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Efficacy of Ethno-Veterinary Medicinal plants from Nicobarese community on Sero-cytokine biomarkers in Native Nicobari fowl

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Abstract

A study was designed to assess the therapeutic efficacy of ethno-veterinary medicinal herbs of Nicobarese tribes on sero-cytokine biomarkers against LPS of *E. coli* using Nicobari fowl as a biological model under tropical humid island ecosystem of Andaman and Nicobar Islands. Information on ethno veterinary medicinal practices for common gastro intestinal ailments was collected from Nicobarese tribe. Herbal antibiogram was studied with extracts of medicinal plants used in the ethno veterinary tribal medicinal practice (EVTMP) *viz*., *Taberna montanacrispa*, *Psidium guajava* and *Lee indica*. *In vivo* trial was carried out with decoction out of formulation with the dry leaf powder of *Tabernamontana crispa*, *Psidium guajava* and *Lee indica* @ 10:5:1, respectively. Each of three groups of birds was received decoction mixed water (3 mL per bird; twice in a week), negative control with plain water and positive control (Oxytetracycline in water). On day 22, all the birds were injected intramuscularly with LPS of *E. coli (*100 µg/kg body weight). Serum cytokine biomarkers (IL-6, IFN-γ and MHC) were quantified. Aqueous herbal extracts have antibacterial activity against gut *E. coli* isolates of cloacal swab. Strong antibacterial activity was recorded with *Psidium guajava* and *Taberna montanacrispa. Lee indica* had moderate antibacterial activity. Highly potent antibacterial activity was exhibited by the extract of *Psidium guajava* against all *E. coli* isolates at 165.0 µg per µL. Pharmacological evaluation of EVTM herbal formulation in terms of sero cytokines biomarkers with composition of *Tabernamontana crispa* (10 g) + *Psidium gujava* (5 g) + *Lee indica (2.5* g*)* @ 3ml per bird twice in a week in Nicobari fowl reported lower serum level of IL -6 and higher serum level of IFN-γ and MHC.

Key words: *Ethno veterinary tribal medicinal plants, Herbal antibiogram, Sero-cytokine biomarkers*

Introduction

Cytokines are important biomolecules to boost the host immune response against the pathogens through regulation of both innate and adaptive immune responses. These pleotropic proteins act either in close proximity to their production sites or get into the circulation in large quantity to act in systems. Quantification of sero-cytokine levels helps to confirm the possible inflammatory mechanisms of diseases occurred and to assess physiological processes as well. Hence, cytokines can be defined as sero-biomarkers to diagnose the severity of inflammatory diseases (Mack, 2007) for deciding therapies to cure them (Chen *et al*., 2015) based on the immune status of the host. Colibacillosis, the most common infectious poultry bacterial disease is caused by *Escherichia coli* (Bai *et al*., 2018, Nolan *et al.*, 2020). Lipopolysaccharide (LPS), the cell wall component of this Gram-negative bacterium is one of the most potent stimuli to activate the pro-inflammatory cytokines and inflammatory chemokines in inflammatory diseases (Qi *et al*., 2017). LPS of *E. coli* is a potent antigen in chickens (Shimizu *et al.,* 1988; Sunwoo *et al*., 1996). In recent time, various studies have been conducted on efficacy of various medicinal herbs to mitigate the inflammatory diseases due to prevalence of multiple-antibiotic resistant pathogenic *E. coli* in poultry (Sargeant *et al*., 2019, Jai Sunder *et al*., 2021). Medicinal plants based feed additives in the feed decrease the gut inflammation by down regulation of pro-inflammatory cytokines through immune stimulatory effect of phytochemicals in the medicinal plants (alkaloids, flavonoids, glycosides, polysaccharides, diterpenoids, lactones, etc) in the animal immune system (Hartady *et al*., 2021, Jai Sunder*et al.,* 2016, Sujatha *et al.,* 2017a, Sujatha *et al.,* 2017b). Herbal extracts as additives in the poultry feed had improved the production performance

