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PHYTOCHEMICAL AND ANTI-LIPIDEMIC EVALUATION OF METHANOL EXTRACT OF *CALYPTROCHILUM CHRISTYANUM* (RCHB.F.) SUMMERH (ORCHIDACEAE) AND ITS FRACTIONS ON ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Hyperlipidemia is defined as an elevation of one or more of the following: cholesterol, cholesterolesters, phospholipids, or triglycerides in the body. Such triggers a lot of cardiocasvular dieasses with a high mortality. Herbal drugs containing active ingredients, plant parts or plant materials in the processed or crude state with certain anti lipidemic properties are useful agents used to treat hyperlidemia. *Calyptrochilum christyanum* though not widely documented, it's association with the Orchidaceae family suggests it may share similar anti lipidemic properties as other orchid species. The study evaluated the quantitative phytochemical profile and the antilipidemic activity of the methanolic extract of *Catlyptrochilum christyanum* and its fractions. Quantitative assay was done using standard methods ad the atilipdemic assay was doe usig spectrophotometric analysis. The secondary metabolites present in the leaves of *Calyptrochilum christyanum* are in decreasing order are flavonoids >, phenols> tannins > glycosides> alkaloids> terpenoids > saponins > steroids > hydrogen cyanide. The anti-lipidemic results showed that the extracts demonstrated significant anti-lipidemic activity, increasing HDL levels and reducing LDL levels in a dose-dependent manner with the dichloromethane extract giving the highest percentage reduction. These findings confirms that *Calyptrochilum christyanum* has potential as a natural treatment for hyperlipidemia.

KEYWORDS: *Catlyptrochilum christyanum.* Quantitative, phytochemical, anti-lipidemic.

INTRODUCTION

Hyperlipidemia is defined as an elevation of one or more of the following: cholesterol, cholesterolesters, phospholipids, or triglycerides. Hyperlipidemia, in particular elevated LDL (hypercholesterolemia), is one of the most prevalent risk factors contributing to the evolution of atherosclerosis and consequent vascular disease(Angamo et al., 2013). It is simply defined as elevated concentrations of lipids or fats within the blood. Numerous factors contribute to the development of atherosclerosis, including endothelial damage, hyperlipidemia, inflammatory and immunologic factors, plaque erosion or rupture, hypertension, and smoking (FREDRICKSON, 1971). Hyperlipidemia is considered one of the major risk factors causing cardiovascular diseases (CVDs). CVDs accounts for 1/3 of total deaths around the total world, it is believed that cardiovascular diseases will turn out to be the main cause of death worldwide by the year 2020(Uloko et al., 2018). Hyperlipidemia is subdivided into two broad classifications: primary (familial) or secondary (acquired) hyperlipidemia. Primary hyperlipidemia derives from a plethora of genetic disorders that a patient may inherit through birth, while secondary hyperlipidemia typically originates from an alternate underlying etiology, such as an unhealthy diet, medications (amiodarone, glucocorticoids), hypothyroidism, uncontrolled diabetes, and/or a poor lifestyle regimen(Grundy et al., 2019). Herbal drugs contain active ingredients, plant parts or plant materials in the processed or crude state with certain excipients, i.e., dilutions, solvents or preservatives (Bent, 2008; Calixto, 2000). These active ingredients protect plants from damage and diseases and contribute to the plants aroma, flavor and color. Scientifically, they are known as phytochemicals which include several classes such as saponins, flavonoids, glycosides, tannins, alkaloids and terpenoids (Anulika et al., 2016) .

Calyptrochilum christyanum is an epiphytic orchid species native to West and Central Africa, known for its adaptation to the tropical rainforest canopy. The plant features thick, leathery leaves and pseudobulbs that help it retain moisture in its humid environment. Its small, fragrant, white or pale green flowers, equipped with a spur, are likely pollinated by specialized insects. The orchid thrives on the bark of trees, relying on air moisture and decaying organic matter for nutrients. The flowers bloom on short, erect racemes, and though small in size, they are noted for their aesthetic appeal and pleasant fragrance. While the specific ethnobotanical uses of *Calyptrochilum christyanum* are not widely documented, the

plant's association with the Orchidaceae family suggests it may share similar ethnomedicinal properties with other orchid species. In many parts of the world, orchids are valued for their healing properties, spiritual significance, and ornamental beauty (Iwu, 2014; Sandberg et al., 2005).

