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The virucidal potentials of *Tapinanthus globiferus*: A *Vitellaria paradoxa* C.F. Geartn epiphytes (whole plant) extract in experimental bronchitis in broiler chicken

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ABSTRACT: Infectious Bronchitis Virus (IBV) belongs to the family *Coronaviridae*; genus *Coronavirus*, together with turkey coronavirus and nine species of mammalian viruses. The *Tapinanthus globiferus*: A *Vitellaria paradoxa* C.F. Geartn epiphytes (whole plant) was prepared and cold extracted using 70% methanol. Acute toxicity studies using set of 36 number of four weeks old apparently healthy chickens grouped into six categories (A-F) was performed. Total numbers of 30, four weeks old broiler chickens were grouped into six (I-VI) with each group consisting of five (n = 5) chickens. Infected birds manifesting all the signs and symptoms of infectious bronchitis (IB) and confirmed through alkaline phosphatase (ALP) and creatinine phosphor kinase (CPK) assay was isolated and provided with 300 ml of drinking water over a period of 24 hours. The contaminated water was used as the inoculum. The infection was carried out by administering 3 ml of the infective dose to groups I-IV. The treatment with the extract at doses of 100, 200, 300 and 400 mg/bw was given to groups I-IV while groups V and VI were the standard and negative controls respectively. Monitoring of the viral load was done at a week interval through the ALP and CPK enzyme assay. Doses of 1000 to 3000 mg/kgbw did not cause any mortality while LD50 was not found even at the highest dose of 5000 mg/kgbw. The doses of 300 and 400 mg/kgbw were found to maintain a very low viral load. Prophylaxis with 400 mg/kgbw for five consecutive days prior to infection does not show any prophylactic effect. Result from this study reveals that methanol extract of *Tapinanthus globiferus*: A *Vitellaria paradoxa* C.F. Geartn epiphytes has pharmacological effect against IBV with a very low level of toxicity, though, no prophylactic effect.

Keywords: Epiphytes, IBV, phytoagents, poultry, *Tapinanthus globiferus,* virucidal, *Vitellaria paradoxa.*

INTRODUCTION

Avian infectious bronchitis (IB) is a highly contagious and acute viral disease of chickens caused by avian infectious bronchitis virus (IBV), which is an Office International des Epizooites (OIE) list B disease (Arthur et al., 2005). It is an important disease of chickens causing respiratory problems, reduced performance, decreased hatchability and fertility, nephrosis, irreparable damage to reproductive organs, reduced egg quality and increased susceptibility to secondary infections leading to decreased profit at slaughter and costly vaccination programmes (European Pharmacopoeia 9th 2017a; De Wit, 2000).

IBV belongs to the family *Coronaviridae*; genus *Coronavirus*, together with turkey coronavirus and nine species of mammalian viruses (Cavanagh and Naqi, 2003).

It differs from turkey coronavirus, which is more closely related to mammalian coronaviruses (Cavanagh, 2005; Cavanagh, 2000). IBV is pleiomorphic, but generally round, possessing an envelope of 60 to 220 nm in diameter with club shaped surface projections (spikes) of about 20 nm in length (Cavanagh and Naqi, 2003; Cavanagh, 2005). The virion envelope contains phospholipids, glycolipids, cholesterol, di and triglycerides and free fatty acids (Cavanagh, 1995).

Diseases are the main constraints of indigenous poultry production in most rural areas of Nigeria where Indigenous poultry farming is an integral part of mixed farming (Jordan, 2017). The poultry industry has been one of the most dynamic and ever expanding sectors, contributing much to the global economy. Meat consumption, all over the world, is increasing exponentially and the demand for safe and cheap protein source, free of infectious agents, is on the rise as the day progress (Jordan, 2017). But the emergence of diseases like BSE, SARS and highly pathogenic avian influenza questions the concept of 'safe protein source' (Liang et al., 2019).

