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In Vitro Antioxidant Bioassay Guided Fractionation and Evaluation of Protective Effect of Methanol Extract of Baphia Longipedicellata Leaves Against Cadmium Chloride-Induced Testicular Damage in Rats

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Abstract- ***The present study evaluated bioassay-guided fractionation, the in vitro antioxidant activity, and protective effects of the methanol leaves extract of Baphia longipedicellata against cadmium chloride–induced testicular damage in Wistar rats. Phytochemical screening revealed the presence of key bioactive compounds: flavonoids; tannins; alkaloids; phenolics; and terpenoids, with fractionation indicating higher concentrations of phenolic constituents in the ethyl acetate and butanol fractions. Antioxidant assays showed significant concentration-dependent activities in lipid peroxidation inhibition, nitric oxide and DPPH radical scavenging. Crude extract and ethyl acetate fractions showed superoxide inhibition and ferric reducing power effects comparable to catechin. Cadmium chloride exposure induced marked oxidative stress, hormonal imbalance, and impaired sperm parameters evidenced by reduced levels of testosterone, follicle-stimulating hormone, luteinizing hormone, and endogenous antioxidants, with elevated malondialdehyde levels. Treatment with the extract (200 and 400 mg/kg) significantly ameliorated these effects by restoring antioxidant enzyme activities, improving reproductive hormone levels, and enhancing sperm quality in a dose-dependent manner. Acute toxicity test results indicated relative safety at lower doses. In conclusion, Baphia longipedicellata leaves extract exhibits potent antioxidant and protective properties, supporting its potential as a natural therapeutic agent against cadmium-induced testicular toxicity and oxidative stress–related reproductive disorders.***

Keywords: Baphia longipedicellata, Infertility, Cadmium Chloride, Bio-guided Fractionation, Free radicals

I. INTRODUCTION

The utilization of medicinal flora for therapeutic purposes predates recorded human history; recently there has been a renewed shift in focus toward employing these plants as alternatives to conventional pharmaceuticals in the treatment and management of various ailments (Oyedeji et al., 2025).

This resurgence of interest may have stemmed from the acknowledgment of the historical applications of certain plants in both ancient and traditional medicinal practices, including acupuncture and Chinese herbal treatments (Huangdi neijing) dating back approximately 2,200 years, as well as the Ayurvedic system of ancient India (Akshatha et al., 2022), which continues to underpin current traditional medicine. Numerous scholarly investigations have demonstrated that plants harbor an extensive array of secondary metabolites, which confer upon them significant medicinal properties, particularly in terms of their antioxidant and antimicrobial efficacy (ref).

At present, the growing understanding of free radicals and reactive oxygen species (ROS) is catalyzing a pivotal transformation in medical science, heralding potential breakthroughs in health promotion and disease management. While oxygen is vital for life, under certain conditions it can harm the body by forming ROS, which trigger damaging physiological effects (Yalçın & Kaya, 2024).

Free radicals and antioxidants are central to these processes: free radicals cause cellular damage, while antioxidants counteract them by donating electrons and neutralizing their harmful activity (Akoro et al., 2025).

Antioxidants include both naturally occurring compounds in humans (like glutathione, ubiquinol, and uric acid) and plant-derived substances such as nutraceuticals and secondary metabolites (Badriyah et al., 2023).

Baphia longipedicellata is a leguminous species classified within the Fabaceae family. This plant is indigenous to Kenya and is also found in the Republic of Congo, Nigeria, and several West African nations, although it is at risk due to habitat degradation (Ibitoye et al., 2023).

The leaves of B. longipedicellata have a documented history of use in traditional medicine for the treatment of ailments such as dry coughs, whooping cough, bronchial catarrh, asthma, indigestion, gastritis, diarrhea, malaria, and male infertility (Ntore et al., 2021). However, there exists a paucity of information regarding this species for its use to treat male mammal infertility as a result of testicular dysfunction, as there are no documented medicinal properties associated with B. longipedicellata in this regard.

Literatures abound on the adverse health effect of cadmium (Cd) especially on the testicular health of various mammalian species, including human (Maisetta et al., 2019).

Issues pertaining to male infertility, which encompass conditions such as erectile dysfunction, low sperm count, and premature ejaculation, have been addressed through the administration of various synthetic pharmacological agents, including proviron, prolistem, clomiphene citrate, and anastrozole, albeit often accompanied by side effects. Consequently, the exploration of botanical-derived drugs and dietary supplements has emerged as a matter of significant priority in recent years (Pietrzak, & Nowak, 2021).

Pharmacologists, microbiologists, botanists, and chemists specializing in natural products are diligently investigating terrestrial ecosystems for phytochemicals and therapeutic compounds that could be developed for the treatment of diverse medical conditions, including infertility, with minimal or no adverse effects. In fact, as reported by the World Health Organization, approximately 25% of contemporary pharmaceuticals in use have their origins in plant sources (WHO, 2008).

