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Antibacterial Spectrum of Aspartic Acid Complexes

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ABSTRACT

Background: The study reports the antibacterial spectrum of aspartic acid complexes that with reported antimicrobial activities.

Methods: Five antimicrobial complexes of aspartic acid: $Cd(asp)_2$, $Na[Mn(asp)_3]$, $Na_2[Mn(asp)_2]$, $Mn(asp)_2$, and $Na[Cu(asp)_3]$ were screened for activity against 72 bacterial strains representing aerobic and facultative bacteria. The panel of test strains included reference organisms, as well as clinical and environmental isolates from a wide variety of sources.

Results: All the agents inhibited all the organisms at 10-20mg/mL with the MIC of 10mg/mL for more that 80% of the test strains. $Mn(asp)_2$ (MIC = 10mg/mL) was the most active. $Cd(asp)_2$ [MIC = 10mg/mL for 62/72 (86.11%) and MIC = 20mg/mL for 10/72 (13.9%)] was the least active. *S. aureus* (MIC = 10mg/mL) was most susceptible while *Proteus* spp [MIC = 10mg/mL for 11/14 and MIC (20mg/mL) for 3/14 strains] was the least. Best activities were shown against Gram positive bacteria strains.

Conclusion: The complexes have broad spectrum of activities against both susceptible and intrinsically resistant strains of bacteria including those known to be opportunistic pathogens. The complexes thus have potential for development as chemotherapeutic agents.

1. INTRODUCTION

The antimicrobial activities of the coordination compounds of aspartic acid had been reported[1-3]. The compounds have been found to have good inhibitory activities against the organisms screened in those studies. Higher activities were reported for Gram positive over Gram negative bacteria. However the number of organisms employed in the reported studies were limited in view of the respective objectives of those studies. Thus the spectrum of antimicrobial actions of these promising antimicrbial compounds are yet to ascertained.

In some studies the activities were found to be comparable or better than some standard drugs currently being used for the management of infectious diseases[2-3,4]. It has also been shown that these compounds are simple and relatively cheap to synthesize with good yield indicating the good potential of the compounds for developments into clinically useful formulations[2].

Complexes of aspartic acid that have been reported to exhibit promising activities include $Cd(asp)_2$, $Na[Mn(asp)_3]$, $Na_2[Mn(asp)_2]$, $Mn(asp)_2$, and $Na[Cu(asp)_3]$[3,5,6]. In this report the activity of these complexes were screened against a broad range of bacteria obtained from a wide variety of sources including reference strains, clinical isolates and organisms from environmental sources. These organisms represented those to be

encountered in the potential in-use conditions of these compounds. The screenings were done with the objective of establishing the spectrum of activities of the compounds with the ultimate aim of examining the possibility of employing any of them for chemotherapeutic purposes.

MATERIALS AND METHODS

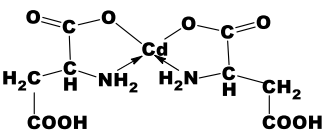
Test organisms

Test organisms were from wide variety of sources (Table 1) and they include strains of *Escherichia coli, Pseudomonas aeruginosa, Proteus spp*, *Klebsiella spp* and *Enterobacter spp* which are Gram negative organisms while *Staphylococcus aureus* and *Enterococcus spp* were the Gram positive organisms.

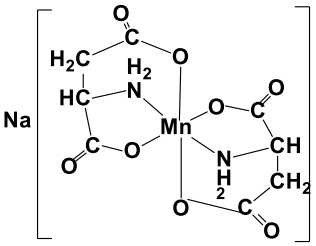
Reference strains were from stocks of culture collections maintained in the Laboratory of the Department of Pharmaceutical Microbiology of the Obafemi Awolowo University, Ile-Ife, Nigeria. Clinical isolates were obtained from specimens from human infections (including diarrhoea, urinary tract infections and wounds infections), environmental isolates were from water samples collected from various sources in the community, All isolates were identified by conventional biochemical tests and were maintained by cryopreservation according to the method of Gibson and Khoury[7] as earlier reported[8]. A total of seventy-two bacteria strains were used in the study.

The aspartic acid complexes

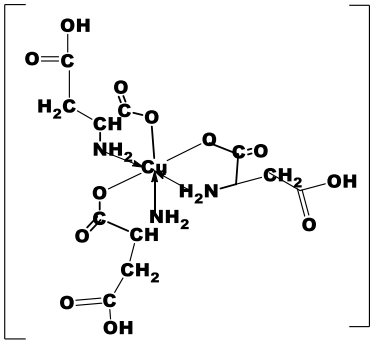
The complexes were prepared as previously reported[3,5]. They include: bisaspartatocadmium(II), $Cd(asp)_2$; bisaspartatomanganese(II), $Na_2[Mn(asp)_2]$; sodium trisaspartatomanganese(II), $Na[Mn(asp)_3]$; bisaspartatomanganese(II), $Mn(asp)_2$ and sodium trisaspartatocupprate(II) $Na[Cu(asp)_3]$ [Figure 1].



