

CHEMICAL ACTIVE INGREDIENTS IN SUPERLIV® (LIQUID HERBAL SUPPLEMENT) AND ITS ANTIMICROBIAL IN-VITRO ASSAY

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ABSTRACT: *An experiment was carried out to evaluate the chemical active ingredients present in Superliv®, a liquid herbal mixture produced by Ayurved India and marketed in Nigeria by Animal Care Konsult, using the essential oil method involving the extraction process, the gas chromatography and the mass selective detector (MSD) technique. Results from this study shows that the major active ingredient in the herbal supplement is Pyrimidine (83.33%) which has been considered as an effective antioxidant. The other part of the experiment assessed the In-vitro antimicrobial effect of Superliv®. The herbal supplement (Superliv®) was incorporated into MacConkey agar medium which was prepared by suspending 47g in 1 litre of distilled water. Dissolved completely and then sterilized by autoclaving at 121°C for 15 minutes. After cooling to about 55°C, it was poured into Petri-dish and sensitive Escherichia coli and Staphylococcus aureus were inoculated into the medium and incubated. The herbal supplement had no significant antimicrobial in-vitro effect on the gram positive and gram negative bacteria at the manufacturers recommended dosage. However at an increased concentration of 60 -100% it had inhibitory growth effect on the gram positive and while at 90-100% on negative bacteria.*

KEYWORDS: Antimicrobial, Chemical, Herbal, Bacterial, Antioxidant

INTRODUCTION

Derived product from herbs and spices is in no doubt a good alternative to inorganic (synthetic) antibiotics and antimicrobial feed additives. Nowadays, the use of herbal mixtures is receiving much attention particularly in broiler chickens (Alçiçek *et al.*, 2003, 2004; García *et al.*, 2007) and laying hens (Çabuk *et al.*, 2006). Herbal extracts assist in colonization of the beneficial microbial population within the gastrointestinal tract to more balanced levels (Zaika, 1988; Jang *et al.*, 2007). Besides their antimicrobial properties (Ultee *et al.*, 2002), they also exhibit antioxidant (Basmaciolu *et al.*, 2004), antifungal (Bang *et al.*, 2000; Shin and Lim, 2004), digestion-stimulating, and enzymatic (Jamroz *et al.*, 2003, 2005; Hernandez *et al.*, 2004) activities.

Although, certain limitations are also reported in the published literature (Oyagbemi *et al.*, 2008) regarding the use of botanical preparations; one of the critical claims is the lack of scientifically validated safety information of the herbal formulations. Attempts to collate toxicity data on poultry herbal supplements revealed not only the scanty information available in this aspect but also the fact that the scientific investigations were not carried out uniformly throughout the world.

With the rate at which herbal products are enjoying so much patronage from the poultry industry for the past few decades, it is very important for continuous and well detailed

researches to be carried out on these herbal mixtures from time to time, particularly on the chemical active ingredients present in these products. These will not only give a comprehensive documentation of what the herbal products is composed of, but will also give a guide line towards understanding the mode of operation of these herbal product in the animal body system.

MATERIALS AND METHODS

Evaluation of Chemical Active ingredients Present in the Herbal Supplement (Superliv®).

Experimental site; this experiment was carried out in the laboratory of the Department of Chemistry, University of Lagos, Lagos-state, Nigeria.

Procedures

The method used for this analysis is called the “essential oil method” which involved the extraction process, the gas chromatography procedure and the mass selective detector (MSD) technique.

Extraction Procedure: the extraction method involved the following procedure:

- 10mls of sample (herbal supplement) was taken into a separation funnel
- Then 30mls of hexane was measured and the sample was extracted with the 30mls of hexane three times.
- The extract was collected and exposed into the atmosphere for about 30minutes
- Later, it was evaporated and placed into water bath to concentrate the sample
- The remaining concentrate was then filtered with the aid of Pasteur pipette; using cotton-wool as the separating medium and anhydrous sulphate for adsorption
- It was then filtered into the vial bottle, ready for gas chromatography (GC) procedure as described in the user manual from Agilent technology, USA.

