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Proximate Analysis and Time Killing Kinetics of *Calotropis procera* Extracts on Some Selected Pathogens

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OSA, KOO and AOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OSA and KOO managed the analyses of the study. Authors OSA, KOO and AOO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The proximate composition and time killing kinetics of the leaf and stem extracts of *Calotropis procera* were carried out. The proximate composition showed moisture content of (10.45 and 9.78%), protein (16.20 and 8.15%), fat (1.99 and 0.96%), ash (14.32 and 6.39%), crude fibre (6.73 and 23.23%) and carbohydrate (49.49 and 51.49%) for leaf and the stem respectively. Twelve pathogenic bacteria and five fungi species were obtained from the Department of Microbiology, Federal University of Technology, Akure, Ondo-State and typed cultures of the organisms were collected from National Institute of Medical Research (American type culture collection centre (ATCC), USA). The time-kill studies are important because comprehensive information about pharmacodynamics of a putative antibacterial agent may not be gained simply through endpoints such as Minimum Inhibitory Concentration. This study is done to examine the time-frame required for the microbes to be killed. It was determined on each isolates with the extracts taken at their

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Minimum Inhibition Concentration values. The study was evaluated in hours of 0 hr, 6 hrs, 12 hrs, 24 hrs, 48 hrs, 72 hrs and 96 hrs, the methanol leaf extract kill most of the organisms within 24 hrs while aqueous leaf extract was unable to kill most of the organisms under 48 hrs.

Keywords: Calotropis procera; proximate; killing; extracts; analysis; composition.

1. INTRODUCTION

Calotropis procera holds a reputed position as a medicinal plant. *Calotropis* was formerly placed in the family of Asclepiadaceous (the milkweed family), which is now considered a subfamily of the Apocynaceae [1]. This is a large family of plants including 415 genera and about 4555 species distributed largely throughout the tropics. It is often dominant in areas of abandoned cultivation, especially on sandy soils in areas of low rainfall. It can be used as an indicator of over-cultivation [2]. The plant can spread rapidly from the base of the plants, and can regrow from the root system is favorable conditions even if the aboveground plant has disappeared [3]. All parts possess valuable medicinal properties. According to Ayurvedic medicine, the whole plant is alexipharmic and cures leprosy, ulcers, and spleen and liver diseases. The juice is laxative, anthelmintic and cures piles. Root bark is diaphoretic and cures asthma and syphilis. Flowers are analgesic, astringent and cure inflammations ad tumors. [4] found anti-inflammatory activity in rats of a chloroform-soluble fraction from the roots [5] investigated antimicrobial activity of *C. procera*. Various cultures and civilizations from ancient times to the present day have depended either fully or partially on herbal medicines because of their effectiveness, affordability, low toxicity and acceptability [6]. The main purpose of this investigation is to determine the proximate composition and time-killing kinetics assay.

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation

The plant parts were collected from Elekute quarters, Ado-Ekiti, Ekiti State, Nigeria. The plant parts (leaves and stem) were adequately washed with clean water and air dried at room temperature (25±2˚C). They were then pulverized (crushed) separately with grinding machine to obtain smooth powder.

2.2 Preparation of Crude Extracts

2.2.1 Aqueous extraction

One hundred and fifty grams (150 g) of the ground powder of each of the plant parts were soaked separately in 750 ml sterile distilled water for 72 hours at room temperature.

2.2.2 Organic solvent extraction

One hundred and fifty grams of the ground powder of each of the plant parts (leaf and stem) were soaked separately with the solvents in 750 ml capacity flask for 72 hours. They were then filtered through a 3-layered sterile muslin cloth. The solutions were concentrated *in vacuo* in a rotary evaporator to remove the solvent. This was reconstituted with 5% aqueous $DMSO_4$ before has been used for antimicrobial analysis [7].

2.2.3 Sources of bacterial cultures

Test microorganisms: They includes typed cultures of bacteria and fungi (American type culture collection centre (ATCC) USA and microbial isolates from the stock cultures of the Department of Microbiology, Federal University of Technology, Akure and Department of Biological Sciences, Afe Babalola University, Ado Ekiti.

