

Full Length Research Paper

The antimicrobial pattern and phytochemical properties of the leaf extracts of *Senna podocarpa*

O. A. Ogundare

Department of Microbiology, Federal University of Technology Akure, Ondo State. E-mail: ayodeleogundare@yahoo.com.

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Methanol extract of *Senna podocarpa* leaf was obtained using the cold extraction method. The extract was tested for antimicrobial activity against ten organisms, namely *Pseudomonas aeruginosa*, *Streptococcus feacalis*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus leutus*, *Salmonella typhi*, *Bacillus cereus*, and *Candida albicans*. The agar well diffusion method was used to carry out this test. Antimicrobial activity was indicated by appearance of zones of inhibition around wells. Results showed that the extract inhibited the growth of all the organisms except *E. coli*, with zones of inhibition ranging from 3 mm in *S. aureus* and *M. leutus*, to 28 mm in *C. albicans*. The Minimum inhibitory concentration was also determined using four different concentrations of the extract (30, 25, 20, 15 mg/ml). The death rate of the susceptible microorganism by 30 mg/ml concentration of the extract was investigated, whereby *P. aeruginosa*, *K. pneumoniae* and *S. typhi* continually reduced from 420, 350 and 600 cfu/ml respectively to zero after 12, 9 and 8 h of respective interaction between them and the extract. However, *S. feacalis* reduced gradually from 550 cfu/ml to zero at the 10th h. Mechanism of action of the extract on the organism inhibited was also carried out. Results showed that potassium ions were continuously leaked throughout the time of interaction (24 h) between the extract and the susceptible organisms. Result of the phytochemical screening showed the presence of saponins, tanins anthraquinone, phlobatanin, and alkaloids. The percentage yield of the plant as recorded is 4.6%.

Key words: *Senna podocarpa*, antimicrobial, minimum inhibitory concentration, zone of inhibition, rate of killing, extract organisms.

INTRODUCTION

Plants have been used since antiquity for shelter, firewood and food. Herbal medicine, which is the use of medicinal plants in the treatment and cure of sicknesses and diseased conditions, has been with man since the beginning of time. Primitive people have used plants to cure a variety of human ailments, and early men have used concoction of various plants for curing disease. In recent times, herbal medicines have become an integral part of the primary health care system of many nations (Fajimi and Taiwo, 2000).

In Africa, herbal medicines are often used as primary treatments for many diseases including HIV/AIDS and HIV-related problems. These African herbs are being recommended by the ministry of health in South Africa and member states for use in HIV (Edward et al., 2005). There are some indications that the roots of *Hypoxis* (*Hypoxis* is a well known plant of the family hypoxidaceae) are found to contain sterols and sterolins, hence have the

potential to enhance immunity (Edward et al., 2005).

For the past two decades, there has been an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agent (Bonjar and Farrokhi, 2004). For example Mubashir et al. (2008) has investigated on the antibacterial activity of whole plant extract of *Marrubium vulgare*, Iwalokun et al. (2007) also reported on the comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus* and Ogundare (2007) reported on antimicrobial effect of *Tithonia diversifolia* and *Jathropa gossipifolia* leaf extracts.

Senna podocarpa formerly *Cassia podocarpa* guill. et Perr (leguminosae - Caesalpinioideae) is a glabrous shrub up to 5 m high. It is widely distributed in West Africa and could be found in the Savannah forest of the region. The plant is locally found on old farmlands in both

Western and Northern parts of Nigeria (Riley, 1963; Dalziel and Hutchinson, 1958). It is extensively used in folklore medicine for the treatment of skin diseases (Sofowora, 1993). The leaves are known for their antigonorrhoeal and purgative properties as well as a guinea worm and sore healing remedy among the Igbos and Yoruba speaking tribes of Nigeria (Akanmu, 1999; Elujoba et al., 1994; WHO, 1999), where it is known locally as Agelo-ogala and Asunwon, respectively (Adefemi et al., 1988). The ripe fruits are brownish-black, shiny, flat beaked, and slightly curved with small transverse ridges, indehiscent, 10 - 12 cm long and 1.5 cm broad.