TAXONOMIC CLASSIFICATION OF *CALYPTROCHILUM CHRISTYANUM*

Kingdom: Plantae

Phylum: Streptophyta

Class: Equisetopsida

Subclass: Magnoliidae

Order: Asparagales

Family: Orchidaceae

Genus: *Calyptrochilum*

Specie: *C. christyanum*

Common name: Orchids

Figure 1: **Pictorial representation of *Calyptrochilum christyanum.***

AIM OF THE STUDY

To evaluate the quantitative phytochemical profile and the antilipidermic activity of the methanolic extract of *Catlyptrochilum christyanum* and its fractions.

METHODS

Calyptrochilum christyanum (leaf) was collected and air dried for ten days under a shade. The plant sample was pulverized to fine powder and weighed. The extraction process was carried out by cold maceration using methanol and dicloromethane in 1:1 for 48 hours ad concentrated using rotary evaporator at 40◦c. The concentrated extract was further dried in hot water bath at 40˚c. Fractionation was done using column chromatography using silica gel

as adsorbent the following solvents N-Hexane, Dichloromethane, ethyl acetate, polar methanol, 40% aqueous methanol and water. Quantitative and qualitative phytochemical analysis for saponins , alkaloids, tanninis, flavonoids, Terpenoids, Steroids, Glycosides and Reducing sugars were done according to standard procedures(Sofowora, 1982). Rats within the weight range of 150-200g were grouped into four animals per treatment group namely: Non-Induced, Positive control, Negative control, Crude extract, and the various fraction groups. The rats were fasted for 12 hours before inducing diabetes by injecting Alloxan at a dose of 130mg/kg Intra-peritoneally. The plant extract fractions at 100mg, 200mg ad 300mg dosing and standard drug (Glibenclamide 5mg) were dissolved using 12% Tween 80 and treatments administered orally using a gavage. Blood samples were collected from the tail vein and lipid profile parameters were done spectrophotometrically according to the method of(Roeschlau P, Bernt E, n.d.) (1974).

RESULTS

Table 2: Result of percentage yield of *Calyptrochilum christyanum*.

Weight of Sample (g)	Weight of Extract (g)	% Yield
1000g	45.8	4.5%

Table 3: Showing results of percentage yield of the different fractions of *Calyptrochilum christyanum*.

S/N	Solvents	% Yield
1	N-Hexane	22.3
2	Ethyl acetate	37.1
3	Dichloromethane	30
4	Methanol	3.2

Table 1: Phytochemical constituent of *Calyptrochilumchristyanum*.

Phytoconstituent	Composition (mg/100g)
Flavonoid	1650.62
Phenol	1195.81
Tannin	1021.79
Glycoside	721.05
Alkaloid	643.02
Terpenoid	450.26
Saponin	0.77
Steroid	0.65
Hydrogen cyanide	0.37

Table 2: Anti lipidemic properties of *Calyptrochilum christyanum*.

Lipid Parameter	CHOLESTEROL	HDL	TG	LDL LDL = TC-HDL-(TG/5)
SAMPLE	Mg/dl	Mg/dl	Mg/dl	Mg/dl
Control	122.26	54.95	148.63	37.58
Standard	127.04	65.40	157.78	30.09
Toxic	131.57	65.69	166.31	32.63
Crude 100mg	131.70	61.71	161.20	37.75
Crude 300mg	132.33	64.54	161.20	35.55
Crude 600mg	132.58	64.05	167.25	35.08
Methanol 100mg	133.59	72.65	169.62	27.01
Methanol 300mg	133.59	73.14	183.29	23.79
Methanol 600mg	133.84	70.35	177.24	28.05
Ethyl Acetate 100mg	134.72	73.76	182.50	24.46
Ethyl acetate 300mg	135.10	65.52	165.92	36.39
Ethyl acetate 600mg	135.10	71.42	175.14	28.65
N-Hexane 100mg	135.73	67.68	159.89	36.07
N-Hexane 300mg	136.11	70.81	178.82	29.53
N-Hexane 600mg	137.37	69.34	171.98	33.63
Dcm 100mg	137.49	69.83	172.77	33.11
Dcm 300mg	137.49	67.00	171.46	36.20
Dcm 600mg	188.69	75.60	381.25	47.04