IBV has led to severe losses in the poultry industry (Jordan, 2017), the direct losses are due to high mortality, poor egg quality, and meat production, and the indirect losses result in increased costs and challenges in IBV prevention (Liang et al., 2019). Farmers usually have to start with new flock after heavy losses incurred due to disease outbreaks. The existing conventional disease control programs favor the high investment intensive systems of production with birds in confinement and not small-scale farmers with less than 100 birds. Due to high cost of conventional medicines and vaccines coupled with the lack of knowledge on their use, these drugs are usually out of reach of the small-scale farmers (Jackwood and De Wit, 2014). There is therefore need for cheap easy to use and sustainable local poultry disease control programs.

Since ancient times, plants and plant parts have an indispensable source of medicine for indigenous poultry production systems (Lelešius et al., 2019). Although modern medical science has developed to a great extent, many farmers in most rural areas of Nigeria and Africa in general, depend on plant parts and herbal remedies for indigenous poultry health management. Unfortunately, local medical traditions are being lost because they are communicated orally from generation to generation and are largely undocumented. Very little has been done to verify and validate information gathered (Mahmood et al., 2011).

Biological products derived from plants are used in medicine for different pharmacological reasons, including the treatment of infectious and non-infectious diseases (Shayganni et al., 2016). This class of antimicrobial plants is acknowledged and well investigated, and classes of active compounds have already been identified (Kama-Kama et al., 2016). The investigation of antiviral substances derived from plants is insufficient in comparison with the investigation of antimicrobial properties. Fortunately, several experiments have shown that plants have positive antiviral activity *in vitro* and *in vivo* (Gandhi et al., 2016). However, the same plants can have different antiviral activity against RNA or DNA viruses, either enveloped or non-enveloped, and even against different types or strains of a virus (Jaime et al., 2013; Rady et al., 2018).

In this work, the potency of the 70% methanol extract from the epiphyte (*Tapinanthus globiferus*) of a tree species *Vitellaria. paradoxa* C.F. Geartn was evaluated in experimental broiler chickens.

MATERIALS AND METHODS

Experimental site

The research was conducted at both the teaching/ research farm and the Central Laboratory of the Federal College of Wildlife Management, New Bussa. The experimenttal station (New Bussa), Niger State, Nigeria, geographically located between latitude 9° 53'N and 9° 83'N and longitude 4° 31'E and 4° 51'E (Garba et al., 2020).

Experimental animals

Hundred (100) day old, broiler chicks (to cover for the anticipated mortality) were purchased from a reputable farm at Ibadan and raised under good management, feed and water was supplied *ad-libitum* for about 4 weeks before the commencement of the experiment.

Plant materials

The plant *Tapinanthus globiferus* (an epiphyte of *Vitellaria paradoxa* C.F. Geartn) was sourced from Federal College of Wildlife Management New Bussa Estate and the surrounding villages where it is in abundance. Its identity was confirmed at the Department of Forestry, Federal College of Wildlife Management, New Bussa. It was deposited and assigned voucher No: FCWM/Gab/2093.

Preparation of extract

The whole plant sample was sliced and chopped into smaller pieces to hasten drying. It was then pulverized using pestle and mortar. Cold extraction was performed using 70% v/v methanol/water as solvent. The preparation of the crude extract was based on the method described by Garba et al. (2015). Briefly, one hundred gram (100 g) of the dried sample was pulverised to powdered form and cold extracted in 400 ml of methanol/water. Extraction lasted for 48 hours. The extract was then filtered using muslin cloth and the solvent was removed and recovered

using rotary evaporator. The extract was then transferred into a sterile universal bottle and stored at 4°C until required for use. The yield of the extract was calculated as the percentage of the dry sample.

Phytochemical analysis

The phytochemical analysis of the extract of *V. Paradoxa* Epiphyte was carried out based on coloration and precipitation test as described by Sofowora (1982) and Trease and Evans (2002).

Test for alkaloids

Zero point five gram (0.5 g) of extract was diluted into 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia, 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for phenols

One millilitre (1 ml) of crude extract and 1 ml of Iron (III) chloride solution was mixed for 2 minutes. Formation of a deep bluish green colouration of the mixture indicates the presence of phenols.

