Research Article**Antibacterial activity of *Cannabis sativa* extracts against *Salmonella* Gallinarum isolated from Poultry**

Yunusa Umar Abubakar^{1*}, Dalha Wada Taura², Abdulhamed Baba Njobdi², Patience O. Ihedigbo¹, Miracle Uwa Livinus³

¹Department of Animal Health, College of Agriculture, Jalingo, Taraba State, Nigeria

²Department of Microbiology, Bayero University, Kano, Nigeria

³Department of Biochemistry, Bayero University, Kano, Nigeria

Received: 7 November 2020

Revised: 25 December 2020

Accepted: 26 December 2020

Abstract

Background: *Salmonella* Gallinarum is a non-motile gram-negative rod and host-specific bacteria for poultry with very high economic implications. **Objective:** This study was carried out to determine the antibacterial activities of *Cannabis sativa* plant extracts (petroleum ether and methanolic, seed and leave extracts) against *Salmonella* Gallinarum isolated from poultry. **Materials and Methods:** The antibacterial activity of the plant extracts was conducted by assessing the activity of the plant extracts on *Salmonella* Gallinarum isolated from poultry (chickens) using agar well diffusion method. **Results:** The antibacterial activity of *C. sativa* plant extracts against *Salmonella* Gallinarum ranged between 8.50±0.71 and 23.50±0.71mm. Petroleum ether seed extract had the highest antibacterial activity of 23.50±0.71mm at 2000µg/ml. The antibacterial activities of *C. sativa* plant extracts were in the order; PSE > PLE > MSE > MLE. All plant extracts inhibited the activities of *Salmonella* Gallinarum at varying concentration. The minimum inhibitory concentration (MIC) of petroleum ether leave and methanol seed and leave extracts were 125µg/ml respectively while the MIC of petroleum ether seed extract was the least at 62.50µg/ml. The findings show significant antibacterial activity of *C. sativa* plant extracts against *Salmonella* Gallinarum ($P<0.05$). **Conclusion:** The findings of the present study shows that *C. sativa* seed and leave extracts can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities that brings about breakthrough in the treatment of Fowl typhoid (FT) caused by *Salmonella* Gallinarum.

Keywords: Antibacterial, *Cannabis sativa*, poultry, *Salmonella* Gallinarum

Introduction

Salmonella is a bacterial enteric pathogen capable of infecting a guest host. Depending on the serotype of the infected host, *Salmonella* can induce various diseases, gastroenteritis type, and systemic infection, through the asymptomatic carriage (Youssef et al., 2017). Fowl typhoid (FT) caused by *Salmonella enterica* subsp. *enterica* serovar Gallinarum biovar Gallinarum (*Salmonella* Gallinarum) is a severe systemic disease, producing high mortality rates (up to 90%) in poultry birds of all ages, thus causing heavy economic loss (Kumari et al.,

2013; Lawal et al., 2015).

Chickens are the natural hosts for *S. Gallinarum*. However, in natural outbreaks, FT has been discovered in turkeys, guinea-fowl, quail, pheasants, ring-necked doves, ostriches, peafowl, sparrows and parrots (Shivaprasad, 2000). The susceptibility of ducks, geese and pigeons to *S. Gallinarum* varies, but all of these birds generally appear to be resistant (Kang et al., 2012). A greater percentage of females appear to remain as reactors, probably due to the sequestered nature of localised infection of the ovarian follicles. *Salmonella* Gallinarum could be cultured for up to 121 days from the faeces of experimentally infected rats (Badi et al., 1992).

Salmonella Gallinarum rarely cause clinical cases in people. Self-limiting enteritis is thought to be the most

***Address for Corresponding Author:**

Yunusa Umar Abubakar

Department of Animal Health, College of Agriculture, Jalingo, Taraba State, Nigeria

Email: yunusamara2@gmail.com

DOI: <https://doi.org/10.31024/apj.2020.5.6.1>

2456-1436/Copyright © 2020, N.S. Memorial Scientific Research and Education Society. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

common presentation, although other syndromes have been reported. Their threat to immune-compromised individuals is still unclear (PHAC, 2010). A urinary tract infection caused by *Salmonella* Gallinarum was reported in a kidney transplant patient in 2017, and it is possible that some infections might have been missed (Ahamed et al., 2017). Organisms identified only as group D *Salmonella* have occasionally been recovered from people who were infected with HIV or had other immunosuppressive conditions (Abuhasna et al., 2012).

