

# Antitrypanosomal Activity of Leaf Extracts of *Acalypha Wilkesiana*

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## Abstract

*Acalypha wilkesiana* (Red Hot Cat's Tail) has reportedly being used to treat a number of fungal (Eukaryotic) infections and so, might also have trypanocidal (parasitic- eukaryotic) properties. In this study, Ethanolic and water extracts of Red Hot Cat's Tail plant were graded into concentrations of (30, 15, 7.5 and 3.73mg/ml) and tested against actively motile *Trypanosoma brucei brucei*. The extracts were then screened for *alkaloids, tannins, saponins, cardiac glycosides, amino acid, reducing sugar, steroids, triterpanoids, and anthraquinones*. At 30mg/ml, complete cessation of motility of the parasite occurred within 25min (for aqueous extract) and 30min (for ethanolic extract). At 15mg/ml, 7.5mg /ml, 3.72mg/ml of both the extracts, complete cessation of motility of the parasites within 40mins, 45mins and 50mins was observed respectively. High activity of the leaf extracts against *T. brucei brucei* might not be unconnected with the presence of efficient bioactive compounds in it. Therefore, this plant can locally be used against *T. brucei brucei* infestation.

## Keywords

*Acalypha Wilkesiana*, *Trypanosoma Brucei Brucei*, Extract, Concentration, Phytochemicals

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## 1. Introduction

A lot of people in Nigeria live below the average living conditions and as such, expensive drugs including some antibiotics are generally not affordable. The use of locally sourced plants as antimicrobial agents is a common practice in such environments. These plants are worthy of scientific investigations and as such this research becomes valuable. Trypanosome diseases are among the ailments treated using some of these plants. The most common trypanosome diseases encountered in Africa are sleeping sickness and nagana.

Herbal preparation of *Acalypha wilkesiana* (Red Hot Cat's Tail) plant was used for the remedies of many diseases in

Nigeria such as fungal and bacterial infections; and some parasitic infestations.

*Acalypha wilkesiana* is an evergreen shrub which grows 3m high and spreads 2m across. The stem is erect with many branches which have fine hairs and form closely arranged crown. The leaves are large and broad with teeth around the edge and can be flat or crinkled. The flowers are reddish with spikes at the end of the branches and their stalks are 10–20cm long.

*A wilkesiana* plant is both tropical and subtropical, which grows naturally in Vanuatu and occurs in the Pacific Islands.

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However, it prefers light, well-drained soil.

Oyelami *et al.*, (2003) carried out a non-comparative study to evaluate the safety and efficiency of ointment extracted from *Acalypha wilkesiana* using 32 subjects with clinical symptoms of mycoses. The ointment successfully controlled the mycoses in 73.3% of the affected patients. It was very effective in treating *Pityriasis versicolor*, *Tinea pedis* and *Candida intetrigo*, with 100% cure. Oyelami *et al.*, (2003) concluded that *Acalypha wilkesiana* ointment can be used to treat superficial mycoses. Akinyemi *et al.* (2005) evaluated crude extracts from six important medicinal plants, namely *Phyllanthus discoideus*, *Ageratum conyzoides*, *Terminalia avicennioides*, *Bridella ferruginea*, *Acalypha wilkesiana* and *Ocimum gratissimum*, to find activity against methicillin resistant *Staphylococcus aureus* (MRSA).

Both ethanolic and aqueous extracts of this plant showed effects on MRSA with minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of 30.4-37.0 µg/ml and 18.2-24.0 µg/ml respectively. A high MBC value was found in two plants and the other four contained traceable amounts of anthraquinones. This study provided scientific support for the use of *Acalypha wilkesiana* against MRSA (Nagarajan *et al.*, 2005).

*Trypanosoma brucei (gambiense)* is a species of salivary trypanosome which causes African Trypanosomiasis, also known as sleeping sickness in humans and nagana in animals. *T. brucei* has traditionally been grouped into three subspecies: *T. brucei brucei*, *T. brucei gambiense* and *T. brucei rhodesiense*. Only rarely can the subspecies *T. brucei brucei* infect a human (Deborggraeve, *et al.*, 2008).

