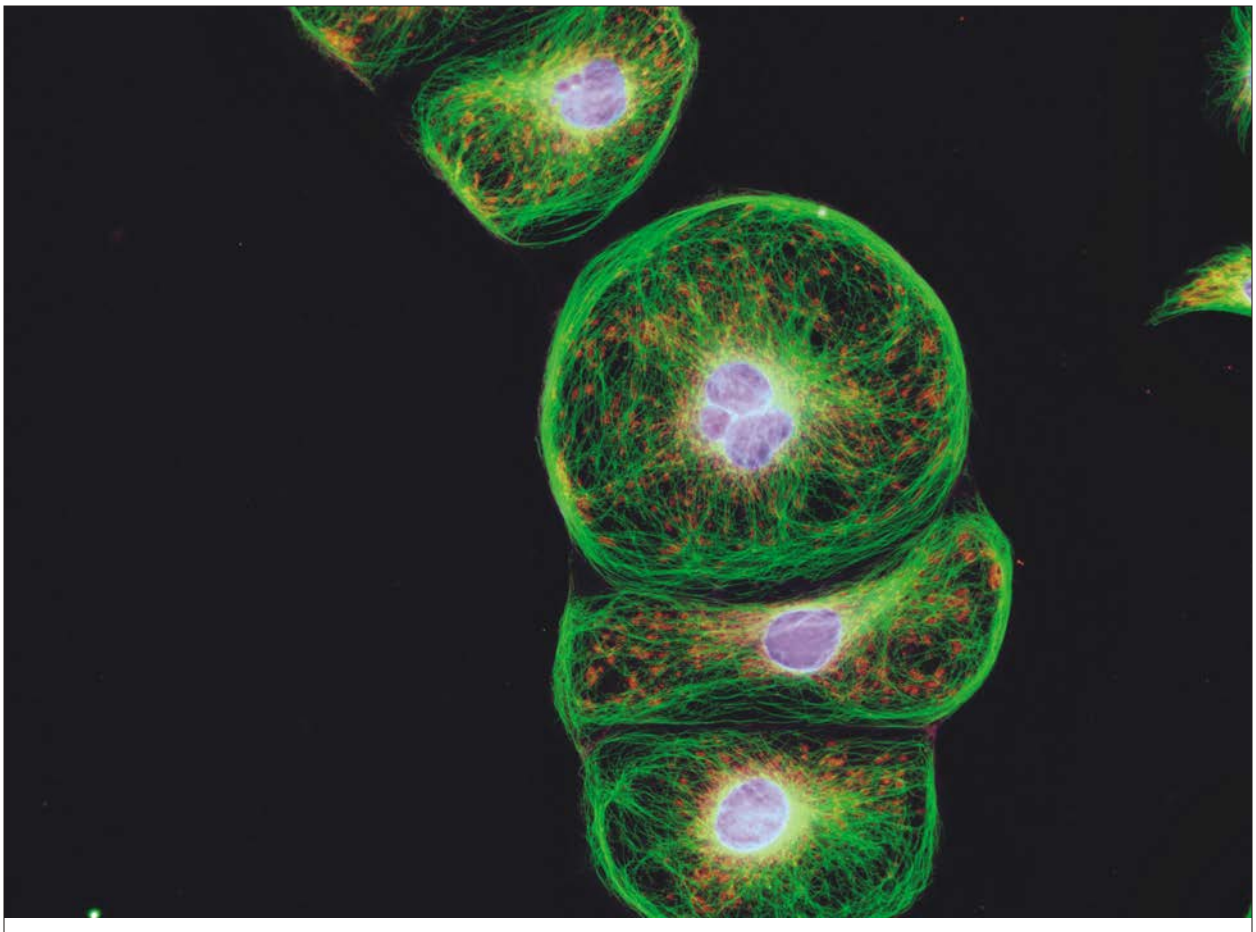

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Comparative Studies on the Amount of Protein, Sodium and Potassium Ions Released by Methanolic Extracts from Six *Cassia* Species

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Abstract: The amount of protein, sodium and potassium ions released by some pathogenic bacteria in broth cultures containing methanolic extracts from leaves of six *Cassia* species was investigated using absorption spectrophotometer. The amount of protein leaked ranged between 4-60 mg mL^{-1}. Generally, the amount of protein released into the growth medium was found to vary between the different bacteria species and also differs among the various cassis species used. The quantity was higher among the gram positive organisms like *Bacillus*, *subtilis*, *Staphylococcus* and *Clostridium species* than the gram negative ones like *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* and *Shigella*. A similar trend was observed for the sodium and potassium ions.The amount that was released was between 1.0-74 for sodium and 1.00-65 for potassium. However, the amount of potassium ion released was lower than that of the sodium ions for a particular organism with a given plant specie. Also, *Cassia auriculata* demonstrated the greatest potential to cause the leakages of these intracellular materials followed by *C. alata*; whereas *C. mimosoides* was the least. The mechanism of antimicrobial activity of extracts from these plants is due to leakages of these cellular constituents and thus justifies their use in ethnomedicine.