and the quality of end products (Dhama *et al*., 2014, Jai Sunder *et al*., 2013, Jai Sunder *et al*., 2014). Ethno-medicinal plants are used in invention of new drugs for therapeutic purposes (Asma Bibi *et al*., 2016). In crude form, medicinal herbs express a combination of activities (El-Mahmood *et al*., 2008).Various literatures revealed that medicinal plants used in traditional medicine have antibacterial activity against gut pathogens both in *in-vitro* and *in-vivo* studies in poultry production system (McMurray *et al*., 2020, Sujatha *et al.,* 2017c). Andaman and Nicobar Islands (ANI) is a botanical paradise with thousands of endemic plant species; out of which, 52 plant species are used in the medicinal formulations (Asma Bibi *et al*., 2016). Tribals of ANI primarily depend on these medicinal herbs for maintenance of the health and improvement of productivity and reproduction of the livestock. It is important to document the ethno-veterinary medicinal practices of tribals to conserve the knowledge of medicinal herbs in treatment of livestock which in turn facilitate for formulation of new anti-microbial agents. Further, validation of ethno-veterinary medicines is required for complete documentation of their biological functions; which in turn improve the health and productivity of poultry, similar studies are lacking in Andaman and Nicobar Islands (Travel *et al*., 2021). Lipopolysaccharide (LPS) is a natural adjuvant synthesized by gram-negative bacteria that stimulates cells through Toll-like receptor 4 (TLR4), causing the release of inflammatory cytokines. Moreover, anti-inflammatory are possibility recruited in response to live infections, where as it is avoided by purified challenges such as LPS or cytokines. Hence, infection and inflammation induced by LPS of *E. coli* is a widely used model to assess the biotic stress on immunity of animals (Klaudia and Alina, 2015). Therefore, this present study was designed to assess the therapeutic efficacy of ethno-veterinary medicinal herbs of Nicobarese tribal on concentration of sero-cytokine biomarkers against LPS of *E. coli* using Nicobari fowl as a biological model under tropical humid island ecosystem of Andaman and Nicobar Islands.

Methodology

Documentation of Ethno-veterinary medicinal practice

Information on ethno-veterinary medicinal practices for common gastro intestinal ailments was collected from Nicobarese tribe at Car Nicobar (9.1573° N, 92.7581° E), Nicobar Islands and Harminderbay, Hutbay (10°42'04.0"N; 92°28'25.0"E)**,** South Andaman of ANI. Botanical name was identified by using flower and leaf parts of plants with the help of Botanical Survey of India, ANI.

Preparation of plants extraction

Medicinal plants used in the ethno veterinary tribal medicinal practice (EVTMP) viz., *Taberna montanacrispa*, *Psidium guajava* and *Leea indica* were collected in the month of March where in Temperature (30°C) and humidity (81%) was recorded. The matured leaves at the mid stem of plants were collected, shadow dried and powdered. Extraction was done as per the method described by Handa *et al.* (2008). Herbal powders (3 g) were mixed with hot distilled water (40°C; 30 mL) for 24 h to make decoction. Extract was then filtered using Whatman's No. 1 filter paper. The extract was filled in the glass vials and placed under a hot stream of water (35°C) to dry completely, taken weight of the crude extract and finally stored at 4°C for further use.

Herbal Antibiogram study

Preliminary *in-vitro* screening was done to study the efficacy of the extracts against poultry gut *Escherichia coli*. A total of 25 cloacal swabs were collected from 25 weeks old of black Nicobari fowls that were maintained under the intensive system of management. Those swabs were inoculated in the nutrient broth and incubated at 37°C overnight. The cultures were then next day streaked on Eosin Methylene Blue agar plates. A total of 20 *E. coli* (80%) isolates were identified based on colonial morphology and biochemical tests. The crude extracts of EVTMP were reconstituted with 10 ml of triple distilled water for antibacterial activity against *E. coli* isolates. The bacterial suspension turbidity was compared with

McFarland standard solution (containing 10^7cfu per mL) (Murray *et al*., 1999). The antibacterial activity of extracts was recorded using conventional disc diffusion method. The isolated inoculums of *E. coli* were applied aseptically on the solidified nutrient agar (NA) using applicator. The plate was allowed to dry for 5 min. The impregnated disks (Himedia) of known concentration were placed aseptically on the agar surface. The activity was determined by the presence of clear zones of inhibition around the disks after incubation for overnight at 37°C. The diameter (mm) of the clear zone of inhibition was measured that was classified as per Harun *et al*. (2016). Disk of Gentamicin (10 µg) was used as a standard antibacterial or positive control and the solvent and empty discs were used as a negative control.

In-vivo trial

A total of 108 day-old Nicobari fowl chicks with a mean initial body weight of 36.15 ± 0.05 g (mean ± SD) were housed in wire cages. The temperature was recorded between 28 °C and 30°C throughout the experimental period. The relative humidity varied from 80 to 85 %. The chicks were provided with the lighting schedule of 23 L: 1 D (23 h light and 1 h dark per day) upto day 42. All the birds were provided with *ad libitum* potable clean water and feed in mash form (corn-soybean meal as per ISI, 1994).