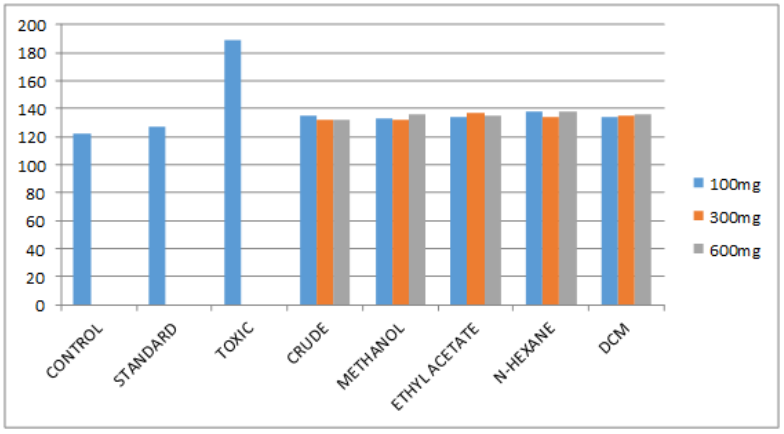

Figure 1: Cholesterol activity of *Calyptrochilum christyanum*.

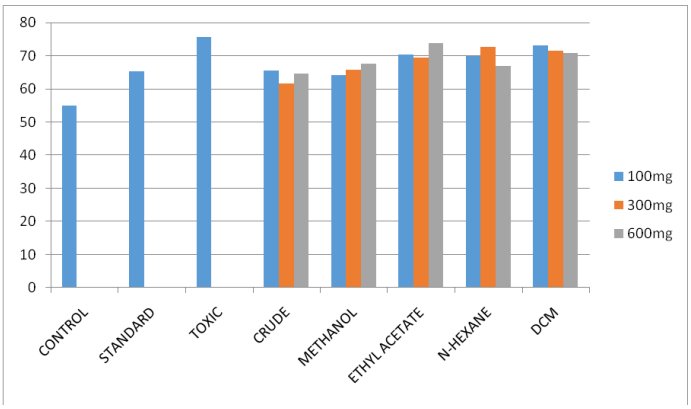

Fig. 2: HDL activity of *Calyptrochilumchristyanum*.

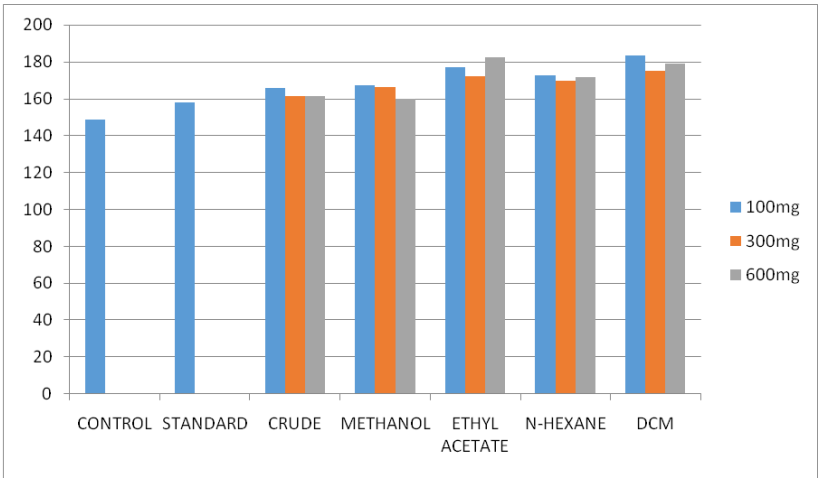

Fig 3: Tryglyceride activity of *Calyptrochilum christyanum*.

