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# ANTI-TUBERCULAR ACTIVITIES OF *CURCUMA LONGA* EXTRACT AND FRACTIONS AGAINST *MYCOBACTERIUM TUBERCULOSIS* ($H_{37}R_v$)

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## ABSTRACT

Tuberculosis is one of the major human diseases primarily caused by *Mycobacterium tuberculosis* with other members of *Mycobacterium* complex. The disease is complicated due to the emergence of multi-drug resistant and extensively drug resistant strains of *M. tuberculosis.* The anti-tubercular activities of crude extract and fractions of *Curcuma longa* (turmeric) was evaluated against a clinical isolate of *M. tuberculosis* ($H_{37}R_v$) using microbroth dilution method. The activity of the extract and fractions were comparable to Rifampicin (0.04 µg/ml). Qualitative phytochemical components were determined using standard methods. The Minimum Inhibitory Concentration (MIC) was determined by tetrazolium dye assay using a microbroth dilution technique. Cold maceration of powdered turmeric (rhizome) with 70% ethanol yielded a crude extract (E), successive and exhaustive partitioning of E with petroleum ether (Ep), ethyl acetate (Eea) yielded soluble fractions as well as residual fraction (Eaq). The crude extract and fractions inhibited the growth of *M. tuberculosis* at 5000 µg/mL. The MIC of crude ethanol (E), aqueous (Eaq) and petroleum ether (Ep) were at 39.06±0.58 µg/mL and 0.78±0.02 %v/v while ethyl acetate (Eea) was at 19.53±0.50 µg/mL. The extract and all the fractions were bactericidal on *M. tuberculosis.* The minimum bactericidal concentration (MBC) of crude ethanol extract and aqueous fractions were at 78.13±0.51 µg/mL while the MBC of petroleum ether and ethyl acetate fraction were at 1.56±0.10 %v/v and 39.06±0.57 µg/mL. The crude extract and fractions contained alkaloids, flavonoids, tannins and saponins. The results obtained suggest that turmeric will be a good bio resource for anti-TB drug development.

**Keywords:** Tuberculosis, *Mycobacterium tuberculosis, Curcuma longa*, Phytochemical, MIC**.**

## INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by a slow-growing, acid-fast bacilli known as *Mycobacterium tuberculosis*. An estimation of 10.5 million people fell ill with tuberculosis and 1.2 million people died globally; it was also recorded that there was an additional 208,000 death among HIV-positive people (WHO, 2020). Nigeria is ranked the sixth country with TB burden with an estimate of 440,000 TB new cases. Patients do not exhibit any symptom of disease except when impairment of immunity arises due

to malnutrition, diabetes, malignancy and AIDS (Ogbo *et al.,* 2018). However, about 10% of healthy individuals may develop active tuberculosis in their life time due to genetic factors. The organisms reside in the granuloma in dormant state for decades by utilizing the host lipids, slowing down their replicative genes and waiting for an opportunity such as a weakened immune system to reactivate and cause active disease (Assam *et al*., 2020).

*Mycobacterium tuberculosis* is an intracellular pathogen with an unusual thick, waxy cell wall and a complex life cycle. The bacteria are transmitted by aerosol droplets and often infect the lungs and can also infect cells of bones, genitourinary tract, skin and joints (Gouzy *et al.*, 2021).

Treatment of tuberculosis is globally known as Directly Observed Treatment Short-course (DOTS). It is a multidrug and a long-term therapy comprising of numerous antibiotics with significant level of toxicity. After approximately 4 weeks of treatment with antibiotics, the patient's condition improves significantly, causing patients to discontinue the treatment regimen, which gives rise to drug-resistant persistent populations.

The global emergence of multidrug resistant (MDR), extensively drug resistant (XDR) and more recently totally drug resistant (TDR) strains of *M. tuberculosis* has become a major problem and cause of drug ineffectiveness (Ojo *et al.,* 2018; Samreen *et al.,* 2021). *Bacillus Calmette-Guerin* (BCG) is the only validated vaccine against pulmonary tuberculosis. Vaccination with BCG prevents the most severe forms of childhood tuberculosis by reducing the risk of meningeal and miliary TB by 85% and TB-associated deaths by 66%. The BCG vaccine protects children against TB for up to 10 years, but its efficacy declines with each subsequent year. It causes complications in immune-compromised individuals. *Bacillus Calmette- Guerin* does not provide protection against adult pulmonary TB and lacks any additional benefit upon revaccination of healthy and active TB cases. Furthermore, there is safety issues related to the BCG vaccine, as it is a live attenuated vaccine with a risk of becoming virulent (Whitlow *et al*., 2020).

