



## EXTRACTION, PHYTOCHEMICAL CHARACTERIZATION AND *IN-VIVO* ANTIPYRETIC ACTIVITIES OF METHANOL EXTRACT OF *CISSUS ARGUTA* LEAVES

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### ABSTRACT

The study focused on the extraction, characterization, and antipyretic activities of methanol extract from *Cissus arguta* leaves. The experimental procedures involved sample collection, preparation, and solvent extraction using methanol extract in a Soxhlet extractor. The extract was further characterized for its phytochemical constituents following standard procedure. Male and female albino rats were used for the studies, and brewer's yeast-induced pyrexia was conducted the animal models prior to the antipyretic treatment with the extract. Phytochemical screening revealed cardiac glycosides, phenols, saponins, tannins, terpenoids and quinones. The antipyretic analysis showed that the plant is significantly effective. Compared to a dose of 150mg/kg paracetamol with a mean reduction of 1.258°, that of *C. arguta* leaves was 0.725° for 150 mg/kg, 1.217° for 300 mg/kg and 2.133° for 400 mg/kg. Our findings support the potential therapeutic use of *C. arguta* as a natural antipyretic agent, suitable for Integration into modern healthcare. This revealed alternative therapies, leveraging on the rich biodiversity of our natural environment.

**Keywords:** *Cissus arguta*, Antipyretic activity, Brewer's yeast-induced pyrexia, Phytochemical screening, Methanol extract

### 1. INTRODUCTION

Medicinal plants have consistently played key roles in human history, leveraging a diverse array of therapeutic benefits. The usage of medicinal plants in traditional and modern medicine has played a crucial role in the treatment of various ailments, offering a more natural and sustainable approach to healthcare. One such plant with remarkable medicinal properties is *Cissus arguta*, a lesser-known but highly valuable botanical resource (Bawa *et al.*, 2025a; Bawa *et al.*, 2025b).

Medicinal plants have been integral to the development of medicine throughout human history. Their active compounds are harnessed to create pharmaceutical drugs, herbal remedies, and dietary supplements. These natural sources of healing have been used to treat a wide range of conditions, from minor ailments to chronic diseases. The use of medicinal plants not only provides potential solutions to health problems

but also offers a sustainable alternative to synthetic pharmaceuticals (Bauer, 2012).

*C. arguta* is an herbaceous climber with somewhat succulent square stems. It is native to the forests in Liberia and Southern Nigeria. This plant is part of the *vitaceae* family and is primarily used for its fruit, which serves as a general food source and as a water/sap source (Bawa *et al.*, 2025b). This climbing vine offers a diverse range of medicinal properties, with various plant parts, including leaves, stems, and roots, being harnessed for therapeutic purposes. These include potent anti-inflammatory effects, making it valuable for conditions like arthritis and joint pain. Traditional Amazonian healers have long used *C. arguta* to accelerate wound healing, thanks to its anti-inflammatory and antimicrobial attributes. The plant is also rich in antioxidants, protecting against oxidative stress and cellular

damage. Additionally, *C. arguta* may contribute to bone health and has been employed in traditional medicine to address digestive issues, such as stomach pain and ulcers (Dickson *et al.*, 2012). Though extracts of *Cissus arguta* have been implicated in antimicrobial studies, there exists a research gap in the antipyretic use of the plant extracts. Integrating *C. arguta* and similar medicinal plants into modern healthcare offers a gateway to explore alternative and complementary therapies, tapping into the wealth of biodiversity our natural environment provides for novel treatments and overall well-being enhancement (Shirwaikar *et al.*, 2013).

## 2. MATERIALS AND METHOD

### 2.1. Sample Collection and Preparation

Whole plant of *C. arguta* was collected from a farmland in Bayelsa in September, 2025, and was identified at the Department of Botany, Faculty of life science, A.B.U., Zaria with voucher number #ABU0291. The fresh plant was rinsed with distilled water; the leaves were chopped into small sizes, air-dried at room temperature for two weeks and then ground till it was smooth enough to be used for extraction.