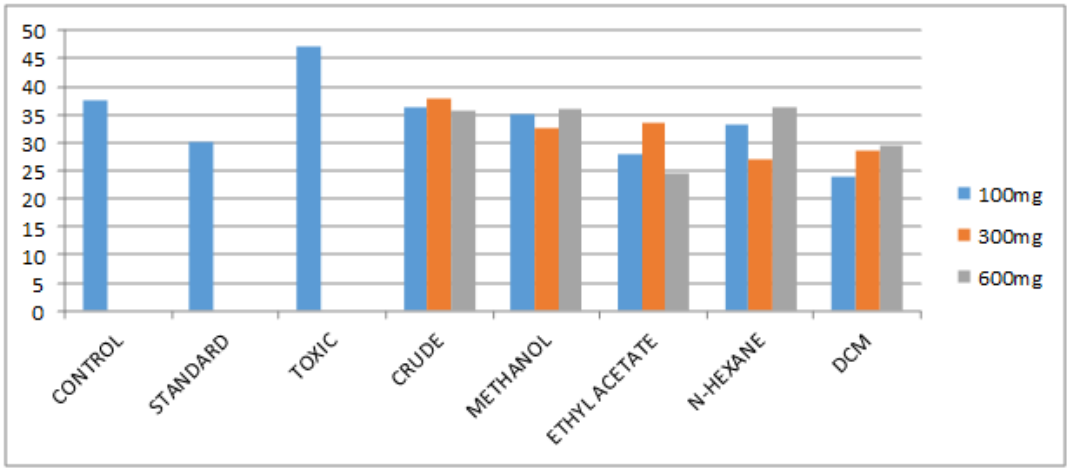

Fig 4: LDL activity of *Calyptrochilum christyanum*.

DISCUSSION

Calyptrochilum christyanum demonstrated various yields using different solvents with ethyl acetate giving the highest yield of 37% followed by Dicloromethane (DCM) giving a 30% yield. This reveals an abundance of mid polar compounds in the extract. N- Hexane gave the lowest yield which implies that very low non polar compounds are present in the extract.

The secondary metabolites present in the leaves of *Calyptrochilum christyanum* in decreasing order are flavonoids >, phenols> tannins > glycosides> alkaloids> terpenoids saponins > steroids > hydrogen cyanide. These secondary metabolites will be responsible for the antilipidermic activity of this leaf, they act either synergistically or independently to enhance the antilipidermic activity of the leaf(Prasain et al., 2010).

The lipid profile for the control group represents normal lipid metabolism without any intervention. These values are within the normal range for rats, serving as a benchmark to compare how different treatments affect lipid levels. The cholesterol, HDL, LDL, and triglyceride levels are balanced, showing no signs of hyperlipidemia.

The toxic group represents untreated hyperlipidemia induced by chemical induction. The cholesterol level is notably higher than in the control group, which suggests an elevated risk of cardiovascular diseases. Interestingly, the HDL is also significantly higher, which may indicate a compensatory mechanism by the body to counteract the elevated LDL levels. Despite the increase in HDL (which is protective), the high LDL level (47.04 mg/dl) remains a concern as it is linked to an increased risk of atherosclerosis due to cholesterol deposition in arterial walls (Grundy et al., 2019).

The standard group treated with a known antilipidemic drug shows improved lipid parameters compared to the toxic group. The LDL value is significantly reduced to 30.08 mg/dl, which reflects the efficacy of the drug in reducing bad cholesterol levels. Additionally, the HDL level is higher than the control group, signifying the protective role of the standard drug in enhancing the body's ability to remove cholesterol from the bloodstream(Ford et al., 2016).

The crude extract, across the three doses, shows improvement over the toxic group in terms of lipid profile. The cholesterol levels are slightly elevated compared to the control group but are much lower than in the toxic group, indicating that the crude extract has some antilipidemic activity.