Test for tannins

Zero point five grams (0.5 g) of the extract was boiled with 10 ml of distilled water in a test tube and then filtered. A few drops of 10% of ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for terpenoids (Salkowski test)

To 0.5 g of the extract was added 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for cardiac glycosides

One gram (1 g) of the extracts was treated with 2 ml of glacial acetic acid, a drop of 10% $FeCl_3$ and 1 ml of concentrated H_2SO_4. The appearance of brown coloration indicates the glycosides.

Test for flavonoids

Five millilitres (5 ml) of dilute ammonia was added to the aqueous portion of the extract followed by concentrated sulphuric acid (1 ml). A yellow coloration that disappears on standing indicates the presence of flavonoids.

Test for saponins

To 0.5 g of extract was dissolved in 5 ml of distilled water in test tube. The solution was shaken and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken after which it was observed for the formation of an emulsion.

Test for anthraquinones

One millilitre of the plant crude extract was mixed with 1 ml of chloroform, then 10% NH_3 solution was added to the mixture. A brick red precipitate indicate the presence of anthraquinones.

Test for phlobatannins

Zero point two grams (0.2 g) of the crude extract was mixed with 5 ml of 1% HCl in a test-tube and heated for 2 minutes. A red precipitate indicates the presence of phlobatannins.

Test for steroids

Five drops of concentrated H_2SO_4 was added to 0.2 g of the extract. A reddish brown colour indicates the presence of steroids.

Standard drug

The standard IBV antiviral drug (Toroclox^R) was purchased from State Veterinary Clinic, Bosso, Minna, Niger State, Nigeria as oral solution.

Pharmacological studies

Acute toxicity studies

Thirty six (36), four weeks old broiler chickens were grouped into six (A-F) of six (6) chicken each. To the first group was administered dose of distilled water (control A), then, to subsequent groups (B-F) were administered doses of 1000, 2000, 3000, 4000 and 5000 mg/kgbw in increasing order. The bird's reaction, body condition and number of mortality were monitored and recorded over 24 hours period in each group.

Harvesting of the viral particles (IBV)

A local chicken with the symptoms of IBV (and confirmed through ALP and CPK enzymes analysis) was sourced from community around New Bussa, water was provided in a clean container to which the chicken was forced to drink several times from it, such that the virus containing saliva contaminates the water. The water was preserved in a sample bottle.

Preparation of the infective dose

Infection of animals: The contaminated water was supplied to the birds in each experimental group, which was allow to be fend upon for a period of 24 hours.

Antiviral effect: Thirty (30) grower birds at the fourth week of age were grouped into six (6) which each group consisting of 5 birds as thus:

Group I was administered the extract at 100 mg/L.
Group II was administered the extract at 200 mg/L.
Group III was administered the extract at 300 mg/L.
Group IV was administered the extract at 400 mg/L.
Group V is the positive control (infected and administered the standard drug).
Group VI is the negative control (Infected and not treated with either the standard drug nor the extract).

Treatment regime: The treatment was sustained for a period of three weeks after which blood samples from the wing vein, was collected in a non-heparinised sample bottle from the animals in each group for enzyme analysis. Also, some selected animals from each grouped were sacrificed for post mortem.

Prophylactic activity test: The highest effective dose of 400 mg/kgbw was administered to a group of five chickens for seven consecutive days, thereafter; they were infected as described previously.

Blood biochemical analysis: Blood samples were collected into tubes without anticoagulant for serum analysis on alkaline phosphatase (ALP) and creatinine phosphor kinase (CPK) was determined based on the method of Trevor (2013).

Post mortem pathological examination of some key organs

On day 29th, the final mean weight of the animals in each group was determined, while two animals from each treatment group was randomly selected and sacrificed. They were de-feathered and carefully dissected under the strict instruction and guidance of an expert. The weights and lesion characteristics of lungs portrayed by the experimental birds in each treatment groups were determined/examined and compared with the positive and negative controls;

Statistical analysis

Significant differences between the groups were determined using analysis of variance (ANOVA) SPSS package version 21, while post-test analysis was conducted with Duncan's multiple comparison tests. Values were calculated for each analysis as mean ± SEM. Values were regarded as statistically significant when $p \leq 0.05$ Yalta, (2008).

