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# In Vivo Adaptogenic Lipidomics of Essential Oil Extract of Thaumatococcus Daniellii Leaf

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**Abstract**
*Thaumatococcus daniellii* leaf had been described as being very functional biochemically, due to the essential biomolecules it contains in very regulated quantity. This study was aimed at evaluating the dietary effect of the *T. daniellii* leaf essential oil on lipid profile in selected tissues. Anti-stress activity was evaluated by lipid profile modulation (HDL, LDL, TRIG. and T. CHOL.). Quantitative and qualitative analyses of essential oil profile of the *T. daniellii* leaf extract were determined by Gas Chromatography and Mass Spectrophotometer (GC-MS). The GC-MS analyses revealed the amount of some volatile bioactive compounds, with total yield of 0.12% from leaf via hydrodistillation. 0.5ml daily dose of the essential oil revealed effective lipid modulating activity, thus could be of therapeutic importance at this dose.
**Keywords:** *Thaumatococcus daniellii* leaf, Essential oil, Lipidomics, Adaptogenic, HDL, LDL, Total Cholesterol, Triglyceride

## INTRODUCTION

Adaptogens or adaptogenic substances, compounds, herbs or practices refer to the pharmacological concept whereby administration results in stabilization of physiological processes and promotion of homeostasis, an example being by decreased cellular sensitivity to stress. Stress is considered to be any condition which results in perturbation of the body's homeostasis [1]. Stress refers to a situation in which demands are perceived to exceed one's personal resources [2].

Also stress has been reported to elevate the level of total cholesterol in the body and total cholesterol has been declared a culprit behind various life threatening diseases like hypertension, cardiovascular diseases, atherosclerosis, obesity, hypercholesterolemia, metabolic syndrome, and even diabetes [3] [4] [5]. Due to its hydrophobic nature, cholesterol is transported by lipoproteins, and various types of these lipoproteins have been identified with the two most abundant known as; low-density lipoprotein (LDL) and high-density lipoprotein (HDL) [3]. Hypercholesterolemia resulting from stressors is usually characterized by both abnormal serum and hepatic triglyceride and cholesterol levels [6]. Increase serum total cholesterol may have caused impairment in the triglyceride metabolism leading to the accumulation/deposition of free fatty acids in the liver, thus triggering a condition known as fatty liver [6]. This expanded liver fatty acid pool leads to increased mitochondrial and peroxisomal β-oxidation, which produces reactive oxygen species. This may, in turn, promote a local proinflammatory state leading to progressive liver injury [7].

*Thaumatococcus daniellii* leaf is often used for packaging among the Nigerian natives, its preference is often as a result of its sweetness due to sweetener-thaumatin.

Most of the previous studies on *Thaumatococcus daniellii* leaf were non physiological because they relied on phytochemical screening and proximate analysis carried on the plant. Therefore, it is expedient to demonstrate biochemical effect of the essential oil extract on physiologically stressed animals. The composition of the essential oil had been investigated [8], however, the composition had to be confirmed in-line with the anti-stress activity.

## METHODOLOGY

### Sample Collection and Preperation

*T. daniellii* leaves (large sized about 45cm and 30cm broad) were obtained from a farm Wanikin in Ile-Ife, southwest Nigeria and sun dried. The leaf sample was dried using oven at $50^0$C for 9 hours. The 500g of the dried leaves were ground to powdered form and preserved on the bench for aeration before the extraction procedures.

### Extraction and estimation of essential oils

Air-dried and crushed leaves (250 g) of *T. danielliii* were subjected to hydrodistillation using Clevenger-type apparatus for 8 h in accordance with the British Pharmacopoeia specification. The distillate isolated was preserved in a sealed sample tube and stored under refrigeration until analysis.