Key words: Protein, sodium ions, potassium ions, ethnomedicine, *Cassia*

Introduction

A systematic survey of antimicrobial agents shows that they are general nomenclature for all drugs or chemical substance that acts on microorganism either to kill or suppress their growth (Prescott *et al*., 2000).These antimicrobial agents occur from different sources either as natural, produced by microorganism biochemical pathways, extracts from both plant and animal origin and synthetics (Tortora *et al*., 2002). A long time before the scientific research into the development of antimicrobials agents began, plants have been used for curative purpose without knowing that these agents are directed against some microorganism that are causative agents for such diseases. (Kaufman *et al*., 1999). Invention of the art of writing and advances in the science of chemistry made man to begin to identify, isolate and document the bioactive chemicals contained in various plants (Blinking, 2000).

Medicinal plant is any plant in which one or more of its organs contain substance that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (Stanjner*et al*., 2006). During the early years of human existence, thousands of years ago, many plant materials by instinct, intuition or trials by error were used to combat ailments. As ideas of different tribes, communities and culture developed, the use of various plants becomes wide spread in accordance with their belief. Traditionally, the use of plant preparation is passed from one generation to the other. Those relating to drugs plants with dangerous effect remained the secret property of trustworthy initiated members of the community such as medicine men, witch doctors and herbalists (Blinking, 2000).

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The medicinal plants played a vital role among the illiterate and highly civilized men in the folklore, superstition connected with spreading of disease or washing of evil spirit and changing one's favors all of which are still practiced by people in both developed and developing countries. The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethnopharmacologits, Botanist, Microbiologist and Natural product chemists are combing the earth for phytochemicals and lead which could be developed for treatment of infectious diseases. (Kaufman *et al*., 1999).

Most species in the genus *Cassia* has been used in ethnomedicine and their *in vitro* antimicrobial activity has been documented (Oladunmoye and Akinyosoye, 2004). Release of sodium and potassium ions by ethanolic and aqueous extract of *Cassia occidentalis* have also been reported (Oladunmoye *et al*., 2007). But so far, no studies have been carried out on the release of protein, sodium and potassium ions of the six most commonly used species in ethnomedicine. The present investigation was carried out to quantify the amount of protein, sodium and potassium ions leaked into the growth medium of pathogenic bacteria containing leaf extract from these six *Cassia* species.

Materials and Methods

Plant Samples: Sources, Extraction and Fractionation

The plants used for the study were six species of the genus *Cassia*. They are *Cassia alata, C. auriculata, C. hirsuta, C. mimosoides, C. occidentalis* and *C. obtusifolia*. They were collected at various locations in Shagari village, Akure, Ondo State, Nigeria except *C. auriculata* that was collected from Forestry Research Institute of Nigeria (FRIN) Ibadan, Oyo State, Nigeria.

Mr. S.O. Aduloju of Crop, Soil and Pest Management Department, the Federal University of Technology, Akure authenticated the plants. Confirmation was also made by comparison with herbarium specimens of Department of Forestry and Wild Life, Federal University of Technology, Akure. The fresh leaves of the six *Cassia* species were air dried for 5 days, grounded into fine powders by blending in a high-speed electric blender (Binatone Model) and kept in airtight container to avoid absorption of moisture.

Each sample (400 g) was weighed with metler balance (A.G. Switzerland) into the different solvents. The solvent used was methanol. The resultant mixtures were left in the laboratory for 72 h. This was then filtered with muslin cloth. The solvent was allowed to dry and the crude extract obtained. The extract was then concentrated in vacuo using rotary evaporator.

Exactly 10 g each of the crude extract of the six *Cassia* species were absorbed on silica gel of 60-120 mesh (BDH) and chromatographed on a column of silica gel-60 slurry packed in petroleum ether. The column was gradients eluted first with petroleum ether and then with ethyl acetate: methanol 40: 1 and finally with 100% methanol.

About 100 mL of the fraction was collected into a 500 mL flask. The fractions collected were analyzed by thin layer chromatography (TLC) on precoated plates Merck, silica gel 60 F254, 0.2 mm thickness using ethyl acetate: methanol (40: 1) as the mobile phase. The fractions collected were numbered. Fractions showing the same TLC characteristic are bulked together and later concentrated *in vacuo*. Visualization of the spots on plates were by observing under ultra violets light and by spraying separately with vanillin-sulphuric acid reagent followed by heating at 100°C for 5 min.