Fowl typhoid disease can be transmitted orally (e.g., in food and water or by cannibalism) and via the respiratory tract (Basnet et al., 2008). The causative organisms may also enter the body at other sites, such as in wounds. *Salmonella* Gallinarum can be found in the feces of sick birds; however, they do not seem to colonize the intestinal tract in the absence of disease (Xu et al., 2018). Clinical signs of FT in chickens include anorexia, diarrhea, dehydration, and decreased egg production (Kwon and Cho, 2011). The morbidity and mortality of FT are highly variable in poultry and are influenced by host age, host susceptibility, nutrition, flock management, and virulence of *Salmonella* Gallinarum, which is a major factor affecting the severity of the disease (Lee et al., 2007). Due to the detrimental effect of virulent bacterial diseases to poultry production and the entire national economy, this research was embarked to determine the antibacterial activities of *Cannabis sativa* plant extracts (petroleum ether and methanolic seed and leave extracts) against *Salmonella* Gallinarum isolated from poultry.

Materials and Methods

Collection and processing of plant sample

One kilogram (1kg) of dried leaf and seed of *Cannabis sativa* (Indian hemp) were obtained from Taraba State Command, National Drug Law Enforcement Agency (NDLEA), Jalingo, Taraba State. The samples were confirmed at the Department of Plant Biology, Bayero University Kano. The samples were grounded into powder using mortar and pestle as described by Mukhtar and Tukur (1999).

Extraction of plant materials

A procedure by Redfern et al. (2014) was followed for extraction using Soxhlet apparatus (Pyrex Company, United Kingdom). Petroleum ether and methanol solvents were used for the extraction. One hundred and eighty grams (180g) of the powdered plant leaf and seed material was filled in cotton sacked material and introduced inside the Soxhlet extractor. Following this, five hundred milliliters (500ml) of petroleum ether was also introduced into the Soxhlet extractor. The side arm was lagged with glass wool, the solvent was heated using hotplate, the condenser on the isomantle beginning to evaporate moving through the apparatus to condenser, the condenser then dripped into the reservoir containing the plant

materials. As the level of solvent reached the siphon, it poured back into the flask and the circle continued until the plant material colour turned to colourless, and the extracted material poured into the beaker. The process was done for methanol solvent, with the same gram of the plant material. The extracts were left to air dry for the solvents to evaporate and the fraction was later obtained.

Phytochemical Screening

Phytochemical screening of petroleum ether and methanol seed and leaf extracts of *Cannabis sativa* was carried to find the presence or absence of active secondary metabolites such as alkaloids, tannins, flavonoids, saponin, glycoside, phenols and steroid/triterpenoid as described by Edeoga et al. (2005) with modifications by Abubakar et al. (2020a).

Collection of chicken samples

In this study, a total of 50 chicken visceral organs and intestinal contents were randomly pooled from freshly slaughtered diseased and healthy chicken of different abattoirs in Jalingo town, Taraba State, Nigeria. The samples were collected between September and November 2019, from five poultry abattoir. The collected samples were introduced into sterile polythene bag and transferred immediately in an ice pack to the laboratory for bacteriological analysis. Following transport in ice packs ($<8^{\circ}\text{C}$) to the laboratory, visceral organs and intestinal contents samples were homogenised in buffered peptone water within 24h post collection from abattoirs as described by Abraham et al. (2019).

Bacteriological Study

Isolation and identification of bacterial isolate

About 2g of the homogenized chicken sample was immediately inoculated into Rappaport Vassiliadis Soya (RVS) peptone broth for selective enrichment of *Salmonella* organism of Veterinary Importance and was incubated at 37°C for 24hrs as described by OIE (2012). After 24hrs, one loopful of RVS peptone broth culture was streaked into MacConkey lactose agar (MLA) and blood agar (BA) and was incubated at 37°C for 24hrs and the growth colony was observed after 24hrs incubation and the result was recorded. The identification of the organisms was performed by the tests as described by Merchant and Packer (1997) and Cheesbrough (2006). On the basis of growth morphology and Gram staining characters, and biochemical tests the organism was isolated and identified.