The extracted sample was thrice extracted with redistilled n-hexane. The content was concentrated to 1ml for gas chromatography analysis and 1µl was injected into the injection port of GC. The GC equipment used was HP 6890 powered with HP chemstation Rev. A09.01 (1206) software. The split ratio was 20:1, the carrier gas was nitrogen at inlet temperature of $250^0$C with a column type of HP INNOWax and column dimensions of 30m x 0.25mm x 0.25µm. the oven program parameters include initial temperature at $60^0$C, first ramping at

$12^0$C/minutes for 20minutes, maintain for 2minutes and second ramping at $15^0$C/min for 3minutes, maintained for 8minutes. The detector used was FID at $320^0$C at hydrogen pressure 22psi and compressed air of 35psi.

**Experimental Design**

**Immobilization stress (IS) combined with cold restraint stress (CRS)**

Male albino rats were used according to the standard guidelines of the Care and Use of Experimental Animal Resources. 25 male albino rats weighing 200 ± 10 g were used to evaluate the ability of the *T. daniellii* leaf oil extract to combat stress and were obtained from standard animal house. The rats were housed 5 per cage under constant environmental conditions (20–24 °C; 12 h light/dark cycle), and were given ad libitum access to standard pelleted food and water. After the administration of extracts for 21 days, combined IS and CRS test was performed by immobilizing animals in the cold chamber at 4 ± 0.3 ºC; the plexiglass cage volume was adjusted to the size of the animal, to restrain completely their movements [9] for 2 hours, except group 1. Group 2 animals were treated after stress with diazepam (5 and 10 mg/ml BW) [10].

Group 1: Untreated, unstressed group-Negative control group (C-)
Group 2: Untreated, stressed group-Positive control group (C+)
Group 3: Diazepam treated (5.0 mg/ml/BW/0.2ml ip), stressed group (D1)
Group 4: Diazepam treated (2.5 mg/ml/BW/0.2ml ip), stressed group (D2)
Group 5: Treated (extract-0.5ml orally), stressed group (T)

**Preparation of Serum and Tissue Homogenates**

The rats were sacrificed by cervical dislocation. Blood samples were collected by ocular punctures into plain bottles. Serum was prepared by aspiration of the clear yellowish liquid after clotting and centrifuged for 10 minutes at 3000 g in a bench centrifuge. The clear supernatant was used for the estimation of serum lipids. The animals were quickly dissected and the lung, brain, heart, liver, and kidneys removed and rinsed with ice-cold 1.15% potassium chloride. The tissues were then homogenized in ice-cold 0.1M phosphate buffer solution using a Teflon homogenizer.

**Lipid Profile Evaluations**

**Determination of Triglyceride Concentration**

The triglyceride concentration was determined using colorimetric method [11]. Briefly, 10 µl of the sample was mixed with 1 ml of Pipes reagent (40 mM phosphate buffer, 5.5 mM 4-chlorophenol and 17.5 mM $mg^{2+}$) and enzyme reagent (4-aminophenazone, adenosinetriphosphate, lipase, glycerolkinase, glycerol-3-phosphate oxidase and peroxidase). Thereafter the mixture was incubated for 5 min at 37 ºC and the absorbance at 546 nm was taken against reagent blank within 60 min. The triglyceride concentration was subsequently calculated against the standard.

**Determination of plasma total cholesterol concentration**

The cholesterol was determined according to the principle described by [12]. 1 ml of the reacting mixture containing 4-aminoantipyrine, phenol, peroxidase, cholesterol esterase, cholesterol oxidase and 80mM Pipes buffer pH 6.8 was mixed with 10µl of plasma and incubated for 5 min at 37ºC. The absorbance at 546 was then taken against the reagent blank within 60min. The concentration of cholesterol in the sample was subsequently calculated against a standard.

**Determination of plasma HDL-cholesterol concentration**

The precipitation was carried out according to the method of [13] as described in the kits manufacturer (Randox Laboratories Ltd) manual. Briefly, 200 µl of plasma was mixed with 500 µl of the precipitant (0.55mM phosphotungstic acid and 25mM magnesium chloride) and allowed to sit for 10 min at room temperature. Then, the mixture was centrifuged for 10 min at 800 × *g*. Thereafter, the clear supernatant was separated off and subjected to the same procedure for the determination of cholesterol described above.