Medicinal plants have been discovered and used traditionally in medicine practice since prehistoric times. Plants synthesize hundreds of chemical compounds for functions including defense against insects, diseases, and herbivorous mammals. The interest in plant derived drugs is mainly due to the current widespread belief that green medicine is safe and more dependable than expensive synthetic drugs, which have adverse side effects. Traditional treatment is practiced mostly on a local scale, therefore the plants used for treatment, vary considerably based on the country or place where it is used. Researchers from high TB burden countries have reported different plant species used locally by traditional healers for the treatment of tuberculosis and other diseases caused by mycobacteria (Miggiano *et al.,* 2020). These plants have become one of the remarkable alternatives for treatment since they are rich in numerous varieties of secondary metabolites such as alkaloids, flavonoids, tannins and phenolic compounds (Ngadino *et al.,* 2018).

Turmeric is a spice that is spread throughout the world's tropical and subtropical regions, with a short stem that bears 8-12 leaves. Curcumin (a flavonoid) is found abundantly in this plant. Curcumin enhances phagocytic activity of macrophages (Ong *et al*., 2020). The plant measures up to 1 meter long, it can be planted throughout the year preferably in the north and it is an important spice throughout the world. *Curcuma longa* (Turmeric) is a member of the *Zingiberaceae* family. It is known as *Ataile pupa* by Yorubas, *Gangamau* by the Hausas, *Turi* by Nupe, *Iblue* by the Urhobos, *Girgir* by the Tivs and *ntu ntu* in Igbo. In terms of medicinal properties, the plant has a lot of promise. It is anti-inflammatory, anti -tubercular, hepatoprotective, blood-purifying, antioxidant, liver tissue detoxifier and regenerator (Dejene, 2021). Due to urgent need for new anti-tubercular agents, it is particularly appropriate at this time to explore plants for the development of new anti-tubercular drugs. As such turmeric plant was selected for study of anti-tubercular potentials against *Mycobacterium tuberculosis* in the present study.

## MATERIALS AND METHODS

### Collection and Identification of Plant Materials

Fresh turmeric rhizomes were purchased from Kure market, Minna, Niger State, Nigeria in

November, 2019. Samples were transferred into sterile plastic containers and transported to the Laboratory of the Department of Biological Sciences, Federal University of Technology, Minna, for identification by an Ethnobotanist. The specimen was assigned Voucher number FUT/PLB/ZIG/004. Turmeric rhizomes were identified as *Curcuma longa* (*Zingiberacea*). Voucher specimens was deposited in the Herbarium Unit of Department of Plant Biology for future reference.

**Preparation and Extraction of Plant Materials**

The plant materials were washed in slow running tap water to remove dirt. They were diced into smaller pieces with a sterile knife and dried for two weeks at room temperature (28 ± 2°C). The air-dried materials were pulverized to powder with an electric blender (Brentwood JR-20OR).

Specifically, 400g of *C. longa* powder was extracted by cold maceration with 70% ethanol (2000 mL) for 5 days to yield a crude extract (coded E). Successive and exhaustive partitioning of extract E with petroleum ether and ethyl acetate gave rise to petroleum ether (coded Ep), ethyl acetate (coded Eea), soluble fractions as well aqueous residual fraction (coded Eaq) which were freeze dried using a freeze dryer (model: LGJ18) at -20 °C. The resulting weight was 21.7 g for crude ethanol, 5.62 g for petroleum ether fraction, 10.6 g for ethyl acetate fraction and 1.78 g aqueous residual fraction respectively. Extract and fractions were stored in well labelled airtight sterile bottles at 4 °C until required for use (Olatunji *et al.*, 2021). Prior to experiment, the crude extract and fractions were oven dried at 40 ˚C to remove any residual solvent of extraction that may interfere with test results.