Determination of antibacterial activity of *Cannabis* extracts

Salmonella Gallinarum isolates were subcultured onto sterile nutrient agar plates and incubated at 37°C for 24

hours. A sterilized wire loop was used to ensure absolute dilution of the subculture in normal saline (0.85% w/v) such that their turbidity matches with 0.5 MacFarland standards which give a mean of 1.0×10^8 cfu/ml microbial population density (Abdulrashid et al., 2018). Agar well diffusion method was used for the antibacterial assay as described by Nester et al. (2004). Mueller Hinton agar was prepared as specified by the manufacturer. The media was autoclaved at 121°C for 15 minutes, poured aseptically into sterile petri-dishes and allowed to gel. A loopful of standardized isolate suspension was streaked evenly on each agar plate. A 0.004g of the leaf and seed extract were separately dissolved in 1ml dimethyl sulphoxide (DMSO) to obtain 4000µg/ml stock solution of leaf and seed fractionated extracts of *Cannabis sativa*. From this, the working concentrations of 2000µg/ml, 1000µg/ml and 500µg/ml were made. Then 0.1ml of each fractionated extract was inoculated into three wells (6mm diameter) bore with a sterile cork borer to each plate. The fourth (4th) well contains a commercial oxytetracycline HCl (0.03 µg/ml) and served as positive control. The plates were then allowed to stand for 30mins on the table for pre-diffusion of the extract and were inoculated at 37°C for 24hrs. After incubation, the antibacterial activity of the extract was determined by measuring the mean diameter zones of inhibition produced by each of the extracts against the bacterial species and result was recorded in millimeter (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) of the petroleum ether and methanol, seed and leave extracts against the test organisms were determined using the broth dilution method (Clinical Laboratory Standard Institute, 2015). To each concentration of the extracts i.e. 500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml and 31.25µg/ml, a loop full of culture adjusted to 0.5 MacFarland standard was inoculated into it and all the tubes were incubated at 37°C for 24 hours. The tube containing broth and extract serve as the positive control while the tube containing broth and inoculum serves as negative control. The lowest concentration of the extract that inhibited the growth of each organism was considered as minimum inhibitory concentration (MIC).

Minimum Bactericidal Concentration (MBC)

This was conducted by the method described by CLSI (2015). To determine this, the tubes that showed no visible bacterial growth from the MIC tubes were streaked onto Mueller Hinton plates and incubated at 37°C for 24 hours. The lowest concentration in which no growth occurred was taken as the MBC.

Statistical Analysis

All data were analyzed using the statistical package SPSS (Version 20) and Microsoft Excel 2013. Descriptive statistics

were carried out to determine the mean zone of inhibition of *Cannabis sativa* extracts against *Salmonella* Gallinarum and expressed in millimetre (mm). One Way Analysis of Variance and Duncan Multiple Range test were used to show significant difference between mean variables. The $P < 0.05$ were considered statistically significant.

Results

Phytochemical analysis of *C. sativa* plant extracts

The result of the phytochemical analysis of *C. sativa* plant extracts using petroleum ether and methanol solvents is presented in our earlier study (Abubakar et al., 2020b).

Cultural and biochemical characterization of *S. Gallinarum*

The result of the cultural and biochemical characteristics of the test organism indicated that *S. Gallinarum* was observed to be a Gram negative, rod-like and non-motile bacteria and reacted positive to oxidase, methyl red and hydrogen sulphide tests. Also, the bacterial isolate reacted negative to catalase, coagulase, Vogues Proskauer, indole, citrate, urease and Triple Sugar Iron tests (Table 1).

Antibacterial activity of *C. sativa* extracts on *Salmonella* Gallinarum

The results from the sensitivity test showed that the plant extracts had remarkable activity against the tested microorganism with inhibition zones ranging between 8.50 ± 0.71 and 23.50 ± 0.71 mm at various concentrations. Petroleum ether seed extract (PSE) had the highest activity of 23.50 ± 0.71 mm at 2000µg/ml while methanol leave extract had the least activity of 8.50 ± 0.71 mm at 500µg/ml. The antibacterial activities of *C. sativa* plant extracts were in the following order, PSE > PLE > MSE > MLE (Table 2).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *C. Sativa* plant extracts

The MIC values ranged between 62.50µg/ml and 125.00µg/ml while the MBC values ranged between 125.00µg/ml and 250.00µg/ml (Table 3). Petroleum ether seed extract had the least MIC value of 62.50µg/ml at MBC of 125.00µg/ml. Petroleum ether leave, methanolic seed, and leave extracts had MIC values of 125µg/ml respectively.