RESULTS AND DISCUSSION

The major constraint to massive poultry production and hence cheaper protein supply source in developing countries of Africa and some Asian countries is the prevalence of diseases. The drugs/vaccines available for the management of these myriad diseases are either too expensive, unavailable, unaffordable or inaccessible to (most especially) rural farmers. Added to the aforementioned issues is the resistance to the drug molecules developed by the causative organisms and the seeming chronic toxicity experienced from the use of the drugs over a long period of time. Acute toxicity studies of the methanol extract of the studied sample revealed that it is relatively safe even at a higher dose of 5000 mg/Kgbw (Table 1). The non-toxicity of the extract (at acute stage) from this plant has also been reported by Akoma et al. (2018) and Ndukwe et al. (2007). This potential drug source being of organic origin, may not be expected to bio accumulate and be of any chronic toxicity threat.

The high level of mutations of the IB virus (IBV) leads to the emergence of new serotypes and genotypes, and limits the efficacy of routine prevention. Medicinal plants, or substances derived from them, are being tested as options in the prevention of infectious diseases such as IB in many countries. Qualitative analysis of *Tapinanthus globiferus* revealed the presence in high concentration of flavonoids, alkaloids and tannins (Table 2) all which have been reported to act against viral particle through either: Particle aggregation (tannins) (Lelešius et al., 2019), intercalation of the DNA strand (flavonoids) (Worthington et al., 2008) and/or inducing mutations in the DNA (alkaloids) (Zhang et al., 2014).

Determination of serum alkaline phosphatase activities allows for an inference to be made with regard to the viral load. This becomes even more pronounced in situations where availability of equipment for viral isolation and subsequent determination of its load is unavailable particularly, in less sophisticated developing countries such as Nigeria. As revealed in Table 3, the initial ALP

Table 1. Toxicological studies of the various doses of *Tapinanthus globiferus*: A *Vitellaria paradoxa* C.F. Geartn epiphytes extract on experimental birds.

Dose	Number of animals	Observations	Death recorded
0 mgkg^{-1}bw (Distilled water)	6	The birds remained normal and there is no reduced feed intake	0
1000 mgkg^{-1}bw	6	The birds remained normal and there is no reduced feed intake	0
2000 mgkg^{-1}bw	6	The birds remained normal and there is no reduced feed intake	0
3000 mgkg^{-1}bw	6	The birds were momentarily sluggish with feathers becoming erect but become active after a while	0
4000 mgkg^{-1}bw	6	The birds were momentarily sluggish with feathers becoming erect but become active after a while	1
5000mg/kg^{-1}bw	6	The birds were momentarily sluggish with feathers becoming erect but become active after a while	1

Table 2. Qualitative phytochemical constituents of methanol extract of *Tapinanthus globiferus*: A *Vitellaria paradoxa* C.F. Geartn epiphytes extract.

Phytochemical constituents	Inference
Saponins	+
Flavonoids	++
Alkaloids	+++
Tannins	++
Anthraquinones	+
Carbohydrayes	++
Steroids	+++
Cardiac glycosides	++

Key: $^{++}$ = High concentration, $^{+++}$ = Very high concentration and $^{+}$ = Moderate concentration.

Table 3. Means and standard deviation of serum alkaline phosphatase activities of experimental birds treated with various doses of *Tapinanthus globiferus*: A *Vitellaria paradoxa* C.F. Geartn epiphytes extract.

Group	W0	W1	W2	W3
Group I 1000mg/L	17.164±6.6	13.38±4.2	8.41±2.32	10.36±3.42
Group II 2000mg/L	18.132±5.321	14.231±1.97	6.126±3.16	6.926±3.29
Group III 3000mg/L	15.241±5.31	11.926±4.52	9.214±4.31	11.812±5.16
Group IV 4000mg/L	20.461±7.62	16.581±5.31	10.961±2.93	18.491±6.32
Group V Standard Drug	16.146±5.91	12.241±4.39	9.232±4.21	11.816±5.36
Group VI Infected Not Treated	18.164±6.67	14.181±5.86	16.362±5.01	23.681±8.21

W_O = Before the treatment commence, W_1 = A week into the treatment, W_2 = Two weeks into the treatment and W_3 = Three weeks into the treatment.

values (W_O) were all observed to be higher with slight reduction in the first week into the treatment (W_1) and a further reduction in the second week (W_2) and a subsequent slight increase in the third week (W_3). However, in the negative control, it could be observed that there was no significant difference ($p \leq 0.05$) in the values from W_0 across to W_3 with a particular reference to W_2. The mean values obtained in this study are within the range cited by Thrall (2007).