The microorganisms used in this study include *Shigella dysenteriae, S. dysenteriae* ATCC 24162*, Escherichia coli, E. coli* ATCC 35218, *Staphylococcus aureus, S. aureus* ATCC 25923, *Samonella typhi, S. typhi* ATCC 22648*, Pseudomonas aeruginosa, P. aeruginosa* ATCC 27853*, Klebsiella pneumonia, and K. pneumonia* ATCC 34089, *Aspergillus flavus, Aspergillus flavus* ATCC 204304*, Malazessia furfur* ATCC 44349*, Candida albicans and C. albicans* ATCC 10231.

2.3 Proximate Analysis

The crude protein, lipids, fiber and ash were determined in triplicate using the methods described by [8].

2.4 Analysis of Time-Kill Kinetic Assay

The rate of bacteria and fungi killing were determined using leaf and stem extract at its MIC value for each bacterial and fungal isolates by time-kill kinetic assay as described by [9]: Overnight (24 hours) of nutrient broth bacterial cultures were diluted to 5 × 10^5 cfu (colony forming unit)/ml with freshly prepared nutrient broth and 1ml MIC of the plants leaf extract for each bacterial isolate was added. The broth cultures were incubated at 37°C with agitation at 160 rpm in a shaker water bath. 5 ml aliquot of each culture was collected at different time intervals (0 h, 1 h, 6 h, 12 h, 24 h, 36 h and 48 h), serially diluted to 1.0 × 10^5 with nutrient broth and 1 ml was plated onto nutrient agar plates. After incubating the plates at 37°C for 16 hours viable colonies were enumerated. The bacterial population was recorded in terms of log^{10} cfu and plotted against time for each bacterial isolate.

3. RESULTS

The proximate analysis of stem and leaves samples of *Calotropis procera* was performed for ash, protein, fat, crude fibre, carbohydrate and moisture. Generally the leaf has the highest values in all the parameters except crude fibre which was higher in stem (23.30%) than leaf (6.80%) and crude protein of the leaf was 16.53±1.01 and for stem 8.25±0.04 (Table 1).

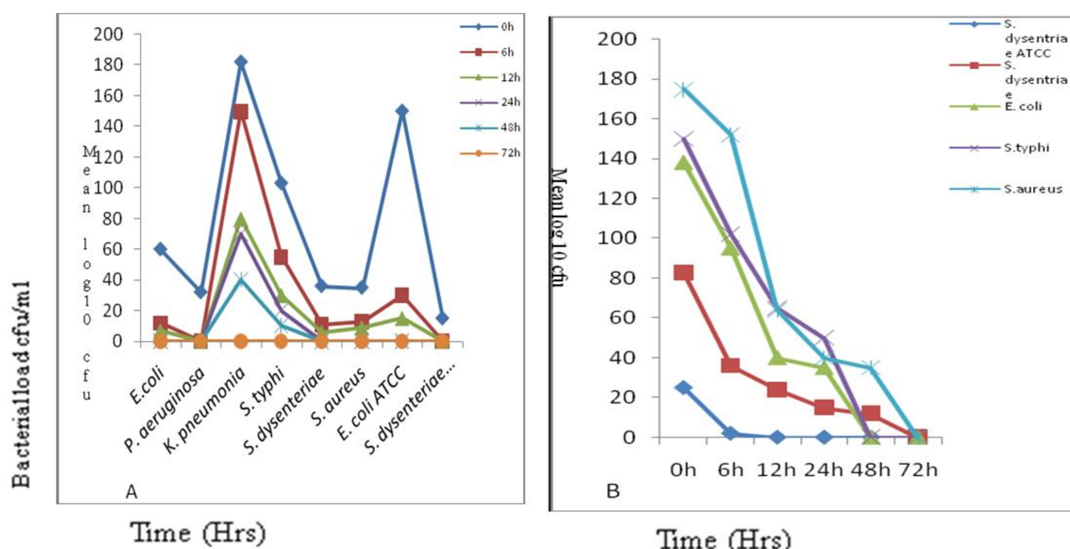
Table 1. Proximate composition of stem and leaf of *Calotropis procera* (%)

Parameters	Leaf	Stem
Moisture	10.45±0.50^c	9.78±0.10^d
Protein	16.20±1.0^e	8.15±0.04^c
Fat	1.99±0.05^a	0.96±0.01^a
Ash	14.32±0.50^d	6.39±0.03^b
Crude fibre	6.73±0.02^b	23.23±2.23^f
Carbohydrate	49.49±5.67^f	51.49±5.34^e