**Phytochemical Screening of Turmeric Extract and Fractions**

Qualitative phytochemical screening was carried out on all the turmeric extract and fractions to detect the presence or absence of various secondary metabolites (alkaloids, saponins, flavonoids, tannins, phenolic acids) using standard method (Sayantani and *Ramachandra*, 2021).

**Anti-tubercular Assay**

**Source of test organism**

The microorganism used for this study was the clinical isolates of *Mycobacterium tuberculosis*($H_{37}R_v$). The organism was obtained from sputa of TB patients from Directly Observed Treatment Strategy (DOTS) Unit of the Diagnostic Laboratory of National Institute for Pharmaceutical Research and Development (NIPRD, 2020) Idu, Abuja, Nigeria.

**Digestion and Decontamination of *Mycobacterium tuberculosis* Clinical Isolate**

Two grams (2g) of sodium hydroxide was dispensed into 50 ml of sterile distilled water labeled A. The mixture was homogenized, in a separate conical flask. Specifically, 50 ml of the sterile distilled water, 1.45 g of sodium citrate was added and homogenized, and the reagent N-acetyl-L-cysteine was added labeled B. Equal volume of the reagents A and B above and the positive acid-fast bacilli (AFB) sputum sample was measured and homogenized with a votex mixer and allowed to stand for 15 minutes to digest and decontaminate the bacilli. Phosphate buffer was added to the broth to neutralize it and was centrifuged at 3000 rpm for 15 minutes (Izebe *et al.*, 2020).

**Identification and confirmation of the organism**

Identification and confirmation of the organism was carried out according to the method described by NIPRD (2020). *Mycobacterium tuberculosis* stock solution obtained from centrifuged sputum mixture above was inoculated into 10 ml sterile Middle Brook 7H9/Tween/ADC (Becton Dickinson and company, France) broth. The setup was incubated at 37˚C for 7 days with daily observation for turbidity monitored. Confirmation and identification of the organism was done using the hot Ziehl-Neelsen staining technique.

**Ziehl-Neelsen acid fast staining**

Ziehl-Neelsen staining technique was adopted to identify and confirm the acid-fast bacilli from other upper respiratory tract microflora in samples. The sputum was smeared on a clean slide and heat-fixed, carbol-fuchsin was poured on the smeared slide, heat fixed and allowed to remain for 3 to 5 mins before washing it off with water. An acid alcohol decolourizer was added in drops continuously on the smeared glass until the carbol-fuchsin faded away. The smeared glass

was then washed with water and methylene blue was applied and allowed to stay for 10-30 seconds. Furthermore, the methylene blue was washed off from the smeared glass and allowed to dry. The slide was observed under microscope (x 100) using oil immersion objective lens (CDC, 2012).

**Standardization of *Mycobacterium tuberculosis***

A measured quantity of 50 µl of *Mycobacterium tuberculosis* stock culture was inoculated into 50 ml of sterile 7H9/Tween/ADC broth. The setup was incubated at 30˚C for 7 days to obtain optical density of 0.2-0.3nm using UV-spectrophotometer at 650 nm. The turbidity of the culture was compared with 0.5 McFarland turbidity standards (approximately $1.5 \times 10^7$ cfu/ml). The culture was standardized to $10^6$ cfu/ml. The standardized culture was then used for anti-tubercular screening (NIPRD, 2020).

**Screening of extract and fractions for Anti-tubercular Activity**

About 100 µg/ml of each of the extract and fractions labelled E, Ep, Eea and Eaq was dissolved in 0.5 ml DMSO to aid dissolution and 0.5 ml sterile Middlebrook 7H9/ADC broth to obtain a stock concentration of 100 µg/ml. The stock concentration of samples was further diluted 1:10 by diluting 0.1ml of stock to 0.9 ml 7H9/ADC broth to obtain a final concentration of 10 µg/ml. Extract and fractions in oil form were dissolved with tween-80 in place of DMSO that was used for paste.

