A RATIONAL IN VITRO EVALUATION OF 53 MEDICINAL PLANTS USED IN THE TREATMENT OF DIARRHOEA AND THE POTENTIAL USE OF *DEINBOLLIA OBLONGIFOLIA* (SAPINDACEAE) EXTRACTS

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DECLARATION

This represents an experimental record for the work carried out in the Department of Pharmacology, University of Pretoria, under the supervision of Prof. J.N. Eloff and Dr L.J. McGaw.

I, the undersigned Gabriele Würger, present this document as my authentic material and acknowledge that it has not been submitted in any other form to any other institution. I also acknowledge that I have consulted many publications in compiling this work and the references are all listed.

Gabriele Würger

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LIST OF ABBREVIATIONS

A/G	Albumin/Globulin ratio
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BEA	Benzene/Ethanol/Ammonia
C	crude extract
Ca	Calcium
CEF	Chloroform / Ethyl acetate/ Formic acid
CF	Chloroform Fraction of *Deinbollia oblongifolia*
CNMR	Carbon nuclear magnetic resonance
COSY	Correlated spectroscopy
Crea	Creatinine
CSIR	Council for Scientific and Industrial Research
DB	*Deinbollia oblongifolia* n-butanol fraction
DC	*Deinbollia oblongifolia* chloroform fraction
DEPT	Distortionless enhancement by polarization transfer
DH	*Deinbollia oblongifolia* hexane fraction
DO	*Deinbollia oblongifolia*
DM	*Deinbollia oblongifolia* 35% water in methanol fraction
DMSO	Dimethyl sulfoxide
DW	*Deinbollia oblongifolia* water fraction
EA	Ethyl acetate
E. coli	*Escherichia coli*
EMW	Ethyl acetate/Methanol/Water
F	Fraction
GGT	Gamma glutamyl transferase
GLOB	Globulin
H	Hexane

HMBC	Heteronuclear bond correlation
HMQC	Heteronuclear multiple quantum correlation
HNMR	Proton nuclear magnetic resonance
INT	p-Iodonitrotetrazolium violet
K	Potassium
LC_{50}	Lethal celltoxicity
MEM	Minimum essential medium
MIC	Minimum Inhibitory Concentration
MTT	3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide
Na	Sodium
NIR	Near-Infrared Reflectance
NMR	Nuclear magnetic resonance
OECD	Organisation for Economic Cooperation and Development
P.a.	*Peltophorum africanum*
R_f	Retention factor
S. aureus	*Staphylococcus aureus*
SB	*Spirostachys africana* n-butanol fraction
SC	*Spirostachys africana* chloroform fraction
SD	Standard Deviation
SH	*Spirostachys africana* hexane fraction
SI	Selectivity Index
SM	*Spirostachys africana* 35% water in methanol fraction
SW	*Spirostachys africana* water fraction
TA	Total activity
T.e.	*Trichilia emetica*
TLC	Thin Layer Chromatography
TSP	Total serum protein
Urea	Urea

UV	Ultraviolet
WHO	World Health Organization
Z.m.	*Ziziphus mucronata*

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ABSTRACT

Antibiotic Feed Additives (AFA) have been used to prevent many bacterial infections during weaning of livestock. The use of these AFA's resulted in the development of multiresistant bacterial strains and was therefore banned by the European Union. The United States also restricted the use of these feed additives considerably. Many scientists have started to search for alternatives in the prophylactic and therapeutic treatment of bacterial infections. Plants have been used traditionally by resource poor people all over the world to treat many infections. Diarrhoea not only causes many problems in the high intensity production of livestock but also leads to many human deaths. A large number of plants have been used to treat diarrhoea in humans and animals. Several authors have selected one or two species based on traditional use to evaluate in depth. In this project several different relevant parameters of 53 plant species used traditionally to treat diarrhoea were investigated in order to develop a model that would identify the species with the highest chance of delivering a useful antibacterial product.

