



## The Screening for the Antitrypanosomal Potentials of the Extracts of *Curcuma longa* L.

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### Abstract

**Background:** Extracts of *Curcuma longa* rhizome extracts were investigated for their biological activity.

**Methodology:** The presence of phytochemicals and antitrypanosomal activities were investigated.

**Results:** The extracts contained the phytochemicals (Alkaloid, Anthraquinone, Carbohydrates, Cardiac Glycosides, Flavonoid, Glycosides, Phenols, Saponin, Steroid, Tannin and Triterpenes) in table 1 and 2. The extracts showed very good activity against the parasite from figure 1, 2 and 3.

**Discussion:** The presence of the phytochemical confirms their medicinal potentials. The crude extracts of *Curcuma longa* were analyzed *in vitro* for trypanocide activity on *Trypanosoma brucei brucei* at concentration 100.0 mg/ml, 50.0 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml. The best activity was seen in Petroleum ether extract, followed by the ethanol extract, then Chloroform which was still impressive.

**Conclusion:** The results obtained from this study show that the turmeric contains potential antimicrobial components which will be of great use for the advancement of remedies by pharmaceutical industries as a therapy against various diseases.

**Keywords:** *Curcuma longa*; Petroleum Ether; Chloroform; Ethanol; Antitrypanosomal Activity; *Trypanosoma brucei brucei*

### Abbreviations

A.B.U.: Ahmadu Bello University; AAT: Animal African Trypanosomiasis; ABUCAUC: Ahmadu Bello University Ethics Committee on Animal Use and Care; AT: African Trypanosomiasis; *C. longa*: *Curcuma longa*; CPCSEA: Committee for the Purpose of Control and Supervision on Experiments on Animals; HAT: Human African Trypanosomiasis; NARICT: National Research Institute for Chemical Technology; RBC: Red Blood Cells; *T. b. brucei*: *Trypanosoma brucei brucei*.

### Introduction

African trypanosomiasis (AT) are extracellular protozoan parasites that cause chronic infections in Humans, and their livestock, and are predominantly transmitted by *Glossina* sp. *Trypanosoma congolense* and *Trypanosoma brucei brucei* (*T. b. brucei*), are among

the most common trypanosomes, which causes livestock infections [1]. Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT) form the two main forms of AT. And it is projected about 70 million population in around 36 countries of sub-Saharan Africa may be at the menace of HAT while AAT looms over the lives of millions of cattle every year. HAT is caused by two strains of *T. b.* that infect either humans (*T. b. gambiense*) or animals (*T. b. rhodesiense*), while AAT portends the lives of several million herds of cattle every year, thereby necessitating new approaches of contending the disease [2]. Primary diagnosis of the presence of *T. b.* at the first stage of infection can partake a significant impact on a patient's outcome by permitting timely and adequate treatment before the disease progresses into a second stage. This is significant because, at this later phase, the parasite infiltrates the central nervous system, which leads towards the neuropsychiatric

manifestations. such as deep sensory disturbances, derangement or sleep disorders, that sternly compromise the quality of life of the patient. This second stage is fatal if untreated and treatments used to treat it are expensive and/or highly toxic. In contrast, drug therapy for early-stage HAT is effective and only mildly toxic [3].

The name 'turmeric' has originated from the Feudal Latin name *terramerita*, which became *terre merite* of French, meaning deserved earth or meritorious earth, a reputation by which powdered turmeric was known in commerce [4]. Turmeric (*Curcuma longa* L., family: *Zingiberaceae*) is one amongst the foremost important and ancient spices of India and a conventional item of export. It's used as condiment, dye, drug and cosmetics additionally to its use in religious ceremonies. The rhizome is that the economic part of turmeric and is well-known for its medicinal properties together with its application in cosmetics and as a natural dye within the textile industry [5]. Altogether South Asian countries, turmeric has been in use from ancient time as a spice, food preservative, colouring agent, and cosmetic and within the traditional systems of drugs (*Ayurveda*, *Sidha*, *Tibetan* and *Unani*) [4].