Transmission of *T. brucei* between mammal hosts is usually by an insect vector, the tsetse fly. *T. brucei* parasites undergo complex morphological changes as they move between insect and mammal over the course of their life cycle. *T. brucei* is one of only a few pathogens that can cross the blood brain barrier. (Masocha, *et al.*, 2012). There is an urgent need for the development of new drug therapies as current treatments can prove fatal to the patient.

*T. brucei gambiense* causes slow onset chronic Trypanosomiasis in humans. Most common in central and western Africa, where humans are thought to be the primary reservoirs. (Barrett, *et al.*, 2003). *T. brucei rhodesiense* causes fast onset acute Trypanosomiasis in humans. Most common in southern and eastern Africa, where game animals and livestock are thought to be the primary reservoir. (Barrett, *et al.*, 2003). *T. brucei* causes animal African Trypanosomiasis, along with several other species of Trypanosoma. *T. brucei brucei* is not human infective due to its susceptibility to lysis by Trypanosome Lytic Factor-1 (TLF-1), (Stephens, *et al.*, 2012). However, as it is closely

related to, and shares fundamental features with the human infective subspecies, *T. brucei brucei* is used as a model for human infections in laboratory and animal studies.

## 2. Research Methodology

### 2.1. Experimental Animals

White albino rats were obtained and kept in animal house of the department of vector and parasitology, Nigerian Institute for Trypanosomiasis Research (NITR) Kaduna, Nigeria.

### 2.2. Test Parasite

*T. brucei brucei* were collected from the blood of an infected rat in Vector and Parasitology Department NITR, Kaduna. The infected blood was inoculated into healthy rats for transportation and propagation. The in vitro study was conducted in research Laboratory, of the Department of Microbiology, Bayero University Kano, Nigeria.

### 2.3. Determination of Parasitemia Level

*Trypanosoma brucei brucei* were cultured using 96 wells micro titre plate and maintained at 37°C. Rapid matching wet examination technique as described by Herbart & Lumsten, (1976) was performed by examining a drop of the blood under 40x magnification of light microscope and counting the number of the parasites in each field, and matched with log figure obtained from the reference table (Atawodi *et al.*, 2003).

### 2.4. Plant Material

The leaves of *A. Wilkesiana* leaves were harvested from botanical garden of the Department of Plant Biology, Bayero University Kano and identified in the herbarium of the same department where an accession number was obtained. The leaves were air dried at room temperature and grinded into powder using Laboratory mortar and pestle.

### 2.5. Extraction

Percolation method was used as described by Wang *et al.*, (2006). 40g of the plants powder was transferred into each of two (2) separate bottles either of which containing 600ml of absolute ethanol and the same volume of distilled water respectively. The set up was kept for 72 hours with periodic agitation. The content from each bottle was filtrated using Wattman's no. 1 filter paper. The filtrate from both solvents were evaporated using laboratory water bath.

### 2.6. Standard Drug

Diminazane diaceurate drug, manufactured by Dimiga Plus Company France, was used as positive control and 10%

Dimethyl sulphoxide (DMSO) was used as negative control.

### 2.7. Preparation of Test Concentration

Stock solution of the test concentration was prepared by dissolving 60mg of the plant extract in 2ml of 10% DMSO to obtain test concentrations of 30, 15, 7.5 and 3.7mg/ml by serial dilution method.

The same concentration was prepared with Diminazane diaceurate for the control.

### 2.8. In Vitro Test

20µl of each of the prepared test concentrations of the plant extract and 5µl of the prepared blood were mixed and incubated at 37°C. The parasitemia level was determined every 5minutes for the period of one hour. The effect of the plant extract on *T. brucei* was observed and determine base on the motility of the parasites (Atawodi *et al.*, 2003).

### 2.9. Preparation of Extract for Phytochemical Screening

Both ethanolic and aqueous extracts of *A. wilkesiana* leaves were prepared in 20g/200ml and dried at 60°C protected from light. The residue was weighed and dissolved in 10% known volume of *dimethyl sulphoxide* (DMSO). The extracts were used for the detection of qualitative phytochemical analyses. (Poongothai *et al.*, 2011)

### 2.10. Screening Procedure

Simple standard chemical tests were carried out for the qualitative phytochemical screening of *A. wilkesiana*. These tests were used to detect the presence of bioactive agents such as alkaloids, tannins, saponins, cardiac glycosides, amino acid, reducing sugar, steroids, triterpanoids, anthraquinones, etc. The phyto-constituents were assayed for, using standard method as described by (Akintobi *et al.*, 2013).