Microorganisms Used in the Bioassay

The clinical isolates were obtained from the stock cultures of the State Specialist Hospital, Akure Ondo State, Nigeria. The identities of the organisms were confirmed using standard and conventional methods of the morphological and biochemical characteristics of each organism (Brock and Madigan, 2000).

Studies on the Leakages of Potassium Ions from Bacteria by Active Fractions of the Extracts

The method of Oladunmoye *et al*. (2007) but with slight modification was used for the determination of the leakages of potassium ions from the susceptible microorganism. Eighteen hour old cultures were used. The cells were washed twice in physiological saline by centrifugation before use. The inoculums 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 which gave 0.50, 0.25, 0.12, 10.0 and 0.03% w/v. The supernatant solution obtained after centrifugation at 7000 rpm was analyzed for potassium ion using flame photometer. Triplicate readings were made for each supernatant.

Studies on the Leakages of Proteins by the Active Fractions

The modified method of Stajiner *et al*. (2006) was used to determine the quantity of protein leakage from the organism. Washed suspension of the organism (10^6 cell mL^{-1}) was treated with various concentrations ranging between 0.03-0.50% w/v at intervals of 30 min, 1, 2, 3 and 4 h. Each suspension was then centrifuged at 7,000 rpm and 0.1 mL of each supernatant was added to Bradford rd reagents prepared as follows: 100 mg of Coomasie brilliant blue G-250 was dissolved in 50 mL of 95% ethanol. To this solution 100 mL of 845% (W/V) phosphoric acid was added. The resulting solution was diluted to final volume of 1 L with distilled water. Bovine Serum Aluminum (BSA) was used to distilled water. Bovine Serum Albumin (BSA) was used to construct a standard protein curve by weighing out the BSA and dissolving in 0.1 mL of 0.15M NaCl. To this sample, 5 mL Bradford reagent was added and the content was mixed by inversion. The varying concentration of BSA used ranged from 10 ug to 100 ug. The absorbance of the mixture of the supernatant and the Bradford reagent at 595 mm was measured in duplicate after 2 min and 1 h against a blank prepared from 0.1 mL of 0.15M NaCl and 5 mL Bradford reagents. The weight of protein was plotted against the corresponding absorbance resulting in a BSA standard curve used to determine the protein in unknown samples.

Results and Discussion

Table 1 results indicated that all the organisms released varying amount of protein which was found to also depend on the specie of the *Cassia* under consideration. Gram positive organisms were found to induce higher leakage of protein than the Gram negative ones. The observed variation may be explained in terms of difference in the chemical composition on the cell walls that ultimately determine the structural integrity of the organism cell (Brock and Madigan, 2000). The cell wall of the Gram

Table 1: Amount of protein (mg ML^{-1}) released by methanohic extract from six *Cassia* species on some pathogenic bacteria

Organisms	*Gram Reaction*	*C. alata*	*C. occidentalis*	*C. auriculata*	*C. obtusifolia*	*C. hirsute*	*C. mimosoides*
B. subtilis	+	60.0	56	80	45	38	20
C. diphtheriae	+	25	35	42	50	25	14
C. sporogenes	+	18	10	70	38	23	16
S. aureus	-	58	60	95	34	29	30
S. epidermidis	+	40	10	44	18	30	16
S. faecalis	+	20	06	56	50	27	23
E. coli	-	15	12	32	24	20	09
K. pneumoniae	-	0	12	19	12	13	08
P. vulgaris	-	21	08	22	06	08	12
P. aeruginosa	-	12	17	31	18	10	15
S. typhi	-	06	13	30	12	08	08
S. marcense	-	28	11	14	08	04	06
S. dysenteriae	-	14	08	24	26	05	04

+ = Present; - = Absent

negative organisms has been shown to be more complex than that of the Gram positive due to the presence of additional moiety called lipopolysaccharride (Tortora *et al*., 2002). The complexicity and interwoven nature of the Gram negative cell wall may be responsible for the lower values of protein leaked arising from trapping of the molecules at the cell surface level. However the Gram positive organisms' cell wall with less interlocking structures might permit leakages of more protein molecules (Jawetz *et al*., 2004). Among the two main categories of tested organisms based on Gram reaction, there was difference in the amount of protein released by various organisms in the different genera or different species in the same genus. Variation in genetic composition which may be plasmid coded (Prescott *et al*., 2000) or chromosomal may be responsible for this observed trend.

