Release of sodium and potassium ions of Calotropis procera extract on selected pathogenic organisms

Oluwakemi-Sola, Asoso[1]; K.O., Oladunmoye M.K.[2] ; A.O., Ogundare[2]
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[1]Department of Biological Sciences, Afe Babalola University, Ado-Ekiti, Nigeria, E-mail address:oyesolakemi@gmail.com
[2] Department of Microbiology, School of Sciences, Federal University of Technology, Akure, Ondo State, Nigeria. E-mail: chourlar@yahoo.com.

Abstract

The rate of release of sodium and potassium ions by aqueous, ethanol, methanol and acetone extract of leaves and stem of *Calotropis procera* were investigated on selected pathogenic microorganisms in the genera of *Salmonella typhi, Escherichia coli, Shigella dysenteriae, Staphylococcus aureus, Candida albicans, Malazzesia furfur* ATCC 44349 and their typed isolates using flame photometer. The aqueous extract was found to be more effective in some organisms in the leakage of sodium and potassium ions whereas other solvent like acetone and methanol extract also were more effective in other type of organisms. For example; the amount of sodium released by stem extracts showed that *S. typhi and C. albicans* had the highest amount of 23 and 24mg/ml respectively while *E. coli* had the highest amount of sodium released by stem extracts of 20mg/ml. The comparison of the amount of sodium and potassium ions released by the plant extracts with two commercial antibiotics (Tetracycline and Amoxycillin) showed that the amount of sodium released by both amoxicillin and tetracycline *of Calotropis procera* using selected pathogenic organisms were higher than the amount of potassium ion released.

Keywords: bacteria; *Calotropis procera*; pathogenic organisms; sodium ions; potassium ions; plant extracts.

Liberación de iones sodio y potasio en extracto de *Calotropis procera* Linn en determinados microorganismos patógenos

Resumen

Se investigó la tasa de liberación de iones de sodio y potasio para los extractos acuoso, etanólico, metanólico y en acetona de hojas y tallo de *Calotropis procera procera* L en microorganismos patógenos seleccionados de los géneros *de Salmonella typhi, Escherichia coli, Shigella dysenteriae, Staphylococcus aureus, Candida albicans, Malazzesia furfur* ATCC 44349 y sus tipos de aislantes, usando la técnica de fotometría de llama. Se encontró que el extracto acuoso era más efectivo para algunos organismos en la salida de sodio e iones del potasio; mientras que otros extractos con solventes como acetona y metanol, también eran eficaces para otro tipo de organismos. Por ejemplo, la cantidad de sodio liberado por los extractos de tallo, mostró que *S. typhi y C. albicans* presentaron la mayor cantidad de 23 y 24 mg/ml respectivamente. A diferencia de *E. coli,* que tuvo la mayor cantidad de sodio liberada de 20 mg/ml por extractos de tallo. La comparación de la cantidad de iones de sodio y potasio liberados por los extractos de la planta con dos antibióticos comerciales (tetraciclina y amoxicilina), mostró que la cantidad de sodio liberado por amoxicilina y tetraciclina de *Calotropis procera,* usando organismos patógenos seleccionados, fue mayor que la cantidad de iones de potasio liberados.

Palabras Clave: *Calotropis procera;* bacterias; extracto de plantas; iones de sodio; iones de potasio; organismos patógenos.

INTRODUCTION

Calotropis. procera holds a reputed position as a medicinal plant in different systems of medicine In India. 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 ad cures asthma and syphilis. Flowers are analgesic, astringent and cure inflammations ad tumors. Basu and Chaudhuri (1) found anti-inflammatory activity in rats of a chloroform-soluble fraction from the roots. Jain et al. (2) investigated antimicrobial activity of *C. procera.* The maximum inhibitory activity was observed in ethanol extracts of root bark against Enterobacter *cloaceae* and of stem against *Fusarium moniliforme*. The Latex of *C. procera* is used in traditional medicine as a purgative, antisyphylitic and antiodontalgic agent and as a cure for verrucas.

Extracts from latex, leaves and flowers in Morocco had more effect on yeasts than on fungi. According to the Unani system of Medicine (3), this plant is useful against leprosy, scabies, and ringworm of the scalp, piles, asthma, liver and spleen enlargement and dropsy. *Calotropis procera* extracts can be used as a coagulant in cheese-making (4) (5). The uses of this plant from NIF (National Innovation Foundation) database for Asthma; the flower powder is been used, Ear Ache; the latex of the plant is used, according to literature the plant extract is used as bronchodilator (6); flower buds of *Calotropis*, along with black pepper seeds and salt, are crushed to make pills the size of small peas.

