# SERUM BIOCHEMICAL ASSAY OF BROILER CHICKENS ADMINISTERED WATER CONTAINING VARIOUS MEDICINAL PLANT LEAF METHANOL EXTRACT

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**Abstract**
Bans on the use of antibiotics as feed additives have accelerated and led to investigations of alternative feed additives in animal production. To this end, the response of broiler chickens fed various medicinal plants methanol extract as a replacement for antibiotics was investigated. A total of 180 unsexed Ross strain broiler chickens were randomly assigned to four available plant leaf extract namely; *Gercinia kola* (Bitter Kola), *Alcornea cordifolia* (Christmas bush), *Pterocarpus santalinoides* (Red scandal wood) and *Chromolera Odorata* (Hagony or Siam weed). Each treatment group had 30 birds each. The treatments were replicated thrice with 10 birds per replicate in a Completely Randomized Design (CRD). Feed and water were provided *ad libitum* throughout the experiment which lasted for 56 days. Serum biochemical assay of broiler chickens were evaluated. Significant differences ($p<0.05$) were observed in the mean values of all the parameters measured with exception of total protein and globulin. However, the values obtained did not reveal any health problem. In conclusion, the findings of this study showed that the medicinal plant methanol extracts have considerable potentials as component of broiler chicken diet. *Alcornea cordifolia* plant methanol extract can successfully be used to replace antibiotics for broiler production. Further research should be carried out on *Alcornea Cordifolia* and other medicinal plants to examine their potentials and inhibitory characteristics.
**Keywords**:Serum biochemical assay, antibiotics, medicinal plant, methanol extract, *Gercinia kola, Alcorneacordifolia, Pterocarpus santalinoides, Chromolena odorata*

## Introduction

The use of antibiotics as growth promoters in animal nutrition is facing reduced social acceptance due to the appearance of residues and resistant strains of bacteria; antibiotic use has been banned in the European Union since January 2006.Natural feed additives of plant origin are generally believed to be safer, healthier and less subject to hazards for humans and animals. Many herbs and plant extracts have antimicrobial activities and antioxidant properties which make them useful as natural animal feed additives (Faixova and Faix 2008).

Many of the medicinal plants commonly available have not been scientifically studied to validate the efficacy and to identify the phytochemical constituents that may be responsible for their medicinal values. Only limited studies have been conducted to investigate the biochemical profile of broiler chickens administered water containing medicinal plant (*Gercinia kola, Alcornea cordifolia, Pterocarpus santalinoides* and *Chromolera odorata*) methanol extract. In consequence present study was arranged to evaluatethe serum biochemical assay of broiler chickens to four medicinal plant (*Gercinia kola, Alcornea cordifolia, Pterocarpus santalinoides* and *Chromolera odorata*) methanol extract.

## Materials and methods

This study was carried out in the Poultry unit of the Teaching and Research farm, Michael Okpara university of Agriculture, Umudike, Abia State. Umudike is located on latitude $05^{o}$ 21' N and longitude $07^{o}$ 33' E, with an elevation of about 112m above sea level. The location has an annual rainfall of 177 - 2,000mm per annum, (April to October) and a short period of dry season (November to March) with a relative humidity of about 50-90% and monthly temperature range of $17^{o}$C – $36^{o}$C (NRCRI, 2019).

The fresh leaves of the medicinal plants investigated (*A. cordifolia, C. odorata, P. Santalinoides* and *G. kola*) were collected within Michael Okpara University of Agriculture, Umudike and air dried for

two weeks under shade. The dried parts were pulverized to fine powder using a mechanical grinder, sieved and weighed. A total of 500g of each of the powdered plant materials was soaked in 1500ml of methanol for 24h at room temperature. The extracts were filtered using non-adsorbent muslin cloth into a clean beaker. The filtrate was dried by evaporating off the solvent at 50$^{o}$C in a hot air oven over a period of one to two days. The experimental design for the experiment was Completely Randomized Design (CRD) with medicinal plant methanol extract as the only factor of interest. The study lasted for 56 days.

A total of 180day-old Ross strained unsexed chicks were weighed and randomly allotted to six equal treatment groups (T1, T2, T3, T4, T5 and T6) each having 30 chicks. Each treatment was replicated three times of 10 chicks per replicate. T1 was the positive control, T2 (Negative control), T3 (*Chromolaenaodorata)*, T4 (*Pterocarpussantalinoides)*, T5 (*Alchorneacordifolia)*and T6 (*Garcinia kola)*. Administration of medicinal plant methanol extract in their drinking water (1g/l of water) commenced the first day of the experiment. Feed and water were given *ad libitum* throughout the experiment. The gross composition of the experimental diet is presented in Table 1.