Experimental design

Decoction was prepared out of formulation with the dry leaf powder of *Tabernamontana crispa*, *Psidium guajava* and *Leea indica* @ 10:5:2.5, respectively. The final concentration of decoction was 1.5 mg/mL of aqueous extract. Experimental birds were divided into three groups with three replications each and 12 birds in each replicate in a completely randomized design. Birds in G1 were received decoction mixed water (3 mL per bird; twice in a week). Birds in G2 were kept as a negative control with plain water. Birds in G3 were treated as positive control (Oxytetracycline in water). On day 22, all the birds were injected intramuscularly with LPS of *E. coli (*100 µg/kg body weight) (lipopolysaccharides from escherichia coli o128:b12). Blood samples (3 mL) were collected from the wing vein from each replicate group after 24 h and day 7 (stress period). Blood samples were centrifuged at 3000 ×g for 15 min at 4°C and stored the serum samples at –20°C until further analysis. Serum cytokine biomarkers (Interleukin-6; IL-6, Interferon gamma; IFN-γ and Major-histocompatibility complex (MHC B class) were quantified using commercially available ELISA kits of Arsh Biotech Pvt. Ltd. India (Kit Nos.LTHC4000EA (IL-6-pg/ml); LT30008EAYQ (IFN—γ: pg/ml); LT23008EAYQ (MHC-B: ng/ml) with the instruction of the manufacturers. Standard curves were prepared using software product by absorption values measured at a specific wavelength (450nm) in spectrophotometer. The means of cytokine concentrations were calculated and duncan's new multiple range test was used to compare the mean values were compared at P□0.05.

Results and discussion

Ethno Veterinary Tribal Medicinal Practice

Three ethno veterinary medicinal plants and their therapeutic use were recorded from Nicobarese tribal farming community for the ailment of bloody diarrhea and other gut problems in poultry. Common name, botanical name (Botanical Survey of India, Andaman and Nicobar Islands) and clinical applications of documented plants were presented in Table 1. Various authors reported that *Leea indica* was used in traditional practice to treat the diarrhea and dysentery (Rajakumar and Shivanna, 2009; Ranjithn *et al*., 2010; Rahman *et al*., 2012; Bais, 2013; Tareq*et al*., 2017; Khuniad *et al*., 2022). Extensive review on *Leea* species (Kekuda *et al*., 2018; Hossain *et al*., 2021) supported the present report on ethno veterinary use of *L. indica* as a traditional medicine. Leaf extract of guava is being used as herbal medicine to treat gastro intestinal ailments (Braga et al., 2016). The present ethno veterinary medicinal composition containing *Tabernamontana crispa* might have been used to relieve stomachache during diarrhoea according to the report of Asma Bibi *et al*. (2016) that *Tabernamontana crispa* as an endemic medicinal plant of Andaman and Nicobar Islands with antimicrobial potential is used for stomachache. Similarly, the present ethno veterinary medicinal

composition containing *Tabernamontana crispa* might have been used to relieve stomachache during diarrhea.

Herbal antibiogram

Aqueous extraction methods are inexpensive and its bioactive compounds are less toxic compared to other extraction methods; therefore, aqueous herbal extract is more suitable and useful as poultry feed supplement. The present findings showed that the aqueous herbal extracts have antibacterial activity (Table) against *E. coli* isolates of cloacal swab. Strong antibacterial activity was recorded with *Psidium guajava* and *Taberna montanacrispa. Leea indica* had moderate antibacterial activity. Highly potent antibacterial activity was exhibited by the extract of *Psidium guajava* against all *E. coli* isolates at 165.0 µg per µL. This evaluation study has reported that the documented ethno veterinary tribal formulation might have inhibited the gut colonization of *E. coli.*

Anayochukwu *et al*. (2019) recommended that aqueous extracts of *Psidium guajava* (20 mg/mL) with other plants (synergistic action) had higher antibacterial activity compared to alone against *E.coli*. Lannaon *et al.* (2010) reported that herbal combinations containing guava as one of herbs can be a potential antibiotic for broiler chickens; which performed better than existing antibiotics. The present work justified the previous research recommendations on *Psidium guajava*. Phyto-chemical components of the *P. guajava* extracts such as tannins, saponins, flavonoids, and terpenes attribute to its effectiveness to reduce the growth of different gut associated bacteria (Anayochukwu *et al*., 2019; Naseer *et al*., 2018, Maysarah *et al*., 2016). Earlier studies reported that *L. indica* extracts had higher antimicrobial activities (Wiart *et al*., 2004; Chander and Vijayachari, 2016, 2018). The antibacterial properties of leaves *L. indica* might be due to possession of alkaloids, flavonoids, glycosides, phenols, terpenoids, and steroids (Khuniad *et al*., 2022). Aqueous extracts of *L. indica* leaves in combination of silver nanoparticles exhibited antimicrobial activity against *E. coli* (Rokhade and Taranath, 2016; Rokhade and Taranath, 2017). Major bioactive compounds present in the *Tabernamontana crispa* species are indole alkaloids that are responsible for antibacterial and anti-inflammatory activities (Marinho, *et al*., 2016; Garga and Das, 2017).

