

BIOAVAILABILITY OF THE ANTISALMONELLAL ACTIVE INGREDIENTS CONTAINED IN ALLIUM SATIVUM BULBS

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ABSTRACT

The evaluation of the bioavailability of the antisalmonellal principle (s) contained in *Allium sativum* bulbs was done using the serum antimicrobial activity test and guinea pigs. Two hours after administration of *A. sativum* extract, the serum antimicrobial activity against *S. typhi* significantly increased, ($p < 0.05$) as compared to the activity of serum without extract (SWE). For *S. paratyphi* A and *S. paratyphi* B, significant increases in the serum activity were observed as from 5 hours after administration. ($p < 0.001$ and $p < 0.01$, respectively). The "apparent peak" of serum antimicrobial activity against the three bacteria strains used was observed at 8 hours after administration. The serum minimum inhibitory concentrations (serum MICs) were 16 for *S. typhi*, 16 for *S. paratyphi* A and 8 for *S. paratyphi* B, whereas the serum minimum bactericidal concentrations (serum MBCs) were 2 for *S. typhi* *S. paratyphi* A only showed activity with the neat serum, while *S. paratyphi* did not show any activity. These data suggest that *A. sativum* bulbs contain antisalmonellal principles which are biologically available and which can be developed into useful antimicrobial agents for the treatment of typhoid and paratyphoid fevers.

Keywords: enteric fever, *Allium sativum*, antisalmonellal, and bioavailability.

INTRODUCTION

Typhoid and paratyphoid fevers (enteric fevers) are caused by *Salmonella typhi* and *Salmonella paratyphi* (A and B), respectively (Cheesbrough, 1991). Worldwide, there is an estimated 16 million cases of typhoid fever causing 600,000 deaths each year (WHO, 1996). The overwhelming majority of infections and deaths occur in developing countries in general and in Sub-Saharan Africa in particular, where typhoid fever is endemic. Typhoid fever continues to be a marked public health burden (WHO, 1996). Conventional antimicrobial drugs are becoming more and more unavailable to the common man in Africa due to increased cost. Also, there is a greater prevalence of resistance to chloramphenicol, ampicillin, tetracyclines, and sulphonamides in developing countries (WHO, 1981). The increasing ineffectiveness of these drugs combined with the unavailability of alternative antimicrobials in developing countries, are contributing to the spread of major infectious diseases among which are enteric fevers (Cheesbrough, 1991).

In a previous work, *Allium sativum* bulb extract was reported to show antimicrobial activity against *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella paratyphi* B (Gatsing

et al., 2003). The extract was also found to contain flavonoids, cardiac glycosides, polyphenols and steroids (Gatsing *et al.*, 2003), in addition to the organosulfur compounds, (Iwu, 1993; Kamel & Saleh, 2000; Hyeong-Chan & Young-Hoi, 2001).

In a search for therapeutic agents from natural sources with potential for the treatment of typhoid and paratyphoid fevers, *Allium sativum* was selected and tested *in vitro* and *in vivo*. This was with a view to assessing the bioavailability of the active ingredients and the therapeutic efficacy of the extract of this plant.

MATERIALS AND METHODS

Plant sample, experimental animals and bacteria strains

The bulbs of *Allium sativum* (garlic) were purchased from Dschang main market and authenticated at the Cameroon National Herbarium (HNC), Yaoundé. Male guinea pigs were used in the study. The ages of the animals varied between 3 and 4 months, and they weighed between 327g and 402g. The bacteria strains, including *Salmonella typhi*, *Salmonella paratyphi* A, and *Salmonella paratyphi* B, were obtained from the Bacteriology Laboratory, Pasteur Centre, Yaoundé, Cameroon.

Preparation of the extract

Fresh cloves of *A. sativum* (500g) were thoroughly ground and 500ml of water were added; then the suspension was warmed in a water bath at 45°C for 15 minutes, with constant stirring. The mixture was filtered while still warm, and the filtrate was concentrated in a drying oven at 45°C.

Evaluation of the bioavailability of the antisalmonell ingredients.

This was done using the serum antimicrobial activity test, as described by Youmans *et al.* (1975). To monitor the effect of *A. sativum* extract *in vivo*, after oral administration of the extract (4g per kg body weight) to guinea pigs, the inhibitory or lethal action of the animal serum against a standard inoculum of the test bacteria was measured using dilution technique, as described by Cheesbrough (1991).

Statistical analysis

Statistical analyses were performed with the aid of SPSS for Windows software programme (Release 10.0.7). Group comparisons were done using the Student's t-test. A p value of < 0.05 was considered statistically significant.

RESULTS

The serum dilutions and percentages of inhibition of *S. typhi*, *S. paratyphi* A and *S. paratyphi* B, with *A. sativum* extract and male guinea pigs are as shown in Table 1.

