COMPARISON OF THE ANTIMICROBIAL EFFECTS OF CRUDE EXTRACTS OF SOME ANTIDIARRHOEAL HERBS

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ABSTRACT

An investigation was conducted to compare the in vitro and in vivo antimicrobial effects of the crude extracts of six antidiarrhoeal decoctions strictly derived from traditional herbal preparations. The in vitro study showed that the extracts were active against some of the tested bacteria. Zones of inbibition were observed for five extracts namely Sclerocarya birrea Hochst, Annona senegalensis Pers, Monodora tenuifolia Benth, Mitragyna africana Korth and Butyrospermum paradoxum Geartn. Though Escherichia coli (K99) was resistant in vitro, complementary in vivo study on diarrhoea induced rabbits using this microbe was effectively controlled by the water extracts of Annona senegalensis Pers, Sclerocarya birrea Hochst, Mitragyna africana Korth and Monodora tenuifolia benth. From the result of this study, it was considered that the potency of these extracts is dependent on their extractable chemical constituents and that their varying degrees of potency as well as the interchanged rate of in vitro and in vivo activities between individual extracts is influenced by the interaction of these constituents with the target enzymes within the operative medium.

INTRODUCTION

From earliest times, man has used herbs in the treatment of diseases and ailments. Most of these herbs, especially the higher ones, have been known to show bacteriostatic or bactericidal effects on disease causing bacteria[1,2].

As a result of research into the active components contained in some of the higher plant extracts, considerable information has surfaced within chemotherapy. For instance, a naturally occurring 2-methoxy - 1-4-naphthoquinone which is active against several phytopathogenic organisms has been isolated from the crude extract of garden blossom, *Impatiens balsamina*[3]. Until now, a great number of these agents exist for various purposes and the search for new ones should be vigorously pursued since the target microbes may often evolve into new genetic variants which could subsequently become resistant to existing agents. But with the current trend in the biotechnology of plant tissue cultures[4], the possibility that man may soon have to depend on the higher plants as a source of a number of antimicrobial agents cannot be underscored since these plants will most likely continue to produce antimicrobial agents that could be used against infections by microorganisms[5].

Admittedly, plant extracts from different parts of the world have been demonstrated in the last couple of decades to possess antimicrobial properties because of the presence of antimicrobial agents[6-11].

Results of scientific investigations have shown that microorganisms such as *Bacillus anthracis, Shegella, Salmonella, Clostridium, Vibrio chlocholerae,* enteropathogenic *Escherichia coli* etc, are responsible for diarrhoeal diseases[12-15]. A number of plants have been reported to be effective in the traditional management and treatment of the disease. In Nigeria, *Terminalia* species have been demonstrated to be effective in both the trado-medicinal and clinical treatment of diarrhoea[6]. *Ardisia colorata* Roxb growing in Malaysia has been shown in Thailand to have antidiarrhoeal properties[16].

The potency of plant extracts or drugs could be enhanced or reduced on the whole animal pharmacology (*in vivo*) and on living cultured bacteria cells (*in vitro*). As a result, *in vitro* experiments can be used in screening and later extrapolated to *in vivo* situations[17,18]. The extrapolation to *in vivo* situations is always necessary in order to simulate the modifying effects of other

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biological agents which, in the system, will affect the overall activity of the compound being tested[19]. This study was therefore undertaken to compare the antimicrobial effects of the crude extracts of some antidiarrhoeal herbs on living cultured bacteria cells *in vitro* and on the whole animal pharmacology *in vivo*.

EXPERIMENTAL

In vitro assay

Cultures

The microorganisms employed in this study were obtained from the Department of Veterinary Microbiology and Parasitology, University of Maiduguri, Borno State, Nigeria with the exception of *E. coli* (K99) which was obtained from Dr. Tekdek of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. All the microorganisms were propagated and stored on nutrient agar slants. The nutrient agar medium was obtained in dehydrated powder form from 'Oxoid' and was prepared according to the manufacturer's specifications. All stock cultures were maintained in nutrient agar slants at 4°C and subcultured in nutrient broth (Oxoid) at 37°C for 24 hours prior to each antimicrobial testing.