Scientists have estimated that over 250,000 species of angiosperms exist on earth; most of these plants are yet to be explored for their medicinal properties (Judith, 2000). It may be that in these unexplored plants lies the answer to the treatment of many diseases.

MATERIALS AND METHODS

Collection of sample

The plant material used for this research work which is *S. podocarpa* was harvested in April, 2008 from a forest in Obanla on the grounds of the Federal University of Technology Akure, Nigeria, where they were found growing naturally. This plant was identified by Mr. Hassan, a laboratory technologist in the department of crop science and production, Federal University of Technology, Akure.

Extraction

The leaves were plucked, air dried for three weeks, ground to fine powder and sieved. Exactly 400 g of the finely grounded leaves were soaked in methanol for 72 h. The solution was then sieved using first, muslin cloth and then number one Whatman filter paper. The filtrate was collected and concentrated *in-vacuo* using rotary evaporator. The crude extract was kept in the dessicator to dry.

Test organisms

The extract was tested against clinically isolated samples from the Obafemi Awolowo University, Teaching Hospital Complex, Ile-Ife, Nigeria. The organisms isolated included *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Candida albicans*, *Salmonella typhi*, *Bacillus cereus*, *Escherichia coli*, *Micrococcus luteus*, and *Staphylococcus aureus*.

Antimicrobial activity assay

The antimicrobial activity of the crude extract (*C. podocarpa*) on the test organism was done using the agar diffusion method. About 1 ml of 18 h broth culture of each of the test organisms was introduced into separate sterile Petri dishes and labeled accordingly. About 20 ml of sterile molten nutrient agar was poured into each Petri dish containing the test organism (bacteria) and 20 ml of sterile molten Saboraud dextrose agar was poured into a petri dish containing the test organism (yeast) and was mixed together gently by swirling the petri dish in one direction to ensure even distribution of the organism. The agar was allowed to set and holes were bored into the plates using sterile cork borer of 7 mm in diameter

aseptically. The well bored on each plate was filled with the crude extract at a concentration of 30 mg/ml and a plate had the well filled with sterile water. This serves as control. The Petri dishes inoculated were incubated upright at 37°C for 18 - 24 h. The relative susceptibility of the organisms to the crude extract is indicated by clear zones of inhibition around the wells, which were observed, measured and recorded in millimeters.

Determination of minimal inhibition concentration

The minimum inhibitory concentration is the lowest concentration of a drug that prevents growth of a particular pathogen (Prescott, 2008). Different concentrations (15, 20, 25 and 30 mg/ml) of the leaf of *S. podocarpa* crude extract were prepared and introduced into the different holes bored on agar plate seeded with test organism. The plates were incubated at 37°C and room temperature (28°C ± 2°C) for bacteria and fungi respectively.

Phytochemical screening

The crude extracts obtained were subjected to phytochemical screening, to determine the presence of bioactive agents such as alkaloids, tannins, phlobatannin, anthraquinones and cardiac glycoside (Sofowora, 1993).

Determination of death rate

The number of viable organisms killed by 30 mg/ml of extract at one hour interval was also determined. A 5 ml broth culture of the test organism was centrifuged at 2000 revolution per minute (rpm) for 10 min. The supernatant was decanted and the sediment (That is, the cells) were twice washed with normal saline by centrifuging at 2000 rpm for 10 min. The cells were made up with normal saline. Serial dilution was done to the power of 6 that is, 10⁻⁶.

One milliliter each of dilution 10⁻², 10⁻⁴, 10⁻⁵ were plated and incubated to determine the number of colony forming units per milliliter. Exactly 5 ml of the standardized test organism was mixed with 5 ml of 30 mg/ml crude extract of *S. podocarpa* leaf. The suspension was thoroughly mixed and held at room temperature (28 ± 2°C), and the killing at every one hour of interaction up till the 24th was determined.

Determination of mechanism of action

A 30 mg/ml of the crude extract was prepared. Exactly 1 ml of the prepared test organism was added to 1 ml of the 30 mg/ml concentration of crude extract. Two sterilized curvetts were used. The first one contains 1.0 ml of the prepared test organism and 1 ml of the 30 mg/ml crude extract. The second curvet serves as the control and has only 2 ml of the test organism.

