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Release of Sodium and Potassium Ions by Aqueous and Ethanolic Extract of *Cassia occidentalis* on Some Selected Bacteria

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Abstract: The rate of release of sodium and potassium ions by aqueous and ethanolic extract of leaves of *Cassia occidentalis* was investigated for some selected pathogenic bacteria in the genera *Bacillus*, *Staphylococcus*, *Echerichia*, *Streptococcus*, *Klebsiella*, *Pseudomonas* and *Salmonella* using flame photometer. The aqueous extract was found to be more effective in the leakage of Na⁺ and K⁺ ions than the ethanolic extract for all organisms investigated except *Salmonella*. The aqueous extract released 2.66 ppm sodium ions on *Pseudomonas aeruginosa*, whereas ethanolic extract released 13.3 ppm while the K⁺ ions released are 9.282 and 49.980 ppm for ethanolic and aqueous extract, respectively. It was found that only *Salmonella typhi* gives higher leakages of sodium and potassium ions for ethanolic extract than the aqueous extract. Comparison of the amount of Na⁺ and K⁺ ions release by the plant extract with two commercial antibiotic (Chloramphenicol and tetracycline) showed that the latter gives a higher value than the former. For sodium ion, *Bacillus subtilis* gives 167 ppm and 164 ppm for chloramphenicol and tetracycline respectively where as 2.28 and 3.42 ppm was measured for ethanolic and aqueous extract of the *Cassia occidentalis* respectively. There was no significant difference in the amount of leaked Na⁺ ions and potassium ions between the two antibiotics. For Na⁺, *Salmonella typhi* has 164 ppm for chloramphenicol and 163 ppm for tetracycline while the value for *Proteus vulgaris* was 160 and 163 ppm, respectively.

Key words: Sodium ions, potassium ions, *Cassia occidentalis*

INTRODUCTION

Cassia occidentalis belong to the family Fabaceae and it has been found to posse's antibacterial, antifungal, laxative analgesic, choleric and diuretic properties (Cowan, 1999). It has been accepted world wide due to its properties and actions to cure a lot of diseases and illness. It has hence been classified as a medicinal plant with antimicrobial activity.

A number of mechanisms exist for the ant microbial activities of many antimicrobial agents including plant extracts (Mailard, 2002).

These include prevention of cell wall biosynthesis, inhibition of protein synthesis, interference with nucleic acid biosynthesis and interaction with cytoplasmic membrane and its disruption as well as leakage of cytoplasmic constituent like Na⁺ and potassium ions. There has not been any information on the release of sodium and potassium ions by extract from *Cassia occidentalis*. The present studies was aimed at determining the leakage of sodium ions by extracts of *Cassia occidentalis* 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 otherwise in order to explain and establish possible mechanism of action (Rio and Recio, 2005).

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MATERIALS AND METHODS

Leaves of *Cassia occidentalis* was obtained from Shagarri Quarters in Akure, Nigeria and identified by Mr. S.O. Aduloju of Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure, Nigeria. All analysis was carried out in the Department of Microbiology, Federal University of Technology, Akure, Nigeria, in 2004. The leaves were air dried and ground into powder and dissolve in 60% ethanol and water and the mixtures allowed to stand for 72 h. The mixtures were filtered and the extracts obtained were concentrated in a vacuo using rotary evaporator. The extract was partially purified using column chromatography (Harbone, 1984).

The methods of Davidson (1980) and Ayres and Payne (1984) were adopted for the determination of the leakages of potassium ions from the determination of the leakages of potassium ions from the susceptible microorganism. Eighteen hour old culture was used. These cells were washed twice in physiological saline by centrifugation before use. The inoculum's size was then adjusted to contain approximately 10^6 organisms per mL. Exactly 0.5 mL of the cell suspension of each organism was added to 4.5 mL of the prepared concentrations of the fraction give 0.50, 0.25, 0.12, 10.0 and 0.03% w/v. the supernatant solution obtained after centrifugation at 7000 rpm at intervals of 5, 10, 20, 25 and 30 min were analyzed for potassium and sodium ion using flame photometer. Triplicate readings were made for each supernatant.

