurities were washed with n-hexane through a 2.4 x 50 cm bed of silica gel. The column was eluted with n-butanol: acetic acid: water (1:1:1 v/v/v). The fractions were collected and aliquots applied as a series of spots to a strip of TLC plate, dried, sprayed with Lieberman- Burchard reagent and heated. Positive fractions were pooled together and used for the experiment.

### 2.3. Experimental procedure

Albino rats (n = 36) were divided equally into 6 groups. Group I served as normal control, groups II-VI were administered with Saponin from *T. diversifolia* at doses of 20, 40, 60, 80 and 100 mg/kg body weight respectively for 21 days.

The rats were sacrificed by cervical dislocation and the blood collected into clean dry beakers and serum was prepared as described by Akanji and Nlumanze (1987). The serum levels of cholesterol, triglyceride, HDL-c and LDL-c were assayed by Randox commercial kit according to the manufacturer's direction. Ethical clearance was obtained from the ethical and legal unit of Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.

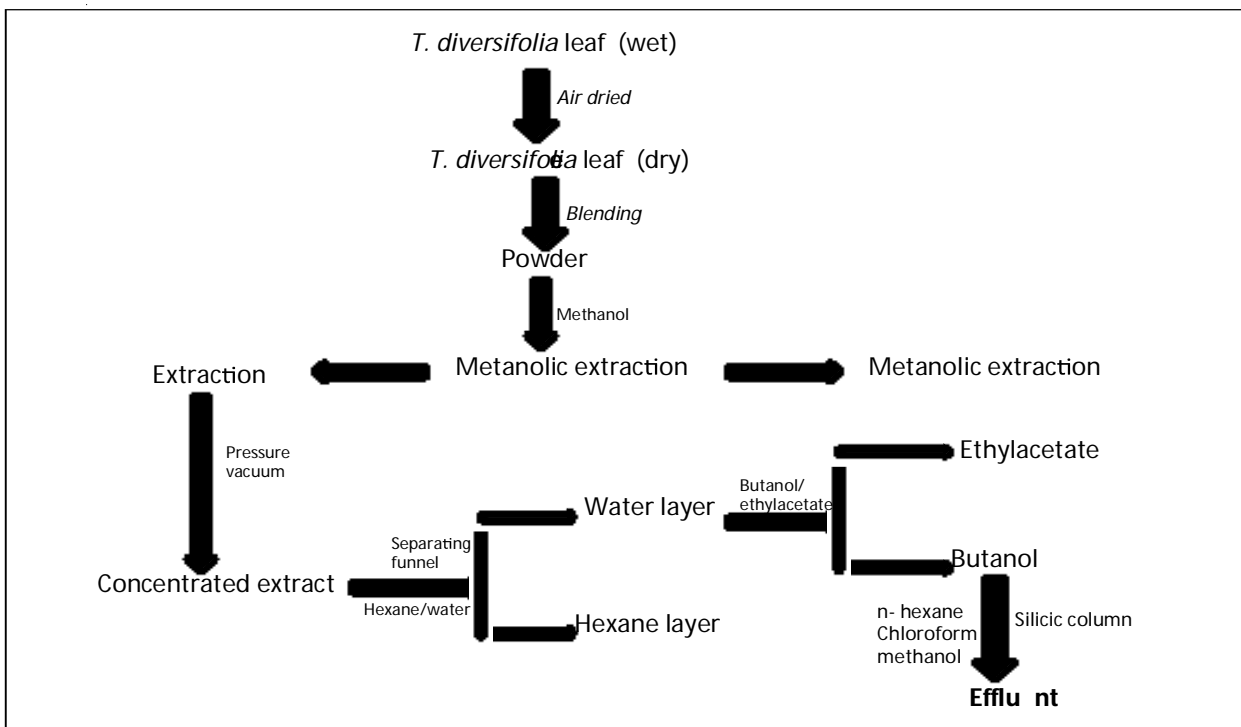


Figure 1: Flowchart showing Saponin extraction adapted from Elekofehinti et al. (2013)