This, therefore, form the need for this research to find a credible, scientifically proven evidence for the use of B. longipedicellata as a natural remedy for male infertility caused as a result of Cadmium poison.

II. MATERIALS AND METHODOLOGY

a.Chemicals and Reagents

All chemicals and reagents used were of analytical grade (Analar) and biochemical reagents like Randox kits, including those for Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), total cholesterol, and triglycerides were procured from Seglor Nigeria Limited. They were all used without any further purification.

b. Aquisition of Baphia longipedicellata leaves and Preparation of Extracts

Fresh foliage of B. longipedicellata was gathered from the botanical garden at the Cocoa Research Institute of Nigeria (CRIN), situated in Ibadan, Oyo State. The leaves were air-dried in cool dark room at the room temperature. The dried leaves were size reduced and made into powder using a house-hold kitchen blender, and subsequent extraction via cold maceration. 5 g of the powder were soaked in 2 liters of 80% methanol for a duration of 72 hours at 25°C. The resultant mixture was filtered through No. 1 Whatman filter paper, and the filtrate concentrated using a rotary evaporator.

c.Bioassay-Guided Fractionation

The crude extract was fractionated following the method of Hernández-Álvarez et al. (2024). The extract was dissolved in double distilled water (100 mL) followed by sequential partitioning with 400 mL (in four extraction batches of 100 mL each) of n-hexane, chloroform, ethyl acetate and n-butanol in that order of polarity. Each fraction obtained was

evaporated to dryness in a rotary evaporator and subsequently analyzed for phytochemical constituents and in vitro antioxidant activity.

d. Phytochemical Constituents Determination

The phytochemicals in the plant were determined using standard procedure in accordance with the methodology established by Mani et al. (2024); Ibitoye et al. (2023); Harbone & Baxter (1993) which is designed to provide baseline data regarding the potential active phytochemicals present in the fractions of the extract.

Test for Alkaloids: The Dragendoff's reagent method, as delineated by Harbone & Baxter (1993), was used for the detection of alkaloids. A portion of the extract was dissolved in distilled water and about 1 mL of the solution taken. 3 to 4 drops of Dragendoff's reagent were added. The formation of a reddish-brown precipitate is taken as a positive result of the presence of alkaloids.

Test for Saponin: The frothing and blood hemolysis tests, as documented by Ibitoye et al. (2023), was used for the evaluation of saponin. About 1 ml of the extract was taken, and 1 ml of distilled water was added and shaken well. The formation of persistent froth was observed, confirming the presence of saponin.

Test for Anthraquinones: The Borntrager's test as described by Harbone and Baxter (1993) was used for the determination of anthraquinones presence. An aliquot of the extract in distilled water was boiled with 5 mL of 10% sulphuric acid in a water bath for 5 minutes. The solution was filtered and allowed to cool. The filtrate was extracted into chloroform and organic layer separated. 3 to 6 drops of 10% ammonia solution were added and shaken slightly. The appearance of violet colour at the lower aqueous layer of ammonia confirmed the presence of anthraquinones.

Test for Glycosides: A test procedure used by Harborne and Baxter (1993) was employed to test for the presence of glycosides. 2 mL of the extract in distilled water was added 1 mL glacial acetic acid, 2 drops of 2% ferric chloride solution and about 1 mL of concentrated sulphuric acid carefully added down the side of the test tube. A reddish-brown ring at the junction of the two layers indicates the presence of glycosides.

Test for Flavonoids: The assessment of flavonoids involving lead acetate, ferric chloride, and sodium hydroxide, as described by Mani et al. (2024) was used. About 1 ml of the extract was taken. Two ml of 2% NaOH solution and 3 to 4 drops of dilute HCl were added. The colour initially turned to an intense yellow colour with NaOH solution and later became colourless. This colour change in appearance confirmed the presence of flavonoids

Tannin Assay: The evaluation of tannins was performed using the lead acetate test as outlined by Mani et al. (2024). About 1 ml of the extract was taken. Three to five drops of 10% lead acetate solution were added. The formation of a gelatinous precipitate confirmed the presence of tannin.

e. In-vitro Bioassays on Antioxidant potentials of Baphia longipedicellata extracts and its fractions

Animals: A total of fifty-six (56) male Sprague-Dawley rats, aged between four and five weeks weighing between 145 and 185 grams, were sourced from the Animal House of the Physiology Department, University of Ibadan, Nigeria.

The animals were accommodated in adequately ventilated enclosures within the Pharmaceutical Technology Departmental Animal House, maintained at a temperature range of 28 to 30 degrees Celsius, and subjected to controlled light cycles of 12-hour light and 12 hours darkness. They were provided with a diet consisting of standard laboratory chow (Ladokun Feeds, Ibadan, Nigeria) along with access to water for a period of 7 days for acclimatization before the experiment began.