**Determination of plasma LDL-cholesterol concentration**

The LDL-cholesterol concentration of the plasma samples was determined according to the equation of [14].

LDL Cholesterol (mg/dl) = Total Cholesterol – Triglycerides/5 – HDL Cholesterol (APPENDIX)

All values are expressed as mean and error bar. Statistical evaluation was done using One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using SPSS 17.0 for windows. The significance level was set at $p<0.05$.

 

## RESULTS AND DISCUSSIONS

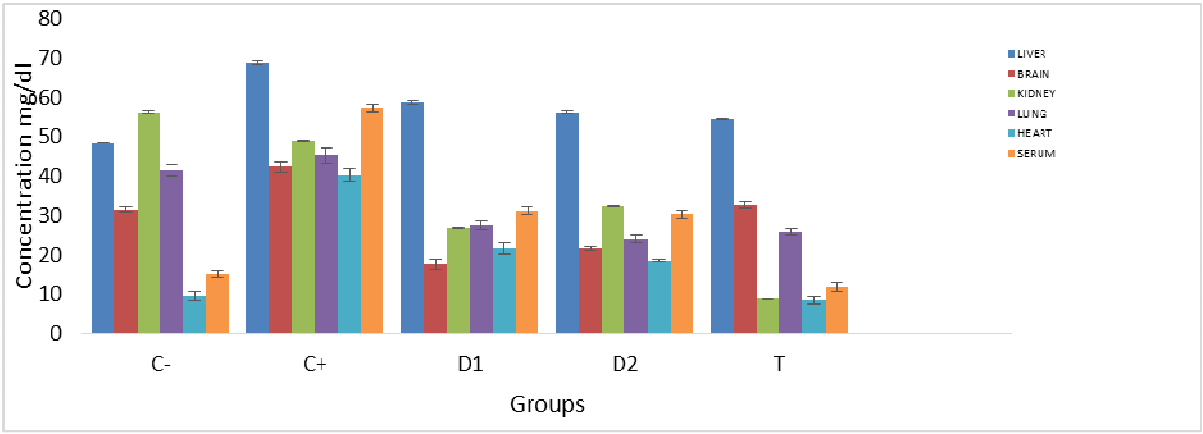


Figure 1: Effects of the extract on triglyceride profiles in liver, brain, kidney, lung, heart and serum (mg/dl). Each value represents mean (n=5).

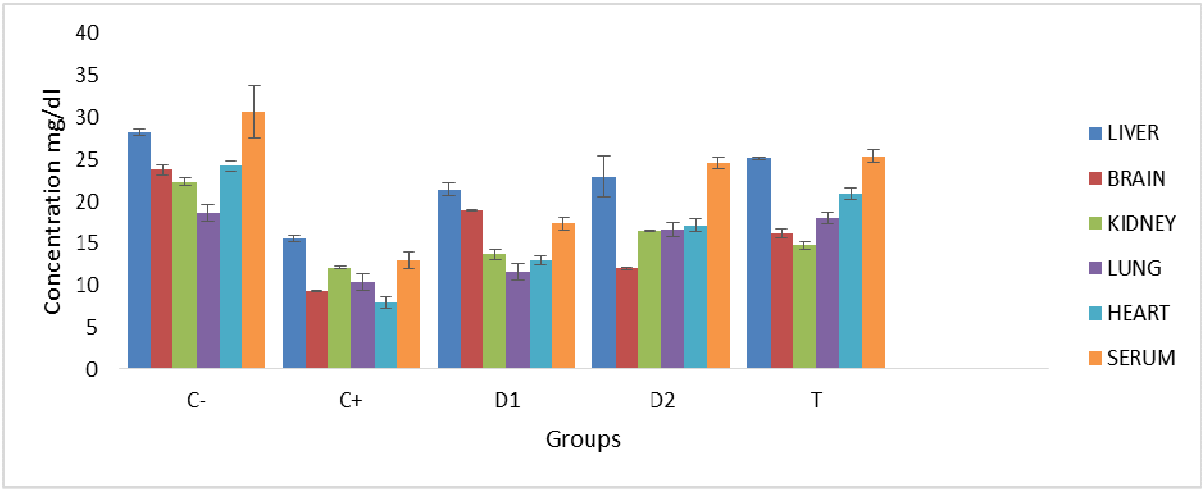


Figure 2: Effects of the extract on high density lipoprotein profiles in liver, brain, kidney, lung, heart and serum (mg/dl). Each value represents mean (n=5).