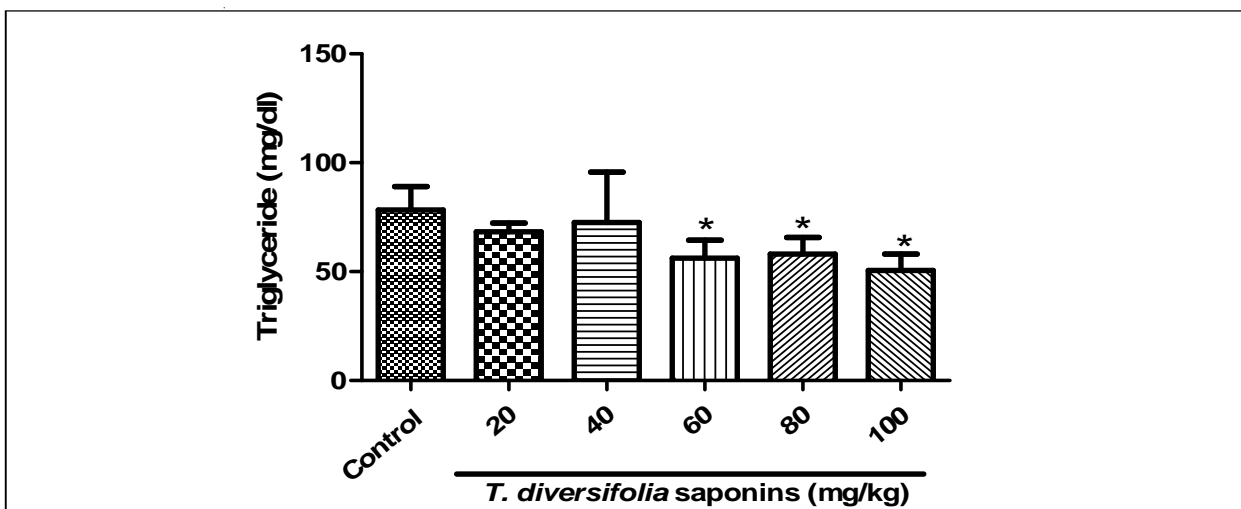
### 3. Statistical analysis

The data are expressed as mean ± SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) using SPSS version 23. Differences were considered to be statistically significant when  $p < 0.05$ . Graph pad prism was used to plot the graph.

### 4. Results

#### 4.1. Effect of saponins from *T. diversifolia* leaf on triglyceride (triacylglycerol) of rats

The effect of Saponins from *T. diversifolia* leaf on triglyceride concentration in normal rats is presented in Figure 2. Saponin (60-100 mg/kg) significantly reduced ( $p < 0.05$ ) the level of triglyceride when compared with control group.



Note: \* indicates statistical difference ( $p < 0.05$ ) compared to control.

Figure 2: Effect of Saponins from *Tithonia diversifolia* leaf on serum triglyceride concentration in normal rats

#### 4.2. Effect of saponins from *T. diversifolia* leaf on cholesterol level of normal rats

The effect of saponins from *T. diversifolia* leaf on cholesterol level of normal rats is presented in Figure 3. Saponin (40, 60, 80 and 100 mg/kg) significantly reduced ( $p < 0.05$ ) the level of cholesterol when compared with control group.