Antibacterial activities against two pathogens important in diarrhoea (*Staphylococcus aureus* and *Escherichia coli)* were positive parameters in selecting species. Because tannins frequently have antibacterial activity, but are not useful as prophylactic agents due to their effect on production, high tannin content was considered to be a negative indication. Cellular toxicity was also used as a negative parameter at a later stage.

In addition to the *in vitro* assays there are also other parameters to be investigated to evaluate the potential use of plants. The influence of season of collection was determined on the antibacterial activity and tannin content of acetone leaf extracts of five plant species traditionally used to treat diarrhoea. They were *Acacia karroo*, *Acacia sieberiana* var. *woodii*, *Peltophorum africanum*, *Trichilia emetica* and *Ziziphus mucronata*.

The antibacterial activity varied depending on the season of collection with the best activity generally in the months of late summer to autumn (January to April). The activity of *Acacia karoo* against *E. coli* was best in the month of April (MIC = 0.11 mg/ml (TA = 332 ml/g)) and against *S. aureus* in the month of March (MIC = 0.06 mg/ml (TA = 334 ml/g)). *Acacia sieberiana* subsp. *woodii* extracts had the best activity against *E. coli* (MIC = 0.10 mg/ml (TA = 303 ml/g)) in March against *S. aureus* in April (MIC = 0.08 mg/ml (TA = 303 ml/g)). *Peltophorum africanum* extracts were most active against *E. coli* in February (MIC = 0.05 mg/ml (TA = 1188 ml/g)) and against *S. aureus* in February and March (MIC = 0.04 mg/ml (TA = 1188 ml/g and 1075 ml/g)). *Trichilia emetica* extracts were generally not very active against the bacterial strains (best activity: MIC = 0.22 mg/ml (TA = 74 ml/g) against *E. coli* in May and MIC = 0.28 mg/ml (TA = 26 ml/g) against *S. aureus* in December). *Ziziphus mucronata* was most active

against both bacterial strains and in May (*E. coli*: MIC = 0.10 mg/ml (TA = 589 ml/g); *S. aureus*: MIC = 0.04 mg/ml (TA = 1099 ml/g)). The tannin content varied in the extracts as well. The antibacterial activity however did not seem to be directly correlated to the tannin content.

Another important parameter in the use of plant species is to determine the interspecies variation of plants based on genetic or environmental influences. Leaves from 42 plants of *Combretum molle* were collected at different locations during the same season. The average MIC against *E. coli* was 0.227 mg/ml. The low standard deviation of 0.07 indicates that there was very little variation in activity. The average value against *S. aureus* was 0.399 mg/ml with a slightly higher standard deviation of 0.16. However due to the fact that the samples from different areas extracted different amounts, the total activity varied. The tannin assays revealed that there was with one exception no correlation between the antibacterial activity and the tannin content. So it can be safe to assume that genetic variation does not influence the activity too much at least in *C. molle* leaves

Fifty three plant species traditionally used to treat diarrhoea in published literature were then ranked using a novel system in order to determine which species had the most potential value. Ranking was based on the lowest MIC value against *E. coli*, lower activity against *S. aureus* (to limit selecting for general metabolic toxins), low tannin concentration and high extract yield.

From this ranking, five plants were chosen to investigate their potential value further: *Acacia sieberiana* var. *woodii* (*E. coli*: MIC = 0.13 mg/ml, TA = 108 ml/g; *S. aureus*: MIC = 0.13 mg/ml, TA = 108 ml/g; Yield = 14 mg), *Albizia adianthifolia* (*E. coli*: MIC = 0.14 mg/ml, TA = 239 ml/g; *S. aureus*: MIC = 0.04 mg/ml, TA = 765 ml/g; Yield = 34 mg), *Deinbollia oblongifolia* (*E. coli*: MIC = 0.17 mg/ml, TA = 158 ml/g; *S. aureus*: MIC = 0.08 mg/ml, TA = 338 ml/g; Yield = 27 mg), *Spirostachys africana* (*E. coli*: MIC = 0.13 mg/ml, TA = 300 ml/g; *S. aureus*: MIC = 0.09 mg/ml, TA = 438 ml/g; Yield = 38 mg) and *Tetradenia riparia* (*E. coli*: MIC = 0.09 mg/ml, TA = 214 ml/g; *S. aureus*: MIC = 0.13 mg/ml, TA = 149 ml/g; Yield = 20 mg). None of the plants contained any tannin.