Preparation of crude extracts

The specified parts of plant materials were collected from Maiduguri Metropolis in Borno State of Nigeria. The plants were number coded as *Albizia chevalieri* Harms (Leguminosae) (1); *Annona senegalensis* Pers (Annonaceae) (2); *Butyrospermum paradoxum* Gaertn (Sopotaceae) (3); *Mitragyna africana* Kenth (Rubiaceae) (4); *Monodora tenuifolia* Benth (Annonaceae) (5); *Sclerocarya birrea* Hochst (Anacardiaceae) (6). The stem bark of the plant was coded (C) and the leaf (L). The plant specimens were washed with clean water and air-dried in the laboratory. A 10g sample each was powdered and 5g each was weighed out into a sterilised volumetric flask. Each was extracted with 20cm^3 of distilled water according to herbal specifications. It was filtered on Whatman (W and R Balston Ltd, England) and kept in a refrigerator at 4°C when it was not used immediately after preparation. Hence, extracts are identified by a code containing a digit and a letter indicating the plant and the plant part extracted respectively. The control, as indicated in Table 3, was just distilled water.

Test for antimicrobial activity

Using a sterile loop, a 24 hour subculture of each tesst organism was uniformly lawn inoculated over the surface of a sterile nutrient agar and allowed to dry. Six equally spaced wells of about 8mm were cut in the inoculated plates and filled with about 3 x $10^{10}\mu g/cm^2$ of the aqueous extracts of the plant containing 2.5mg/cm^3. The plates were aerobically incubated overnight and were subsequently examined for zones of inhibition around the wells. The zones, where present, were quantified by direct linear measurements of their diameters. The results are presented in Table 1.

Table 1: *In vitro* activity inhibition by the water extracts of the plants.

Micro-organisms	Inhibition zones diameter(mm) for					
	1C	2C	3C	4L	5C	6C
E. coli (animal)	0	10	0	0	0	10
Salmonella gallinarum	0	0	0	0	0	0
Salmonella pullorum	0	0	0	0	0	0
Klebsiella species	0	0	0	0	0	10
Pasteurella multocida	0	10	0	0	0	9
Pasteurella hamolytica	0	0	0	0	0	11
Staph. aureus (human)	0	10	0	0	16	16
Stap. aureus (animal)	0	0	8	0	0	14
Staph. aureus (oxford)	0	0	0	0	0	11
Staph. hyicus (human)	0	10	0	10	8	16
E. coli (K99)	0	0	0	0	0	0

In vivo assay

A total of 14 rabbits obtained from a rabbitary farm located at 35 Bama Road, Maiduguri were used and each was identified by a number in the range 1-14. The microorganisms were the standard strain of *E. coli* (K99). Saline solution was prepared by dissolving 1.7g of sodium chloride in 200cm^3 of distilled water and autoclaving for 15 minutes. The solid medium was also prepared by dissolving 7.5g of Eosin Methylene Blue (EMB) agar in 200cm^3 distilled water and sterilised by autoclaving for 15 minutes. The medium was allowed to cool to a temperature of 55°C in a water bath (Gallenkamp, U.K.) and 20cm^3 portions were poured into glass petri dishes. The medium was allowed to solidify overnight in an incubator.

Using a sterile loop, the EMB plates were inoculated with the standard strain of *E. coli* (K99) and incubated overnight at 37°C. The growth of the organism was scraped off with a microscope slide cover and suspended in 10cm^3 saline solution and

mixed thoroughly to obtain a homogenous suspension. Diarrhoea was orally induced in the laboratory rabbits denied food for a day prior to the experiment using the K99 microbe containing 6.3 x 10^8 colonies of bacteria. The bacteria colonies in the diarrhoea rabbits were determined by inserting sterilised swabs into their anus and making a ten-fold dilution, of each of 10^1 - 10^{10} of the *E. coli* solution using ten different test tubes each containing 9cm^3 of saline solution. The last three dilutions 10^8, 10^9, and 10^{10} were plated out and plates of dilution showing single colonies were counted according to standard methods[20]. This process was repeated successively for 3 days before and after each treatment and further dilutions made and plated out as the experiment progressed according to the Brown's Opacity Standards[21] The temperature of the rabbits was measured for 3 days before and after each treatment because of the pyrogenic nature of the *E. coli* extract.