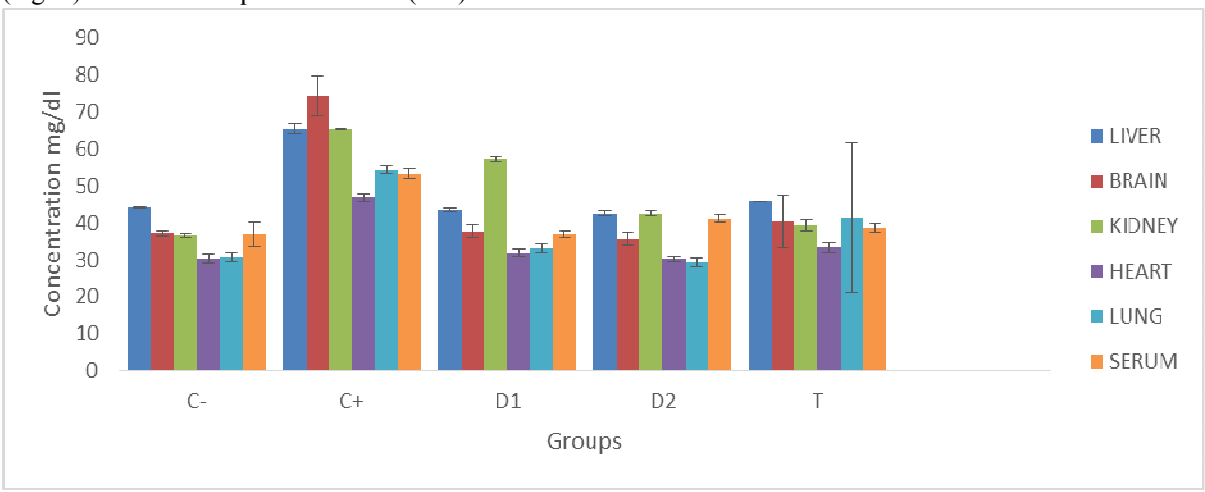


Figure 3: Effects of the extracts on total cholesterol profiles in liver, brain, kidney, lung, heart and serum (mg/dl). Each value represents mean (n=5).

 

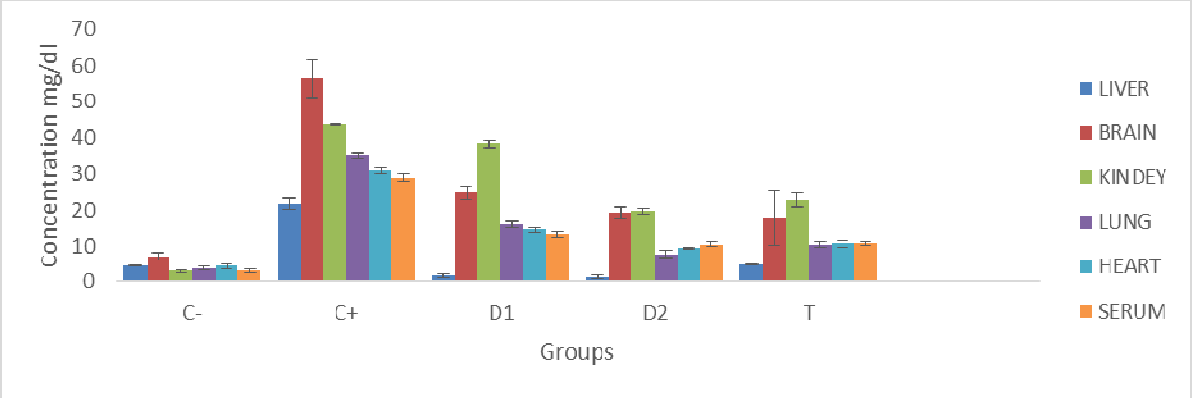


Figure 4: Effects of the extracts on low density lipoprotein profiles in liver, brain, kidney, lung, heart and serum (mg/dl). Each value represents mean (n=5).