The number of mechanisms exists for the antimicrobial activities of many antimicrobial agents including plant extracts (7).There has not been any information on the release of sodium and potassium ions by extract from *Calotropis procera.* The present studies was aimed at determining the leakage of sodium ions by extracts of *Calotropis procera* on some selected pathogenic bacteria so as to determine whether the antimicrobial activities and medicinal properties of the plants is due to leakage of protoplasmic materials or not (8)(9).

Materials and method

Collection of plant samples

Apparently healthy plant namely *C. procera* were collected from Ado-Ekiti, Ekiti State Nigeria.

Preparations of Plant Extracts

The plants parts leave and stem were air-dried for 5 weeks at room temperature (25 + 2 oC) and then ground to powder with a mechanical grinder (Thomas Wiley machine, model 5 USA). Powders (200gs) of each plant were extracted with 1litre of sterile aqueous water, ethanol, methanol and acetone separately at room temperature (25 + 2 oC). They were labeled as crude extracts.

Sources of test isolates

They include type cultures of bacteria and fungi from American type culture collection center (ATCC) at NIMR (Nigeria Institute of medical research) and the clinical isolates from the stock

Bacteria

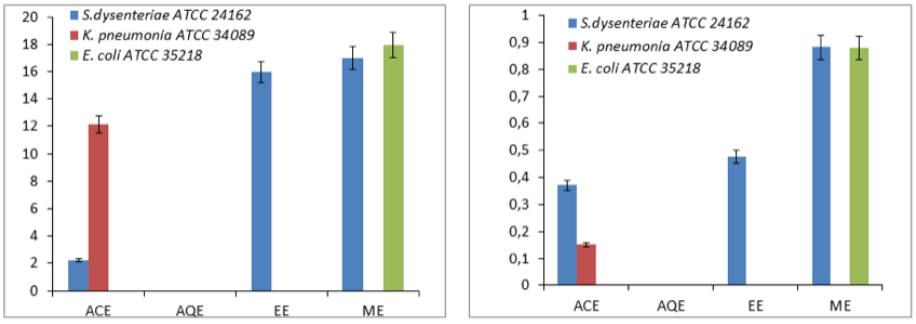
Shigella dysenteriae, S. dysenteriae ATCC 24162, *E. coli, E. coli* ATCC 35218, *Staphylococcus aureus, S. aureus* ATCC 25923, *Salmonella typhi, S. typhi* ATCC 22648, *Pseudomonas aeruginosa, P. aeruginosa* ATCC 27853, *Klebsiella pneumoniae, and K. pneumoniae* ATCC 34089

Determination of sodium and potassium ion leakage

Exactly 0.5 ml each of the standardized organism was added to 4.5 ml of the prepared concentration of the leaf extracts and then incubated for 18 hours. The solution was centrifuged at 7000 revolutions per minute (r. p. m.) and the supernatant analyzed using a flame photometer at 589 nm and 766 nm for sodium and potassium ion leakage respectively. The sodium and potassium ion leakage was determined using the method of Oladunmoye et al. (10).

RESULTS

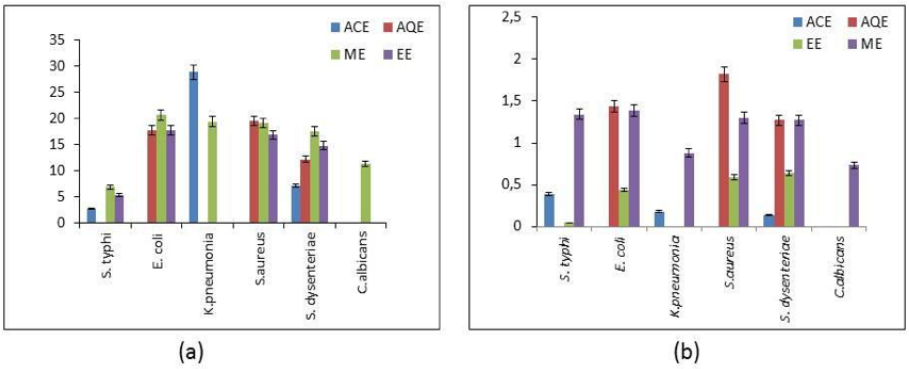
The amount of sodium released by leaf extract using some typed isolates namely *S. dysenteriae* ATCC 24162, *K. pneumonia* ATCC 34089 *and E. coli* ATCC 35218; *E. coli* ATCC 35218 had the highest amount of sodium in methanol-leaf extract (Figure 1a). The content of potassium released by leaf extracts especially for methanol leaf extracts was higher for both *E. coli* ATCC 35218 and *S. dysenteriae* ATCC 24162 with value of 0.9 mg/ml (Figure 1b).