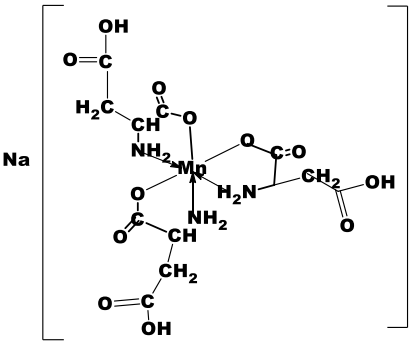
bisaspartatocadmium(II), $Cd(asp)_2$



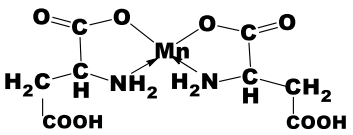
Sodium bisaspartatomanganese(II) $Na_2[Mn(asp)_2]$;



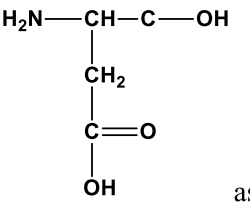
Sodium trisaspartatocupprate(II) $Na[Cu(asp)_3]$



sodium trisaspartatomanganese(II), $Na[Mn(asp)_3]$



bisaspartatomanganese(II), $Mn(asp)_2$



aspartic acid

Figure 1. Structures of the aspartic acid complexes

Preparation of agents

A 1.6 grams of each agent was weighed and suspended in 4ml of sterile water in a test tube and shaken thoroughly using the Rotamixer Electric Shaker to give a 400mg/ml stock solution. The stock solution was then diluted using sterile water to obtain 400mg/ml, 200mg/ml and 100mg/ml concentrations.

Determination of Minimum Inhibitory Concentrations (MICs)

MICs were determined by the agar dilution method on the Mueller Hinton Agar as earlier described[9,10]. Briefly, stock suspensions of the 400 mg/mL, 200mg.mL and 100mg/mL of compounds were prepared and from these, plate concentrations containing 40, 20 and 10mg/mL of compounds in the test medium were made. Plates containing streptomycin (1µg/mL) were similarly prepared and employed as controls. The bacteria were grown overnight in nutrient broth. The cultures were diluted to a final density of 2 x 10^5 cfu/mL in normal saline and applied to the surface of the Mueller Hinton Agar plates containing dilutions of the compounds, streptomycin, or solvent alone employing a multi-point inoculator. Plates were incubated at 37°C for 48 h. All plates were observed for growth and the minimum dilution completely inhibiting the growth of each organism was taken as the MIC.

RESULTS

The minimum inhibitory concentrations of the complexes of aspartic acids for the test organisms have been determined [Table 1]. All agents were found to possess broad spectrum of antimicrobial activity. Each compound however differs in their minimum inhibitory concentrations. Most of the compounds demonstrated the MIC of 10 mg/mL while few had MIC of 20 mg/mL. None of the compounds had MIC of 40 mg/mL.

$Mn(asp)_2$ had an MIC of 10 mg/mL against all the organisms making it the most active of all. $NaCu(asp)_3$ had the MIC of 10 mg/mL to all the organisms except one clinical strain of *P. aeruginosa* which had an MIC of 20 mg/mL. $Na(Mn(asp)_3$ had the MIC of 10 mg/mL to all except to one strain each of *E. coli* and *Enterococcus* spp with the MIC of 20 mg/mL. $Na_2[Mn(asp)_2]$ had MIC of 10 mg/mL to all except for three strains of *Klebsiella*, one strain of the *Enterobacter* spp and the *Proteus vulgaris* environmental strain with MIC of 20 mg/mL each. The least activity was shown by $Cd(asp)_2$ with ten of the tested strains having the MIC of 20 mg/mL.