RESULTS AND DISCUSSION

Table 1 results indicated that the extracts of *Cassia occidentalis* causes leakages of sodium and potassium ions from the cytoplasm of tested bacteria. The release of these ions might be responsible for the bactericidal and bacteristatic activities of the extract. Sodium and potassium ions had been shown to activate enzyme which in turn mediate the biosynthetic processes in bacteria. The leakage of these ions might be responsible for the inhibitory activity of the extract. Mailard (2002) has reported that one of the mechanisms of action of antimicrobial agents is due to release of cytoplasmic materials like sodium and potassium ions. The rate of leakage of these ions varies from one organism to the other. The variation might be due to the difference in the nature of the cell wall composition of the different bacteria. It has been shown that the leakage of intracellular materials from bacterial is dependent on the nature, type and composition of the cell wall (Ayres and Payne, 1999). Another reason for this may be due to variation in the genetic composition of the different organism which may be chromosomal or plasmid coded (Prescott *et al.*, 2005). This affect some physiological properties of organisms like solute uptake, active transport ant facilitated diffusion of molecules across the cell membrane.

The amount of sodium ion released was also different from the amount of potassium ions leached into the growth medium. The variation might be due to difference in the molecular size of these two ions. The amount of release intracellular constituents have been found to be dependent on the size of pores on the cell wall and membrane as well as the molecular size of ions (Manosroi *et al.*, 2004).

Table 1: Amount of sodium and potassium ions in part per million released by *Cassia occidentalis*

Microorganisms	Ethanol (ppm) Na ⁺	Hot water (ppm) K ⁺	Ethanol (ppm) Na ⁺	Hot water (ppm) K ⁺
<i>Bacillus subtilis</i>	2.28	3.42	1.428	41.412
<i>Escherichia coli</i>	1.90	6.46	2.142	67.116
<i>Klebseilla</i> sp.	9.12	8.36	12.852	7.140
<i>Proteus vulgaris</i>	14.06	20.90	0.714	52.836
<i>Pseudomonanas aureginosa</i>	13.30	21.66	9.282	49.980
<i>Staphylococcus aureus</i>	15.50	21.28	0.714	68.544
<i>Salmonella typhi</i>	13.30	2.442	4.276	14280

Table 2: Amount of sodium and potassium ions in part per million released by chloramphenicol and tetracycline

Microorganism	Chloramphenicol (ppm) Na ⁺	Tetracycline (ppm) K ⁺	Chloramphenicol (ppm) Na ⁺	Tetracycline (ppm) K ⁺
<i>Bacillus subtilis</i>	10.02	9.84	9.46	8.36
<i>Escherichia coli</i>	9.96	9.90	8.80	1.98
<i>Klebsiella</i> sp.	9.78	9.60	7.92	3.63
<i>Proteus vulgaris</i>	9.60	9.78	9.13	7.70
<i>Pseudomonas aureginosa</i>	9.84	9.96	8.25	10.00
<i>Staphylococcus aureus</i>	10.08	9.42	9.02	8.58
<i>Salmonella typhi</i>	9.84	9.78	8.69	9.02

Generally, Na⁺ ions are readily released into the growth medium than the K⁺. This might be due to the fact that sodium ions are much smaller (24 g) than potassium (34 g). (Trease and Evan, 2002).

The amount of ions release also varies with the two extraction solvents used. Aqueous extract was found to give higher values than the ethanolic extracts. The cause of this might be related to the difference extractive capacity of the solvent in relation to the molecular bonding and polarity. Water is a polar solvent and the biologically active compound in the leaves of *Cassia occidentalis* might be polar permitting more of these pharmacologically active compounds to be present in the aqueous fractions than in the ethanolic extracts. This might aid faster rate of leakage of these ions in aqueous fraction than the ethanolic extracts.

The antibiotics were found to cause higher leakages of the ions than the plant extracts in some organisms (Table 2). The reason may be due to fact that the antibiotics are better purified and hence has a higher efficacy and potential to dislodge the protoplasmic materials in the bacteria concerned. However chloramphenicol was found to give higher values than tetracycline for potassium ions whereas the values were not significantly different for sodium ions. The observed trends may be explained in terms of concentration of the active principles in the drugs as well as the target organs during formulation.

CONCLUSION

The release of these ions may explain the antimicrobial activity of *Cassia occidentalis* and thus justify its use in ethno medicine.

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