Figures on the same column are having the same alphabet which means they are not significantly different from each other and those with different alphabet are having different column and are significantly different from each other

3.1 Time Killing Kinetics

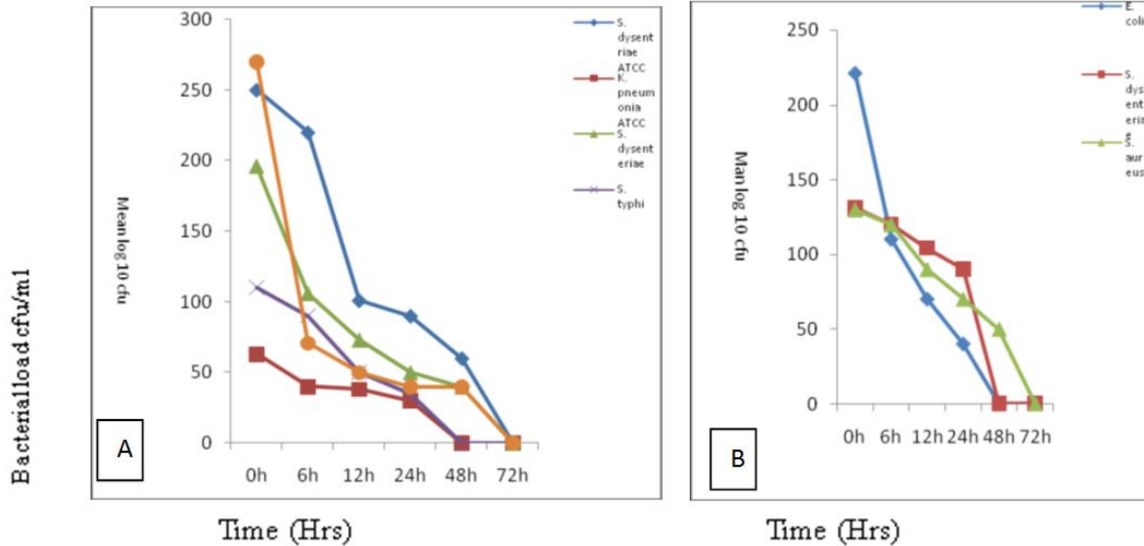
The time-killing kinetics of the extracts on the organisms was carried out. The rate of killing of the methanol leaf extract on bacterial isolates like *E. coli*, *K. pneumoniae*, *S. aureus*, *P. aeruginosa, S.typhi, S. dysenteriae, E. coli* ATCC 35218 and *S. dysenteriae* ATCC 24162 was carried out (Fig. 1). Most of the isolates were killed before 48hrs-72hrs for instance comparing two isolates; *S. typhi* and *S. dysenteriae* ATCC 24162 were killed at 24 hrs. This study also include the rate of killing of the ethanol leaf extract on the same bacterial isolates (Fig. 2) where *S. dysenteriae* ATCC 24162 was killed at 12 hrs and *S. typhi* was killed at 48 hrs.



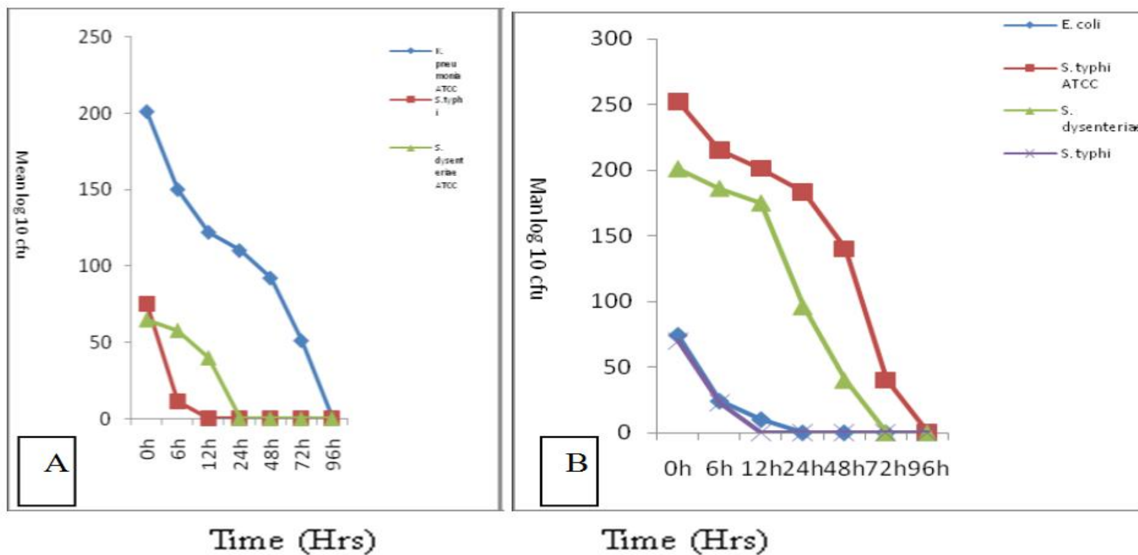
A) Fig. 1. Rate of kill of the methanol- leaf extracts on bacterial isolates
B) Fig. 2. Rate of kill of the ethanol-leaf extract on bacterial isolates