Table 1: Compounds with designated letters

| COMPOUNDS | |
|---|---|
| 6,10,14-trimethyl-2-pentadecanone | A |
| Methyl pentadecyl ether | B |
| 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol | C |
| n-Hexadecanoic acid | D |
| (E)-3-Eicosene | E |
| Phytol | F |
| Geranylgeraniol | G |
| Octadecamethylcyclononasiloxane | H |
| Squalene | I |
| Tetratetracontane | J |
| Tetradecanal | K |
| 13-Tetradecen-1-ol acetate | L |
| Oleic acid | M |
| 9-Hexadecenoic acid, octadecyl ester | N |
| 2- Chloropropionic acid, hexadecyl ester | O |
| 3,7-dimethylnonane | P |
| 3,6-dimethyl undecane | Q |
| Dodecamethylcyclohexasiloxane | R |
| 2,3,5-trimethyl decane | S |
| Tetradecamethylcycloheptasiloxane | T |
| Decamethylcycloheptasiloxane | U |

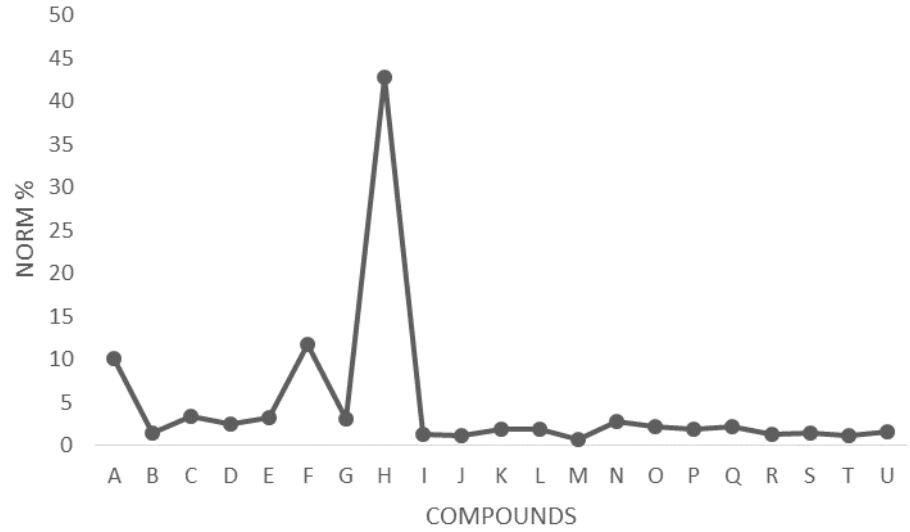


Figure 4: % availability of compounds in essential oil of leaf extract of *T. daniellii* Using Gas Chrmatography-Mass Spectroscopy

There were mark increases in triglycerides and total cholesterol levels for all the experimented tissues in the untreated stressed group (C+) of animals when compared to the unstressed and untreated group (C-), and the experimental groups as shown in Fig. 1 and 3 respectively. The marked increase is probably due to stimulation of hypothalamus-pituary axis (HPA) and sympathetic system, resulting in, liberation of catecholemines and glucocorticosteriods, which inhibits the immune system at multiple sites like liver, kidney [15]. Cortisol, the major glucocorticoid, is rapidly synthesized and secreted in response to ACTH; this is part of a response to stress and increases circulating levels of energy-providing compounds: glucose, free fatty acids and free amino acids. This entire metabolic pathway produces a burst of energy to prepare the animal for an emergency situation [16]. However, it cannot be said if this same biochemical mechanism is same in the entire tissues. This result is similar to lipid modulation evaluation of quail egg extracts administration [10]. Effects of *T. daniellii* leaf essential oil extracts could be described as responsible for the reduction in cholesterol and triglycerides levels owing to its anti-cholesterolemic activity of the extracts or/and inhibition of stimulation of sympathetic nervous system by the extracts as revealed by the untreated unstressed groups for cholesterol and triglyceride level values.