Discussion

Findings of our previous study on the phytochemical constituents of *C. sativa* plant extracts revealed that *C. sativa* leaves and seeds extracts are rich source of alkaloid, flavonoid, tannins, phenol, steroids, glycoside, saponins

Table 1. Cultural and Biochemical characterization of *S. Gallinarum*

Test	Inference	Test	Inference
Microscopic Morphology	Rods	Oxidase	+
Gram stain	–	Vogues Proskauer	–
Catalase	–	Methyl red	+
Coagulase	–	Indole	–
Hydrogen sulphide	+	Citrate	–
Urease	–	Triple Sugar Iron	–
Motility	–		

Positive (+), Negative (–)

Table 2. Antibacterial activity of *C. sativa* plant extracts against *Salmonella* Gallinarum

Plant extracts	Concentration (µg/ml) / Zone of Inhibition (mm)			
	2000	1000	500	Control (+ve) (0.03µg)
PSE	23.50±0.71 ^a	19.50±0.71 ^b	14.50±0.71 ^c	29.10±1.51 ^d
PLE	20.50±0.71 ^a	16.50±0.71 ^b	13.50±0.71 ^b	32.00±1.00 ^c
MSE	12.00±0.00 ^a	10.50±0.71 ^a	8.50±0.71 ^a	23.00±1.51 ^b
MLE	9.50±0.71 ^a	9.50±0.71 ^a	8.50±0.71 ^a	22.00±0.75 ^b

Abbreviations: Petroleum ether seed extract (PSE); petroleum ether leave extract (PLE); methanol seed extract (MSE); methanol leave extract (MLE). Values are means ± SD of triplicate observation. Control (oxytetracycline HCl). Values (Means±SD) ($n = 3$) followed by different letters across the rows are significantly different at $P < 0.05$.

Table 3. MIC and MBC of *C. sativa* plant extracts on *S. Gallinarum*

Plant extracts	Concentration (µg/ml)	
	MIC	MBC
Petroleum ether seed extract (PSE)	62.50	125
Petroleum ether leave extract (PLE)	125.00	250
Methanolic seed extract (MSE)	125.00	125
Methanol leave extract (MLE)	125.00	250

and triterpenoid (Abubakar et al., 2020b). The presences of these secondary metabolites that are biologically active are responsible for the antibacterial activities of *Cannabis Sativa*. The selection of these plants was due to their bioactive constituents that produces some biological activity in man and animal (Simon et al., 2015). These compounds also serve defensive functions for the plant against insect attack, herbivores, and microbial infection (Patel, 2010). According to Sihoglu and Tepe (2015), plants have naturally occurring chemicals that are non-nutritive and for their self-protection. They have therapeutic values, protective or diseases preventive properties which can be synthesized into useful drugs (Heinrich et al., 2016).

The test organism, *Salmonella* Gallinarum was isolated and identified through standard cultural and biochemical techniques. Since the isolation and correct identification of *Salmonella* are very crucial for the characterization, the colonies having typical cultural characteristics were selected as presumptive for *Salmonella* serovars. Cultural morphology on Rappaport Vassiliadis Soya (RVS) peptone broth and Eosine Metheylene Blue (EMB) was in accordance with the findings of Park et al. (2012) and Ferdous et al. (2013). In this study, the colony characters of the isolate, the production of hydrogen sulfide gas with black color colonies on TSI agar was corresponded with the findings of others (Shrama and Katock, 1996; Hossain et

al., 2006). In Gram's staining, the morphology of the isolated bacteria was small rod shape, non-motile, Gram negative, Vogues Proskauer negative which also corresponded with morphological characters of *Salmonella* as described by several authors (Freeman, 1995; Jones et al., 1997; Hossain et al., 2006). Biochemical reactions reported during present investigation were classical findings of *S. Gallinarum* (Mir et al., 2015; Dey et al., 2016). The prevalence of *Salmonella* Gallinarum infections in chickens could be associated with the accumulation of *Salmonella* Gallinarum aerosols in the atmosphere of chicken barns that are inhaled by chickens into the respiratory tract.