Blood samples were collected from one bird randomly selected from each replicate per treatment for the evaluation of serum biochemical profile. Blood collection was carried out by using a sterile needle to puncture the right jugular vein, and blood drawn into the syringe. Serum was separated for determination of albumin globulin ratio, total cholesterol, creatinine, urea, ALT and AST using commercial kits. SOD activity was determined using the Bio Vision-Superoxide Dismutase Activity Assay Kit. This sensitive SOD assay kit utilizes WST-1 to produce a water-soluble Formosan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to xanthine oxidase (XO) activity and is inhibited by SOD. Therefore, the inhibitory activity of SOD can be determined via a colorimetric method. The results are expressed as the inhibition rate (%). Other biochemical parameters were measured using a Roche Cobas Integra 400 Plus autoanalyzer (Roche Diagnostics, GmbH,Mannheim Germany).

All data generated were subjected to Analysis of Variance (ANOVA) and treatment means that were significantly different were separated using Duncan's Multiple Range Test (Duncan, 1955) according to Steel and Torrie (1980) using computer software IBM SPSS Statistic version 20 (SPSS, 2012).

**Table 1: Percent ingredients and nutrient composition of experimental diet**

| **Parameters** | **Starter** | **Finisher** |
|---|---|---|
| Maize | 48.00 | 57.00 |
| Soyabean meal | 31.00 | 23.00 |
| Fishmeal | 3.00 | 3.00 |
| Palm kernel meal | 10.20 | 9.30 |
| Wheat offal | 4.00 | 4.00 |
| Bone meal | 3.00 | 3.00 |
| Salt | 0.25 | 0.25 |
| Lysine | 0.20 | 0.10 |
| Methionine | 0.10 | 0.10 |
| Vit/Min premix | 0.25 | 0.25 |
| **Total** | 100 | 100 |
| **Nutrient composition** | | |
| **Crude protein (CP) (%)** | 22.98 | 19.63 |
| **Metabolizable energy (ME) (kcal/kg)** | 2883.22 | 2940.96 |

**Results and Discussion**

The serum biochemical profile of broiler chickens fed various plants methanol extract is presented in Table 2. Significant differences ($P<0.05$) were observed in Albumin, urea, creatinine glucose, Aspartate transference and Alanine triphosphatase while in total protein, Globulin and total cholesterol significant difference were not recorded ($P>0.05$).

Albumin count is within the range of 2.23 – 3.24 g/dl obtained from treatments 1 and 4 respectively. Though significantly differences were not observed in treatments 2, 3, 4, 5 and 6 (P>0.05) but significantly different from treatment 1 (P<0.05). Except for treatments 3 and 6, this has a value slightly higher than the normal range of values, the other treatment groups' fall within the range of 1.3 – 2.3 g/dl (Aiello and Mays, 1998). When protein intake exceeds the amount required for growth and maintenance, these probably result in increased level of serum albumin in indigenous chicken (Aiello and Mays, 1998),

The level of urea in the serum is an excellent indicator of kidney function (Sakas, 2002). Significant differences were not observed (P>0.05) in treatments 1, 2, 3, 5 and 6 but significantly different (P<0.05) from treatment 4. However, the values obtained ranged from 22.73 – 31.33 mg/dl were above the normal range (2.5 – 8.1 mg/dl) as reported by Aiello and Mays, (1998). In all the treatment groups, it shows that this effect cannot be attributed to the test feedstuff. However, Ajagbonna *et al.* (1999) stated that high urea level suggests increase in urea enzymes activities (ominthine, Carbonyl transferase and originase) which results in prerenal azotemia (observed in dehydrated birds).

Serum creatinine and urea are waste products used to assess kidney function. The level of creative and blood protein depends on the quality of dietary protein (Awosanya *et al*., 1999). However, significant differences were not observed (P>0.05) in treatment groups of 1 and 4 but significantly different (P<0.05) from treatments 2, 3, 5 and 6 which in turn did not record any significant difference (P>0.05) within them. Furthermore, the values obtained were within the range of 0.34 – 0.89 mg/dl which was slightly lower than the normal range of a healthy chicken of 0.9 – 1.8 mg/dl (Aiello and Mays, 1998). Campbell (2013) reported that lower value could also indicate good absorption and utilization of protein in the diet.