Sero-biomarkers

LPS of *E. coli* being a pathogenic antigen stimulates the production of immune cytokines (Yousefi *et al*., 2021) activating intracellular signaling pathways (Alber *et al*., 2021) to initiate the inflammatory response. LPS mediated the secretion of cytokines such as IL-6, IFN-γ and MHCs. ELISA based quantification of these cytokines in serum is a specific and reliable test. This quantitative ELISA specific for chicken cytokines has been developed based on anti-chicken IL-6, IFN-γ and MHC- II mAb produced from immunized mice (Lambrecht *et al.*, 2000). The efficacy of ethno veterinary poly-herbal extracts on cellular and humoral immunity of Nicobari fowl injected with LPS *E. coli* were given in Table 2. Significantly higher serum concentrations of IL-6 on 24 hours and d 7 ($p<0.05$) was recorded in plain water supplemented group. The diet supplemented with poly herbal extracts significantly decreased the sero-concentration of IL-6 on 24 hours and d 7 post LPS injection. There is no significant difference in the sero-level of IL-6 between poly-herbal and antibiotic supplemented groups. Supplementation of ethno-veterinary poly-herbal extracts significantly increased sero concentration of IFN-γ and MHC-B, at 24 hours and day 7 of LPS injection as compared to plain water and antibiotic supplemented groups. High level of sero-IL-6 parameter characterizes LPS induced inflammation model in broiler chickens. LPS-induced inflammation might have increased the mRNA level of IL-6 in white blood cells which in turn stimulated higher secretion of IL-6 at 1 h and 3 h after LPS administration (Boever *et al*., 2009, Peng *et al*., 2018) where as sero-level of IL-6 has been lowered in the EVTM poly herbal supplemented group that indicated low inflammation (Hartady *et al*., 2021). Chicken Interferon gamma (IFN-γ) has crucial role against invading pathogens in poultry (Kaiser and Staheli, 2014). Increased sero-IFN-γ concentration with poly herbal formulation comprising of *Taberna montanacrispa*, *Psidium guajava* and *Lee indica* might have increased the proportion of phagocytes in blood of *E. coli* infected birds and subsequently increased the secretion of MHCB cells (Janardhana *et al*., 2007) due

to up-regulation of its genetic expression and activated macrophages, T cell responses to terminate intracellular pathogens (Kogut *et al.,* 2005). IFN-γ enhanced MHC (Mack, 2007) which in turn determined the immune-competence of poultry (**Saxena** *et al*., 2012, Kaufman 2013, Miller and Taylor 2016). The MHCB concentration with EVTMP supplementation has improved from its normal serum level (Sujatha *et al.,* 2020) in Nicobari fowl. Thus, ethno-tribal veterinary medicinal practices enhanced the secretion of the potential cytokines and suppressed mediators of inflammation which in turn might eliminate the microbes (Mack, 2007). Leaf of *Psidium guajava* contains higher amount of secondary metabolites of anti-inflammatory compounds (Naseer *et al*., 2018) that attributed to better immunity against LPS *E. coli*. Similar observation was reported by Shah *et al.* (2018) that *L. indica* mitigated the Dinitrobenzene sulfonic acid induced inflammatory bowel disease in rats as a potent anti-inflammatory agent. Similarly, *L. indica* showed a strong inhibitory activity on nitric oxide (pro-inflammatory cytokine) production in LPS induced cell lines (Saha *et al*., 2004) and IFN-γ induced macrophages in these cell lines. Therefore, ethno-veterinary medicine herbal supplementation alleviated the LPS-induced inflammatory response by inhibiting the production of IL-6 (Bai *et al*., 2019). Similar findings were reported in the present study that in-vivo treatment of ethno-veterinary medicine herbs enhanced the immune responses due to higher production of ChIFN-γ in poultry. *In-vitro* and *in-vivo* studies in animal models with use of the aqueous extracts of *L. indica* revealed that this herb has therapeutic applications in treatment of inflammatory disease (Khuniad *et al.,* 2022). The pharmacological evaluation study could infer that this Ethno veterinary tribal medicinal practice could attribute to the immunological trait of protection against colibacillosis through mechanisms mediating IL-6, IFN- and MHC (Bagheri *et al*., 2022). The presence of certain bioactive compounds in the extracts and fractions could further support therapeutic potential and traditional medicinal usage of these plants. Therefore, well-designed pre-clinical and clinical studies are essential before any recommendations could be made on the efficacy and safety of EVTM-based therapeutic interventions. With the growing concerns about the use of antibiotics in animal production, natural biomolecules like cytokines through herbs of the present study may be considered as an alternative for enhancing the health by stimulating the immune system.