### 2.11. Alkaloid Test

Five grams each of the *Acalypha wilkesiana* extract and 5ml of honey was stirred with 5ml of 1% aqueous hydrochloric acid (HCL) at 60°C for 5min. The sample was filtered a 3 layered muslin cloth. One millilitre of the filtrate was treated with few drops draggendoff's reagent. Blue black turbidity serves as preliminary evidence of alkaloids. (Akintobi *et al.*, 2013).

### 2.12. Saponins Test

Five grams each of the extract and 5ml of honey were shaken separately with distilled water in a test tube. Frothing which persists on warming was taken as an evidence for the presence of saponins. (Akintobi *et al.*, 2013).

### 2.13. Tannins Test

Five gram each of the extract and 5ml of honey were stirred separately with 100ml of distilled water and filtered. 1ml of ferric chloride reagent was added to the filtrate. A blue black or blue green precipitate was an indication of presence of tannins. (Akintobi *et al.*, 2013).

### 2.14. Flavonoids Test

Five millilitres of dilute ammonia solution was added to aqueous filtrate of the test sample followed by the addition of 1ml concentrated H<sub>2</sub>SO<sub>4</sub>. Yellow coloration indicates the presence of flavanoids (Akintobi *et al* 2013).

### 2.15. Cardiac Glycosides (Keller-Killiani Test)

Five grams of each of the extract and 5ml of honey were dissolved separately in 2ml of glacial acetic acid containing a drop of ferric chloride solution. This was underplayed with 1ml of concentrated H<sub>2</sub>SO<sub>4</sub>. A brown ring at the interface indicates a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a green ring may form which just gradually spreads throughout the layer (Akintobi *et al.*, 2013).

### 2.16. Salkoski Test

Five grams of the extract and 5ml of honey were dissolved in 20ml of chloroform. Few drops of sulphuric acid were carefully added to form a layer at the lower part. A reddish brown colour at the interface indicates the presences of steroids nucleus (Akintobi *et al.*, 2013).

### 2.17. Test for Amino Acids

1ml of the extract was treated with few drops of ninhydrin reagent. Appearance of purple colour shows the presences of amino acid (Poongothai *et al.*, 2011).

### 2.18. Test for Anthraquinones

Five millilitres of the extract solution was hydrolysed with dilute concentration of H<sub>2</sub>SO<sub>4</sub> extracted with benzene and 1ml of dilute ammonia was added. Pose pala colouration suggests positive result for anthraquinones (Poongathai *et al.*, 2011).

### 2.19. Test for Triterpenoids

10mg of the extract was dissolved in 1ml of chloroform. 1ml of acetic acid anhydrous was added following the addition of 2ml of concentrated H<sub>2</sub>SO<sub>4</sub>. Formation of reddish violet color indicates the presence of triterpenoids. (Poongathai *et al.*, 2011).

## 2.20. Phlobotannins Test

Deposition of red precipitate when an aqueous extract of the test samples was boiled with 1% hydrochloric acid (HCL) indicates the presence of phlobotannins (Akintobi *et al.*, 2013).

## 3. Results

After observing the negative control at intervals of 5min incubation, at 37°C, the parasites survived more than 4 hours in the absence of the extract. For positive control, at 3.72mg/ml of diminazene diaceturate, complete cessation of

movement and death of the parasites occurred within 10-15mins.

About 20-25 parasites per field, were dosed with 3.72mg/ml, 7.5mg/ml, 15mg/ml, and 30mg/ml of both aqueous and ethanolic extracts of the plant. At 30mg/ml, complete cessation of motility of *T. brucei* within 25min and 30min was observed for aqueous and ethanolic extracts respectively. At 15mg/ml, 7.5mg /ml, 3.72mg/ml of both ethanolic and aqueous extract, complete cessation of motility of the parasites within 40min, 45mins and 50mins was observed respectively. Table 1 and 2 below show the summary of the result.