Standard stock solution of sodium and potassium ions (200 ppm) was used for preparing 100, 50 and 25 ppm solutions of standard sodium and potassium ions. From the 200 ppm stock solution of sodium ion, 1.0 ml was dispensed into 2.0 ml of deionized water to make 100 ppm solution. From the 100 ppm solution, 1.0 ml was dispensed into 2.0 mls of deionized water to make 50 ppm and 1.0 ml of 50 ppm solution was dispensed into 2.0 ml of deionized water to make 25 ppm of sodium standard solution. The same was done to obtain 100, 50, 25 ppm standard stock solutions of potassium.

The first curvet was placed in the atomizer orifice and readings were taken at 0, 10, 20, 30, 40, 50, 60, 120, 360, 540, 720, and lastly at 1440 min. The control readings were also taken at the same time intervals.

Table 1. Antimicrobial sensitivity test of leaf extract of *Senna podocarpa* on the test organism.

Test organisms	Zone of inhibition (mm)
<i>Streptococcus faecalis</i>	13.0
<i>Klebsiella pneumoniae</i>	13.0
<i>Shigella dysenteriae</i>	8.0
<i>Pseudomonas aeruginosa</i>	12.0
<i>Candida albicans</i>	28.0
<i>Salmonella typhi</i>	20.0
<i>Bacillus cereus</i>	10.0
<i>E. coli</i>	Nil
<i>Micrococcus luteus</i>	3.0
<i>S. aureus</i>	3.0

Table 2. Minimum inhibitory concentration (MIC) of the leaf extract of *Senna podocarpa*.

Test organisms	Concentration (mg/ml)
<i>Streptococcus faecalis</i>	30.0
<i>Pseudomonas aeruginosa</i>	20.0
<i>Klebsiella pneumoniae</i>	15.0
<i>Shigella dysenteriae</i>	15.0
<i>Candida albicans</i>	15.0
<i>Salmonella typhi</i>	5.0
<i>Bacillus cereus</i>	15.0
<i>Micrococcus luteus</i>	20.0
<i>Staphylococcus aureus</i>	15.0

RESULTS AND DISCUSSION

The antimicrobial activity of the leaf extract of *S. podocarpa* at a concentration of 30 mg/ml showed zones of inhibition that ranged from 3 mm in *S. aureus* and *M. luteus* to 28 mm in *C. albicans*. It also showed inhibitory activities against the following organisms *S. faecalis* having 13 mm, *K. pneumoniae* 13 mm, *S. dysenteriae* 8 mm, *P. aeruginosa* 12 mm, *S. typhi* 20 mm, and *B. cereus* 10 mm (Table 1).

The minimum inhibitory concentration was 30 mg/ml for *S. faecalis*, 20 mg/ml for *P. aeruginosa* and *M. luteus*, 15 mg/ml for *S. dysenteriae*, *K. pneumoniae*, *C. albicans*, *S. aureus* and *B. cereus* and 5 mg/ml for *S. typhi* as shown in Table 2.

Table 3 shows the phytochemical constituents namely: flavonoids, tannins, phlobatannins, saponins alkaloids, and anthraquinones.

Figures 1 - 7 shows the rate at which the extract killed the microorganisms. The extract became bactericidal to *S. faecalis* at the 6th h, *P. aeruginosa* and *M. luteus* at the 12th h, *K. pneumoniae* at the 9th h, *S. aureus* at the 11th h, *Bacillus cereus* at the 24th h, and *Salmonella typhi* at the 8th h.

Figures 8 and 9 shows the potassium ion leakage pat-

Table 3. Phytochemical Compounds of leaf extract of *Senna podocarpa*.

Compounds	Results
Saponnin	+
Tannin	+
Anthraquinone	+
Phlobatannin	+
Alkaloids	+
Luberman's test	+
Salwoski test	-
Keller killani test	-
Legal's test	-

Note keys: - : Negative +: Positive.

tern of the organisms by the extract, while Figures 10 and 11 shows the sodium ion leakage pattern of the organisms by the extract. This result has shown that within 24 h of interaction between the organisms and the extract, both potassium and sodium ions are leaked from the cells (and the continuously) of the organisms.