Table 1: Ethno veterinary medicinal plants of Nicobarese tribal farming community

Sl.No	Local name	Botanical name	Use
1	TöKURòTòNG	*Taberna montanacrispa*	Treatment gastro intestinal problems
2	Tokurotong, Kuyavo, Tokiteuny	*Taberna montanacrispa* *Psidium guajava* *Leea indica*	Treatment of dysentery

Table 2*:* Antibacterial activity of extracts of medicinal plants of Nicobari tribal farming community against *E. coli* isolates

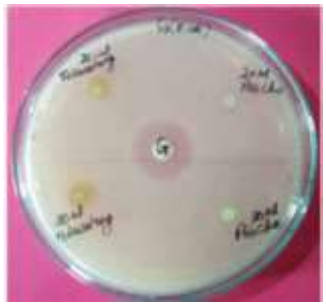
Sl. No	Common name (Nicobari language)	Plant name	Zone of Inhibition (mm)	Minimum Inhibitory concentration (µg per µl)
1	TöKURòTòNG	*Taberna montanacrispa*	16±1.0	475.0
2	KUYAVö	*Psidium gujava*	16±1.0	165.0
3	Tokiteuny	*Leea indica*	15±1.0	770.0
4	Gentamicin (10 µg) (Positive control)		25	
5	Distilled water (Negative control)		Nil	

Inhibitory zone: 0-6 mm (no activity), 7-10 mm (weak inhibition), 11-15 mm (moderate inhibition), more than 16 mm (strongly inhibited) (Harun *et al*., 2016).

Table 3: Sero -cytokine biomarkers with supplementation of poly herbal extract in Nicobari fowl challenged with lipopolysaccharide

Groups/post challenge	Control	Poly herbal	Antibiotic
Interleukin - IL6 (pg/ml, mean ± SD)			
24h	355.10± 49.17[a]	298.9± 40.32[b]	324.36± 76.10[b]
1 week	326.42± 45.13[a]	279.78± 34.42[b]	290.35± 25.70[b]
Interferon Gamma - IFN-γ (pg/ml, mean ± SD)			
24h	627.82± 98.805[b]	753.93± 146.73[a]	658.82± 83.97[b]
1 week	507.20± 61.28[b]	771.91± 66.83[a]	640.27± 79.63[ab]
Major Histo-compatibility complex- MHCB (ng/ml, mean ± SD)			
24h	2.22± 0.180[b]	3.650± 0.70[a]	2.29± 0.073[ab]
1 week	1.995± 0.140[b]	3.091± 0.077[a]	2.44± 0.0558[ab]

Plate: Zone of inhibition of medicinal plants of Nicobari tribal farming community against *E.coli* isolates



TöKURòTòNG (*Tabernamontanacrispa)*

Tokiteuny (*Leeaindica)*

KUYAVö (*Psidiumgujava)*

Conclusion

Aqueous herbal extracts have antibacterial activity against gut *E. coli* isolates of cloacal swab. Strong antibacterial activity was recorded with *Psidium guajava* and *Taberna montanacrispa. Leea indica* had moderate antibacterial activity. Highly potent antibacterial activity was exhibited by the extract of *Psidium guajava* against all *E. coli* isolates. Pharmacological evaluation of EVTM herbal formulation in terms of sero cytokines biomarkers with composition of *Tabernamontana crispa* (10 g) + *Psidiuma gujava* (5 g) + *Leea indica* (2.5 g) @ 3ml per bird twice in a week in Nicobari fowl reported lower serum level of IL -6 and higher serum level of IFN-γ and MHC. It has up regulatory action on innate immune homeostasis, resistance to invading pathogens and improved immune function. However, investigation on the spectrum of antimicrobial activity and immunity enhancing properties of this tribal ethno veterinary poly herbal formulation is needed based on other specific pathogen challenge study.

References

Alber, A., Stevens, M. P. &Vervelde, L. (2021). The bird's immune response to avian pathogenic *Escherichia coli*. Avian Pathol. 9 https://doi.org/10.1080/03079457.2021.1873246, 1-0.