All experimental procedures were carried out without the administration of anesthesia, adhering strictly to the guidelines established by the National Institutes of Health (NIH). Ethical approval was secured from the Moshood Abiola Polytechnic institutional committee concerning the use and care of laboratory animals.

f. Experimental Design and Administration of Extract

Randomly, the rats were distributed into seven (7) groups of eight (8) animals each. The schedule of the extract and drug treatment is depicted in Table 1 below.

The animals were pre-treated with oral administration of the methanol extract of Baphia longipedicellata leaves (MLBL) and Quercetin for thirty (30) days while Cadmium chloride (CdCl2) was administered to the animals in the last 3 days. The vehicle used for the drug delivery was olive oil.

g. Induction of testicular damage in wistar rats

The testes of the animals were damaged using cadmium chloride as described by Oyedeji et al. (2025). The rats were administered with cadmium chloride (2.5 mg/kg) intraperitoneally and were observed for the first 12 hrs for any display of any abnormal physiological, behavioral or neurological signs and/or death.

h. Assay methods

Testicular and hepatic antioxidant assays which include glutathione-S transferase (GST) activity, Superoxide dismutase (SOD), reduced glutathione (GSH) level, nitric oxide level, glutathione peroxidase (GPx), malonaldehyde (lipid peroxidation and myeloperoxidase activity were all carried out following the method of Sarikaya et al. (2023); Hawash et al. (2022); Tijani et al. (2021).

Furthermore, serum testosterone level, luteinizing hormone, follicle stimulating hormone and sperm analysis (sperm motility, count, sperm volume and sperm live-to-death ratio) were carried out according to the method of Oyedeji et al. (2025); Yehia et al. (2024). Serum triglycerides (TG) level, Total cholesterol (TC) level, low- and high-density lipoprotein (HDL) were estimated by using RANDOX kits according to the method described by Fuchs et al. (2023).

Table 1: Schedule of the extract and drug treatment

Grouping	Group name	Treatment
1	Positive Control	Rats were given olive oil (per oral)
2	Toxicant ($CdCl_2$)	Rats were given 2.5mg/kg of cadmium chloride ($CdCl_2$) by i.p
3	$CdCl_2$ + $MLBL_{200}$	Rats were given 2.5mg/kg of $CdCl_2$ by i.p alongside with 200mg/kg body weight of MLBL orally
4	$CdCl_2$ + $MLBL_{400}$	Rats were given 2.5mg/kg of $CdCl_2$ by i.p and subsequently
5	$CdCl_2$ + QUER	Rats were given 2.5mg/kg of $CdCl_2$ by i.p and quercetin at 50mg/kg body weight orally
6	$MLBL_{400}$	Rats were given 400mg/kg body weight of MLBL orally
7	QUER	Rats were given quercetin at 50mg/kg body weight orally

STATISTICAL ANALYSIS

All values were expressed as the mean ± SD. Data were analysed using one-way ANOVA followed by the post hoc Duncan multiple range test for analysis of biochemical data using SPSS (10.0; SPSS Inc., Chicago, IL, USA) at 0.95 confidence level.

III. RESULTS

Table 2: Phytochemical constituents of Crude and Fractions of Baphia longipedicellata Leaves extracts.

Phytochemicals	MLBL	HFBL	BFBL	EAFBL	CFBL	HEFBL
Cardiac glycosides	++	–	–	++	++	–
Phlobatannis	–	–	++	–	–	–
Alkaloids	++	++	–	++	–	++
Anthraquinone	++	–	–	++	++	–
Terpenoids	++	–	++	–	++	–
Tannis	++	++	–	–	_	–
Saponins	–	–	–	–	++	–
Flavonoids	++	–	++	++	++	–

MLBL- Methanol extract of Baphia longipedicellata leaves, HFBL- Hexane fraction of Methanol leaves extract of Baphia longipedicellata, BFBL- Butanol

fraction of Methanol leaves extract of Baphia longipedicellata, CFBL- Chloroform fractoin of Methanol leaves extract of Baphia longipedicellata, EAFBL- Ethyl acetate fraction of Methanol leaves extract of Baphia longipedicellata, HEFBL- Hydro-Ethanol fraction of Methanol leaves extract of Baphia longipedicellata; ++ = present, -- = absent.