DISCUSSION

The antimicrobial activity of medicinal plants and drugs varies in their inhibitory effect, depending on the concentration of crude extracts or synthetic drug, size of inoculums, temperature, nature of organism, and rate of diffusion (Prescott, 2008).

The results obtained in this study has shown that the leaf extract of *S. podocarpa* possess antimicrobial (especially antibacterial properties) property.

S. podocarpa leaves are extensively known for their antigonorrhoeal and purgative properties as well as a guinea worm and sore-healing remedy among the Igbos, in Nigeria (Akanmu, 1999).

From Table 1, all the bacteria used, except *E. coli* showed sensitivity to the leaf extracts of *S. podocarpa* with zones of inhibition ranging from 3.0 mm in *M. luteus* and *S. aureus* to 20 mm in *S. typhi*. *C. albicans* recorded the highest sensitivity in all with a zone of inhibition of 28 mm. Thus the leaf extract of the plant has exhibited a broad spectrum activity. The susceptibility of *S. aureus* to the plant extract justifies the traditional use of this plant in sore-healing and is also in agreement with the findings of Daziel (1937), which says that the fresh leaves are used as poultices for swellings and wounds. At the same time the sensitivity of some organisms such as *S. dysenteriae*, *S. typhi*, and *P. aeruginosa* is indicative that the extract contains bioactive components against some enteric organisms and hence can be employed in the treatment of diseases caused by such.

The phytochemical compounds detected in the leaf extract included alkaloids, flavonoids, tannins, phlobatannins, saponins and anthraquinones. Cardiac glycosides were

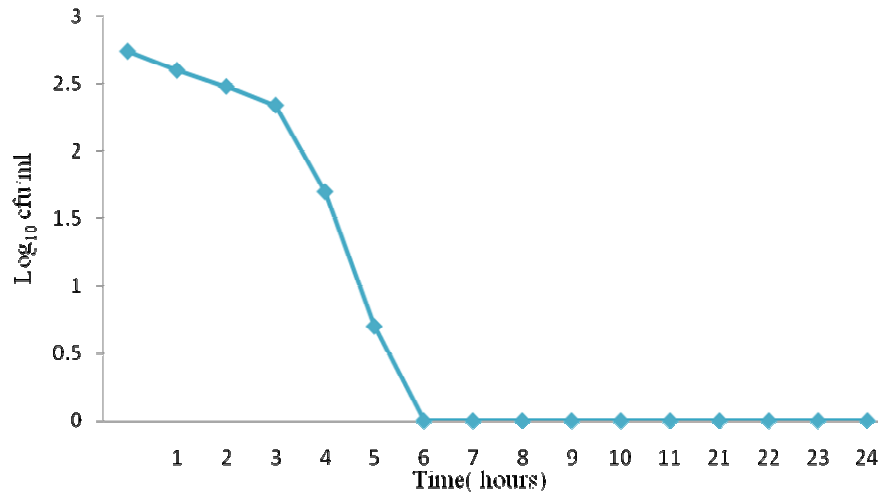


Figure 1. Rate of killing of *Streptococcus feacalis* by the leaf extract.

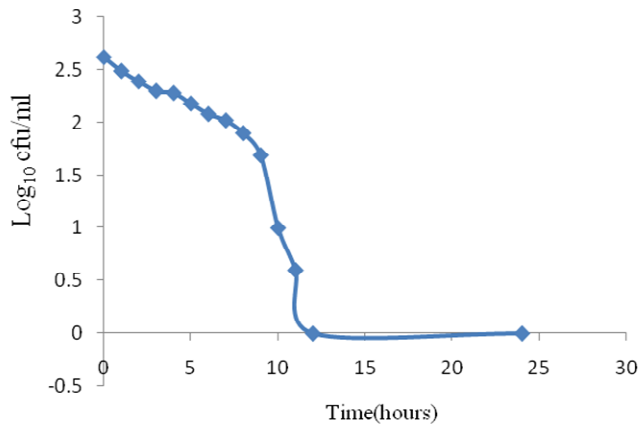


Figure 2. Rate of killing of *Pseudomonas aeruginosa* by the leaf extract.