**Table 1.** Activity of Ethanolic Extract of *A. wilkesiana* Leaves on Test Organisms.

Contact Time (min)	30mg/ml	15mg/ml	7.5mg/ml	3.75mg/ml
1 – 5	AM	AM	AM	AM
6 - 10	ARM	AM	AM	AM
11 – 15	SM	ARM	AM	AM
16 – 20	VSM	ARM	ARM	ARM
21 – 25	CCM	SM	SM	ARM
26 – 30	CD	VSM	SM	SM
31 – 35	-	CCM	VSM	SM
36 – 40	-	CD	CCM	VSM
41 – 45	-	-	CD	CCM
46 – 50	-	-	-	CD
51 – 55	-	-	-	-
56 – 60	-	-	-	-

Key: AM = Active motility. ARM = Reduced motility. SM = sluggish motility. VSM = very sluggish motility. CCM = complete cessation of motility. CD = complete death.

**Table 2.** Activity of Aqueous Extract of *A. wilkesiana* Leaves on Test Organisms.

Contact Time (min.)	30mg/ml	15mg/ml	7.5mg/ml	3.75mg/ml
1 – 5	AM	AM	AM	AM
6 - 10	ARM	AM	AM	AM
11 – 15	SM	ARM	ARM	AM
16 – 20	CCM	SM	ARM	ARM
21 – 25	CD	SM	SM	ARM
26 – 30	-	VSM	VSM	SM
31 – 35	-	CCM	VSM	SM
36 – 40	-	CD	CCM	VSM
41 – 45	-	-	CD	CCM
46 – 50	-	-	-	CD
51 – 55	-	-	-	-
56 – 60	-	-	-	-

Key: AM = Active motility. ARM = Reduced motility. SM = sluggish motility. VSM = very sluggish motility. CCM = complete cessation of motility. CD = complete death.

**Table 3.** Presence of Phytochemicals in *A wilkesiana* Leaf Extracts

Phytochemical	Aqueous extract	Ethanolic extract
Saponins	+	+
Flavonoids	+	+
Tannins	+	+
Amino acids	-	-
Reducing sugar	+	+
Cardiac glycosides	+	+
Steroids	+	+
Triterpenoids	+	+
Anthraquinones	+	-

Key: + = presence. - = absence.

## 4. Phytochemical Screening

Investigation of phytochemicals from both the extracts shows presence of alkaloids, reducing sugar, flavonoids, saponins, tannins, cardiac glycoside, steroids, triterpenoids (in both ethanolic and aqueous extract) and anthraquinones (in the aqueous extract only). However, test for amino acid indicated a negative result (Table3).

## 5. Discussion

Both the water and ethanolic extracts were found to show trypanocidal activity especially at higher concentrations and longer contact time. The protective or killing effects of the plant have been attributed to its phytochemicals which are the non-nutrients plant compounds.

Different phytochemicals have been found to possess a wide range of activities, which may help in protection against many infections. Gull *et al.*, (2012) reported that the main factors that determine the antimicrobial activities of a plant are the type and composition of the plant, amount used, type of the organism, composition of the plant's compounds, pH value and temperature of the environment

Furthermore, glycosides, saponins, flavonoids, tannins, and alkanoids have hypoglycemic and anti-inflammatory activities (Augusti and cherian, 2008).

Reports show that saponins possess hypocholesterolemic and antidiabetic properties (Rupasinghe *et al.*, 2003).

The triterpenoids have also been shown to decrease blood sugar level in animal studies (Mandal *et al.*, 2009).

Steroids, triterpenoids, and saponins showed analgesic properties on central nervous system activities (Shaikh *et al.*, 2010).

In this study a broad classification of chemical extracts from the plants was made into alkaloids, phlobatannins, cardiac glycosides, tannins, triterpenoids, saponins, flavonoids which were present in varying concentrations (Table 3). The presence of these compounds indicates that the plant has medicinal values and these vary with the type of a particular compound and their concentration.

## 6. Conclusion

Exposure of the leaf extract of *Acalypha wilkesiana* to *Trypanosoma brucei* (for around five minutes) resulted in no activity on the parasites at all concentrations. However, complete cells' death occurred when the time was increased to at least 26 min. especially at higher concentrations. Hence, the leaf extract can be used as a potential drug candidate against *Trypanosoma* infestations.

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