Turmeric is one amongst the species which has been used an extended time as a medicinal plant for antibacterial activity because it contains active curcumin. However, curcumin isn't enough to create this compound widely utilized in the clinical field because of its low bioavailability [6]. Turmeric is thought to be the "golden spice" also as of the "spice of life." It's stood employed in India as a medicinal plant and held sacred from historical time. Turmeric has a sturdy relationship with the socio-cultural life of the individuals of the Indian subcontinent. This "earthy herb of the Sun" with the orange-yellow rhizome was well-thought-out as the "herb of the Sun" by the people of the Vedic period. No wonder the historians regarded turmeric as the *Oushadhi*: "the healing herb", 'the most outstanding herb', 'the one herb above all others', etc. Turmeric has a minimum of 6000 years of documented history of its practice as medicine and in many socio-religious usages [4]. Recent researches on turmeric are focused on its Antibacterial [7-9], Anticarcinogenic [10-12] Antidiabetic [13-15], Anti-Inflammatory [16-18], Antimicrobial [19-21], Antioxidant [22-24], Antirheumatic [25,26], Antiviral [27-29], Choleric [30,31].

## Materials and Methods

### Preparation of medicinal plants

- **Collection of Plant Materials:** The fresh rhizome part of *Curcuma longa* was collected from Area BZ, A.B.U., Zaria and

authenticated at Herbarium Section, Department of Biological Sciences, A.B.U., Zaria.

- **Preparation of the rhizome extracts:** The rhizome was washed with distilled water, air-dried at room temperature for three days and then, pulverized using a clean electronic grinder. The 100.0g portion of pulverised rhizome was put into 250 ml of petroleum ether for 24 hours. Then the solvent was filtered and the residue was air-dry and repackage. This was repeated for chloroform and ethanol respectively. Each extract was filtered, the excess solvent was recovered using a recovery apparatus. The remaining extract was evaporated to dryness at room temperature in a steady air current over a water bath as described by Abubakar, *et al.* (2020) [32].
- **Phytochemical Studies:** The phytochemical analysis was carried out quantitatively to determine the presence of Alkaloid, Phenols, Flavonoids, Saponins, Tannins, Resin, Glycosides and Steroids using the methods described by Abubakar, *et al.* (2020) [32].
- **Preparation of the test samples:** In the study of the antitrypanosomal activities of this plant, concentrations of 100.00-6.25 (mg/ml) of each extract were used for the screening. This was done by dissolving 10.00 g (1000 mg) of the extracts in 10 ml DMSO solvent i.e., 100.00 mg/ml. And subsequent were made via serial dilutions 50.00 - 6.25 mg/ml using dextrose saline and were freshly prepared as accordance by Fathuddin and Inabo, (2017) [33].
- **Sources and Maintenance of The Test Organisms:** The parasite, *T. b. brucei* was obtained from the NARICT, Basawa, Zaria, Nigeria. The organisms were maintained in the laboratory by the continuous passage in rats with accordance to Fathuddin and Inabo (2017) [33].
- **Laboratory Animals:** Wister strain albino rats were obtained and kept at the animal house of NARICT. The animals have maintained following Fathuddin and Inabo (2017) [33]. The experimental animal was handled per the guidelines of the World Health Organization. (2010) [34]; Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) Guidelines for Laboratory Animal Facility (2010) [35]; A.B.U. Ethics Committee on Animal Use and Care [ABUCAUC] (2010) [36]. All protocols and procedures used in this study were reviewed and approved by the ABUCAUC Policy. The ABUCAUC approval number is ABUCAUC/2012/MICROB/APP/001.

### Antitrypanosomal bioassay

The trypanosome parasitaemia was determined by the use of wet mount according to The Wet and Thick Blood Film Method, and microscopic evaluation at 400x magnification using The Rapid Matching Method Assessment of the *in vitro* trypanocidal activity was conducted according to Fathuddin and Inabo, (2017) [33]. The Positive Control was performed using recommended concentrations of *Diminazine aceturate* (Sequene, PI Drugs and Pharmaceuticals LTD India) and the negative control was just mixed in dextrose saline. The 96 round-bottom well microtiter plate was incubated at room temperature [33].