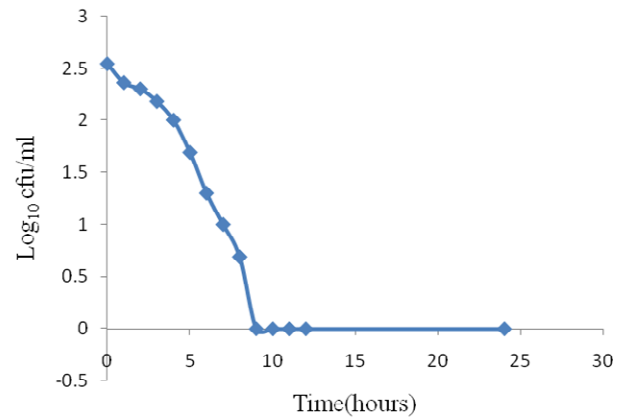


Figure 3. Rate of killing of *Klebsiella pneumonate* by the leaf extract

absent. These phytochemicals may be responsible for the antimicrobial activity of *S. podocarpa* leaf. According to Ogunkunle and Tonia (2006), the presence of saponins, tannin, alkaloids, anthraquinone and phlobatannins are responsible for bioactivity of the plant extract.

Figures 1 - 7 of this report shows a continuous reduction of the colony forming unit per milliliter (cfu/ml) of the test bacteria in the extract over a period of twenty four hours. Thus the action of the extract showed a bactericidal type. Fagbemi et al. (2009) reported cases of rate of killings of unripe banana, turmeric and lemon grass on some pathogens to have a similar trend.

The mechanism of action of the extract showed the leakage of sodium and potassium ions from the cells of the organisms. Again, the trend of the leakage is similar to the pattern of the rate of killing. Both ions were leaked continuously for the period of 24 h of the experiment.

Ogundare (2006) reported a similar case using *V.*

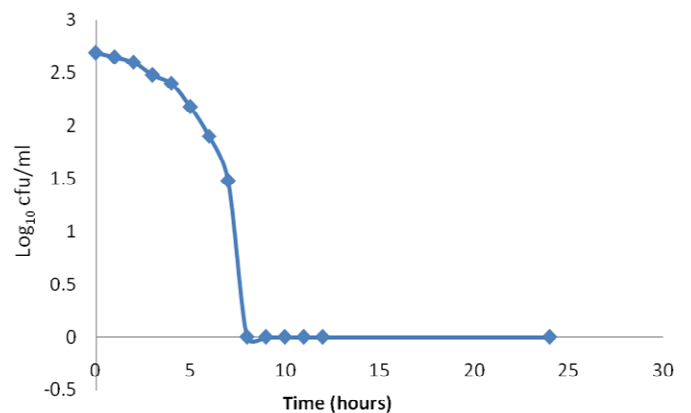


Figure 4. Rate of killing of *Salmonella typhi* by the extract.

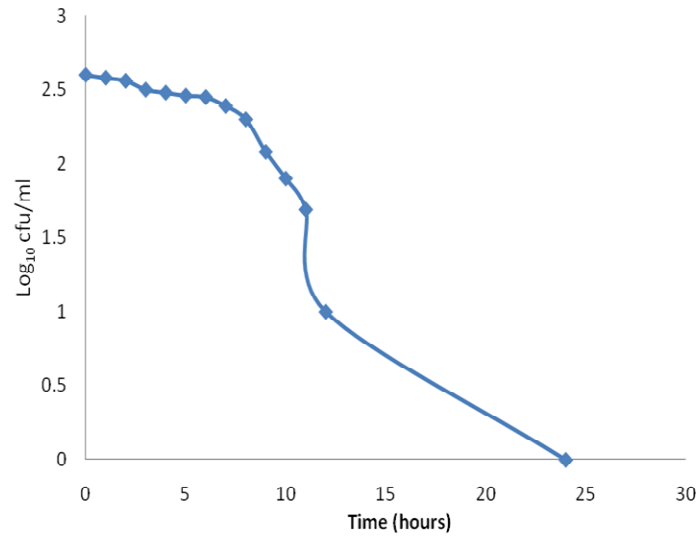


Figure 5. Rate of killing of *Bacillus cereus* by the extract.