Table 3: Inhibitory activity of the Crude and Fractions of Methanol extract of Baphia longipedicellata leaves (MLBL) against Malonaldehyde radicals (index of lipid peroxidation relative to Catechin

CONC. (mg/mL)	% INHIBITION OF LIPID PEROXIDATION					
	MLBL	HFBL	BFBL	EAFBL	CFBL	CATECHIN
Control	0.000	0.000	0.000	0.000	0.000	0.000
100	34.34 ± 2.54^{ab}	-5.34 ± 1.60^{a}	23.24 ± 3.54^{b}	17.43 ± 1.75^{a}	08.47 ± 0.98^{a}	32.42 ± 3.92^{ab}
200	43.23 ± 2.76^{ab}	-12.34 ± 1.35^{a}	26.51 ± 3.61^{a}	31.42 ± 1.23^{a}	13.45 ± 0.78^{a}	43.26 ± 4.39^{ab}
500	57.27 ± 3.11^{ab}	-15.12 ± 2.34^{a}	29.45 ± 4.26^{a}	46.92 ± 3.25^{a}	21.72 ± 2.19^{a}	55.34 ± 4.90^{ab}
750	69.98 ± 3.20^{ab}	09.54 ± 2.81^{ab}	34.22 ± 3.25^{a}	61.48 ± 2.68^{a}	35.28 ± 2.58^{a}	63.43 ± 5.75^{ab}
1000	75.12 ±5.76^{ab}	18.65 ± 1.87^{ab}	38.21 ± 2.62^{a}	73.21 ± 4.27^{a}	52.80 ± 3.56^{a}	76.27 ±4.28^{ab}
1500	76.29 ± 4.65^{ab}	26.48 ± 2.27^{ab}	47.39 ± 3.91^{a}	82.14 ± 5.59^{a}	69.46 ± 3.91^{a}	80.76 ± 5.93^{ab}
IC_{50}^{++}	655.21	445.03	514.25	761.85	1419.59	401.96

MLBL- Methanol extract of Baphia longipedicellata leaves, HFBL- Hexane fraction of Methanol leaves extract of Baphia longipedicellata, BFBL- Butanol fraction of Methanol leaves extract of Baphia longipedicellata, CFBL- Chloroform fractoin of Methanol leaves extract of Baphia longipedicellata, EAFBL- Ethylacetate fraction of Methanol leaves extract of Baphia longipedicellata,, a -Significantly different from the catechin at $P<0.05$, b - Significantly increment at $P<0.05$ in a dose dependent manner

Table 4: Inhibitory activity of the Crude and Fractions of Methanol extract of Baphia longipedicellata leaves against Nitric oxide radicals relative to Catechin

CONC. (mg/mL)	% INHIBITION OF NITRIC OXIDE					
	MLBL	HFBL	BFBL	EAFBL	CFBL	CATECHIN
Control	0.000	0.000	0.000	0.000	0.000	0.000
100	-11.94 ± 2.92^{ab}	-9.23 ± 2.73^{a}	31.17 ± 5.10^{a}	-18.23 ± 3.56^{b}	-43.21 ± 5.64^{a}	14.33 ± 2.92^{ab}
200	-08.33 ± 3.81^{a}	-6.37 ± 1.54^{a}	26.32 ± 6.29^{a}	15.75 ± 4.55^{b}	-36.29 ± 3.91^{a}	24.81 ± 1.82^{ab}
500	-04.10 ± 0.42^{ab}	-16.23 ±4.50^{a}	47.73 ± 3.97^{a}	34.83 ± 6.25^{b}	-54.12 ± 7.19^{a}	33.45 ± 6.48^{ab}
750	10.36 ±	-31.2	-21.3	57.82 ±	-62.1	52.11 ± 4.92^{ab}

	1.87 ± 0.84 [ab]	1 ± 1.83 [a]	6 ± 5.14 [a]	4.71 [a] ± 0.84 [b]	7± 5.92 [a]	
1000	29.63 ± 0.84 [ab]	-45.11 ±2.76 [a]	-13.19 ± 10.12 [a]	61.73 ± 5.22 [a] ± 0.84 [b]	-51.39 ± 6.83 [a]	61.83 ± 8.96 [ab]
1500	53.53 ± 2.25 [ab]	28.82 ± 3.46 [a]	38.03 ± 3.51 [a]	73.83 ± 4.72 [a] ± 0.84 [b]	-25.11 ± 7.32 [a]	72.45 ± 7.57 [ab]
IC_{50}^{++}	705.36	671.43	882.56	479.6	437.54	648.48

MLBL- Methanol extract of Baphia longipedicellata leaves, HFBL- Hexane fraction of Methanol leaves extract of Baphia longipedicellata, BFBL- Butanol fraction of Methanol leaves extract of Baphia longipedicellata, CFBL- Chloroform fractoin of Methanol leaves extract of Baphia longipedicellata, EAFBL- Ethylacetate fraction of Methanol leaves extract of Baphia longipedicellata,. a -Significantly different from the catechin at P<0.05, b - Significantly increment at P<0.05 in a dose dependent manner