Table 1: Serum dilutions and percentages of inhibition of *S. typhi*, *S. paratyphi* A and *S. paratyphi* B, with *A. sativum* extract using male guinea pigs.

	Serum Dilutions and Percentages of Inhibition (%)					
	Neat Serum	1/2	1/4	1/8	1/16	1/32
<i>S. typhi</i>						
SWE	2.7 ± 0.5 ^a	NA	NA	NA	NA	NA
2hrs after ad.	5.6 ± 0.4 ^b	NA	NA	NA	NA	NA
5hrs after ad.	74.5 ± 3.4 ^c	23.3 ± 4.2	8.1 ± 2.1	NA	NA	NA
8hrs after ad.	100 ^d	100	48.0 ± 2.1	23.4 ± 0.3	6.5 ± 0.5	NA
11hrs after ad.	75.8 ± 1.0 ^e	23.5 ± 1.2	8.8 ± 0.8	NA	NA	NA
<i>S. paratyphi</i> A						
SWE	2.8 ± 0.4 ^f	NA	NA	NA	NA	NA
2hrs after ad.	2.9 ± 0.5 ^g	NA	NA	NA	NA	NA
5hrs after ad.	63.01 ± 3.5 ^h	17.5 ± 3.0	6.1 ± 1.0	NA	NA	NA
8hrs after ad.	100 ⁱ	66.0 ± 3.0	29.0 ± 1.3	15.4 ± 2.1	2.1 ± 0.5	NA
11hrs after ad.	53.4 ± 1.8 ^j	15.1 ± 1.5	2.0 ± 0.4	NA	NA	NA
<i>S. paratyphi</i> B						
SWE	2.6 ± 0.6 ^k	NA	NA	NA	NA	NA
2hrs after ad.	2.8 ± 0.4 ^l	NA	NA	NA	NA	NA
5hrs after ad.	48.5 ± 2.4 ^m	16.5 ± 2.0	3.0 ± 1.3	NA	NA	NA
8hrs after ad.	92.6 ± 1.8 ⁿ	38.5 ± 3.2	14.8 ± 1.7	2.0 ± 0.6	NA	NA
11hrs after ad.	47.6 ± 1.7 ^o	14.5 ± 1.8	2.0 ± 0.5	NA	NA	NA

Tabulated values are Mean ± SD of six determinations.

Key: NA = No Activity SWE = Serum Without Extract; ad. = Administration.
 a vs b p < 0.05; f vs g p > 0.05; k vs l p > 0.05;
 b vs c p < 0.001; g vs h p < 0.001; l vs m p < 0.01;
 c vs d p < 0.02; h vs i p < 0.01; m vs n p < 0.01;
 d vs e p < 0.02; i vs j p < 0.01; n vs o p < 0.01.

The serum antimicrobial activity against the three bacteria strains used generally increased with time (i.e. time interval between administration of the plant extract and collection of the blood), to reach a peak, and decreased afterwards. The serum without extract (SWE), obtained from male guinea pigs, was found to be active against the three bacteria strains (% inhibition obtained were $2.7 \pm 0.5\%$, $2.8 \pm 0.4\%$, and $2.6 \pm 0.6\%$, against *S. typhi*, *S. paratyphi A* and *S. paratyphi B*, respectively). Two hours after administration of *A. sativum* extract, the serum antimicrobial activity against *S. typhi* significantly increased, ($p < 0.05$) as compared to the activity of SWE. Five hours after administration, the serum activity showed a significant increase, ($p < 0.001$) as compared to the activity after 2 hours. The serum antimicrobial activity showed a significant increase 8 hours after administration, ($p < 0.02$) as compared to the serum activity after 5 hours, followed by a significant decrease afterwards ($p < 0.02$).

Two hours after administration of the extract, the serum antimicrobial activity against *S. paratyphi A* did not show any significant increase, ($p > 0.05$) as compared to the activity of the SWE. The serum activity 5 hours after administration of extract was significantly higher than that observed at 2 hours after administration ($p > 0.001$). Eight hours after administration, the serum antimicrobial activity showed a significant increase, ($p < 0.01$) as compared to the activity at 5 hours, followed by a significant decrease 11 hours after administration. ($p < 0.01$) The serum antimicrobial activity against *S. paratyphi B*, 2 hours after administration of the extract, did not show any significant increase, ($p > 0.05$) as compared to the activity of the SWE. Five hours after administration, the serum activity showed a significant increase, ($p < 0.01$) as compared to that observed at 2 hours, followed by another significant increase at 8 hours after administration. ($p < 0.01$) This was followed by a significant decrease 11 hours after administration. ($p < 0.01$) The "apparent peak" of serum antimicrobial activity with *A. sativum* extract against the three bacteria strains used was observed at 8 hours after administration. Serum inhibition parameters (serum MICs and serum MBCs) with *A. sativum* extract and male guinea pigs are given in Table 2.