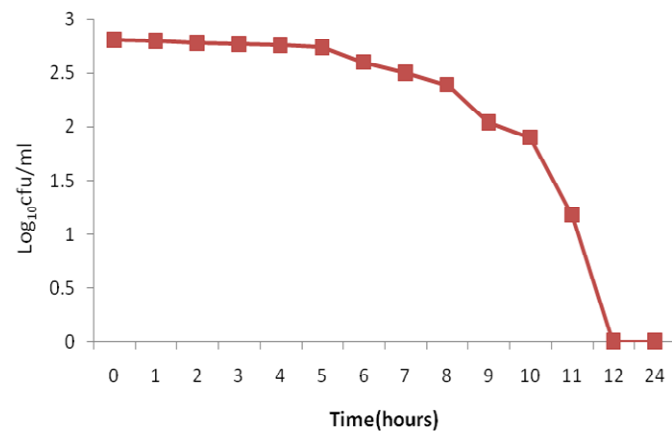


Figure 6. Rate of killing of *Micrococcus luteus* by the extract.

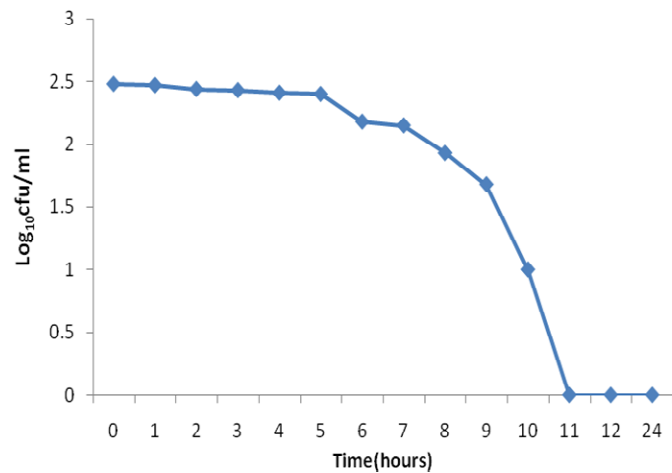


Figure 7. Rate of killing of *Staphylococcus aureus* by the extract.