The HDL levels are higher than the control, especially at the 100 mg dose (65.52 mg/dl), showing that the extract can increase good cholesterol. LDL levels are also reduced compared to the toxic group, though not as much as the standard drug. This indicates moderate efficacy of the crude extract in lowering harmful cholesterol.

The methanol extract across all doses shows consistent improvement in lipid profile. The HDL levels are notably higher than in both the control and toxic groups, especially in the 600 mg dose (67.68 mg/dl), indicating that methanol extract may have a strong capacity to enhance good cholesterol. Cholesterol and LDL levels are reduced compared to the toxic group, though not as much as the standard drug. However, the LDL levels across all doses

remain lower than the toxic group and are similar to the crude extract. The triglyceride levels (TG) show a slight elevation compared to the control, but still lower than in the toxic group.

The ethyl acetate extract demonstrates one of the strongest effects in terms of increasing HDL levels, especially at 600 mg, with HDL reaching 73.76 mg/dl. This suggests a potent ability of this extract to promote the good cholesterol associated with protective cardiovascular effects (Moghadasian, 2000)

The LDL levels are also significantly reduced, particularly at the 100 mg dose (28.05 mg/dl) and 600 mg dose (24.46 mg/dl), bringing LDL close to the levels seen in the standard drug. Cholesterol levels are moderately reduced across the doses, and while triglycerides remain slightly elevated compared to the control, they are still within a reasonable range.

The N-Hexane extract also shows a moderate effect on lipid profiles, with HDL levels increased across the board, especially at 300 mg, where HDL reaches 72.65 mg/dl. LDL levels are lower than in the toxic group, particularly at 300 mg where LDL reaches 27.01 mg/dl. This is close to the effect seen with the ethyl acetate extract at similar doses. Cholesterol levels are relatively stable and moderately reduced compared to the toxic group, while triglycerides (TG) are slightly elevated across all doses but lower than in the toxic group.

The dichloromethane (DCM) extract shows strong effects in terms of raising HDL across all doses, with HDL at 73.14 mg/dl in the 100 mg dose. This makes DCM one of the most potent extracts for promoting good cholesterol. Additionally, LDL levels dare notably reduced across the doses, with values around 28-29 mg/dl, comparable to the standard drug. Cholesterol levels are moderately reduced compared to the toxic group but remain slightly elevated when compared to the control. Triglycerides are slightly higher than the control but still lower than in the toxic group.

The results suggest that *Calyptrochilumchristyanum* extracts, particularly those prepared using ethyl acetate, dichloromethane (DCM), and methanol, exhibit promising antilipidemic properties. These extracts, especially at higher doses, increase HDL levels and reduce LDL, indicating a protective effect against hyperlipidemia. The ethyl acetate extract shows one of the best lipid-lowering effects, particularly at the 600 mg dose, which significantly reduced LDL while raising HDL levels. The standard drug also shows efficacy, but some extracts are

comparable in reducing LDL levels, particularly the ethyl acetate 600 mg and DCM 100 mg. This study's findings support the potential use of *Calyptrochilum christyanum* as a natural treatment for hyperlipidemia, though further research is needed to confirm these results and understand the mechanisms involved.

These findings highlight the potential of *Calyptrochilum christyanum* as a natural source of antilipidemic agents. The strong lipid-modulating properties, particularly of the ethyl acetate, DCM, and n-hexane extracts confirms that the plant has strong anti-lipidemic properties and has a good potential for therapeutic use in managing hyperlipidemia and related cardiovascular disorders.

CONCLUSION

This study confirms the quantitative phytochemical properties and antilipidemic activity of *Calyptrochilum christyanum* leaf extracts. The results showed that the extracts contain flavonoids, steroids, saponins, alkaloids, glycosides, terpenoids, phenols, hydrogen cyanide and tannins. The extracts demonstrated significant antilipidemic activity, increasing HDL levels and reducing LDL levels in a dose-dependent manner. These findings confirms that *Calyptrochilum christyanum* has potential as a natural treatment for hyperlipidemia.

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