Table 2: Serum inhibition parameters (serum MICs and serum MBCs) with *A. sativum* extract and male guinea pigs.

Parameters	Bacteria Strains		
	<i>S. typhi</i>	<i>S. paratyphi A</i>	<i>S. paratyphi B</i>
Serum MIC	1/16	1/16	1/8
Serum MBC	1/2	Neat Serum	ND

Key: ND = Not determined.

The serum minimum inhibitory concentrations (serum MICs) were 16 for *S. typhi*, 16 for *S. paratyphi A* and 8 for *S. paratyphi B*, whereas the serum minimum bactericidal concentrations (serum MBCs) were 2 for *S. typhi*, neat serum (i.e. whole serum, not diluted) for *S. paratyphi A* and was not determined for *S. paratyphi B*.

DISCUSSION

The serum antimicrobial activity technique used in this work was particularly appropriate for the study of antisalmonella drugs. In order to confirm the *in vitro* antisalmonella activity, an *in vivo* study is necessary, since a drug may be very active *in vitro* but may not show the same activity *in vivo* (Youmans *et al.*, 1975, Gatsing *et al.*, 2003). The serum without extract (SWE), from both male and female guinea pigs, was found to exhibit antimicrobial activity against all the three bacteria strains used. This result is in agreement with the report of Youmans *et al.* (1975), who stated that in addition to an antimicrobial drug, there may be numerous other antibacterial substances in the blood, including serum bactericidal activity, circulating opsonins and specific antibody, lysozyme, beta lysin, and other poorly defined components.

Serum antimicrobial activity with the extract against the bacteria strains used generally increased with time to reach a peak, and decreased afterwards. This result suggests that the antimicrobial principles in this plant extract were progressively absorbed from the gastrointestinal tract and that after some time they were progressively excreted or metabolised. It appears that the rate of absorption might have been generally higher than the rate of excretion or metabolism. Consequently, there might be a considerable amount of free-circulating antimicrobial principles from *A. sativum* extract in the plasma (the protein-bound portions of antimicrobial agents are not active) (Youmans *et al.*, 1975).

The serum MIC against the three bacteria strains used varied from 8 to 16, while the serum MBC varied from straight serum to 2. Youmans *et al.* (1975) reported that empirically it has been found that serum lethal levels of 8 or greater have been associated with favourable outcomes, while serum lethal levels lower than 8 are associated with less favourable results. He also reported that response to therapy in patients with urinary tract infections correlated best with the inhibitory level found in the urine; clinical cure was observed in 90% of patients whose urine inhibited growth of the infecting microorganism with dilutions of 1/4 or better.

In the present study, the serum lethal levels were lower than 8. This may be explained by the fact that the work was carried out with crude extract and not with pure compounds (e.g. penicillin, gentamicin, ampicillin). Therefore, the serum lethal levels obtained with *A. sativum* crude extract obtained in this study may suggest that the plant might be used in the treatment of typhoid and paratyphoid fevers. *A. sativum* contains active compounds, with antisalmonella properties, which are biologically available and which can be developed into useful antimicrobial agents for the treatment of enteric fevers.

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REFERENCES

- Cheesbrough, M. (1991). Medical Laboratory Manual for Tropical Countries: Microbiology. ELBS 2, 479 p.
- Gatsing, D., Aliyu, R., Meli, W. B., Adoga, G. I. And Tchouanguep, M. F. (2003). Phytochemical profile and antisalmonellal properties of *Allium sativum* bulb extract. West Afr. J. Biol. Sci. **14**: 29 – 36.
- Hyeong-Chan, J. and Young-Hoi, K. (2001). Analyses of volatile compounds from *Allium* spp. and ovipositional response of *Delia antiqua* to various volatile chemicals. Han'guk Nonghwa Hakhoechi **44** (1): 12 – 19.
- Iwu, M.M. (1993). Handbook of African Medicinal Plants. CRC Press, 500p.
- Kamel, A., Saleh, M. (2000). Recent studies on the chemistry and biological activities of the organosulfur compounds of garlic (*Allium sativum*). Stud. Nat. Prod. Chem. **23**: 455-485.
- WHO (1981). Antimicrobial Resistance. Geneva. WHO Scientific Working Group, p 7.
- WHO (1996). The World Health Report 1996 - Fighting Disease, Fostering Development. Geneva. World Health Organisation, p 39.
- Youmans, G. P., Paterson, P. Y. and Sommers, H. M. (1975). The Biologic and Clinical Basis of Infectious Diseases. W.B. Saunders Company, 855 p.