Each of the animals under treatment was given 3cm^3 of the test extract orally using 5cm^3 syringes at 24, 48 and 72 hours after induction of diarrhoea. The control animals were only given distilled water.

RESULTS AND DISCUSSION

The antimicrobial effects of the aqueous extracts of the six antidiarrhoea herbs at equal concentration was assayed by the growth inhibition of both gram-positive and negative bacteria (Table 1) together with the complementary *in vivo* activity test (Tables 2 and 3). Only five extracts showed some antimicrobial activity against the tested microbes *in vitro* and four extracts in the case of *in vivo* assay when K99 bacteria was used. The lack of both *in vitro* and *in vivo* activity by extract 1C may suggest that this extract has no active compound against bacteria or that the active compound is not water soluble and thus may not be present when extracted with water. The larger zones showed by extract 6C *in vitro* indicate that the extract contains an active compound that is water soluble and highly active against bacteria. The fact that the gram positive bacteria are more sensitive, with larger inhibition zones, than the gram negative for this extract could be that the active principle present in the extract has some affinity towards the cell wall of gram positive microbes and thus was more bactericidal. Although this extract, 6C, like the others, was not active against K99 microorganism *in vitro*, it showed some activity *in vivo*. There was a decrease in body temperature and inoculum size when extract 6C was used *in vivo*, but not more pronounced than was observed for extract 2C comparatively (Tables 2 and 3). Extract 2C exhibited the strongest antidiarrhoeal activity *in vivo* (Figure 1) and the drastic reduction in temperature of the animals when treatment commenced could probably be due to some antipyretic activity of this extract. It also showed some antimicrobial activity *in vitro* but not more pronounced than was observed for extract 6C. Out of the three extracts, 4L, 5C and 3C, that demonstrated little antimicrobial activity *in vitro*, only extracts 4L and 5C exhibited some antidiarrhoeal activity *in vivo*. In the *in vitro* assay,

Table 2. *In vivo* test: result before treatment with extracts.

Animals	1	14	4	6	9	10	11	12	7	8	1	5	2	3
Day 1														
Temperature	39.00	38.90	39.50	39.60	39.50	40.00	38.60	40.00	40.40	39.80	38.80	39.40	39.70	40.00
Colonies x 10^8	6.30	6.30	6.30	6.30	6.30	6.30	6.30	6.30	6.30	6.30	6.30	6.30	6.30	6.30
Log. Count	8.80	8.80	8.80	8.80	8.80	8.80	8.80	8.80	8.80	8.80	8.80	8.80	8.80	8.80
Day 1														
Temperature	41.00	40.20	41.60	42.30	40.00	41.00	39.00	40.60	41.00	40.30	39.80	40.80	40.60	41.80
Colonies x 10^9	1.20	8.30	6.30	10.30	40.00	24.00	1.60	2.50	1.60	3.00	4.20	2.50	4.50	3.41
Log Count	9.10	9.90	9.80	10.00	10.60	10.40	9.20	9.40	9.20	9.50	9.60	9.40	9.70	9.50
Day 2														
Temperature	42.60	40.50	44.30	45.00	40.30	43.40	39.80	41.00	43.00	40.80	43.00	43.80	45.00	44.60
Colonies x 10^{10}	0.25	8.00	5.02	7.80	8.00	9.80	0.63	1.00	0.40	0.60	3.00	0.90	2.53	2.53
Log. Count	9.40	10.90	10.70	10.80	10.90	11.00	9.80	10.00	9.60	10.20	10.50	9.95	10.40	10.40
Day 3														
Temperature	43.00	40.90	45.00	46.80	41.00	44.80	41.00	42.00	43.60	41.30	44.70	47.00	45.90	46.00
Colonies x 10^{10}	4.80	9.00	6.30	2.90	9.00	5.00	1.60	4.80	18.00	2.00	8.00	3.10	8.00	6.30
Log. Count	10.70	11.00	10.80	11.11	11.00	10.70	10.20	10.70	11.30	10.30	10.90	10.50	10.90	10.80