Moreover, infection and subsequent prevalence of the IB viral particles within the system is indicated by the slight increase in the serum activities of creatinine phosphor kinase (CPK). A situation where there is leap in serum creatinine concentration is an indicative of muscular injury due to the viral load (De Wit and Cook, 2014). Comparison of the range of values between the animals treated with 4000 mg/Kgbw (which also happens to be the effective dose) and those treated with the standard drug indicates

Table 4. Means and standard deviations of serum activities of creatinine kinase (CK) U/L in experimental birds treated with various doses of *Tapinanthus globiferus*: A *Vitellaria paradoxa* C.F. Geartn epiphytes extract.

Group	W0	W1	W2	W3
Group I 1000mg/L	2.489±1.19	3.01±0.86	13.019±6.98	12.905±6.99
Group II 2000mg/L	3.142±1.29	4.313±2.672	14.814±8.02	13.827±7.83
Group III 3000mg/L	3.621±1.93	2.624±0.091	10.214±7.93	8.012±3.210
Group IV 4000mg/L	2.981±1.21	5.821±3.82	5.213±3.42	6.731±4.02
Group V Standard Drug	3.431±2.01	3.941±1.98	4.201±3.20	3.928±2.21
Group VI Infected Not Treated	2.926±1.14	4.201±2.78	15.032±8.9	24.814±12.64

W_O = Before the treatment commence, W_1 = A week into the treatment, W_2 = Two weeks into the treatment and W_3 = Three weeks into the treatment.

Table 5a. Lung surface/morphology changes in the experimental birds treated with the various doses of *Tapinanthus globiferus*: A *Vitellaria paradoxa* C.F. Geartn epiphytes extract.

Group	Ballooning degeneration			
	W0	W1	W2	W3
Group I 1000mg/L	None	None	Slightly	Slightly
Group II 2000mg/L	None	None	Very slight	slightly
Group III 3000mg/L	None	None	None	Very slight
Group IV 4000mg/L	None	None	None	None
Group V Standard Drug	None	None	None	None
Group VI Infected Not Treated	None	Slightly	Pronounced	Mottled

W_O = Before the treatment commence, W_1 = A week into the treatment, W_2 = Two weeks into the treatment and W_3 = Three weeks into the treatment.

Table 5b. Lung Multifocal Inflammation/Necrosis observed in the experimental birds treated with the various doses of *Tapinanthus globiferus*: A *Vitellaria paradoxa* C.F. Geartn epiphytes extract.

Group	Multifocal inflammation/necrosis			
	W0	W1	W2	W3
Group I 1000mg/L	Absent	Absent	Absent	Slightly
Group II 2000mg/L	Absent	Absent	Absent	Slightly
Group III 3000mg/L	Absent	Absent	Absent	Slightly
Group IV 4000mg/L	Absent	Absent	Absent	Very slightly
Group V Standard Drug	Absent	Absent	Absent	Absent
Group VI Infected Not Treated	Absent	Slightly	Pronounced	Necrotic

W_O = Before the treatment commence, W_1 = A week into the treatment, W_2 = Two weeks into the treatment and W_3 = Three weeks into the treatment.

no significant difference ($p \leq 0.05$) between the two while a significant difference ($p<0.05$) was observed to exist between these two groups and those treated with the doses of 3000, 2000 and 1000 mg/Kgbw (Table 4).

Ballooning degeneration and multifocal inflammation/ necrosis as revealed by the pathophysiology/morphology studies in Tables 5a and 5b clearly indicates a strong correlation between the efficacy of the standard drug and the effective of 4000 mg/Kgbw and the two differs with the remaining doses of 1000, 2000 and 3000 mg/Kgbw. The absence of these features (Ballooning degeneration and multifocal inflammation/necrosis) is a clear indication of the reduced viral load within the system of the treated animals (European Pharmacopoeia, 2017a; De Wit 2014).

