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Evaluation of a phytogenic product from two western herbal medicines to replace an antimicrobial growth promoter in poultry production

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Declaration

The experimental work described in this thesis was conducted in the department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Phytomedicine Programme under the supervision of Professor J.N. Eloff, Dr J. Picard and Dr S.P.R. Bisschop.

These studies are the result of my own investigations, except where the input of others is acknowledged and have not been submitted in any other form to another University.

Ilse van Heerden

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Abstract

Evaluation of a phytogenic product from two western herbal medicines to replace an antimicrobial growth promoter in poultry production

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Promoter: Prof. J.N. Eloff

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Co-promoter: Dr. S.P.R. Bisschop

Antimicrobial growth promoters (AGPs) are substances that are added to feed in sub-therapeutic levels in intensive animal production to improve weight gain and conversion of feed (FCR) into body mass. AGPs have been used widely as growth promoters in broiler and pig production under high-density growth conditions. Despite the observed efficacy, the use of AGPs has been criticized due to its possible role in the development of antibiotic resistance in human pathogens. Directive 183/2003 of the European Parliament, issued in 2003, banned of the use of all antibiotic agents as growth promoters in the European Union from 2006. The new context caused an increase in the search for alternative growth promoters.

The aim of this study was to produce a commercially viable prophylactic antibacterial phytogenic product from *Ginkgo biloba* and *Hypericum perforatum* with a low potential to develop resistance, as an alternative to AGPs in poultry production.

The first objective of this study based on earlier results of the Phytomedicine Programme, was to evaluate the activity and potentize extracts from *Ginkgo biloba* and *Hypericum perforatum* for optimal activity against relevant bacterial pathogens. Extracts of ethyl acetate (EA), hexane, dichloromethane (DCM) and acetone (in order of activity) from a direct extraction procedure of powdered *G. biloba* leaves were active against *Enterococcus faecalis, Staphylococcus aureus* and *Clostridium perfringens*. The EA, hexane and DCM extracts were 2 to 3 times more active than the acetone extract (average total activity 1728 ml/g dry extract for the 3 pathogens). The DCM-, EA-, acetone- and hexane extracts (in order of activity) from the direct extraction procedure from *H. perforatum* were only active against *C. perfringens* with the first three extracts having a total activity of between 1026 and 1333 ml/g dry material and the hexane extract a total activity of 333 ml/g dry material. The spectrum of activity of *G. biloba* corresponds to that of Zn-bacitracin, which is commonly used an antibiotic growth promoter in the poultry industry.

The second objective in this study was to combine extracts or fractions of extracts of *G. biloba* and *H. perforatum* to optimise activity against selected bacterial pathogens. A synergistic effect could be observed when combining

a ratio of 1:5 of *G. biloba: H. perforatum* (hexane extracts) or 1:15 (acetone extracts) against *E. faecalis* while only an indifferent (neutral) effect was observed against *C. perfringens.*

After elucidation of the quantitative and qualitative aspects involved in the antimicrobial activity, the major antibacterial compound from *G. biloba* was isolated and characterized as ginkgolic acid ($C_{17:1}$). It was also determined whether activity against *E. faecalis* and *C. perfringens* in an extract or fraction of and extract of *G. biloba* can be attributed only to ginkgolic acid or whether synergism or other interactions also play a role in the antibacterial activity. It was shown that synergistic interactions are at play between constituents in the hexane and EA fraction, with the last mentioned fraction not containing any ginkgolic acid. These results support the use of the whole extract as opposed to isolated compounds as antimicrobial agents against pathogenic organisms.

Two important pharmacodynamic parameters were investigated i.e. resistance development to a hexane extract and the isolated ginkgolic acid from *G. biloba* against *E. faecalis* and secondly the time-kill dynamics of this hexane extract over 24 h against *E. faecalis*. The bactericidal nature of the hexane extract from *G. biloba* as well the absence of decreased susceptibility to this extract (and the isolated ginkgolic acid) in the resistance studies against *E. faecalis* indicate that this extract has potential to be exploited as a alternative to AGPs in the poultry industry.

The final objective was to determine the effect of extracts of *G. biloba* alone or in combination with *H. perforatum* extracts on the performance of broiler chickens over a 35 day period. The effect of these extracts on *C. perfringens* in the intestine of broilers was also investigated. No significant differences were found with relation to any of the production parameters studied (FCR, live weight or % survival) although a trend towards more favourable European Performance Efficiency Factor index values were observed for treatments containing *G. biloba* (5% improvement) or a combination of *G. biloba* and *H. perforatum* (2.1% improvement) compared to the untreated control. Similarly, Zn-Bacitracin resulted in a 5.5% improvement compared to the untreated control. There was a general trend (not statistically significant, P=0.05) towards a reduction in *C. perfringens* scores in the feed supplemented with *G. biloba*- in combination with *H. perforatum* extract which can probably be ascribed to the direct antimicrobial effect. The rate of colonization was however too low to cause infection probably due to lack of virulence of the *C. perfringens* challenge and the absence of predisposing factors due to the hygienic growth conditions used. It is necessary for an effective disease model to be developed in order for the efficacy of any new treatment method to be properly evaluated. Such a model will require a much higher incidence of disease and reproducibility than was achieved in this project.

The safety of using extracts of *G. biloba* with ginkgolic acid as the prime antibacterial compound was considered. The active dose was at least 42 times lower than safe dosage recommended in the literature. The combination of extracts of *G. biloba* and *H. perforatum* holds promise as a potential growth promoter in poultry production.

Better results may be achieved if potentized extracts are used and compared with Zn-Bacitracin and a negative control under industrial growth conditions where the birds are stressed and natural infections would take place.

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List of Abbreviations

AGP	Antibiotic growth promoter
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
AUCC	Animal Use and Care Committee
BCA	Blood Columbia Agar
BEA	Benzene/ ethanol/ ammonium hydroxide
BHI	Brain-heart-infusion
BTA	Blood Tryptose Agar
CEF	Chloroform/ ethyl acetate/ formic acid
DCM	Dichloromethane
EA	Ethyl acetate
EMW	Ethyl acetate/ methanol/ water
EPEF	European Performance Efficacy Index
FCR	Feed conversion ratio
FIC	Fractional inhibitory concentration
FOS	Fructo-oligosaccharides
GA	Ginkgolic acid
GIT	Gastro-intestinal tract
GOS	Gluco-oligosaccharides
GRAS	Generally regarded as safe
GRE	Glucopeptide resistant enterococci
HEN	Hen's egg test
INT	*p*-iodonitrotetrazolium violet
MEDUNSA	Medical University of Southern Africa
MH	Müeller Hinton
MIC	Minimum inhibitory concentration
MOS	Manno-oligosaccharides
MRSA	Methicillin resistant *Staphylococcus aureus*
NE	Necrotic enteritis
NMR	Nuclear Magnetic resonance
OD	Optical Density
P	Passage
PAF	Platelet activity factor
SANS	South African National Standards

SEM	Standard error of Mean
SJW	St. Johns wort
TA	Total activity
TLC	Thin Layer Chromatography
UV	Ultra Violet light
VRE	Vancomycin resistant *Enterococcus*
Zn-Bacitracin	Zinc-Bacitracin