The acetone leaf extract of the bacterial isolates were also analyzed (Fig. 3) whereby all the isolates (*S. dysenteriae* ATCC 24162*, K. pneumoniae* ATCC 34089*, S. dysenteriae and S. typhi* were not killed until 48-72 hours.

Rate of kill of the aqueous leaf extract on bacterial isolates were also carried out (Fig. 4); fewer organisms like *S. aureus*, *S. dysenteriae* and *E. coli* were susceptible to the extract and all these isolates were not killed until 72hrs while Fig. 5 shows the rate of kill of the methanol stem extract on the bacterial isolates where *S. typhi* and *S. dysenteriae* ATCC were killed before 24 hrs.



A) Fig. 3. Rate of kill of the acetone-leaf extract on bacterial isolates
B) Fig. 4. Rate of kill of the aqueous- leaf extract on bacterial isolates



A) Fig. 5. Rate of kill of methanol-stem extract on bacterial isolates
B) Fig. 6. Rate of kill of the ethanol-stem extract on bacterial Isolates

Time killing kinetics assay for the ethanol stem extract was presented in Fig. 6, where all the isolates were killed by the extract under 24 hrs. *E. coli* and *S. typhi* ATCC 22648 were killed at 72-96 hrs. Fig. 7 shows killing effects only on *S. dysenteriae* ATCC 24162 and *S. typhi* within 24 hrs.

Most of the isolates were killed under 72 hrs with the aqueous stem extracts (Fig. 8). Fig. 9 shows the rate of kill of the methanol leaf extracts on fungi isolates whereby the clinical isolates of *C. albicans* was killed by the extract earlier than the typed isolate (*C. albicans* ATCC 10231).