Conclusion

It is therefore concluded that the *Tapinanthus globiferus*: A *Vitellaria paradoxa* C.F. Geartn epiphytes, extracted using 70% methanol/ Water (v/v) has virucidal effect at a concentration 4000 mg/Kgbw.

Recommendation

Considering the emergency situation created by the unavailability, inaccessibility, unaffordability and resistance to the conventional drugs developed by the viral particles, phytoagents such as this, could be packaged and commercialized as an alternative remedy while further purification and pharmacological studies on the extract continuous concurrently.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Akoma, O. A. Nma, N. Y. Musa, S. A., & Salihu, A. B. (2018). Nutritional and phytochemical composition of *Vitellaria paradoxa* (shea fruit pulp). *International Journal of Biochemistry Research and Review,* 22(1), 1-7.

Arthur, S. A., Dhama, K., Kataria, J. M., Rahul, S., & Mahesh, M. (2005). Avian infectious bronchitis: A review. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*, 26(1), 1-14.

Cavanagh, D. (1995). Coronaviridae: A family of enveloped, positive-strand RNA viruses which infect amphibians, birds, and mammals. In: Siddell, S.G. (ed.). *The Coronaviridae*. Plenum Press, New York. p. 73.

Cavanagh, D. (2000). Variation in the viral spike protein gives rise to multiple strains of the virus, which may vary regionally. In: Zuckerman, A. Z., Banatvala, J. E., & Pattison, J. R. (eds.). *Principles and Practice of Clinical Virology*, 4th edition. John Wiley and Sons. Chichester, United Kingdom. p. 345.

Cavanagh, D. (2005). Coronaviruses in poultry and other birds. *Avian Pathology*, 34(6), 439-448.

Cavanagh, D., & Naqi, S. A. (2003). Viral Diseases of Poultry: The Tip of the Iceberg. In: Calnek, B. W., Barnes, H. J., Beard, C. W., McDougald, L. R., & Saif, Y. M. (eds.). *Diseases of Poultry,* 11th edition. Ames, IA, Iowa State University Press. p. 101.

De Wit, J. J. (2000). Detection of infectious bronchitis virus. *Avian Pathology*, 29(2), 71-93.

De Wit, J. J., & Cook, J. K. (2014). Factors influencing the outcome of infectious bronchitis vaccination and challenge experiments. *Avian Pathology*, 43(6), 485-497.

European Pharmacopoeia 9th (2017a). Avian infectious bronchitis vaccine (live). European Directorate for the Quality of Medicines and HealthCare (EDQM), Council of Europe, Strasbourg, France, 1008–1010.

Gandhi, G. R., Barreto, P. G., dos Santos Lima, B., Quintans, J. D. S. S., de Souza Araujo, A. A., Narain, N., Quintans-Junior, L. J. & Gurgel, R. Q. (2016). Medicinal plants and natural molecules with *in vitro* and *in vivo* activity against rotavirus: A systematic review. *Phytomedicine*, 23(14), 1830-1842.

Garba, M. H., Ampitan, T. A., Halidu, S. K., Omotugba, S. K. Fajobi, E. A., & Jeje, C. A. (2020). Anticoccidial potentials of *Cuccumis Metuliferus* E. Mey. Ex Naudin methanol extract in experimental broiler chickens *Journal of Forestry Research and Management,* 17(2).57-67.

Garba, M. H., Kabiru, A. Y., Yusuf, A. M., Muhammad, A. H., Lekene, B. J., Kabir, M., & Joseph, A. (2015). *In vivo* trypanocidal activity of Nymphaea lotus Linn. methanol extract against *Trypanosoma brucei brucei*. *Asian Pacific Journal of Tropical Disease*, 5(10), 808-812.

Jackwood, M. W., & De Wit, J. J. (2013). Infectious bronchitis. In: Swayne, D. E., Glisson, J. R., McDougald, L. R., Nolan, L. K., Suarez, D. L., & Nair, V. (eds.). *Diseases of poultry*, thirteenth edition. Blackwell Publishing Professional Ames, Iowa, USA. Pp. 117-135.