Gas chromatography system; the machines used for this analysis were:

- Gas Chromatography system - Model7890A
- Mass Selective Detector - Model5975C
- Injector - Model7683B

The gas chromatography system was set at the following specifications:

- Initial temperature 80°C to hold for 1minute
- Final temperature 310°C to hold for 5minutes at the rate of 8°C/min
- Heater at 250°C
- Pressure - 12.076PSI
- Mode – Split less
- Average velocity – 67.4cm/sec.
- Column - HP 5ms (30m x 320µm x 0.25µm)
- Volume injected - 1µl
- Size of syringe - 10 µl
- Final run time – 34.75mins.

Each element /compound present in the herbal supplement has different retention time; these elements will try to escape from the sample once its retention time had been attained in the gas chromatographic system.

The time at which each compound tries to escape was recorded and traced in the library for its name and identification.

In-vitro assessment of the antimicrobial effect of Superliv®

Experimental site: This procedure was carried out at the microbiology laboratory of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun-state, Nigeria.

Procedures

1ml of the herbal medication (Superliv®) was incorporated into the medium (MacConkey agar). MacConkey agar medium was prepared by suspending 47g in 1 litre of distilled water. This was brought to boil so as to dissolve completely and then sterilized by autoclaving at 121°C for 15minutes. After cooling to about 55°C, it was poured into Petri-dish and sensitive *Escherichia coli* and *Staphylococcus aureus* were inoculated into the medium. The reason for using these two organisms was that, *E. coli* serves as a representative of Gram negative organisms while *Staphylococcus aureus* was for Gram positive organisms and they are both sensitive with no resistant plasmid. The medium was incubated at 37°C for 24hrs. The growth or otherwise of the sensitive organisms was visually assessed (Oxoid, 2011).

RESULTS AND DISCUSSION

Percentage composition of the active ingredients in herbal supplement (Superliv®)

The percentage composition of the active ingredients in the herbal supplement (Superliv®) is presented in Table 1. Out of about 21 active ingredients present in the herbal supplement, 1,2,4, Triazolo (1,5-a) pyrimidine accounted for 83.39% of the entire supplement, followed by Tricyclo [4.4.0.0(3,9)]decan-4-ol Stereoisomer with 2.99% and 1-Aminopyrene with 2.6%. Tricyclo (4.4.0.0(3,9))decan-4-ol, stereoisomer, 4-Acetyl-6-methoxy-2(1H) quinolinone, Lanosta-8,2 4-dien-3-ol, acetate and 24-Noroleana-4(23),12 diene had 1.63%, 1.36%, 1.31 and 1.22% respectively. The remaining active ingredients are present in less than 1% of the composition.

In-vitro effect of herbal supplement (Superliv®) on bacteria

Table 2 shows the result of the In-vitro effect of the herbal supplement (Superliv®) on Gram positive and Gram negative bacteria. It was observed that the different volumes and concentration of the herbal supplement used which ranged from 0.01ml - 0.006ml (within the recommended dosage) had 0mm diameter of inhibition around the agar perforations (well) and filled with the various concentration of the herbal supplement in the bacteria inoculated growth media (nutrient agar), both for *Staphylococcus aureus* ATCC259299 and *Escherichia coli* ATCC259298. However, the well filled with the antibiotic Ciprofloxacin (2.5µg/ml) which served as the negative control had a 30mm and 28mm diameter of inhibition of bacteria growth around it, while the well filled with water, which served as the positive control, had 0mm diameter of inhibition.

In-vitro effect of the herbal supplement at different concentrations on bacteria

Table 3 shows the results of the In-vitro effect of the herbal supplement on *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative) bacteria, based on the concentration of herbal supplement used. At 100% herbal supplement concentration there was 12mm and 10mm bacteria growth inhibition diameter, but at 90% herbal supplement concentration there was increase in the growth of bacteria thereby reducing the diameter of inhibition round the well to 9mm for both bacteria strains. However, at 80% concentration *Staphylococcus aureus* still maintained the 10mm inhibition diameter while there was no more inhibition of the growth of *Escherichia coli*. From 70% concentration of herbal supplement, the growth inhibition diameter for *Staphylococcus aureus* further decreased from 6mm to zero. The negative control i.e. antibiotic (Ciprofloxacin (5µg/ml)) had 19mm and 18mm diameter of inhibition for both *Staphylococcus aureus* and *Escherichia coli* respectively, while there was 0mm inhibition diameter for both bacteria in the positive control i.e. water.