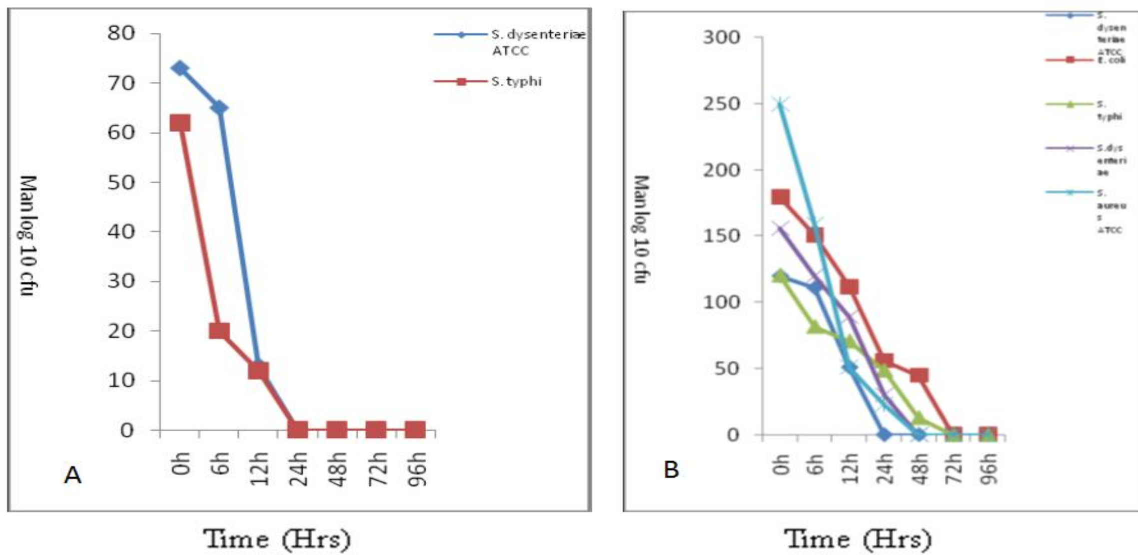
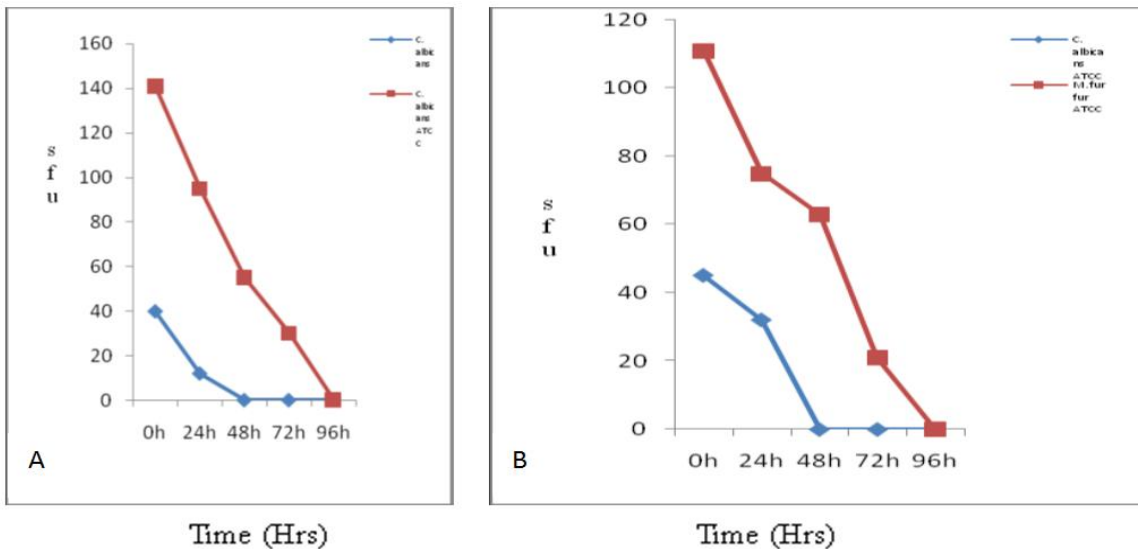


Fig. 7. Rate of kill of the acetone-stem on bacterial Isolates
Fig. 8. Rate of kill of the aqueous-stem extracts on bacterial isolates

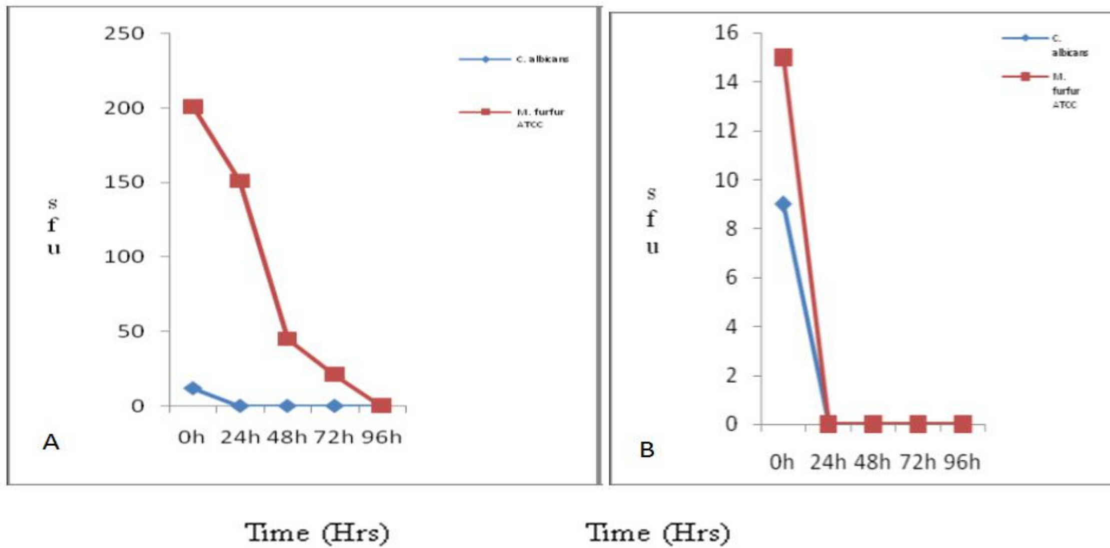


A) Fig. 9. Rate of kill of the methanol-leaf extracts on fungi isolates
B) Fig. 10. Rate of kill of the ethanol-leaf extract on fungi isolates