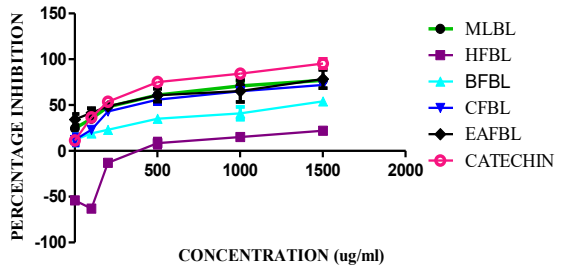

Figure 1: Inhibitory activity of the Crude and Fractions of Methanol extract of Baphia longipedicellata leaves against Superoxide radicals relative to Catechin

MEBL- Methanol extract of Baphia longipedicellata leaves, HFBL- Hexane fraction of Methanol leaves extract of Baphia longipedicellata, BFBL- Butanol fraction of Methanol leaves extract of Baphia longipedicellata, CFBL- Chloroform fractoin of Methanol leaves extract of Baphia longipedicellata, EAFBL- Ethylacetate fraction of Methanol leaves extract of Baphia longipedicellata,.

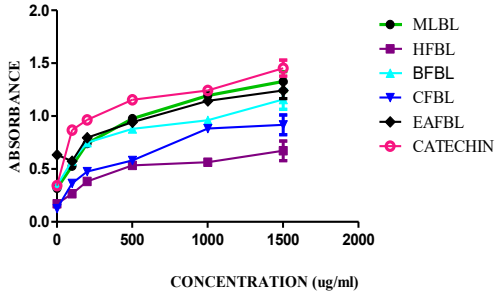

Figure 2: Iron II-Reducing capabilities of Crude and Fractions of Baphia longipedicellata Leaves extracts.

MLBL- Methanol extract of Baphia longipedicellata leaves, HFBL- Hexane fraction of Methanol leaves extract of Baphia longipedicellata, BFBL- Butanol fraction of Methanol leaves extract of Baphia longipedicellata, CFBL- Chloroform fractoin of Methanol leaves extract of Baphia longipedicellata, EAFBL- Ethyl acetate fraction of Methanol leaves extract of Baphia longipedicellata,

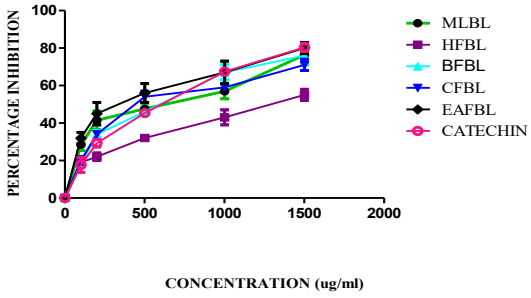

Figure 3: Inhibitory activity of the Crude and Fractions of Methanol extract of Baphia longipedicellata leaves against DPPH radicals relative to Catechin

MEBL- Methanol extract of Baphia longipedicellata leaves, HFBL- Hexane fraction of Methanol leaves extract of Baphia longipedicellata, BFBL- Butanol fraction of Methanol leaves extract of Baphia longipedicellata, CFBL- Chloroform fractoin of Methanol leaves extract of Baphia longipedicellata,

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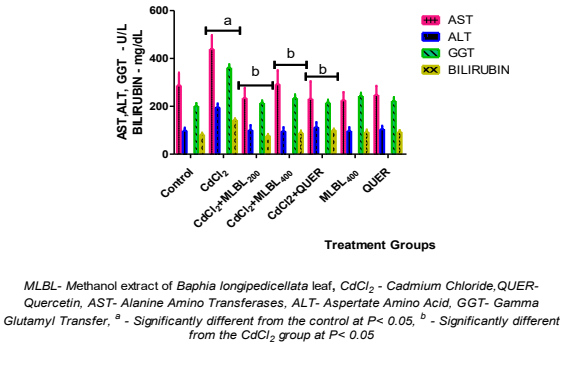
EAFBL- Ethylacetate fraction of Methanol leaves extract of Baphia longipedicellata,

Table 5: Impact of methanol extract of Baphia longipedicellata leaves on the serum level of Testosterone, Follicle Stimulating hormone (FSH), and Luteinizing hormone (LH) lipid profile of wistar rats intoxicated with cadmium chloride

Treatments	Testosterone (U/L)	FSH (U/L)	LH (U/L)
Control	46.17±8.52	63.09±7.53	43.95±4.35
$CdCl_2$	19.81±2.67 [a]	25.16±3.57 [a]	25.04±3.24 [a]
$MLBL_{200}$ + $CdCl_2$	33.65±4.56 [b]	60.12±2.45 [b]	45.65±5.81 [b]
$MLBL_{400}$ + $CdCl_2$	41.02±5.61 [b]	56.58±6.43 [b]	39.54±3.53 [b]
RUTIN + $CdCl_2$	36.51±2.45 [b]	58.74±5.23 [b]	38.51±2.74 [b]
$MLBL_{400}$	43.63±4.07	52.34±4.75	41.63±4.53
QUERCETIN	42.46±5.76	54.61±5.15	39.42±3.54