Fifty microliters of sterile middlebrook 7H9/ADC broth were introduced into well 2-12 of 96 micro broth well plate, 100 µl of extract prepared in middlebrook 7H9/ADC broth was introduced into well one (1), from which 50 µl was taken to the next well using a multichannel pipette repeatedly to well 11, then 50 µl was discarded to have equal volume. The well(s) were inoculated with 50µl of already standardized test organisms [well 1-12]. The plates were incubated for 7 days at 37°C. after the seven days incubation period, the wells were stained by adding 25 µl of tetrazolium dye and allowed to stand for 2 hours. The plates were then observed for presence or absence of microbial growth by colour change in the wells. Colourless wells were recorded as no growth of the test organisms (activity of extract/fraction), while a change in the initial colourless form to pink indicate growth of test organism (no activity of the extract/fractions). Well 12 was the organism viability control (OVC). The efficacy of extract and each fraction was compared with Rifampicin (0.04µg/ml), the procedure was repeated in triplicate (NIPRD, 2020).

**Determination of Minimum Inhibitory Concentration**

Minimum inhibitory concentration (MIC) of the extract and fractions was determined using the micro broth dilution method of NIPRD (2020). The MIC of turmeric extract/fraction was taken as the least concentration that inhibited the growth of *Mycobacterium tuberculosis* after incubation for 7 days.

**Determination of Minimum Bactericidal Concentration**

The minimum bactericidal concentration (MBC) of the extract and fractions was determined using the method of NIPRD (2020) that employ prolonged incubation technique. Specifically, 50µl of the last well before the MIC was diluted in peptone water to neutralize the antimicrobial agent. The diluted solution was inoculated into 10ml 7H9 middle brook broth and incubated for 3 weeks. Post incubation of the sample was observed for turbidity. Absence of turbidity was taken as bactericidal activity at the concentration before the MIC well.

**Statistical Analysis**

Data generated from MIC were expressed as mean values ± standard error of the mean (SEM). Within groups, comparisons were made by analysis of variance using a one-way ANOVA test (SPSS version 23).

## RESULTS AND DISCUSSION

**Qualitative phytochemical components of turmeric extract and fractions**

The phytochemical components in crude extract and fractions of turmeric are shown in Table 1. The extract and all the fractions contained alkaloids. Flavonoids were detected in ethanolic extract, petroleum ether and ethyl acetate fractions. Saponins were also detected in ethanolic extract and ethyl acetate fractions. Tannins were only present in ethanolic extract

while phenols were only detected in ethyl acetate fraction.

**Table 1:** Qualitative phytochemical components of crude ethanolic extract and fractions of turmeric

| Phytochemical components | E | Ep | Eea | Eaq |
|---|---|---|---|---|
| Saponins | + | - | + | - |
| Alkaloids | + | + | + | + |
| Flavonoids | + | + | + | - |
| Phenols | - | - | + | - |
| Tannins | + | - | - | - |

+: present, -: not detected, E: crude ethanol extract, Ep: petroleum ether fraction, Eea: ethyl acetate fraction, Eaq: aqueous fraction

**Anti-tubercular Activity of *C. longa* against *Mycobacterium tuberculosis* $H_{37}R_v$**

Tables 2 and 3 show the anti-tubercular activity of turmeric extract and fractions against *Mycobacterium tuberculosis.* Crude ethanolic extract (E) and aqueous (Eaq) fractions inhibited the growth of *M. tuberculosis* with the MIC at 39.06±0.58 µg/mL while petroleum ether (Ep) and ethyl acetate (Eea) MIC were at 0.78±0.02 %v/v and 19.53±0.50 µg/mL. Furthermore, Crude ethanolic extract (E) and aqueous (Eaq) fractions exhibited bacteriocidal effect on *Mycobacterium tuberculosis* with MBC value of 78.13±0.51 µg/mL while petroleum ether (Ep) and ethyl acetate (Eea) MBC were at 1.56±0.10 %v/v and 39.06±0.57 µg/mL.