Plant extracts: Ace- acetone extract (ACE), Aqe- aqeuous extract(AQE), E- ethanol extract (EE), Me- methanol extract (ME)

Figure 1. Amount of ions (mg/ml) released by leaf extracts of *Calotropis procera* using some typed cultures: (a) sodium (b) potassium.

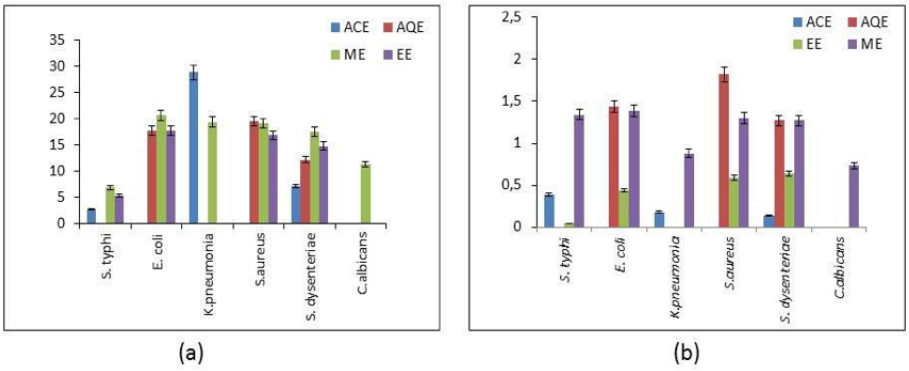
The aqueous leaf extract had the highest amount of sodium especially in K. pneumoniae followed by methanol leaf extract in almost all the organisms (Figure 2a). The amount of potassium leakage by leaf extracts for clinical isolates was also carried out; whereby aqueous and methanol leaf extracts had highest amount of potassium compared to other leaf extracts (Figure 2b).



Ace- acetone extract (ACE), Aqe- aqeuous extract(AQE), E- ethanol extract (EE), Me- methanol extract (ME)

Figure 2. Amount of ions (mg/ml) released by leaf extracts of Calotropis procera by leaf extracts using some clinical isolates: (a) sodium (b) potassium.

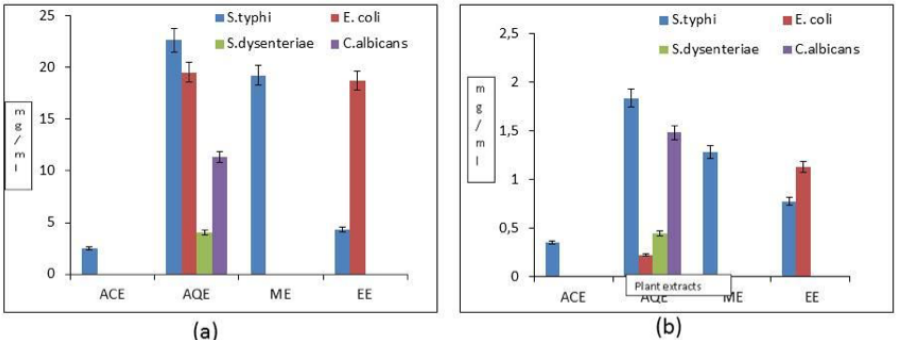
Candida *albicans* ATCC 10231 and other isolates were tested on stem extracts of *Calotropis* procera; the amount of sodium in *C. albicans* ATCC 10231 was higher than other isolates in all the stem extracts (Figure 3a). The amount of potassium released by the stem extracts especially aqueous and methanol had the highest amount of potassium of about 1.8mg/ml in *C. albicans* (Figure 3b).



Ace- acetone extract (ACE), aqe- aqeuous extract(AQE), ee- ethanol extract (EE), me- methanol extract (ME)

Figura 3. Amount (mg/ml) released by stem extracts of *Calotropis procera* using some typed isolates: (a) sodium (b) potassium.