Table 3. Results after treatment with Extracts

Extracts	IC	IC	2C	2C	3C	3C	4L	4L	5C	5C	6C	6C	Controls	
Animals	13	14	4	6	9	10	11	12	7	8	1	5	2	3
Day 0														
Temperature	43.00	40.90	45.00	46.80	41.00	44.80	41.00	42.00	43.60	41.30	44.70	47.00	45.90	46.00
Colonies x 10^{10}	4.80	9.00	6.30	2.90	9.00	5.00	1.60	4.80	18.00	2.00	8.00	3.10	8.00	6.30
Log. Count	10.70	11.00	10.80	11.11	11.00	10.70	10.20	10.70	11.30	10.30	10.90	10.50	10.90	10.80
Day 1				**24 Hours Effect**										
Temperature	42.80	40.80	43.00	43.30	41.00	44.00	39.20	40.00	42.20	40.30	42.50	45.80	45.60	45.70
Colonies	$3.70x10^{11}$	$4.30x10^{12}$	$0.31x10^{8}$	$2.47x10^{8}$	$2.36x10^{12}$	$0.50x10^{13}$	$9.50x10^{8}$	$1.6x10^{9}$	$5.30x10^{8}$	$1.60x10^{9}$	$3.17x10^{8}$	$7.80x10^{8}$	$1.30x10^{12}$	$8.0x10^{11}$
Log Count	11.60	12.63	7.50	8.40	12.40	11.70	9.00	9.20	8.70	9.20	8.50	8.90	12.10	11.90
Day 2				**48Hours Effect**										
Temperature	43.20	41.00	40.20	41.30	43.00	45.00	37.20	38.00	41.20	39.10	40.00	41.20	45.80	45.90
Colonies	$3.70x10^{12}$	$3.80x10^{13}$	$1.02x10^{6}$	$2.48x10^{6}$	$6.30x10^{13}$	$0.60x10^{13}$	$7.30x10^{7}$	$3.0x10^{7}$	$2.0x10^{7}$	$9.0x10^{7}$	$3.10x10^{6}$	$1.50x10^{7}$	$2.0x10^{13}$	$1.30x10^{13}$
Log Count	12.60	13.58	6.00	6.40	13.80	12.80	7.90	7.50	7.30	8.00	6.50	7.20	13.30	13.10
Day 3				**72Hours Effect**										
Temperature	44.30	43.60	39.00	39.20	45.00	46.90	36.20	37.10	40.80	38.10	38.20	39.00	45.80	45.90
Colonies	$3.7x10^{13}$	$2.80x10^{15}$	$1.10x10^{5}$	$2.0x10^{5}$	$1.60x10^{15}$	$6.30x10^{13}$	$1.60x10^{7}$	$8.0x10^{6}$	$2.30x10^{6}$	$2.30x10^{7}$	$3.17x10^{5}$	$2.0 x10^{5}$	$3.0x10^{14}$	$1.60x10^{14}$
Log Count	13.60	15.40	5.00	5.30	15.20	13.80	7.20	6.90	6.40	7.40	5.50	6.30	14.50	14.20

extracts 4L and 5C showed almost equal activity but the *in vivo* study revealed that extract 4L is more potent than extract 5C (Figure 1). The variation in the *in vivo* chemotherapeutic activity between these two extracts could be a result of the difference in the rate at which their bioactive agents interact with the target enzymes capable of decreasing both the temperature of the animals and the inoculum size of the microorganisms.

The fact that these four extracts were not active *in vitro* against the K99 microorganism but showed activity *in vivo* and the varying degrees of activity as well as the interchange rate of potency among individual extracts or drugs within or outside the same medium is not unusual[19,17,18]. For instance, the red azo dye, prontosil, which was active against a systemic streptococcal infection *in vivo* was ineffective against the bacteria in vitro[22]. This was because the chemotherapeutic activity of prontosil was demonstrated to be due to the breakdown product, P-amino-benzene sulfonamide (sulfanilamide), in the body[23,24].