It was observed that Glucose value obtained in this result (9.7.30 - 221.93 mg/dl) were significantly different (P<0.05) though treatments 1, 2 and 4 did not show any significance (P>0.05) but significantly different (P<0.05) from treatments 3, 5 and 6 which in turn did not differ significantly (P>0.05) within them. The values obtained in this result except for treatment 1 were within the range value of a healthy bird that ranged from 126 -204 mg/dl (Campbell, 2013). Treatment 1 which has an increase in the level of glucose above the normal range can be as a result of stress (Hassan *et al*., 2017)

Aspartate transferase is an enzyme present in very high amounts in the liver. It is one of the more reliable indicators of liver disease in birds raised in an enclosure (Agbede *et al*., 2011). The reduction in Aspartate transferase activities when compared to the positive and negative control groups is an indication that the antibiotics in medicinal plant methanol extracts had no toxic effect on the liver of the birds as elevation in the activity of these enzymes is associated with liver disease (Agbede *et al*., 2011) this agrees with the report of Hassan *et al*. (2017).

Cholesterol serves as an intermediate in the biosynthesis of all steroids and this is essential to life. In as much as that total cholesterol did not record any significant difference (P>0.05), the values obtained were ranged from 114-28 – 342.35 for treatments 2 and 3 respectively. The values obtained in this present study were within the range of values reported by Aiello and Mays (1998). Among the treatment groups fed various medicinal plant extract, treatment 5 recorded the least value when compared to the positive control group (treatment 1). This observation is in an agreement with the report of Hassan *et al*. (2017) that the reduction in cholesterol level is associated *Alchornea cordifolia* inclusion in the diet.

Increased activities of aspartrate amino transferase (AST) and Alanine amino transferase (ALT) in the Serum are well known diagnostic indicators of liver injury. However significant difference did not exist (P>0.05) among the treatment groups except for treatment 1 which is significantly different (P<0.05) from the other treatment groups. Furthermore, the values obtained fall within the range of 126.17 – 211.80 U/l from treatments 1 and 5 respectively. Result revealed that they were not significantly affected by the plant methanol extracts included in their water, suggesting that the test material might not pose any serious deleterious health challenges to the animals especially as it relates to liver).

**Table 2:** Serum biochemistry of broiler chicken fed medicinal plant methanol extract

| **parameters** | **T1** | **T2** | **T3** | **T4** | **T5** | **T6** | **SEM** |
|---|---|---|---|---|---|---|---|

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| TP (g/dl) | 5.45 | 4.75 | 6.65 | 5.40 | 4.65 | 5.20 | 0.35 |
| ALB (g/dl) | $2.23^{b}$ | $2.42^{ab}$ | $2.77^{ab}$ | $3.24^{a}$ | $2.89^{ab}$ | $3.03^{ab}$ | 0.13 |
| GLO (g/dl) | 3.22 | 2.33 | 3.88 | 2.16 | 1.90 | 2.17 | 0.32 |
| TCHL (g/dl) | 276.18 | 114.28 | 342.85 | 285.71 | 142.85 | 257.14 | 33.53 |
| UREA (mg/dl) | $28.20^{ab}$ | $26.63^{ab}$ | $29.80^{ab}$ | $22.73^{b}$ | $31.33^{a}$ | $26.63^{ab}$ | 1.07 |
| CREA (mg/dl) | $0.78a^{b}$ | $0.54^{abc}$ | $0.34^{c}$ | $0.89^{a}$ | $0.45^{bc}$ | $0.46^{bc}$ | 0.63 |
| GLU (mg/dl) | $221.93^{a}$ | $194.10^{ab}$ | $97.70^{b}$ | $141.63^{ab}$ | $98.43^{b}$ | $96.20^{b}$ | 15.72 |
| AST (U/L) | $163.75^{a}$ | $182.31^{a}$ | $55.83^{b}$ | $50.89^{bc}$ | $32.27^{bc}$ | $22.48^{c}$ | 15.95 |
| ALT (U/L) | $26.17^{b}$ | $121.18^{ab}$ | $99.93^{ab}$ | $197.47^{a}$ | $211.80^{a}$ | $182.64^{a}$ | 20.19 |

$^{a\text{-}b\text{-}c}$:Means along the same row with different superscripts are significantly ($p<0.05$) different. S.E.M= Standard Error of Mean. TP=Total protein; ALB=Albumin; GLO=Globulin; CREA=Creatinine; GLU=Glucose; AST= Aspartate Transferase; ALT= Alanine Triphosphatase; TCHL=Total Cholesterol

**Conclusion and Recommendation**

The findings of this study suggested that the medicinal plant methanol extracts have considerable potentials as component of broiler chicken diet. *Alcornea cordifolia* plant methanol extract can successfully be used to replace antibiotics for broiler chicken production. Further research should be carried out on *Alcornea Cordifolia* and other medicinal plants to examine their potentials and inhibitory characteristics.

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