Antibacterial activity of *C. sativa* plant extracts against *Salmonella* Gallinarum ranged from 8.50 ± 0.71 to 23.50 ± 0.71 mm. Petroleum ether seed extract had the highest antibacterial activity of 23.50 ± 0.71 mm at $2000 \mu\text{g/ml}$. The antibacterial activities of *C. sativa* plant extracts were in the order; PSE > PLE > MSE > MLE. All plant extracts inhibited the activities of the test organisms at varying concentration. Considering the bioactive contents of *C. sativa*, as previously reported by Khan et al. (2010), the extracts can serve potent and effective therapeutic use. Furthermore, the susceptibility profile of *S. Gallinarum* to the plant isolates was an indication that the isolates can be harnessed as alternative treatment remedy for Salmonellosis and other bacterial avian infection (Marano et al., 2000; Liebana et al., 2013; Ferreira et al., 2014). The minimum inhibitory concentrations (MIC) of *C. sativa* leave extracts against *Salmonella* Gallinarum were higher than those of *C. sativa* seed extracts against *Salmonella* Gallinarum. The higher MIC of the leave extracts could be attributed to the higher prevalence of phytochemicals in the leave extracts than the seed extracts of the plant as recently reported by Kavitha et al. (2017).

Conclusion

The present study has indicated that petroleum ether seed and leave extracts had significant antibacterial effect against *Salmonella* Gallinarum. Thus, *Cannabis sativa* plant extracts are viable therapeutic agents for the treatment of avian diseases. Considering the high antibacterial activity of *Cannabis sativa* petroleum ether seed and leaf extracts against *Salmonella* Gallinarum, the researcher recommends that *Cannabis sativa* plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities that brings about breakthrough in the treatment of Fowl typhoid (FT) caused by *Salmonella* Gallinarum. Further studies on the extracts of the whole plant using Mass Spectroscopy, and Nuclear Magnetic Resonance Techniques is recommended to elucidate the structure for drug synthesis.

Conflicts of Interest

The authors have no conflict of interest to be declared.

References

- Abdulrashid M, Anthony J, Auwalu U. 2018. Antibacterial Activity of *Citrus sinensis* and *Solanum lycopersicum* on Wound Isolated from Hospitals in Kaduna Metropolis Kaduna Nigeria. International Journal of Biomedical Materials Research 6(2):40-49.
- Abraham S, O'Dea M, Sahibzada S, Hewson K, Pavic A, Veltman T. 2019. *Escherichia coli* and *Salmonella* spp. isolated from Australian meat chickens remain susceptible to critically important antimicrobial agents. PLoS ONE 14(10):e0224281.
- Abubakar YU, Taura DW, Muhammad AB, Ibrahim TS, Umar M, Njobdi AB, Hussaini A, Ihedigbo PO, Livinus MU. 2020a. *In vitro* antimicrobial activity of *Cannabis sativa* (Hemp) extracts against avian pathogenic *Escherichia coli* (APEC) isolated from Broilers Chicken. Advance Pharmaceutical Journal 5(5):164-171.
- Abubakar YU, Taura DW, Yushau M, Muhammad AU. 2020b. An *in ovo* investigation on antiviral activity of *Cannabis sativa* extracts against Newcastle Disease Virus (NDV). Advance Pharmaceutical Journal 5(1):21-30.
- Abuhasna S, Al-Jundi A, Rahman MU, Said W. 2012. Non-typhoidal *Salmonella* group D bacteremia and urosepsis in a patient diagnosed with HIV Infection. Journal of Global Infectious Diseases 4:218e9.
- Ahamed I, Nellikode S, Annapandian VM. 2017. Case report of *Salmonella* Gallinarum urinary tract infection in a renal allograft recipient. Transplantation Proceedings 49(7):1591-2.
- Badi MA, Iliadis N, Sarris K. 1992. Natural and experimental infection of rodents (*Ratus norvegicus*) with *Salmonella* Gallinarum. Indian Veterinary Journal 74:731-733.
- Basnet HB, Kwon HJ, Cho SH, Kim SJ, Yoo HS, Park YH, Yoon SI, Shin NS, Youn HJ. 2008. Reproduction of fowl typhoid by respiratory challenge with *Salmonella* Gallinarum. Avian Diseases 52(1):156-9.
- Cheesbrough M. 2006. *Medical Laboratory Practice in Tropical countries*. Part 2, 2nd ed. United Kingdom: Cambridge University Press; Page 157-200.
- Clinical and Laboratory Standards Institute (CLSI) 2015. *Performance standards for Antimicrobial Disk Susceptibility Tests; 364 Approved Standard – Twelfth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
- Dey S, Mahanti A, Batabyal K, Joardar SN, Samanta I, Isore DP, Pakhira MC. 2016. Identification and antimicrobial susceptibility of *Salmonella* Gallinarum isolated from