### Results and Discussion

- Collection of Plant Materials:** The fresh rhizome part of *Curcuma longa* was collected from Area BZ, Ahmadu Bello University Campus, Samaru, Zaria and authentication were issued by Herbarium Section, Department of Biological Sciences, Ahmadu Bello University, Zaria; given as Voucher No.: 1152
- Phytochemical analysis:** The extracts obtained had an appearance of Petroleum Ether - Yellow Oily Liquid; Chloroform - Dark Orange Residue; and, Ethanol -Orange Sticky Residue which is presented in table 1. The phytochemical constituents of Petroleum Ether, Chloroform and Ethanol Extract of rhizome *Curcuma longa* is presented in table 2. The result showed that the extracts contain the following Phytochemicals: Alkaloid, Anthraquinone, Carbohydrates, Cardiac Glycosides, Flavonoid, Glycosides, Phenols, Saponin, Steroid, Tannin and Triterpenes. The finding was similar to what various authors noted [7,10,37-39].
- Antitrypanosomal Bioassay:** The *in vitro* analysis result for the extracts shown in figure 1, 2 and 3. The Petroleum ether, chloroform and ethanol extracts had activity on *T. b. brucei*. From figure 1, it can be seen that the Petroleum ether extract, at 100.00 - 25.00 mg/ml cleared all the parasite in the blood. However, the concentration was too strong that it ended up even lyse the RBC. The concentration of 12.50 cleared the parasite within 10 minutes while the 6.25 mg/ml cleared within 20 minutes. The Chloroform extract had activity between 100.00 and 50.00 mg/ml but it took 25.00 mg/ml - 15 minutes, 12.50 mg/ml - 35 minutes and 6.25 mg/ml - 55 minutes to clear the parasite. The Ethanolic extract like the chloroform extract cleared the parasite but 100.00 and 50.00 mg/ml but it took 25.00 mg/ml -10 minutes, 12.50 mg/ml - 20 minutes and 6.25 mg/ml -30 minutes, which was faster the chloroform extract but slower than petroleum ether. The morphology of the RBC was maintained at lower concentrations i.e., 25.00 - 6.25 mg/ml. The positive control was most effective in clearing all the parasite in less than 5 minutes, while the negative control lasted about 80 minutes. The result of the present study showed that *C. longa* has activity against *T. b. brucei*.

This result is in line with other authors findings, like Le., *et al.* (2019) [40] found that the interesting effect of the *C. longa* EO against *T. b. brucei* bloodstream form with a good selectivity while using essential oil of *C. longa*. Most research works focus primarily on the active ingredient: curcumin. Haddad., *et al.* (2011) [41] stated that some curcumin derivatives have potent trypanocidal activity against the *T. b. brucei* bloodstream form. Even, Jonah and Enoch (2020) [42] found that curcumin from rhizomes of *C. longa* contains active principles with significant *in vitro* antitrypanosomal activity against *T. b. brucei*.

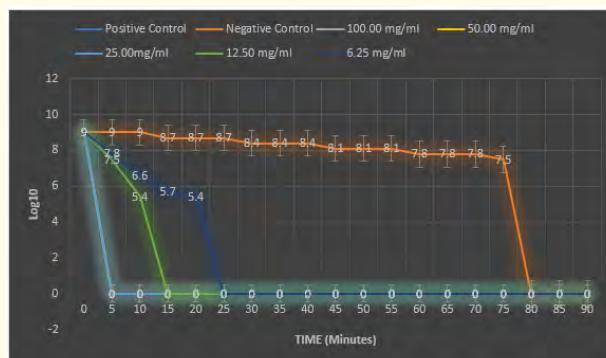


Figure 1: Petroleum ether extract of *C. longa* against *T. b. brucei*.

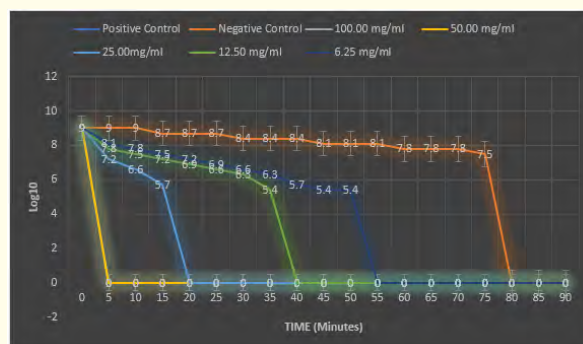


Figure 2: Chloroform extract of *C. longa* against *T. b. brucei*.