Studies by Ross *et al.*[25] to determine the antibiotic substances in some Egyptian plants showed that some of the plants extracts that were ineffective *in vivo* showed some activities *in vitro* and vice versa. The intestinal antisecretory drug, clonidine, which acts by an α_2 - adrenergic mechanism, has been demonstrated to inhibit the increase in intestinal potential difference caused by diarrhoea - induced secretogogues but was ineffective *in vitro*[26,27]. Trifluoromethane sulfoylamidine, currently under investigation in human subjects for the treatment of diabetic complications, retained high *in vitro* potency but was inactive *in vivo*[28,29]. This suggests that the compound does not readily penetrate the peripheral nerve which, presumably, is a factor in the lack of oral activity[30]. As part of the ongoing programme of the synthesis of nucleosides as potential antiparasitic agents, the 5′ - 0 - sulfamoyl nucleoside derivatives of ribavirin namley 1 - (5′ - 0 - sulfamoyl - β - D - ribofuranosyl (1,2,4) triazole - 3 - carboxamide, thioamide sulfamate, 1 - (5′ - 0 - sulfamoly - β - D - ribofuranosyl (1,2,4) triazole - 3 - thiocarboxamide and 1 - (5′ - 0 - sulfamoyl - β - D - ribofuranosyl (1,2,4) triazole - 3 - carbonitrile were synthesised and evaluated for antiparasitic activity *in vitro* and *in vivo*[31]. All these drugs showed significant antiparasitic activity *in vitro*, while only 1 - (5′ - 0 - sulfamoyl - β - D - ribofuranosyl (1,2,4) triazole - 3 - carbonitrile showed significant activity *in vivo* against the tested microbes, *Leishmania donovani* and *Trypanosoma brucei*[32]. This was because this drug was able to inferfere with the adenosine metabolism in the parasites which the other two drugs could not do.

From the foregoing, therefore, it could be seen that the different potencies of these extracts upon oral administration could be a function of the physiochemical properties of the extracts constituents and of a larger number of pharmacokinetic

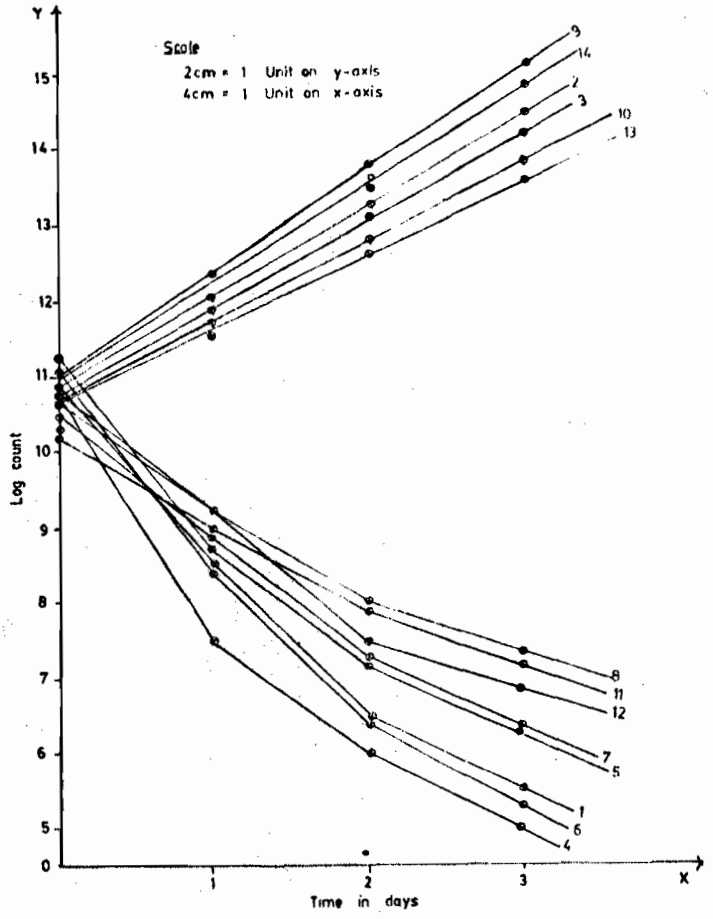

Fig. 1 : Plot of log count against time for diorrhoea rabbits after treatment with extracts

parameters including the interaction of the constituents with the target enzyme as well as the absorption, metabolism, tissue distribution and elimination of these constitutents.

CONCLUSION

The results of this study have shown that the lack of activity, varying degrees of potency and the interchanged rates of activity among these extracts wtihin and outside the medium may be considered to be associated with the difference in sensitivities of the microbes to the bioactive constituents and as well, to a large extent, with the physicochemical, chemotherapeutic and pharmacokinetic properties of these extracts.

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