Anayochukwu, C. N., Aguiyi, J. C., Chibuike, C. J., Ifeanyi, V. O., Ukaegbu-Obi, K. M., Kim, E. G., Ohaeri, U. C. &Onyemegbulem, B, O. (2019). Antibacterial Activity of *Psidiumguajava*Leaf Extract against Selected Pathogenic Bacteria.AdvMicrob. 9:1012-1022.

Asma, Bibi.,Thangamani, A. &Venkatesalu, V. (2016). Some endemic medicinal plants of &amans with antimicrobial potential, J. Appl. Adv. Res. 1(1): 10-15.

Bagheri, S., Paudel, S., Wijewardana, V., Kangethe, R. T., Cattoli, G., Hess, M., Liebhart, D. &Mitra, T. (2022). Production of interferon gamma and interleukin 17A in chicken T-cell subpopulations hallmarks the stimulation with live, irradiated and killed avian pathogenic Escherichia coli. Develop. Comp. Immun.133:104408-104420.

Bai. J., Wang, X., Hao, M., Li, H., Cheng, G., Liu, D., Yang, Y. & Li, Y., (2018). Forsythiaside attenuates *Escherichia coli* lipopolysaccharide-induced liver acute inflammatory response in chicken,European J. Inflam.17:1– 7.

Bais, S. A. (2013). Phytopharmacological Review on an Important Medicinal Plant: *LeeaIndica,* Ethnopharmacology. 1-4.

Boever, S. D., Croubels. S., Meyer, E., Beyaert, S. S. R., Ducatelle, R., & Backer, P. D. (2009).Characterization of an intravenous lipopolysaccharide inflammation model in broiler chickens.Avian Path. 38(5) 403-411.

Braga, M. F.B., Carneiro, J. N. P., Machado, A. J. T., dos Santos, A. T., Sales, D. L., Lima, L. F., Figueredo, F. G. &Coutinho, H. D. M. (2016). *Psidiumguajava* L., from ethnobiology to scientific evaluation: Elucidating bioactivity against pathogenic microorganisms. J. Ethnopharmacol.194:1140–1152. doi: 10.1016/j.jep.2016.11.017

Chander, M. P. &Vijayachari, P. (2016).In vitro antimicrobial and anti-oxidant potentials of selected medicinal plants used by the indigenous tribes of Andaman and Nicobar Islands, India.Bangladesh J. Pharmacol. 11:330-332.

Chander, M. P. &Vijayachari, P.(2017). Vibriocidal activity of selected medicinal plants used by Nicobarese tribe of Andaman and Nicobar Islands, India.J. Pharm. Pharmacol. 5:164-168.

Chen, M.T.C., Mchugh,W., Nidetz, R., Li, Y., Fu, J., Cornell, T.T., Shanley, T.P. &Kurabayashi, K. (2015). ACS Nano. 9: 4173-4181.

El-Mahmood, A.M., Doughari, J. H. &Chanji, F. J. (2008). In-vitro antibacterial activities of crude extracts of Nauclealatifolia and Daniellaoliveri. Sci. Res. Essay. 3(3):102-105.

Garga, D. & Das, T. (2017).Preliminary phytochemical screening and anti-inflammatory effect of the Aqueous extract of *Tabernaemontanadivaricata* flower in wister rats. J. Curr. 9:9–12. doi: 10.22159/ijcpr.2017v9i5.22128.

Handa, S. S., Khanuja, S. P. S., Longo, G. &Rakesh, D. D. (2008).Extraction Technologies for Medicinal and Aromatic Plants.Published by International centre for science and technology, pp. 266.

Hartady, T., Mas Rizky, A., Syamsunarno, A., Priosoeryanto, B. P., Jasni, S. &Balia, R. L. (2021). Review of herbal medicine works in the avian species. VetWorld. 14(11):2889-2906.

Harun, A., Rahim, N. E. A. A., Jalil, M. A. A., Rosdi, N. A. M., Daud, S., Harith, S. S., So'ad, S. Z. M. & Hassan N. M. (2016).Comparative study of antioxidant & antimicrobial activity of root stem & leaves of *leeaindica*species.Malaysian J. Sci.35(2): 259-274.

Hossain, F., Mostofa, M. G. &KhurshidAlam, A. H. M. (2021). Traditional uses & pharmacological activities of the genus leea& its phytochemicals A review. Heliyon. 7. e06222. https://doi.org/10.1016/j.heliyon.2021.e06222

Indian Standard Specification for feed, (2007)

Jai, Sunder., Sujatha,T., Bhowmick,S., Mayuri,S.C., De.A.K., Bhattacharya, D., Perumal,P. & Kundu, A. (2021). Distribution of TET AAC & CTX-M Genes among Antibiotic Resistant Escherichia coli Isolated From Poultry under Various Farming System of A & N Islands. Ind. J. Anim. Res. 55:689-696.