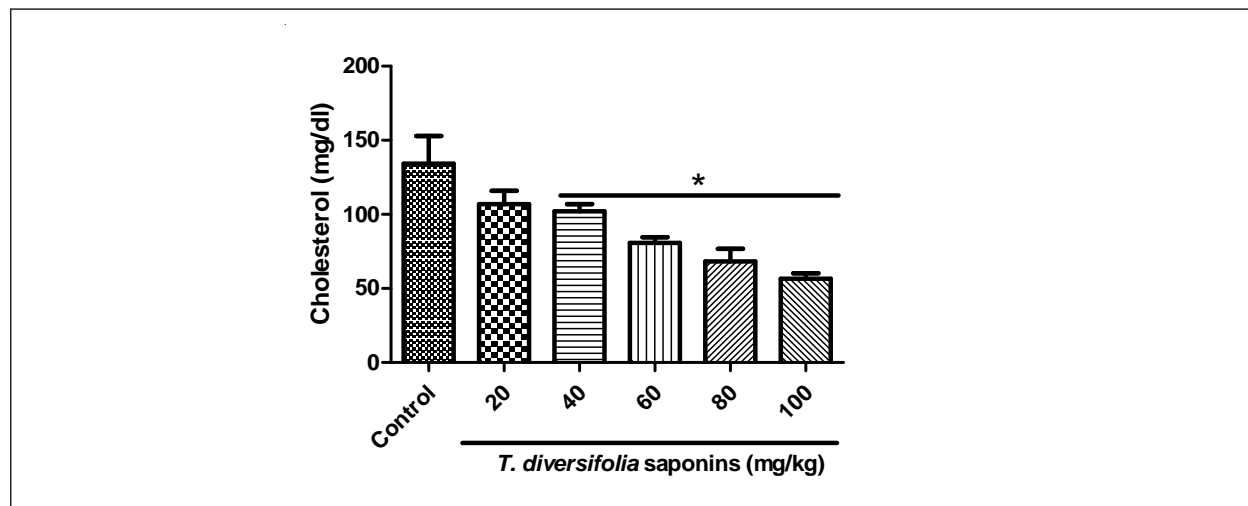


Figure 3: Effect of Saponins from *Tithonia diversifolia* leaf on cholesterol level of normal rats

Note: \* indicates statistical difference ( $p < 0.05$ ) compared to control.

#### 4.3. Effect of saponins from *T. diversifolia* leaf on HDL of normal rats

The effect of Saponins from *T. diversifolia* leaf on HDL level of normal rats is presented in Figure 4. Saponin (20, 40, 60, 80 and 100 mg/kg) significantly increased ( $p < 0.05$ ) the level of HDL when compared with control group.

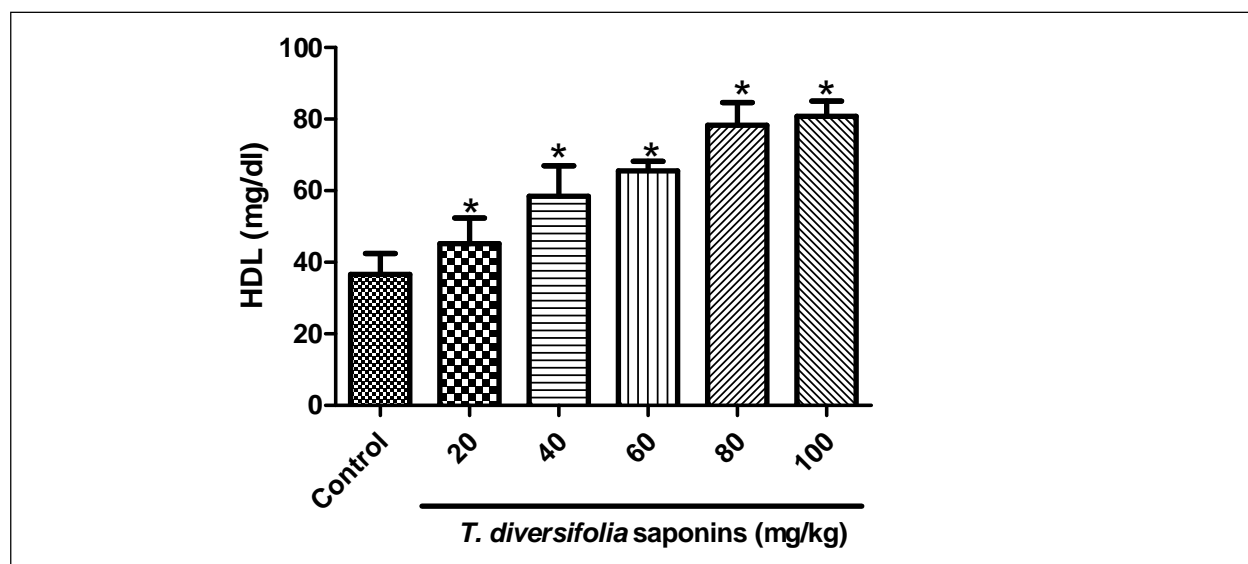
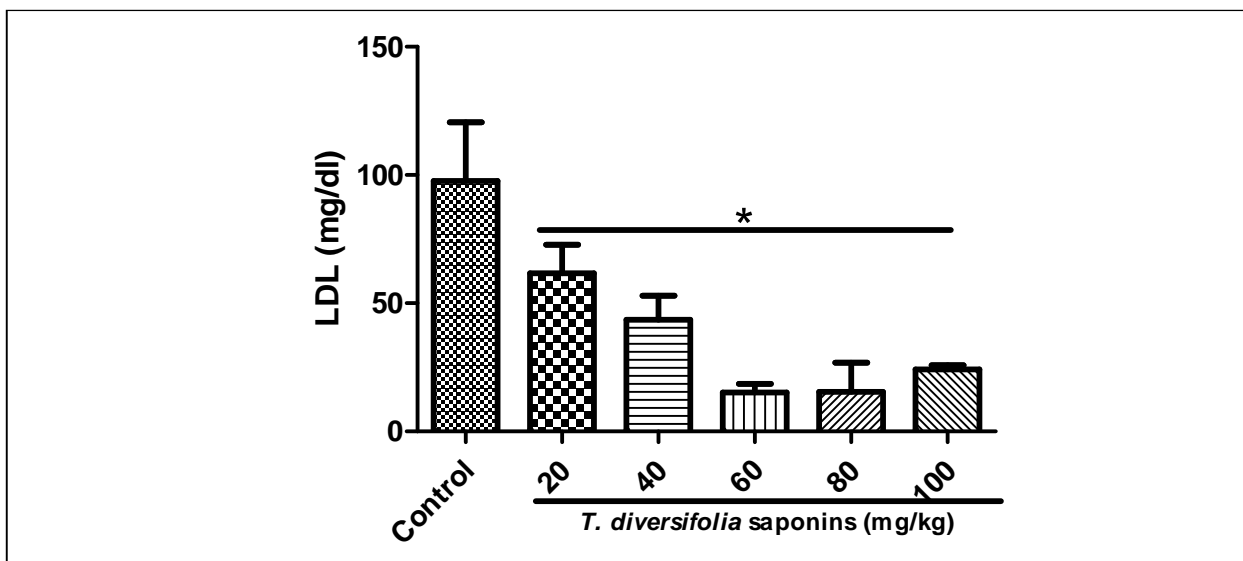