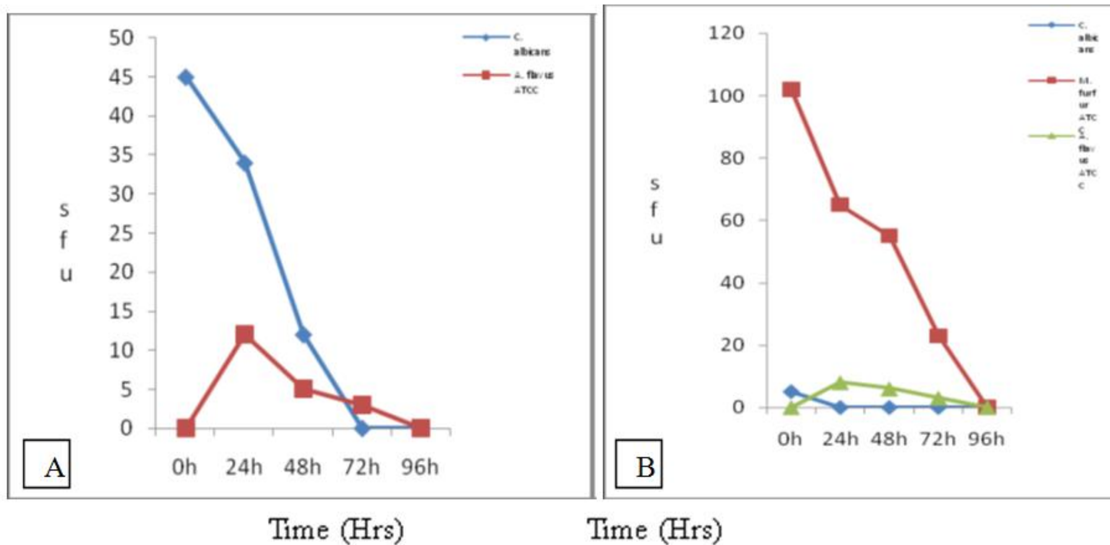
The rate of kill of the ethanol leaf and acetone leaf extract on the fungi isolates in represented in Figs. 10 and 11 respectively where *C. albicans* was killed by ethanol leaf extract within 48 hrs and *M. furfur* ATCC 44349 under 96 hrs. In Fig. 11, *Candida albicans* was killed at 24 hrs and *M. furfur* ATCC 44349 at 96 hrs.

The rate of kill of the ethanol and acetone stem extract on fungi isolates were carried out and showed in Figs. 14 and 15 respectively whereby in Fig. 14, *A. flavus* ATCC 204304 and *M. furfur* ATCC 44349 were not killed until 96 hrs but *C. albicans* was killed at 24 hrs. In Fig. 15, both the two isolates were killed at 96 hrs.