**Table 3: Minimum inhibitory concentration and Minimum bactericidal concentration of turmeric crude extract and fractions against *Mycobacterium tuberculosis* ($H_{37}R_v$)**

| Sample | MIC | MBC |
|---|---|---|
| Crude (Ethanol) | $39.06\pm0.58^{d}$ | $78.13\pm0.51^{d}$ |
| Ethyl acetate | $19.53\pm0.50^{c}$ | $39.06\pm0.57^{c}$ |
| Petroleum ether | $0.78\pm0.02^{b}$ | $1.56\pm0.10^{b}$ |
| Aqueous | $39.06\pm0.50^{d}$ | $78.13\pm0.72^{d}$ |
| Rifampicin | $0.04\pm0.00^{a}$ | $0.04\pm0.00^{a}$ |

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

Values are presented as means ± standard error of mean (SEM) of three replicates. Values with different superscripts along column are significantly different at $p < 0.05$.

## DISCUSSION

In this study, it was observed that saponins, alkaloids, flavonoids, phenols and tannins were present in the extract and fractions of *Curcuma longa* (Table 1). Several authors reported the presence of these phytocomponents in *C. longa* extracts (Ngadino *et al*., 2018; Farrar *et al.,* 2021; Muhammad and Fathuddin, 2021; Pakadang *et al*., 2021). Izebe *et al*. (2020) also reported the presence of saponins, alkaloids, tannins and phenols in *Tetrapleura tetraptera*. Poro *et al.* (2021) detected similar constituents from *Carriss aedulis* extract. Flavonoids induce cell death and inhibit the growth of *Mycobacteria*, by interfering with mycolic acid biosynthesis (Alka *et al*., 2020). According to Anwar and Triyasmono (2018) and Hossain *et al*. (2019), flavonoids also inhibit the cytoplasmic membrane function, DNA gyrase and β-hydroxyacyl-acyl carrier protein.

Arya (2018), reported that alkaloids inhibit ATP-dependent transport of compounds across cell membrane, disrupt the peptidoglycan component of the bacterial cell thereby affecting its integrity and also inhibit the amino acid and DNA synthesis. On the other hand, phenols inhibit efflux pumps (Mazlun *et al*., 2019). Saponins help to lower the surface tension of the bacterial cell wall, thus, damaging the membrane permeability, leading to lysis which makes enzymes and proteins exit the cell, consequently resulting to cell death (Pakadang *et al*., 2021). The inhibitory activity of turmeric extract and fractions could be as a result of the extraction method used, the location in which the plant materials were collected and the type of turmeric used (Farrar *et al*., 2021). Thus, cold maceration employed for the extraction of the plant may be a suitable technique as components of the extract and fractions that may be heat labile are retained. Samreen *et al.* (2021) reported that curcumin is the major component of *Curcuma longa* and is known to prevent anti-TB drug-induced hepatic damage and exhibit dose-dependent inhibition of intracellular growth for *Mycobacterium tuberculosis* $H_{37}R_v$. Bai *et al.* (2016) and Barua *et al*. (2021) demonstrated that *Curcuma longa*

increased the ability of human monocytic cells (THP-I cell line) infected with *Mycobacterium tuberculosis* $H_{37}R_v$ to control infection via inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and induction of apoptosis and autophagy through caspase-3 dependent pathway.

**Table 2: Anti-tubercular activity of turmeric crude extract and fractions against *Mycobacterium tuberculosis* ($H_{37}R_v$)**

| Agents | Concentrations (µg/mL) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 78.13 | 39.06 | 19.53 | 9.77 | 4.88 |
| Crude (Ethanol) | + | + | + | + | + | + | + | + | - | - | - |
| Ethyl Acetate | + | + | + | + | + | + | + | + | + | - | - |
| Aqueous | + | + | + | + | + | + | + | + | - | - | - |
| Rifampicin | + | + | + | + | + | + | + | + | + | + | + |
| | concentration (%v/v) | | | | | | | | | | |
| Petroleum Ether | 50 | 25 | 12.5 | 6.25 | 3.125 | 1.562 | 0.781 | 0.390 | 0.195 | 0.097 | |
| | + | + | + | + | + | + | + | - | - | - | |

- : No activity, + : Activity, µg/mL: microgram per milliliter, %: percentage, v/v: volume per volume.