MLBL- Methanol extract of Baphia longipedicellata leaves, CdCl2 - Cadmium Chloride, a - Significantly different from the control at P< 0.05, b - Significantly different from the CdCl2 group at P< 0.05



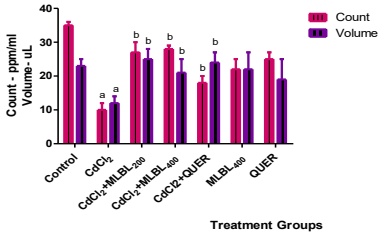
MLBL- Methanol extract of Baphia longipedicellata leaf, $CdCl_2$ - Cadmium Chloride,QUER- Quercetin, AST- Alanine Amino Transferases, ALT- Aspertate Amino Acid, GGT- Gamma Glutamyl Transfer, [a] - Significantly different from the control at P< 0.05, [b] - Significantly different from the $CdCl_2$ group at P< 0.05

Figure 4: Impact of methanol extract of Baphia longipedicellata leaves on the serum liver function of wistar rats intoxicated with cadmium chloride

Table 6: Impact of methanol extract of Baphia longipedicellata leaves on the serum lipid profiles of rats intoxicated with cadmium chloride

Treatments	Cholesterol	Triglycerides	HDL	LDL
Control	2034.60 ±65.17	1266.31 ±46.69	1101.86 ±42.41	118.92±1 4.76
$CdCl_2$	2925.23 ±71.18[a]	3454.62 ±56.67 [a]	626.75±3 6.27 [a]	305.36± 19.91[a]
$CdCl_2$ + *MLBL* 200	2142.56 ±82.67[b]	1615.84 ±64.06 [b]	861.71±3 2.77 [b]	143.91± 17.39[b]
$CdCl_2$ + *MLBL* 400	2048.11 ±68.02 [b]	1426.31 ±78.81 [b]	894.63±4 6.91[b]	138.24± 12.53[b]
$CdCl_2$ + QUER	2245.11 ±59.27 [b]	1528.78 ±46.64 [b]	817.68±3 8.60 [b]	157.68± 14.74[b]
MLBL 400	2126.15 ±67.10	2013.99 ±59.23	765.88±4 9.24	163.91± 15.41
QUER	2365.31 ±83.19	2146.22 ±3.85	804.81±5 1.87	168.35± 10.41

MLBL- methanol extract of Baphia longipedicellata leaves, QUER- Quercetin, CdCl2 - Cadmium Chloride, HDL-High density Lipoprotein, LDL-High density Lipoprotein, a - Significantly different from the control at P< 0.05, b - Significantly different from the CdCl2 group at P< 0.05



MLBL- Methanol extract of Baphia longipedicellata leaf, QUER- Quercetin, $CdCl_2$ - Cadmium Chloride [a] - Significantly different from the control at P< 0.05, [b] - Significantly different from the $CdCl_2$ group at P< 0.05

Figure 5: Effect of methanol extract of Baphia longipedicellata leaves on the Sperm count and volume Ratio in wistar rats intoxicated with cadmium chloride

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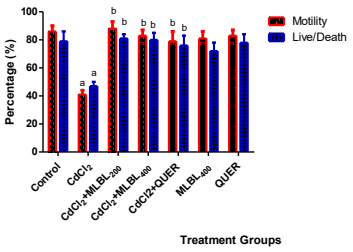
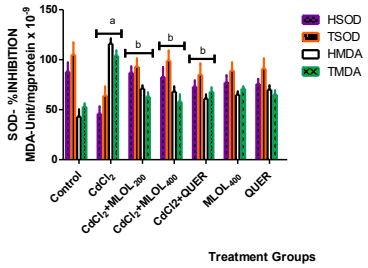


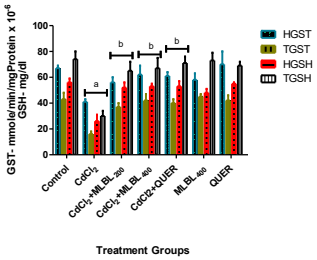
MLBL- Methanol extract of Baphia longipedicellata leaf, QUER- Quercetin, $CdCl_2$ - Cadmium Chloride [a] - Significantly different from the control at P < 0.05, [b] - Significantly different from the $CdCl_2$ group at P < 0.05

Figure 6: Effect of methanol extract of Baphia longipedicellata leaves on the Sperm cells' motility and Live/Death Ratio in wistar rats intoxicated with cadmium chloride