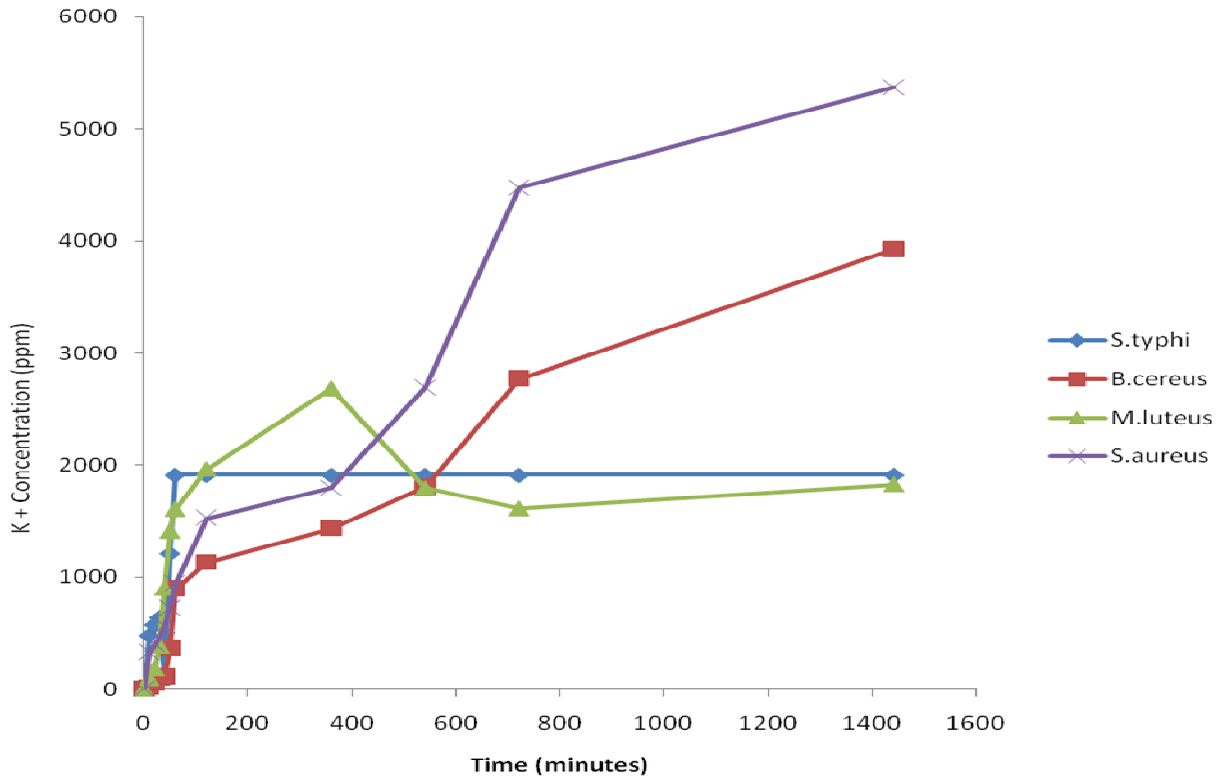


Figure 8. Potassium ion leakage from organisms by *Senna podocarpa* leaf extract.

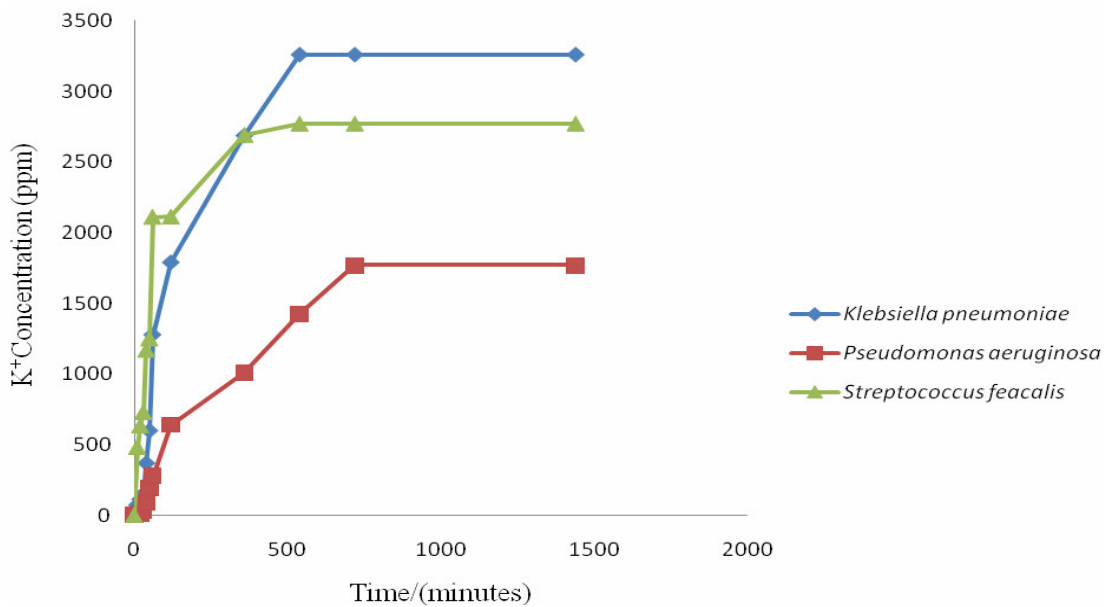


Figure 9. Potassium leakage from organisms by *Senna podocarp* leaf extracts.

tenorrea leaf extracts.

Generally it can be deduced from the figures obtained that the leaf extract of *S. podocarpa* killed the test organisms by leaking potassium and sodium ion from their

cytoplasm, which led to plasmolysis, hence death of the organisms.

This study has not made use of purified extracts of the plant. However, it is possible that purification of the crude

extract might be more effective on the test organisms.

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