The crude extract and fractions of turmeric rhizomes were able to inhibit the growth of *Mycobacterium tuberculosis* at 5000µg/mL (5mg/ml) as shown in (Table 2), which corroborate the findings of Poro *et al*. (2021) on inhibition of *M. tuberculosis* with *Carissa edulis* extract at 5000 µg/ml. This observation supports our findings of turmeric as a potent anti-tubercular extract. The inhibitory potential exhibited by the crude extract and fractions may be due to the presence of phytoconstituents which may be acting singly or in combination to inhibit the growth of the organisms (Array *et al.,* 2019; Izebe *et al.,* 2020).

According to Poro *et al*. (2021) crude extract and fractions are considered to have antimicrobial activity during a susceptibility test if its minimum inhibitory concentration (MIC) is in the range of 100-1,000 µg/mL. The activity is significant when the MIC is less than 100 µg/mL, moderate when the MIC is between 100 - 625 µg/mL and low when is higher than 625µg/ml. The turmeric extract and fractions used in this study were able to inhibit the growth of *M. tuberculosis* with MIC within 19.53 µg/mL (0.02 mg/mL) - 39.06 µg/mL (0.04 mg/mL). Therefore, the rhizomes of *C. longa* extract and fractions have significant activity. This value was comparable to value of *Terminalia catappa* extract that inhibited the growth of *M. tuberculosis* with MIC of 39.06µg/ml as reported by Chaudhari and Badole (2014). Chinsembu (2015) reported that the extract of *Berchemia discolor* exhibited an MIC at 12.5 µg/mL and was effective against *M. tuberculosis,* a value that is slightly lower than the MIC of *C. longa* recorded in this study. Oladosu *et al.,* (2013) studied the inhibitory effects of three antitubercular bioactive compounds from *Acacia nilotica* and reported that they exhibit significant inhibitory effect on *M. tuberculosis* with an MIC of 41±2.65 (Table 3). A value higher than the MIC of 19.53±0.50 obtained from ethyl acetate fraction of *C. long* in the current study.

Safitri *et al*. (2017) reported that *C. longa* rhizomes inhibited the growth of *Mycobacterium tuberculosis* $H_{37}R_v$ with MIC at 187.5 µg/mL. Nagdino *et al.* (2018) showed that ethanolic extract of *Curcuma xanthorrhiza* exhibited an MIC of 1600 µg/mL against *M. tuberculosis* $H_{37}R_v$. The study undertaken by Poro *et al*. (2021) with *Vitex doniana* hydroethanolic crude extract showed MIC at 3125 µg/mL against *M. tuberculosis.* These MIC values obtained are higher than those obtained in the present investigation. This indicates that the ethanolic, ethyl acetate, petroleum ether and aqueous extracts used in this study showed better inhibitory activities against *M. tuberculosis.* The

ethyl acetate fraction was most potent in the present investigation with an MIC of 19.53 µg/mL. This may be due to the ability of ethyl acetate to extract both lipophilic and non-lipophilic compounds. The lipophilic components in the extract enhanced the penetration of hydrophobic outer membrane of *Mycobacterium* species to exert inhibitory effects (Babayi *et al.*, 2022).

In contrast to the findings of this research, Sivakumar *et al.* (2011) reported that ethanolic and aqueous extracts of *C. longa* were not able to inhibit the growth of *M. tuberculosis* $H_{37}R_v$. This might be as a result of the extraction method (boiling of the rhizomes) which might have denatured the bioactive components present in turmeric and also the use of crude aqueous extracts. The difference in the results obtained and other reports, by researchers could be due to different extraction methods, choice of solvents, plant materials used, time of sample collection, soil nutrient available, temperature, water quality, rooting, aeration and the type of turmeric rhizome (Nagdino *et al.*, 2018). The result obtained from this study with MIC ranging from 0.02 mg/ml (19.53µg/ml) to 0.04 mg/ml (39.06 µg/ml) as compared to the standard drug Rifampicin with MIC at 0.04 µg /ml showed that possibly on further purification to eliminate impurities which may interfere with and reduce the potency of the fractions, *C. longa* can serve as a lead in research and development of new drugs to combat resistant strains of *Mycobacterium*.

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**CONFLICT OF INTEREST**

There is no conflict of interest from the co-authors. All the authors cited in the study were duly acknowledged.

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