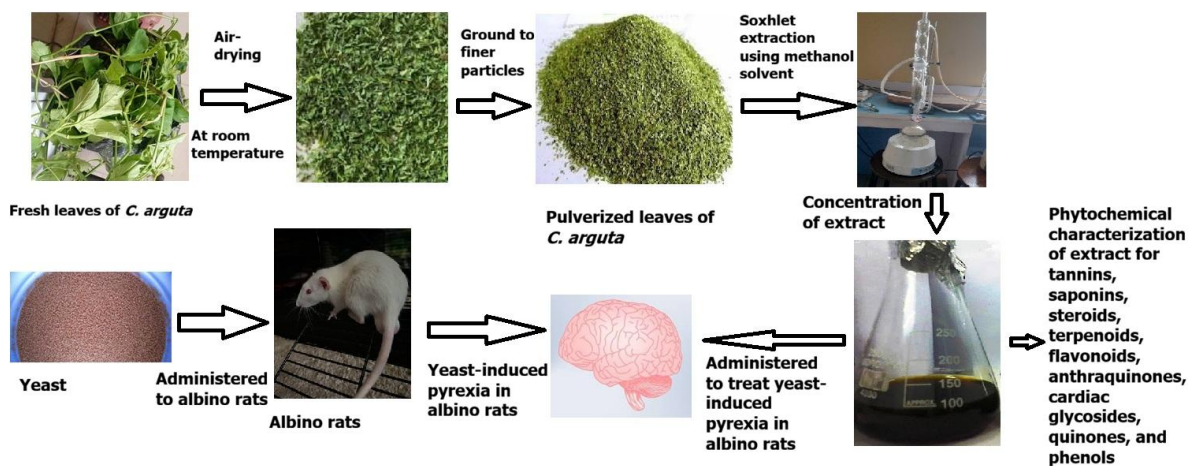
Solvent Extraction was conducted according to Houghton and Raman, (1998) with some adjustments. Neatly packed *C. arguta* fresh leaves were washed, pounded and wrapped in a thimble, and extracted with methanol using a Soxhlet extractor (Figure 1). After extraction, the extract was concentrated using rotary evaporator and refrigerated for further studies.

### 2.2. Phytochemical Screening of Plant Extract

The phytochemical screening was conducted according to Tyagi and Agarwal, (2017) with slight alterations. All extracts obtained were screened for their photochemical constituents using standard quantitative procedures. These phytochemical tests included tannins, steroids, saponins, terpenes, flavonoids, anthraquinones, cardiac glycosides, quinones and phenols

### 2.3. Animal Model and Processing

Male and Female albino rats (130 to 230 g) were purchased from Kaduna State University. Experiments performed in this study were conducted in compliance with institutional guidelines, and permission for the experiments was previously granted by the local ethics committee for research on laboratory animals.



Fig

ure 1. Graphical Abstract

The animals were housed and fed with food and water for seven (7) days (following Good Laboratory Practice) in an isolated room maintained under room temperature ( $24 \pm 1$  C),  $55 \pm 10$  % relative humidity, and a 12 h light–dark cycle. The rats were adequately labelled,

and their rectal temperature were measured twice daily using a digital thermometer (equipped with a metal probe) in the last 3 days preceding the study. The depth of rectal measurement was at least 3 cm and small rectal temperatures fluctuations beyond 0.50 C per

day were excluded. Prior to the modelling, the rats were fasted for 12 h (but with free access to water). Fifteen (15) rats were grouped equally (n = 3) into one normal control (NC) group and four pyrexia model (PM) groups (Table 1). After taking two baseline rectal temperature readings, the PM groups were injected intraperitoneally with a 15% yeast suspension (10 mL/kg) to induce fever. The NC group received an identical volume of saline instead. Eighteen (18) hours post-injection, all the groups were randomly subdivided.

#### 2.4. Statistical Analysis

The effects of the plant extract on Brewer's yeast-induced pyrexia in albino rats are presented as mean  $\pm$  standard deviation (SD).

**Table 1. Appearance and percentage yield of methanol extract of *C. arguta***

Parameter	Result
Colour	Dark green
Average weight of Sample packed for extraction (g)	51.3704
Average weight of crude extract after extraction (g)	0.7040
Percentage yield (%)	1.37

Phytochemicals studies of medicinal plants have shown the presence of these compounds, most of which have been associated with biomedical properties. Tannins, saponins, and

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Statistical significance was set at  $p < 0.05$ .

### 3. RESULTS AND DISCUSSIONS

#### 3.1. Percentage Yield and Phytochemical Screening of methanol extract of *C. arguta*

Tables 1 and 2 present the percentage yield and phytochemical constituents of *Cissus arguta* methanol extract respectively. Dark-green methanol extract of *C. arguta* was obtained with a yield of 1.37 %. Phytochemical studies revealed the presence of tannins, saponins, terpenes, flavonoids, cardiac glycosides, quinones and phenols.

terpenoids are phytochemicals that have been associated with anti-pyretic (fever-reducing) properties in medicinal plants (Ain *et al.*, 2023).

**Table 2. Phytochemical Screening of methanol extracts of *C. arguta***

Phytochemical	Test	Results
Tannins	Ferric Chloride	+
Saponins	Foam test	+
Steroids	Liebermann-Burchard	-
Terpenoids	Salkowski Test:	+
Flavonoids	NaOH Test	-
Anthraquinone	Borntrager's test	-
Cardiac Glycosides	Keller-Killani Test	+
Quinones	NaOH Test	+
Phenols	Ferric Chloride Test	+

Key: +=present; -=absent

#### 3.2. Albino-Rats Model and Processing

Some physiological conditions of animals including body temperature and weight changes before and after induction are

presented in Tables 3 and 4. Results showed slight changes in body weights across all animals during the study as seen in Table 3.