Table 1. MICs of different organisms against the aspartic acid complexes

Species of organisms	Source of organisms	No of strains tested	MIC(mg/mL) /Number of organisms															
			Strep*	$Cd(asp)_2$			$Na(Mn(asp)]_3$			$Na_2[Mn(asp)]_2$			$Mn(asp)_2$			$Na[Cu(asp)]_3$		
			1µg/ml	10	20	40	10	20	40	10	20	40	10	20	40	10	20	40
Gram negative aerobic facultative bacteria																		
Escherichia coli	ATCC 25922	1	1	1	0	0	1	0	0	1	0	0	1	0	0	16	0	0
E. coli	NCTC 8196	1	1	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
E.coli	Faecal	2	2	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0
E. coli	Clinical	12	12	10	2	0	11	1	0	12	0	0	12	0	0	12	0	0
Pseudomonas aeruginosa	ATCC 19429	1	1	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
P. aeruginosa	Water	2	2	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0
P. aeruginosa	Clinical	12	12	12	1	0	12	0	0	12	0	0	12	0	0	11	1	0
Proteus vulgaris	Environments	1	1	0	1	0	1	0	0	0	1	0	1	0	0	1	0	0
Proteus spp	Clinical	13	13	11	2	0	13	0	0	13	0	0	13	0	0	13	0	0
Klebsiella pneumoniae	Water	5	5	4	1	0	5	0	0	4	1	0	5	0	0	5	0	0
Klebsiella spp	Clinical	10	10	8	2	0	10	0	0	8	2	0	10	0	0	10	0	0
Enterobacter spp	Clinical	3	3	2	1	0	3	0	0	3	0	0	3	0	0	3	0	0
Gram positive aerobic facultative bacteria																		
Staphylococcus aureus	ATCC 29213	1	1	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
S. aureus	NCTC 6571	1	1	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
S.aureus	Clinical	2	2	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0
Enterococcus spp	Clinical	5	5	5	0	0	4	1	0	4	1	0	5	0	0	5	0	0

*Streptomycin

DISCUSSION

Generally more that 80% of the test strains from a wide variety of sources were inhibited by 10 mg/mL of all the aspartic acid complexes. All the strains were sensitive at a maximum MIC of 20 mg/ml. The least sensitive strains were strains of *Proteus* and *Klebsiela* spp with three of the fourteen and fifteen strains respectively tested having an MIC of 20 mg/mL to $Cd(asp)_2$ under test conditions. The most sensitive of all the organisms were the *S. aureus* strains with MIC of 10 mg/mL to all the agents. This indicates that despite the considerable activities of these agents to all the organisms screened, the complexes of aspartic acid still demonstrated higher activities to Gram positive than the Gram negative bacteria confirming earlier reports[2,5,6]. The more significant activity against Gram-positive organisms is however to be expected since it is well known that Gram-negative organisms possess a more sophisticated cell envelope and are usually less sensitive to the activity of antibacterial agents[11,12].

The activity of these complexes to the notoriously resistant *Pseudomonas* spp is worth of special note. These organisms are known opportunistic pathogens, found in many environments, responsible for many infections especially bone and soft tissues infections and with propensity for contamination of foods, water and some medicines in the domestic environment. These bacteria remain a significant challenge due to their high morbidity and mortality rates and their potential to develop drug resistance. They are known to have intrinsic lack of susceptibility to most antimicrobial agents in addition to their property of ease of acquiring resistances horizontally. They are thus very troublesome to control in the environments where they are found, especially within the hospital environment[13,14]. That these organisms are susceptible to these complexes *in vitro*, suggests the possibility of using the complexes, especially the most active ones, as sources of cheap and readily available agents against infections caused by the organisms.

Although a Gram negative organisms, *Enterobacter* was susceptible to most of the agents at the MIC of 10mg/mL. The clinical implication of this is that the complexes have potentials use in the management of infections caused by *Enterobatcer spp* such as pneumonia and urinary tract infections[15,16]. This same applications can be inferred for other organisms such as *Klebsiella spp*, *E.coli*, *Proteus spp*, and *P. aeruginosa* as most of these organisms are susceptible to these agents especially the $Mn(asp)_2$ which inhibited all the organisms at 10mg/mL. Indeed, the compound, $Mn(asp)_2$ represents one of the agents whose potential usefulness should be investigated further in *in-vivo* studies and clinical trials.

The infrequent occurrence of variations in MICs within species and between related organisms suggests that resistance to these complexes when it occurs will be due to intrinsic properties of the species involved rather than acquired characters[12,17,18]. For these reasons, it would be useful if the complexes can be exploited for development into antimicrobial chemotherapeutic agents. This is in line with the current search for such substances to augment or replace the presently available antibacterial agents and antibiotics in current clinical use which because of the continuous spread of resistant organisms are becoming much less useful than before[10,19,20].

CONCLUSION

The determination of the spectrum of activities of the complexes of aspartic acid were successfully carried out. Results indicate that the complexes have broad spectrum of activities against both intrinsically susceptible and resistant strains of organisms including those known to be opportunistic pathogens. The maximum MIC was 20 mg/mL with most organisms susceptible at MIC of 10mg/mL. $Mn(asp)_2$ was the most active of all the complexes screened. These complexes have potential for development as chemotherapeutic agents.

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Conflicts of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to infuence the work reported in this paper.

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