Janardhana, V., Ford, M. E., Bruce, M. P., Broadway, M. B., Neil, T. E. O., Karpala, A. J., Asif, M., Browning G F, Tivendale K A, Noormohammadi A H, Lowenthal A J & Bean G D.(2007). IFN-gamma enhances immune responses to E coli infection in the chicken, J Interferon Cytokine Res. 27(11): 937-946.

Kaiser, P. & Staheli, P. (2014). Avian cytokines &chemokines, In: Kaspers B, Schat K (Eds.), Avian Immunology. Academic Press.189–204.

Kaufman, J. The Avian MHC (Chapter 8), In: Schat KA, Kaiser P, &Kaspers B (eds). (2013). Avian immunology, 2nd end. Elsevier Ltd. 149–167.

Kekuda, T. R. P., Raghavendra, H. L., Nitish, A. B. &Akhilesha, S. (2018). Traditional uses chemistry & pharmacological activities of Leeaindica (Burm f) Merr (Vitaceae) A comprehensive review. Int. J. Green Pharm. 12 (1): S71.

Khuniad, C., Ritchie, K. J., Sarker, S. D. &Nahar, L. (2022).Therapeutic potential of leeaindica (vitaceae) Review article, J. Natural Products Discov.1(1) DOI 10.24377/jnpd.article646.

Klaudia, C. &Alina, W. (2015).The influence of enrofloxacin, florfenicol, ceftiofur&*E coli* LPS interaction on T & B cells subset in chicks, Vet. Res. Commun. 39:53-60,

Kogut, M. H., Iqbal, M., He, H., Philbin, V., Kaiser, P. & Smith, A. (2005).Expression & function of Toll-like receptors in chicken heterophils. Dev Comp Immunol. 29 (9): 791–807.

Lambrecht, B., Gonze, M., Meulemans, G. & van den Berg, T. P. (2000). Production of antibodies against chicken interferon-gamma: Demonstration of neutralizing activity and development of a quantitative ELISA. Vet. Immunol Immunopathol. 74: 137–144.

Lannaon, W. J. (2010). Herbal Trees Used as Antibiotics for Broilers. Poultry World. https://www.poultryworld.net/health/articles/2016/5/herbal-trees-used-asantibiotics-for-broilers-2807282W Retrieved on 28-05-2021.

Mack Cara, L. (2007). Serum Cytokines as Biomarkers of Disease & Clues to Pathogenesis Hepatology. 46 (1): 180-188. *DOI 10.1002/hep.21793.*

Marinho, F.F., Simões, A.O., Barcellos,T.&Moura,S. (2016).Brazilian *Tabernaemontana* genus: Indole alkaloids and phytochemical activities. Fetoterapia. 114: 127-137.

McMurray, R. L., Ball M.E.E., Tunney, M. M., Corcionivoschi, N. &Situ, C. (2020).Antibacterial Activity of Four Plant Extracts Extracted from Traditional Chinese Medicinal Plants against *Listeria monocytogenes, Escherichia coli,* and *Salmonella enterica* subsp. *enterica* serovarEnteritidis, Microorganisms. 8(6): 962 – 972.

Miller, M. M. & Taylor, R. L. (2016). Brief review of the chicken Major Histocompatibility Complex: the genes, their distribution on chromosome 16, and their contributions to disease resistance. Poult.Sci. 95(2): 375-392.

Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. & Yolken, R.H. Eds. (1999). Manual of clinical microbiology. 7th ed. Washington.

Naseer, S., Hussain, S., Naeem, N., Pervaiz, M. &Rahman, M. (2018). The phytochemistry& medicinal value of Psidiumguajava (guava).Clinical Phytoscience. 4:32-40.

Nolan, L. K., Vaillancourt, J. P., Barbieri, N. L. & Logue, C. M. (2020). Colibacillosis In: Swayne, D E (Ed.), *Diseases of Poultry*. N.J. John Wiley & Sons Inc., pp. 770–830. https://doi.org/10.1002/9781119371199.ch18.

Peng, L., Matthijs, M.G., Haagsman, H. P. &Veldhuizen, E. J. (2018).Avian pathogenic Escherichia coli-induced activation of chicken macrophage HD11 cells. Dev. Comp. Immunol. 1(87):75–83. https://doi.org/10.1016/j.dci.2018.05.019.

Phan, A. D. T., Netzel, G., Chhim, P., Netzel, M. E., Sultanbawa, Y. (2019). Phytochemical Characteristics & Antimicrobial Activity of Australian Grown Garlic (*Allium Sativum* L.)Cultivars.Food. 8:358. doi: 10.3390/foods8090358.