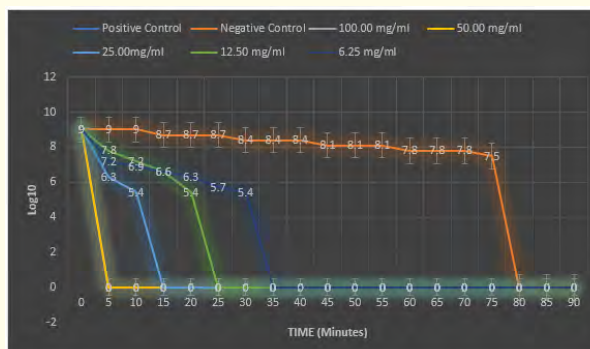


Figure 3: Ethanol extract of *C. longa* against *T. b. brucei*.

| Solvent Used    | Colour and consistency | Percentage Yield (%W/W) on Fresh Weight Basis |
|-----------------|------------------------|---|
| Petroleum Ether | Yellow Oily Liquid     | 0.16  |
| Chloroform      | Dark Orange Residue    | 0.88  |
| Ethanol         | Orange Sticky Residue  | 0.96  |

**Table 1:** The constitute of the various extracts of *C. longa* (rhizome).

| S/ No. | Secondary Metabolites          | Name of Test               | Petroleum ether | Chloroform | Ethanol |
|--------|--------------------------------|----------------------------|-----------------|------------|---------|
| 01     | Carbohydrates                  | Molisch's Test             | +               | +          | +       |
| 02     | Glycosides                     | Fehling's Test             | +               | +          | +       |
| 03     | Phenols                        | Ferric chloride Test       | +               | +          | +       |
| 04     | Antraquinones Derivatives      | Borntreger's Test          | -               | +          | +       |
|        |                                | Modified Borntreger's Test | +               | +          | +       |
| 05     | Cardiac Glycosides             | Kella-Killiani Test        | +               | +          | +       |
|        |                                | Kadde Test                 | +               | +          | +       |
| 06     | Steroid                        | Salkowsk Test              | +               | +          | +       |
| 07     | Saponins                       | Frothing Test              | +               | +          | +       |
| 08     | Steroid (S) or Triterpenes (T) | Leibermen-Burchards Test   | +               | +          | +       |
| 09     | Flavonoids                     | Shinoda Test               | +               | +          | +       |
|        |                                | Sodium hydroxide Test      | +               | +          | +       |
| 10     | Tannins                        | Ferric chloride Test       | +               | +          | +       |
| 11     | Alkaloids                      | Dragendoff's Test          | +               | +          | +       |
|        |                                | Wagner's Test              | +               | +          | +       |
|        |                                | Picric acid Test           | +               | +          | +       |
|        |                                | Tannic acid Test           | +               | +          | +       |

**Table 2:** Phytochemical analysis of various extracts of *C. longa* (rhizome).

## Conclusions

The preliminary phytochemical tests revealed the presence of carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, terpenoids, steroids and tannins within the rhizomes of *C. longa* L. The results obtained from this study show that the turmeric contains potential antimicrobial components which will be of great use for the advancement of remedies by pharmaceutical industries as a therapy against various diseases. The turmeric extract possesses significant activity against *T. b. brucei*. Consequently, it's fairly clear that turmeric being available in pure form, it might be easier to develop new drugs with fewer side effects and more efficiency. Because of being nontoxic with a wide spectrum of biological functions, turmeric may find its application within the formation of several medicinal products which can help in the treatment of various diseases in coming future [43]. In recent years, it has been seen that there is a continuous enthusiasm in treating various diseases with natural products. Further research is needed to supplementary investigate the efficacy of curcumin as a stand-alone drug or as a supplement of current drugs of choice because it has no antagonistic activities but might overcome their drawbacks [44].

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## Contributions and Conflict of Interests

The authors contributed equally and declare no potential conflict of interests.

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