- fowl typhoid outbreak in backyard Vanaraja fowl. *Exploration Animal Medical Resource* 6(1): 63-67.
- Edeoga HO, Okwu DE, Mbaebie BO. 2015. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 4(7):685-688.
- Ferdous TA, Lutful K, Mansurul AM, Mahmud-Hossain KM. 2013. Identification and antimicrobial susceptibility of *Salmonella* species isolated from washing and rinsed water of broilers in pluck shops. *International Journal of Animal Veterinary Advancement* 5(1): 1-8.
- Ferreira JC, Filho RAC, Kanashiro AMI, Darini ALD, Junior AB. 2014. Antimicrobial susceptibility of *Salmonella* Gallinarum and *Salmonella* Pullorum isolated from ill poultry in Brazil. *Ciencia Rural* 46(3): 513-518.
- Freeman BA. 1995. *Burrow's Text Book of Microbiology*. 22nd edn., W. B. Saunders Company, London, UK. pp. 372-472.
- Heinrich M, Kerrouche S, Bharij KS. 2016. Recent Advances in Research on Wild Food Plants and Their Biological—Pharmacological Activity. Sánchez-Mata M de C, Tardío J, eds. *Mediterr. Wild Edible Plants Ethnobotanical Food Composition Tables* (Springer New York, New York, NY). pp. 253-269.
- Hossain MS, Chowdhury EH, Islam MM, Haider MG, Hossain MM. 2006. *Avian Salmonella Infection: Isolation and Identification of Organisms and Histopathological Study*. Bangladesh Journal of Veterinary Medicine 4(1): 07-12.
- Jones TC, Hunt RD, King NW. 1997. *Veterinary Pathology*. 6th edn. Williams and Wilkins Co. Baltimore, USA. Pp. 139-152.
- Kang MS, Kwon YK, Kim HR, Oh JY, Kim MJ, An BK, Shin EG, Kwon JH, Park CK. 2012. Differential identification of *Salmonella enterica* serovar Gallinarum biovars Gallinarum and Pullorum and the biovar Gallinarum live vaccine strain 9R. *Veterinary Microbiology* 160(3-4):491-5.
- Kavitha M, Raja M, Kamaraj C, Karthik Raja R, Balasubramaniam V, Balasubramani G, Perumal P. 2017. *In vitro* Antimicrobial Activity of Azadirachta indica (Leaves) against Fish Pathogenic Bacteria Isolated from Naturally Infected Dawkinsia filamentosa (Blackspot barb). *Journal of Medicinal and Aromatic Plants (Los Angeles)* 6(3):1-7.
- Khan TA, Rue CA, Rehmani SF, Ahmad A, Wasilenko JL, Miller PJ, Afonso CL. 2010. Phylogenetic and Biological Characterization of Newcastle Disease Virus Isolates from Pakistan. *Journal of Clinical Microbiology* 48(5):1892-1894.
- Kumari D, Mishra SK, Lather D. 2013. Pathomicrobial studies on *Salmonella* Gallinarum infection in broiler chickens. *Veterinary World* 6(10):725-729.
- Kwon HJ, Cho SH. 2011. Pathogenicity of SG 9R, a rough vaccine strain against fowl typhoid. *Vaccine* 29:1311-1318.
- Lawal JR, Jajere SM, Mustapha M, Bello AM. 2015. Prevalence of Newcastle Disease in Gombe, Northeastern Nigeria: A Ten-Year Retrospective Study (2004 – 2013). *British Microbiology Research Journal* 6(6):367-375.
- Lee CY, Wey SP, Liao MH, Hsu WL, Wu HY, Jan TR. 2007. A comparative study on cannabidiol-induced apoptosis in murine thymocytes and EL-4 thymoma cells. *International Immunopharmacology* 8(5):732-740.
- Liebana E, Carattoli A, Coque TM, Hasman H, Magiorakos AP, Mevius D, Poirel L, SchuëpbachRegula G, Torneke K, Torren-Edo J, Torres C, Threlfall J. 2013. Public health risks of enterobacterial isolates producing extended-spectrum beta-lactamases or AmpC beta-lactamases in food and food-producing animals: An EU perspective of epidemiology, analytical methods, risk factors, and control options. *Clinical Infectious Diseases* 56: 1030-1037.
- Marano NN. 2000. The National Antimicrobial Resistance Monitoring System (NARMS) for enteric bacteria, 1996-1999: surveillance for action. *Journal of the American Veterinary Medical Association*, 217(12):1829-1830.
- Mir IA, Kashyap SK, Maherchandani S. 2015. Isolation, serotype diversity and antibiogram of *Salmonella* Enterica isolated from different species of poultry in India. *Asian Pacific Journal of Tropical Biomedicine* 5(7): 561-567.
- Mukhtar MD, Tukur A. 1999. *In-vitro* screening for activity of *Pistia stratiotes* extracts. The Nigerian Society for *Pistiastratiotes* Experimental Biology (NISEB) Journal 1(1):51-60.
- Nester EW, Anderson OG, Robert C, Pearsall N, Nester MT. 2004. *Microbiology: a human perspective*, 4th edition, MacGraw-Hill Companies, N.Y. U.S.A pp.635-637.
- OIE 2012. World Organization for Animal Health. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* 6th ed. Paris, France. Pp. 576-589.
- Park SH, Ryu S, Kang DH. 2012. Development of an improved selective and differential medium for isolation of *Salmonella* spp. *Journal of Clinical Microbiology* 50(10): 3222-3226.
- Patel SS. 2010. The constituent of medicinal plants. An introduction to the chemistry and therapeutics of Herbal medicine. Traditional medicine source of new drugs, pharm times. Centre for Agriculture and Bioscience International (CABI) Publishing 34(1):17-18.
- Public Health Agency of Canada (PHAC) 2010. Pathogen