There is an inverse relationship between the stress hormone cortisol and HDL cholesterol which was significant in both men and women and survived stepwise multiple regression analysis and thus, can be concluded that cortisol may affect peripheral cholesterol metabolism to alter HDL cholesterol formation. The result from this study (as shown in Fig. 2) revealed that stressful condition had negative effect and impaired the level of HDL cholesterol in the untreated stressed group (C+) when compared to the condition without stress (C-) with HDL level. This result was in agreement with observations of [10] in their study. The result also revealed that the extract has significant impact on the level of HDL cholesterol as revealed by the group in all the tissues.

Hypercholesterolemia is clinically characterized by an increase of the total and low-density lipoprotein (LDL) cholesterol in plasma. It represents a high risk for the development of atherosclerosis, where also free radicals play a significant role, because they are able to oxidatively damage lipids in the process of lipoperoxidation. It was suggested that cold immobilization stress stimulates free radical generation in the liver of both adult and old rats. Accumulation of LDL cholesterol can lead to various disease states. Fig. 4 revealed the low density lipoprotein (LDL) result. It can be observed that there was a marked increase in LDL levels in the tissues of the untreated stressed group (C+) when compared to the untreated unstressed (C-), and treated stressed groups with values significantly lower in all the tissues than the untreated stressed group. *T. daniellii* leaf extract used gave an impact very similar in ameliorative activity compared to the dose of reference drug used. However by significantly reducing the LDL cholesterol level, it enhances the HDL: LDL cholesterol ratio, and by implication the *T. daniellii* leaf oil extracts can help reduce risk of lipid related diseases. A raised HDL: LDL cholesterol ratio was associated with low risk of diseased events. The ability of *T. daniellii* leaf extracts to significantly reduce the elevated LDL cholesterol may be due to the anti-stress property of the extracts or its anti-lipidemic activity. Advanced lipoprotein analysis had also revealed signs of insulin resistance. Insulin-resistant patients will most often have an elevated triglyceride: HDL-C ratio 89 (suspect insulin resistance when ratio is above 3.5), elevated small-density LDL particles, elevated large triglyceride-rich VLDL particles, smaller HDL particles and increased C-reactive protein levels. The mechanism of stress related increment in LDL level

is unknown, but the oxidation of lipoproteins through oxidative damage has been proposed to be responsible for elevation in LDL, cholesterol, triglycerides and decrease in HDL cholesterol [17, 18, 19].

Hydrodistillation of the leaves of *T. daniellii* gave 0.12 % yield of pale yellow oil. Analysis of the oil was similar to previous discovery [8] while the total availability accounted for was 98.93% of the total oil. The result revealed the amount of the volatile constituent of the oil extract. All of these components are bioactive component with some, contributing immensely to the total antioxidant capacity of the leaf, making it nutritionally relevant, with less toxicity [8].

## CONCLUSION

The present results suggested that administration of *T. daniellii* leaf extract is capable of increasing the capacity to tolerate non-specific stress in experimental animals as evident from the restoration of numbers of parameter studied during induced stressful conditions and act as anti-stress and adaptogenic agent. The plant extract is also able to lower the level of circulating low density lipoprotein in the blood, and also able to improve the antioxidant status of the stress rats, suggesting that the plant can serve as an important therapeutic agent in oxidative and stress related diseases, or management of adverse changes associated with the stressors that alter and impair the normal functioning of the organism. However, care must be exercised when the *T. daniellii* leaf is to be used for any form of treatment.

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