Jaime, M. F. V., Redko, F., Muschietti, L. V., Campos, R. H., Martino, V. S., & Cavallaro, L. V. (2013). *In vitro* antiviral activity of plant extracts from Asteraceae medicinal plants. *Virology Journal*, 10, Article number 245.

Jordan, B. (2017). Vaccination against infectious bronchitis virus: a continuous challenge. *Veterinary Microbiology*, 206, 137-143.

Kama-Kama, F., Midiwo, J., Nganga, J., Maina, N., Schiek, E., Omosa, L. K., Osanjo, G., & Naessens, J. (2016). Selected ethno-medicinal plants from Kenya with in vitro activity against major African livestock pathogens belonging to the "Mycoplasma mycoides cluster". *Journal of Ethnopharmacology*, *192*, 524-534.

Lelešius, R., Karpovaitė, A., Mickienė, R., Drevinskas, T., Tiso, N., Ragažinskienė, O., Kubilienė, L., Maruška, A., & Šalomskas, A. (2019). In vitro antiviral activity of fifteen plant extracts against avian infectious bronchitis virus. *BMC Veterinary Research*, 15, Article number 178.

Liang, J. Q., Fang, S., Yuan, Q., Huang, M., Chen, R. A., Fung, T. S., & Liu, D. X. (2019). N-Linked glycosylation of the membrane protein ectodomain regulates infectious bronchitis virus-induced ER stress response, apoptosis and pathogenesis. *Virology*, 531, 48-56.

Mahmood, Z. H., Sleman, R. R., & Uthman, A. U. (2011). Isolation and molecular characterization of Sul/01/09 avian infectious bronchitis virus, indicates the emergence of a new genotype in the Middle East. *Veterinary Microbiology*, 150(1-2), 21-27.

Ndukwe, I. G., Amupitan, J. O., Isah, Y., & Adegoke, K. S. (2007). Phytochemical and antimicrobial screening of the crude extracts from the root, stem bark and leaves of *Vitellaria paradoxa* (GAERTN. F). *African Journal of Biotechnology*, 6(16), 1905-1909.

Rady, I., Mohamed, H., Rady, M., Siddiqui, I. A., & Mukhtar, H. (2018). Cancer preventive and therapeutic effects of EGCG, the major polyphenol in green tea. *Egyptian Journal of Basic and Applied Sciences*, 5(1), 1-23.

Shayganni, E., Bahmani, M., Asgary, S., & Rafieian-Kopaei, M. (2016). Inflammaging and cardiovascular disease: Management by medicinal plants. *Phytomedicine*, 23(11), 1119-1126.

Sofowora, E. A. (1982). Medicinal plants and traditional medicine in Africa. John Wiley and sons Ltd, New York. Pp. 256-257.

Thrall, M. A. (2007). *Hematologia e bioquímica clínica veterinária*.Philadelphia, Lippincott, Williams & Wilkins, São Paulo: Roca, p. 582.

Trease, G. E., & Evans, W. C. (2002). *Pharmacognosy*. 11th edition. Bailliere Tindll., London, Pytochemistry: Introduction in General Methods. Pp. 227-247.

Trevor, J. W. (2013). *Veterinary Clincal Pathology and proceedings*. Abbey Veterinary Services. Marck and Co. inc. Kendworth, N. J. USA. p. 243.

Worthington, K. J., Currie, R. J. W., & Jones, R. C. (2008). A reverse transcriptase-polymerase chain reaction survey of infectious bronchitis virus genotypes in Western Europe from

2002 to 2006. *Avian Pathology*, 37(3), 247-257.
Yalta, A. T. (2008). The accuracy of statistical distributions in Microsoft® Excel 2007. *Computational Statistics & Data Analysis*, 52(10), 4579-4586.
Zhang, X. L., Guo, Y. S., Wang, C. H., Li, G. Q., Xu, J. J., Chung, H. Y., Ye, W. C., Li, Y. L., & Wang, G. C. (2014). Phenolic compounds from Origanum vulgare and their antioxidant and antiviral activities. *Food chemistry*, 152(1), 300-306.