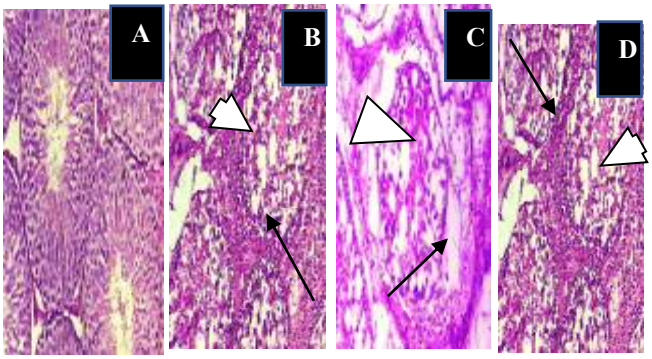
MLBL- Methanol extract of Baphia longipedicellata leaf, QUER- Quercetin, $CdCl_2$ - Cadmium Chloride, HSOD -Hepatic Superoxide Dismutase, TSOD -Testicular Superoxide Dismutase, HMDA- Hepatic Maalonaldehyde, TMDA- Testicular Maalonaldehyde [a] - Significantly different from the control at P< 0.05, [b] - Significantly different from the $CdCl_2$ group at P< 0.05

Figure 7: Effect of methanol extract of Baphia longipedicellata leaves on the hepatic and testicucular activity of Superoxide dismutase and level of Malonaldehyde in wistar rats intoxicated with cadmium chloride



MLBL- Methanol extract of Baphia longipedicellata leaf, QUER- Quercetin, $CdCl_2$ - Cadmium Chloride, HGST- Hepatic Glutathion-S-Transferase, TGST- Testicular Glutathion-S-Transferase, HGSH- Hepatic Reduced Glutathione, TGSH- Testicular Reduced Glutathione, [a] - Significantly different from the control at P< 0.05, [b] - Significantly different from the $CdCl_2$ group at P< 0.05

Figure 8: Effect of methanol extract of Baphia longipedicellata leaves on the Hepatic and testicucular activity of Glutathion-S-Transferase and level of Reduced Glutathione in wistar rats intoxicated with cadmium chloride



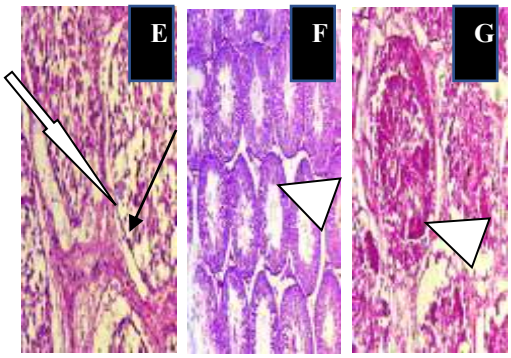


Figure 8: Influence of the methanol extract derived from the leaves of *Baphia longipedicellata* on the testicular histological architecture of *Wistar* rats subjected to cadmium chloride toxicity.

A (Control) - Testicular section exhibiting normal seminiferous tubules alongside germ cells and Sertoli cells.

B ($CdCl_2$) - Testicular section revealing atrophied seminiferous tubules characterized by severely degenerated germ cells and the presence of vacuolations.

C ($CdCl_2$ + $MLBL_{200}$) - Testicular section demonstrating atrophied seminiferous tubules with mildly degenerated germ cells accompanied by multiple vacuolations.

D ($CdCl_2$ + $MLBL_{400}$) - Testicular section illustrating atrophied seminiferous tubules with mildly degenerated

IV. DISCUSSION

The phytochemical screening in Table 2 shows that the methanol leaves extract of Baphia longipedicellata contains a broad spectrum of bioactive compounds, including alkaloids, flavonoids, tannins, terpenoids, cardiac glycosides, and anthraquinones, suggesting that the crude extract retains most of the plant's secondary metabolites.

For instance, flavonoids and terpenoids were abundant in the butanol, ethyl acetate, and chloroform fractions, while alkaloids were prominent in the hexane and hydro-ethanol fractions. Notably, saponins appeared only in the chloroform fraction, and phlobatannins were confined to the butanol fraction.

The results in Table 3 show that the extracts and fractions exhibited varying degrees of inhibition of lipid peroxidation, measured via malondialdehyde suppression. The methanol crude extract and ethyl acetate fraction showed strong, dose-dependent antioxidant activity, with inhibition values increasing significantly as concentration increased and approaching that of the standard antioxidant, catechin. In contrast, the hexane fraction showed poor activity, even negative inhibition at lower concentrations, indicating possible pro-oxidant effects or lack of active constituents.

The ethyl acetate fraction again demonstrated the highest and most consistent activity, with strong dose-dependent increases and an IC value close to that of catechin, indicating effective nitric oxide scavenging ability.