The next step towards the recommendation of a plant for the development of a commercial product was to evaluate the cytotoxicity of the selected five species. The following values were obtained: *Acacia sieberiana* var. *woodii* LC_{50} = 0.026 mg/ml, *Albizia adianthifolia* LC_{50} = 0.068 mg/ml, *Deinbollia oblongifolia* LC_{50} = 0.078 mg/ml, *Spirostachys africana* LC_{50} = 0.025 mg/ml and *Tetradenia riparia* LC_{50} = 0.028 mg/ml.

Deinbollia oblongifolia (for its low LC_{50} value) and *Spirostachys africana* (for its good antibacterial activity and total activity) were potentized by removing inactive compounds through solvent-solvent fractionation. The antibacterial activity against *E. coli* was increased this way (MIC = 0.08 mg/ml for

Deinbollia oblongifolia (chloroform fraction) and MIC = 0.08 mg/ml for *Spirostachys africana* (chloroform fraction)) The LC_{50} values for both chloroform fractions were determined (LC_{50} = 0.188 mg/ml for *Deinbollia oblongifolia* and LC_{50} = 0.062 mg/ml for *Spirostachys africana*). The selectivity index (SI) was also determined and proved that the potentization was indeed successful (*Deinbollia oblongifolia* SI = 2.35 compared to a value of 0.45 for the crude extract; *Spirostachys africana* SI = 0.78 compared to a value of 0.19 for the crude extract). Based on these values, the chloroform fraction of *Deinbollia oblongifolia* was chosen as the less toxic one with similar activity and a higher selectivity index to be worked on further. One of the active compounds was isolated and evaluated for its activity against *E. coli* (MIC = 0.74 mg/ml) The LC_{50} value of 0.042 mg/ml indicated that the activity of the extract was a result of synergism rather than being due to a single active compound (the selectivity index (SI) was 0.06 compared to the values of 0.45 for the crude and 2.35 for the potentized extract).

The plant extracts should of course be as effective against pathogenic strains as they were against the ATCC strains and so the extracts and pure compound of *Deinbollia oblongifolia* were tested for their activity against four different pathological *E. coli* strains. The results showed that the crude extract and the fraction were as active as in the preliminary screening results against only one of the four pathological strains. The pure compound on the other hand was more active against all four pathological strains than against the ATCC strain.

The next step was to test the safety of the extracts of *Deinbollia oblongifolia* in mammals. Unfortunately neither the crude extract nor the chloroform fraction of *Deinbollia oblongifolia* could be used safely in a living organism or in an isolated organ study. A part of the problem may have been caused by the vehicle used in the study despite reports in the literature that an acetone water mixture is safe to use.

In general all the species investigated had good antibacterial activity against *E. coli* this supports the traditional use of these species although we used acetone as extractant rather than the water used traditionally. There were major differences in antibacterial activity over a season indicating that mature leaves were more active than young leaves before senescence started. At least in the case of *Combretum molle* there was little difference in the antibacterial activity of many plants collected at different locations during the same season.

The results obtained in this study could be useful in further studies to develop extracts that can be used to control diarrhoea in animals. Possibly more emphasis should be put on the difference in activity towards *E. coli* and *S. aureus* to eliminate the presence of general metabolic toxins. Such an approach would lead to a different priority order for species to examine. A major first step would probably be to test the *in vitro* and *in vivo* toxicity of selected species.