The major active ingredient in the herbal supplement used for this study is Pyrimidine which has been considered as an effective antioxidant (Prasenjit *et al.*, 2010). Antioxidants are effective because they are willing to give up their own electrons to free radicals. Free radicals are known to react with proteins, lipids, polysaccharides and nucleic acids; thus induce damage to cell membranes, organelles and tissue (Fakoya *et al.*, 1998).

The herbal supplement had no significant antimicrobial in-vitro effect on the gram positive and gram negative bacteria at the manufacturers recommended dosage. However at an increased concentration of 60 -100% it had inhibitory growth effect on both the gram positive and negative bacteria. This finding is similar to that of Nidaullah *et al.*, (2010) and Zaman *et al.* (2011) as they also reported the antimicrobial effect of herbal complex containing similar herbs and plants as that of Superliv®.

CONCLUSION

From the findings of this study; the following conclusions could be drawn.

- The major active ingredient in Superliv^(R) is Pyrimidine which functions as an antioxidant in suppressing the negative effect of the free- radicals in the birds system
- At concentration of 60-100%, Superliv^(R) can inhibit the growth of gram positive and gram negative bacteria in vitro, due to its relative antimicrobial activity.

Table 1: Active Ingredients (%) of the Herbal Supplement (Superliv)

*Active Ingredient	Composition (%)
1,2,4, Triazolo (1,5-a) Pyrimidine(Antioxidant)	83.39
Tricyclo[4.4.0.0(3,9)]decan-4-ol Stereoisomer	2.99
1-Aminopyrene	2.6
Tricyclo(4.4.0.0(3,9))decan-4-ol, stereoisomer	1.63
4-Acetyl-6-methoxy-2(1H) quinolinone	1.36
Lanosta-8,2 4-dien-3-ol, acetate	1.31
24-Noroleana-4(23),12 diene	1.22
Tricyclo(5.2.1.0(2,6) decan-3-one	0.96

6-oxabicyclo(3.1.0)hexane-3-carbonitrile	0.93
4-n-Butylthiane, S,S-dioxide	0.88
Tetrazole	0.45
6-oxabicyclo(3.1.0)hexane-3-carbonitrile	0.37
Ethanone	0.34
1,3-Diphenyl-2-hydroxy-4-ethoxycarbonyl-4H-pyridazino(6,1-a) isoquinoline	0.26
Trans-1,4-cyclohexanedicarbonitril4H-Thiopyran-4-one	0.25
Exo-Norbornyl alcohol	0.24
Trans-1,4-cyclohexanedicarbonitril	0.20
Cyclohexane	0.19
Cyclopentane	0.18
Acrolein	0.15
1-(4-Amino-furazan-3-yl)-5-methyl-1H-(triazole-4-carboxylic acid amide	0.10

*Chemical Structure

Table 2; In-vitro effect of the herbal supplement on Gram positive and Gram negative bacteria.

Herbal supplement* (ml)	<i>Staphylococcus aureus</i> ATCC259299 diameter of inhibition (mm)	<i>Escherichia coli</i> ATCC259298 diameter of inhibition (mm)
0.01	0	0
0.02	0	0
0.03	0	0
0.04	0	0
0.05	0	0
0.06	0	0
0.001	0	0
0.002	0	0
0.003	0	0
0.004	0	0
0.005	0	0
0.006	0	0
Ciprofloxacin (2.5µg/ml)	30	28
Water	0	0

*Manufacturing date of the herbal supplement; November, 2009; Expiry date – November 2012.

Experiment date: 11th January, 2011.

Table 3: In-vitro effect of the herbal supplement at different concentrations on Gram positive and Gram negative bacteria.

Herbal supplement*(%)	<i>Staphylococcus aureus</i> ATCC259299 diameter of inhibition (mm)	<i>Escherichia coli</i> ATCC259298 diameter of inhibition (mm)
100	12	10
90	9	9
80	10	0
70	6	0
60	4	0
50	0	0
40	0	0
30	0	0
20	0	0
10	0	0
5	0	0
Ciprofloxacin (5µg/ml)	19	18
Water	0	0

*Manufacturing date of the herbal supplement; August, 2010: Expiry date – August 2013.

Experiment date: 24th January, 2011.

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