The crude extract showed moderate activity, becoming more effective at higher concentrations, while the butanol fraction exhibited inconsistent behavior, including negative inhibition at some concentrations. The hexane and chloroform fractions largely showed weak or negative activity, suggesting minimal contribution to nitric oxide scavenging. The figure 1 shows a clear concentration-dependent increase in superoxide radical inhibition for most extracts, with the crude methanol extract and ethyl acetate fraction demonstrating strong and consistent antioxidant activity across all concentrations. The standard, catechin, exhibited the highest inhibition overall, though EAFBL closely approached its activity at higher concentrations, indicating the presence of potent antioxidant constituents. In contrast, the hexane fraction displayed poor and initially negative activity, suggesting weak or possible pro-oxidant effects at lower concentrations.

The figure 2 shows a steady, concentration-dependent increase in iron reducing power for all extracts and fractions, indicating their electron-donating capacity. The methanol crude extract and ethyl acetate fraction exhibited strong reducing abilities, closely approaching the activity of the standard, catechin, especially at higher concentrations. In contrast, the hexane and chloroform fractions showed comparatively lower reducing power, suggesting fewer active antioxidant constituents in these fractions.

By Days 3 and 4, these effects became more evident, with consistent occurrences of abnormal urination, diarrhea, and slight disturbances in sleep among rats administered 3000 and 5000 mg/kg, alongside the first recorded deaths at the highest dose.

Collectively, these findings establish cadmium chloride as a potent testiculotoxic agent acting through oxidative stress, apoptosis, hormonal imbalance, and structural disruption of testicular architecture The results presented in Table 4 indicate that administration of cadmium chloride caused a significant reduction in serum levels of testosterone, follicle-stimulating hormone , and luteinizing hormone when compared to the control group, confirming its deleterious effect on the reproductive endocrine system.

However, treatment with the methanol extract of Baphia longipedicellata leaves at both 200 and 400 mg/kg significantly ameliorated these reductions, as evidenced by the marked increase in hormone levels relative to the CdCl2-only group. The result presented in Table 5 shows the effect of cadmium chloride intoxication and treatment with MLBL and quercetin on serum lipid profile, specifically total cholesterol and triglycerides, in Wistar rats.

However, co-administration of MLBL significantly reduced total cholesterol, triglyceride and LDL levels and elevated level of HDL when compared to the CdCl2 group. However, co-administration of the methanol extract of Baphia longipedicellata leaves at both 200 and 400 mg/kg significantly improved all sperm parameters relative to the CdCl2 -only group, indicating a protective and restorative effect.

However, co-treatment with the methanol extract of Baphia longipedicellata leaves at both 200 and 400 mg/kg significantly reduced these elevated enzyme levels relative to the CdCl2 -only group, demonstrating a protective effect against cadmium-induced hepatotoxicity. In figure 4, the 400 mg/kg dose of MLBL showed a more substantial improvement, particularly in reducing GGT and bilirubin levels closer to normal values, suggesting a dose-dependent hepatoprotective activity. Similarly, quercetin, used as a standard antioxidant, also significantly ameliorated the toxic effects of CdCl2 although the effects of MLBL, especially at the higher dose, were comparable in restoring liver enzyme activities. Similarly, treatment with MLBL and quercetin significantly restored SOD activity and reduced MDA levels compared to the CdCl2 group.

Treatment with MLBL and quercetin significantly elevated GST activity and GSH levels compared to the CdCl2 group, demonstrating restoration of antioxidant capacity. A significant decrease in the levels of testicular SOD, GSH, GST and Nitric oxide, alongside an elevation in testicular Malonaldehyde levels, was observed following cadmium chloride intoxication, precisely by 34, 41, 37, 45, 32, and 57%, respectively, in comparison to the control group.

V. CONCLUSION

In conclusion, this study demonstrates that the methanol leaves extract of Baphia longipedicellata possesses significant antioxidant and protective properties against cadmium chloride–induced testicular damage in Wistar rats. The bioassay-guided fractionation revealed that the ethyl acetate fraction and crude methanol extract contain high levels of bioactive phytochemicals, particularly flavonoids and phenolics, which are responsible for strong free radical scavenging, lipid peroxidation inhibition, and reducing power. Cadmium exposure caused marked oxidative stress, hormonal imbalance, and impairment of sperm quality; however, treatment with the extract significantly ameliorated these effects by restoring antioxidant enzyme activities, improving reproductive hormone levels, and normalizing testicular function in a dose-dependent manner.

Overall, these findings highlight the therapeutic potential of Baphia longipedicellata as a natural antioxidant and protective agent against heavy metal–induced reproductive toxicity, supporting its possible application in the management of oxidative stress–related infertility.

VI. FUNDING

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VII. CONFLICTS OF INTEREST

The authors assert the absence of any conflict of interest. The funding entities played no part in the study's design; in the gathering, analysis, or interpretation of the data; in the composition of the manuscript, or in the determination to disseminate the findings.

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