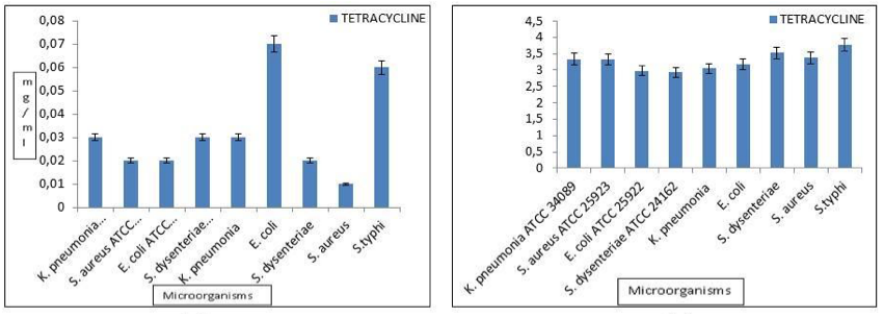
Figure 4a shows that the amounts of sodium released by stem extracts of *C. procera* were higher in *S. typhi* compared to other isolates. The amount of potassium released by stem extracts using some clinical isolates like *S. typhi, E. coli, S. dysenteriae and C. albicans* was also determined; it was observed that aqueous extract had highest amount of potassium in *S. typhi* isolates (Figure 4b).



Ace- acetone extract (ACE), aqe- aqeuous extract(AQE), ee- ethanol extract (EE), me- methanol extract (ME)

Figure 4. Amount of ions (mg/ml) release by stem extracts of *Calotropis procera* using some clinical isolates: (a) sodium (b) potassium.

Antibiotics (Tetracycline) was used as a control, the amount of sodium released was lower in all the organisms compared to the extracts; whereby *S. typhi* had the highest amount of sodium (Figure 5a).



Ace- acetone extract, aqe- aqeuous extract, ee- ethanol extract, me- methanol extract

Figure 5. Amount of ions (mg/ml) released by antibiotics (tetracycline) of *Calotropis procera* using some typed and clinical isolates: (a) potassium (b) sodium.

The amount of sodium ion released by amoxicillin using some isolates was also determined and it was observed that all the isolates had almost the same amount of sodium (Figure 6a).

Potassium leakage released by antibiotics (amoxicilin) shows that *E. coli* ATCC 35218, K. pneumoniae ATCC 34089 and *S. dysenteriae* had the highest amount of potassium (Figure 6b).

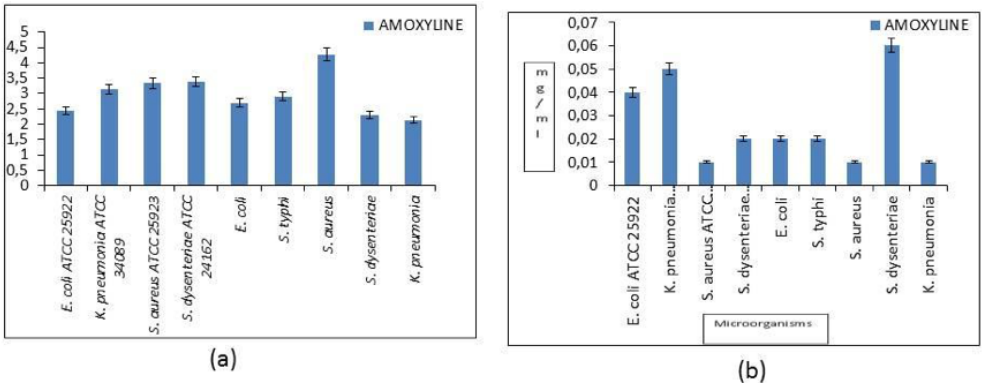


Figure 6. Amount of ions (mg/ml) released by antibiotics (amoxicilin) of *Calotropis procera* using some typed and clinical isolates: (a) sodium (b) potassium.

DISCUSSION

Generally, the release of these ions might be responsible for the bacteriostatic and bactericidal activities of the extract. Na and K ions had been shown to activate enzyme which in turn mediate the biosynthetic processes in bacteria. The rate of leakages of these ions varies from one organism to other and the variation might be due to the difference in the nature of cell wall composition (Oladunmoye et al. (10). The amount of sodium ion released is different from amount of potassium which may be due to the difference in the molecular size of these ions. The leakage of these ions might be responsible for the inhibitory activities of the extracts. Mailard (7) has reported that one of the mechanisms of action of antimicrobial agents might be due to the release of these ions.

The amount of sodium is greater than the amount of potassium which may be due to the differences of the molecular size of the two ions. Generally, sodium ions are readily released into the medium than potassium ions and this might be due to the fact that sodium ions content (24g) is much smaller than that of potassium ions (34g) (11). The amount of ions released varies in different extracts which may be due to the capability of how each of the solvent and aqueous can extract.

Usually, the release of the ions may explain the antimicrobial activity of Calotropis procera and thus may justify its use for ethnomedicine (12).

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