- Safety Data Sheets: Infectious substances – *Salmonella enterica* spp. Pathogen Regulation Directorate, PHAC; 2010 Aug. Available at: <https://www.canada.ca/en/publichealth/services/laboratory-biosafety-biosecurity/pathogensafety-data-sheets-risk-assessment/Salmonella-enterica.html>. Accessed 13 Sept., 2019.
- Redfern J, Kinninmonth M, Burdass D, Verran J. 2014. Using Soxlet Ethanol Extraction to produce and test plant material (Essential oils) for their antimicrobial properties. *Journal of Microbiology and Biology Education* 15(1):45-46.
- Shivaprasad HL. 2000. Fowl typhoid and pullorum disease. *Revue scientifique et technique (International Office of Epizootics)* 19(2):405-424.
- Shrama M, Katock RC. 1996. Deadly outbreak in chicks owing to *Salmonella typhimurium*. *Indian Journal of Poultry Science* 31:60-62.
- Sihoglu A, Tepe B. 2015. Traditional use, biological activity potential and toxicity of *Pimpinella* species. *Indian Crops Production* 69:153-166.
- Simon B, Lidianys M, Lewis-Lujan C. 2015. Solvent effects on phytochemical constituent profiles and antioxidant activities using four different extraction formulation for analysis of *Bucidabuceras*. *BMC Research notes* 1: 17-29.
- Xu L, Liu Z, Li Y, Yin C, Hu Y, Xie X, Li Q, Jiao X. 2018. A rapid method to identify *Salmonella enterica* serovar Gallinarum biovar Pullorum using a specific target gene ipaJ. *Journal of Avian Pathology* 47(3):238-244.
- Youssef M, Mohammed F, Adil M, Abdelhay L, Mostafa E. 2017. Infection caused by *Salmonella* Gallinarum emergent bacteria. *Saudi Journal of Health Sciences* 6:68-70.