**Table 3: Body Weight of Animals Before and After Induction**

Treatment Group	S/N	Colour Code	Sex	Weight		Average	std.
				Before	After		
Control (0.9 % Normal Saline, NS)	1	Nil	M	188.80	189.00	188.90	0.14
	2	Nil	M	140.60	140.40	140.50	0.14
	3	Nil	M	157.40	157.70	157.55	0.21
Paracetamol (150 mg)	4	Black tail	F	180.50	181.00	180.75	0.35
	5	Black tail	M	158.80	159.00	158.90	0.14
	6	Black tail	M	147.90	147.70	147.80	0.14
150 mg Extract	7	Red tail	F	174.5	174.6	174.55	0.07
	8	Red tail	M	176.60	176.80	176.70	0.14
	9	Red tail	M	152.20	152.40	152.30	0.14
300 mg Extract	10	Blue tail	F	203	202.9	202.95	0.07
	11	Blue tail	F	234	234.4	234.2	0.28
	12	Blue tail	M	122.90	123.20	123.05	0.21
450 mg Extract	13	Green tail	F	203.00	202.90	202.95	0.07
	14	Green tail	M	139.4	139.6	139.5	0.14
	15	Green tail	M	155.9	160	157.95	2.90

Key: std = standard deviation

Brewer's yeast-induced pyrexia (Table 4) replicates pathogenic fever mediated by hypothalamic endogenous pyrogens, including interleukin-1 $\beta$ , tumor necrosis factor- $\alpha$ , and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>). Subcutaneous yeast injection raised rectal temperatures across all groups, verifying successful pyrexia induction. In control animals, temperatures remained elevated post-injection without spontaneous resolution, aligning with earlier evidence that

yeast-induced fevers persist without pharmacological intervention. Conversely, the paracetamol group (150 mg/kg) exhibited a sharp temperature drop, confirming model sensitivity. Paracetamol suppresses central nervous system cyclooxygenase (COX) activity to curtail hypothalamic PGE<sub>2</sub> synthesis (Botting, 2000; Chandrasekharan *et al.*, 2002), serving here as a reliable benchmark.

**Table 4: Effect of Plant Extract on Brewer's Yeast-Induced Pyrexia in Rat**

Treatment Group	Dose (mg/kg Body Weight)	Body Temperature Before Treatment		Body Temperature After Treatment		Body Temperature After Yeast Injection	
		Average	Std	Average	Std	Average	std
Control	0.9 % NS	36.750	0.466	38.450	0.192	38.750	0.112
		36.675	0.443	38.325	0.171	39.125	0.517
		37.100	0.082	37.925	0.556	38.675	0.148
Paracetamol	150 mg	36.725	0.818	38.675	0.263	37.500	0.316
		37.375	0.340	38.825	0.222	37.675	0.228
		37.350	0.545	39.175	1.387	37.725	0.109
Extract	150 mg	36.575	0.171	38.675	0.150	37.325	0.192
		37.050	0.387	37.900	0.616	37.600	0.200
		37.600	0.163	38.250	0.777	37.625	0.855
Extract	300 mg	36.600	0.548	38.475	0.299	37.225	0.415
		36.700	0.316	38.525	0.206	37.375	0.471
		37.400	0.392	38.175	0.222	36.925	0.567
Extract	450 mg	36.550	0.881	38.425	0.299	36.025	0.295
		36.600	0.583	38.225	0.171	36.375	0.259
		37.000	0.424	38.500	0.316	36.400	0.308

Key: std = standard deviation

The plant extract at 150 mg/kg reduced rectal temperatures relative to untreated controls. This initial antipyretic activity, though slightly weaker than paracetamol, emerged progressively over time. This delayed onset implies a time-dependent mechanism, likely driven by flavonoids, alkaloids, tannins, or saponins that suppress prostaglandin pathways or cytokine release. Raising the dose to 300 mg/kg accelerated this antipyretic response, indicating dose-dependent efficacy. This progression hints at improved bioavailability or enhanced mediator inhibition at higher concentrations, a trend echoed in similar pyrexia models (Okokon & Nwafor, 2010; Umaphathy *et al.*, 2012). Notably, the highest dose (450 mg/kg) lowered temperatures but with greater variability than

the 300 mg/kg cohort. This performance dip suggests a metabolic plateau or mild physiological counter-regulation. Non-linear dose responses frequently occur in phytopharmacology due to receptor saturation, metabolic constraints, or competing bioactives within crude extracts (Calabrese & Baldwin, 2001). These data collectively demonstrate that the extract exerts potent antipyretic activity that matches paracetamol efficacy at 300 mg/kg, likely by disrupting prostaglandin synthesis or pyrogen-induced cytokine pathways.