Each of the six *Cassia* species caused leakages of protein to different level in a given organism. This can be explained in terms of variation in the concentration of phytoconstituents that are responsible for the antimicrobial activity of the plant extract. The absence of certain biomolecules in a particular species may lower its antimicrobial efficacy and consequently reduced the amount of protein molecule leaked into the growing medium (Blinking, 2000).

The amount also follows a similar trend among the different bacterial species and genera. The explanations for this may be similar to those advanced for protein. Generally, the amount of sodium ions released is more than of potassium ions (Table 2). This may be due to the fact that the atomic number as well as the molecular mass of sodium is less than that of potassium (Tortora *et al*., 2002) and this phenomenon might have aided larger amount of sodium to escaped from the bacterial cell and preventing the bigger potassium ions to leaked out of the cell (Ryan *et al*., 2004). The steady development of permeability trends in sodium and potassium ions could be a consequence of the changes in the formation and distribution of micelles in the membrane after uptake of the extracts (Brock and Madigan, 2000). An alternative explanation would be that the extract initially binds to the non specific surface structure and the redistribution occurs. The release of these ions must be the result of a complex series of events following uptake of the extracts and the immobility of lipid membrane (Prescott *et al*., 2000). The inability of some extract to induce enough leakages of these cytoplasm materials may be that metabolic energy is required to alter the accessibility of sites or move the extract on them.

The mechanism of actions of the antimicrobial activities of the family Casalpinaeceae to which *Cassia* belong may be explained in terms of their ability to induce leakages of these ions (Jawetz *et al*., 2004). Sodium and potassium ions have been known to affect osmotic balances in the cell and their leakages might cause cell lyses and eventual death. These ions are also known to activate enzymes

Table 2: Amount of sodium and potassium ions (ppm) released by bacteria in broth cultures containing extracts from six *Cassia* species

		C. alata		*C occidentalis*		*C. auriculata*		*C. obtusifolia*		*C. hirsuta*		*C. mimosoides*	
Organisms	Gram Reaction	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+
B. subtilis	+	0.90	50.00	6.80	42.00	38.10	52.50	13.00	17.30	16.00	30.00	8.10	26.00
C. diphtheriae	+	14.30	65.00	11.50	37.00	20.50	74.00	9.00	24.00	20.0	26.00	7.00	13.50
C. sporogenes	+	0.6.60	40.00	5.00	15.00	18.40	32.40	25.10	17.40	19.40	30.00	10.00	18.70
S. aureus	-	18.40	07.00	3.00	40.00	14.50	39.40	16.00	16.40	11.00	45.00	9.40	20.50
S. epidermidis	+	13.70	45.00	10.00	18.50	20.40	48.30	14.00	23.70	17.00	20.00	4.20	3.70
S. faecalis	+	08.30	20.00	3.00	19.40	04.70	27.00	15.20	27.40	14.60	25.00	1.00	10.20
E. coli	-	0.420	15.00	3.40	17.00	7.30	5.00	7.80	3.50	9.00	9.00	3.00	19.00
K. pneumoniae	-	6.40	5.00	1.40	12.00	10.50	18.40	10.40	1.40	3.40	5.20	1.00	1.00
Proteus vulgaris	-	10.0	17.20	1.00	15.50	9.30	10.40	7.50	1.70	3.20	7.00	4.30	7.10
P. aeruginosa	-	3.00	14.40	1.80	12.20	7.50	15.50	6.00	1.40	1.00	5.40	1.00	3.00
S. typhi	-	5.60	3.00	2.50	9.40	2.20	12.10	10.00	1.50	6.00	10.00	30.00	4.80
S. marcense	-	40.50	4.00	3.20	3.00	7.50	9.10	11.00	0.70	40.20	1.40	2.20	3.50
S. dysenteriae	-	2.10	7.00	1.00	4.70	3.70	4.70	12.00	0.40	1.40	1.70	1.30	1.20

+ = Present, - = Absent

which are organic catalyst that mediate biochemical reactions (Oladunmoye, 2007). Most cell activity including respiratory and biosynthetic function are under the control of enzymes. The antimicrobial efficacy of the six *Cassia* species may result from damages and inactivation of enzymes due to their ability to induce leakages of these ions (Ryan *et al*., 2004).

Recommendation and Conclusion

The leakages of protein, sodium and potassium ions by methanolic extracts of the six *Cassia* species suggest that the mechanism of their antimicrobial activity may be inherent in these phenomena. This thus justify the use of these plants in ethnomedicine. However further studies should be carried out in order to establish whether the action is bacteristatic or bactericidal. Also the effect on other microbial cells like fungi, viruses and parasites should be investigated.

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