Figure 4: Effect of Saponins from *Tithonia diversifolia* leaf on high density lipoprotein (HDL) level of normal rats

Note: \* indicates statistical difference ( $p < 0.05$ ) compared to control.

#### 4.4. Effect of saponins from *T. diversifolia* leaf on Low Density Lipoprotein (LDL) of normal rats

The effect of Saponins from *T. diversifolia* leaf on LDL level of normal rats is presented in Figure 5. Saponin (20, 40, 60, 80 and 100 mg/kg) significantly decreased ( $p < 0.05$ ) the level of LDL when compared with control group.



**Figure 5: Effect of Saponin from *Tithonia diversifolia* leaf on Low Density Lipoprotein (LDL) level of normal rats**

**Note:** \* indicates statistical difference ( $p < 0.05$ ) compared to control.

## 5. Discussion

Cholesterol is needed by the body in small amount to build cell membrane structure, make hormones, produce vitamin D, produce bile acids which help the body to digest fat and absorb important nutrient. Metabolic studies have shown that high cholesterol in the blood induce both increase LDL synthesis and reduce receptor dependent fractional removal rate of LDL particles (Packard et al., 1983). Reduction of cholesterol concentration by Saponin may reflect both increase cholesterol excretion and inhibition of its absorption. This result suggests that Saponins may be beneficial to prevent atherosclerosis as it reduces the level of normal cholesterol.

Figure 4 shows that Saponins concentration ranging from (20-100 mg/kg) significantly increased ( $p < 0.05$ ) HDL level when compared to the level of HDL of control group. HDL has essentially the opposite effect of LDL as it removes cholesterol from tissues. HDL acquires its cholesterol by extracting it from cell surface membrane and converts it to cholesteryl ester by the action of LCAT.

Figure 5 showed that Saponins concentration (20-100 mg/kg) significantly decreased ( $p < 0.05$ ) the level of LDL when compared with control group. Cholesterol mainly uptake through endocytosis of LDL particles, the cholesteryl ester undergoes hydrolysis by lysosome lipase to yield cholesterol which is subsequently incorporated into the cell membrane.

## 6. Conclusion

This study revealed that Saponins from *T. diversifolia* reduced the level of TG, LDL and cholesterol but increases the level of HDL of a normal rat. This varying concentration of Saponins could be important source of LDL, TG and Cholesterol lowering agent which could be beneficial to reduce the risk of heart diseases.

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