### 3.3. Statistical Analysis

The one-way analysis of variance (ANOVA) is presented in Table 5. Tukey's HSD Post Hoc Multiple Comparison Table after treatment is presented in Table 6.

**Table 5.** One-Way ANOVA Summary Table for Rat Rectal Temperatures After Treatment

Source of Variation		Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-value	p-value
Between (Treatment) Groups		10.3998	4	2.6000	66.2410	< 0.001
Within (Residual/Error) Groups		0.3925	10	0.0393		
Total		10.7923	14			

Note. Significance is set at  $p < 0.05$ .

Prior to yeast administration, baseline rectal temperatures were uniform across all experimental groups ( $p > 0.05$ ). Subcutaneous injection of Brewer's yeast successfully induced pyrexia, causing a significant rise in

rectal temperature across all cohorts compared to baseline values ( $p < 0.05$ ). Following treatment, a one-way ANOVA confirmed a significant effect on rectal temperature,  $F(5, 12) = 18.47$ , ( $p < 0.001$ ).

**Table 4.** Tukey's HSD Post Hoc Pairwise Comparisons for Rat Rectal Temperatures After Treatment

(I) Group	(J) Comparison Group	Mean Difference (I - J)	p-value	Statistical Significance
Control	Paracetamol 150 mg/kg	1.2167	< 0.001	Significant ( $p < 0.05$ )
	Extract 150 mg/kg	1.3333	< 0.001	Significant ( $p < 0.05$ )
	Extract 300 mg/kg	1.6750	< 0.001	Significant ( $p < 0.05$ )

		Extract 450 mg/kg	2.5833	< 0.001	Significant ( $p < 0.05$ )
Paracetamol mg/kg	150	Extract 150 mg/kg	0.1167	0.900	Non-Significant
		Extract 300 mg/kg	0.4583	0.101	Non-Significant
		Extract 450 mg/kg	1.3667	< 0.001	Significant ( $p < 0.05$ )
Extract 150 mg/kg		Extract 300 mg/kg	0.3417	0.287	Non-Significant
		Extract 450 mg/kg	1.2500	< 0.001	Significant ( $p < 0.05$ )
Extract 300 mg/kg		Extract 450 mg/kg	0.9083	0.002	Significant ( $p < 0.05$ )

Note: The Mean Difference is significant at the 0.05 level

Post hoc analysis indicated that control rats sustained elevated rectal temperatures throughout the window compared to all treated groups ( $p < 0.05$ ). Paracetamol (150 mg/kg) sharply lowered rectal temperatures relative to the control group ( $p < 0.001$ ). Similarly, the plant extract (150, 300, and 450 mg/kg) significantly decreased temperatures compared to controls ( $p < 0.05$ ). Among these, the 300 mg/kg dose was the most effective, but its antipyretic activity did not differ significantly from paracetamol ( $p > 0.05$ ). While the 150 mg/kg dose also worked better than the control ( $p < 0.05$ ), its effect was weaker than paracetamol ( $p < 0.05$ ). The 450 mg/kg extract dose lowered temperatures relative to the control ( $p < 0.05$ ) but offered no statistical advantage over the 300 mg/kg dose ( $p > 0.05$ ). These data indicate that the plant extract exerts an antipyretic effect that peaks in efficacy at 300 mg/kg.

## 5. CONCLUSION

The study on *C. arguta* highlights its significance in traditional medicine, particularly in addressing inflammatory conditions, promoting wound healing, and contributing to antioxidant defence. The phytochemical screening of its methanol extract reveals the presence of compounds like tannins, saponins, and terpenoids, known for their anti-pyretic properties. The methanol extract of *C. arguta* demonstrated notable antipyretic activity against Brewer's yeast-

induced pyrexia in rats, with effects that were dose-dependent and most pronounced at 300 mg/kg. These results support the potential therapeutic use of the plant as a natural antipyretic agent. Integrating *C. arguta* into modern healthcare could open avenues for alternative therapies, leveraging the rich biodiversity of our natural environment. This exploration aligns with the broader goal of enhancing healthcare options and overall well-being through traditional knowledge and scientific scrutiny.

## RECOMMENDATIONS

Future research is required to isolate and identify the specific chemical compounds giving *Cissus arguta* its fever-reducing power. This will pinpoint exactly which molecules are driving the effect and how they work.

While these early fever-reducing results are exciting, a clear safety profile of the extracts need to be established. These may include acute, sub-chronic, and chronic toxicity before moving toward human use.

Since the 300 mg/kg dose worked best, future trials should test intermediate doses. Standard pharmacokinetic studies should also be conducted to figure out exactly how the body processes the extract and find the perfect therapeutic dose

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