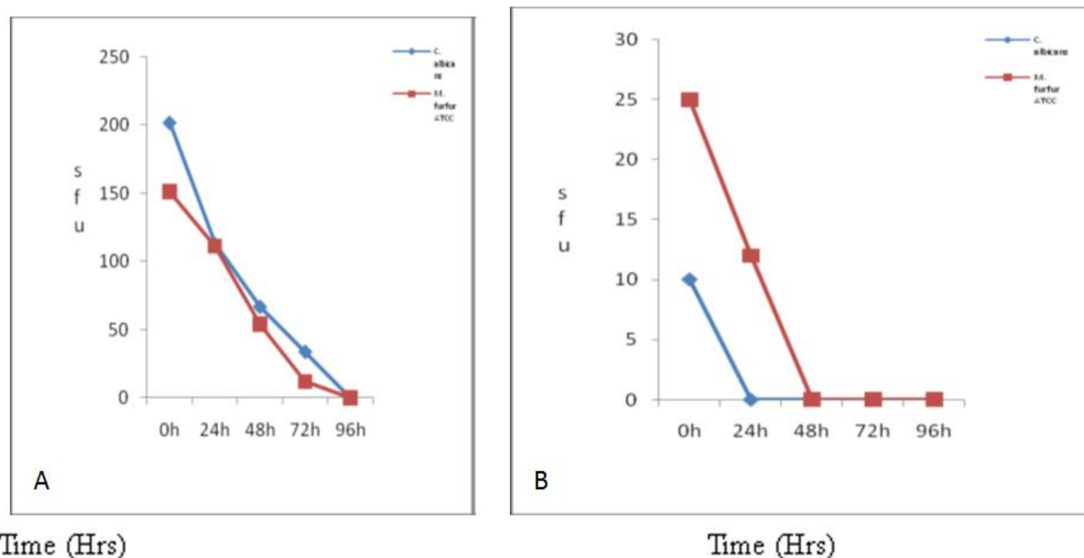
Rate of kill of the aqueous stem extract on fungi isolates were also analyzed in Fig. 16; fewer isolates like *C. albicans* and *M. furfur* ATCC 44349 were susceptible to this extract and *C. albicans* was killed before *M. furfur* ATCC 44349



A) Fig. 11. Rate of kill of the acetone-leaf extract on fungi isolates
B) Fig. 12. Rate of kill of the aqueous-leaf extract on fungi isolates



A) Fig. 13. Rate of kill of the methanol-stem on Fungi isolates
B) Fig. 14. Rate of kill of the ethanol-stem extract on fungi isolates



A) Fig. 15. Rate of kill of the acetone-stem extract on fungi isolates
B) Fig. 16. Rate of kill of the aqueous-stem extract on fungi isolates

4. DISCUSSION

Different literatures and reviews have shown that *Calotropis procera* is a resuscitative plant due to its large number of medicinal properties which also have being proved as potential therapeutic in this study. The proximate composition is an important index to classify the nutritional value of a food material [10]. The proximate composition of the stem and leaves of *C. procera* were carried out and the time-kill kinetic assays were observed in this study. It revealed that the dominant components are protein, ash, crude fibre and carbohydrate. However lipid contents were relatively lower. The concentration of the carbohydrate was higher, a sample with high level of carbohydrate can regulate nerve tissue, Carbohydrate readily accessible fuel for physical performance and regulate nerve tissue and the crude protein content would serve as enzymatic catalyst, mediate cell responses, control growth and cell differentiation [11], the crude fibre content especially the stem implies that they can serve as a source of dietary fibre and can also be used in the treatment of diabetes, obesity and gastrointestinal tract diseases [12]. Ash contents give an idea about the inorganic content and they are also expected to facilitate the metabolic processes, growth and development. The average moisture content was observed and too much of moisture in any sample has been proved to cause caking especially in flour and can also determine the storage and the viability of microorganisms growth [13] and it displayed more information about the storage /shelve life and the viability of microorganisms growth. Therefore, time-killing kinetics assay are required to quantitate pharmacodynamics of a putative antibacterial agent by quantifying the decrease in bacterial growth as a function of time and the drug concentration [14,15]. It was observed that some organisms like *Candida albicans* were killed within 48 hours which shows that the plant has good antimicrobial activities. Herbal medicines are generally believed as safe; however, it is important to evaluate their biological safety before use to avoid fatal consequences. There is no doubt in pharmacological properties of *C. procera* but its toxicological assessment is also indispensable. In view of this, further work on its toxicity will be evaluated.

5. CONCLUSIONS

The present study has investigated the nutritional values of *Calotropis procera* as a source of carbohydrate, ash, fibre and moisture. Due to the time killing kinetics of the *Calotropis procera* extracts, it may be said that it can be effectively used in curing the diseases that could be manifested by the human pathogenic bacteria employed in this study. Therefore, time-killing kinetics assay are required to quantitate pharmacodynamics of a putative antibacterial agent by quantifying the decrease in bacterial growth as a function of time and the drug concentration. The findings in this study

displayed levels of time dependent bacterial inhibition that were different among the tested bacteria and the concentrations, regardless of being gram-negative or gram-positive. These findings might suggest that kinetics of responding to bacterial and fungal strains to the *Calotropis procera* extract during the first 24 hours does not necessarily depend on being gram-negative or gram-positive or the morphology of the fungi.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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