Qi, X., Liu, C. & Li, R. (2017).Modulation of the innate immune-related genes expression in H9N2 avian influenza virus-infected chicken macrophagelike cells (HD11) in response to *Escherichia coli* LPS stimulation. Res. Vet. Sci.111: 36–42.

Rahman, M. A., Imran, T. B. & Islam, S. (2012). Antioxidative Antimicrobial & Cytotoxic effects of the Phenolics of Leeaindica Leaf extract. Soudi.J.Biol. Sci. doi: 10.1016/j.sjbs.2012.11.007.

Rajakumar, N. &Shivanna, M. B. (2009).Ethno-medicinal application of plants in the eastern region of Shimoga district Karnataka India. J. Ethnopharmacol. 126: 64-73.

Ranjith, N. P. &Ramachran, V. S. (2010). Ethnomedicines of kurichyaskannur district western ghats Kerala. Ind. J. Nat. Prod. Resour. 1 (2): 249–253.

Rokhade, V. K. &Taranath, T. C. (2016). Biosynthesis of silver nanoparticles using leaf extract of LeeaIndica (Burm F) Merr& their synergistic antimicrobial activity with antibiotics. Int. J. Pharmaceu Sci. Rev. Res. 40:211-217.

Rokhade, V. K., Taranath, T. C., (2017). Phytosynthesis of silver nanoparticles using fruit extract of Leeaindica (Burm F) Merr& their synergistic antimicrobial activity. Int. J. Pharmaceu. Sci. Rev. Res. 8: 1319-1325.

Saha, K., Lajis, N. H., Israf, D. A., HamzahA. S., Khozirah, S., Khamis, S. &Syahida, A. (2004). Evaluation of antioxidant & nitric oxide inhibitory activities of selected Malaysian medicinal plants, J.Ethno pharm.92:(2–3) 263-267.

Sargeant,J.M., Bergevin,M.D., Churchill,K., Dawkins, K., Deb, B., Dunn, J., Logue,C. M., Novy, A., O'Connor, A.M., Reist, M. &Winder,C. B. (2019). The efficacy of antibiotics to control colibacillosis in broiler poultry: a systematic review. Anim. Health Res. Rev. 20 (2):263–273.

Saxena, R., Stephan, R., Mishra, S. K., Shukla, S., Saxena, D. P. &Pratap, S. O. (2012) Assessment of Immunocompetence Status of Native Breed of Chickens. Biomed Pharmacol J. 5(2): 285-293.

Shah. S., Patel, K. &Rathod, N., (2018). Evaluation of the activity of LeeaindicaMerill.in inflammatory bowel disease using experimental models. Int. J. Pharma Sci. Nano tech. 11:4219-24.

Shimizu, M., Fitzsimmons, R. C. &Nakai, S. (1988). Anti-E.coli immunoglobulin Y isolated from egg yolk of immunized chickens as a potential food ingredient. J. Food Sci. 53:1360-1366.

Shoaib, G., Shah, G. M., Shad, N., Dogan, Y., Siddique, Z., Shah, A. H., Farooq, M., Farooq, K.R. &Nedelcheva, A. (2021). Traditional practices of the ethnoveterinaryplants in the Kaghan Valley, Western Himalayas-Pakistan. Revista de Biología Tropical. 69: (1) 1-11.

Sujatha, T., Sunder, J., De, A. K., Bhattacharya, D., Bhowmick, S. & Kundu, A. (2020). Serum cytokine concentration in native Nicobari fowl of Andaman and Nicobar Islands.Ind. J. Anim. Sci.90 (7): 1002–1005.

Sujatha, T., Abhinaya, S., Sunder, J., Thangapian, M. & Kundu, A. (2017). Efficacy of early chick nutrition with *Aloe vera*&*Azadirachtaindica*on gut health &histomorphometry in chicks.Veterinary World. 10(6): 569-573.

Sujatha, T., Sunder, J. & Kundu, A. (2017). Aloe Vera &Azadirachtaindica (Neem) as antibiotic replacers & immune enhancers in brooding management of Nicobari fowl. JASA. 22 (1): 52-55.

Sujatha, T., Sunder, J., Kannaki, T. R. & Kundu, A. (2017). In vivo effect of Andrographispanniculatanees&Mor indacitrifolia on expression of toll-like receptors in indigenous Nicobari fowl.Ind. J. Poult. Sci. 52:357-360.

Sunder, J., Jeyakumar, S., Sujatha, T. & Kundu, A. (2013). Effect of feeding of Morical(*Morindacitrifolia*) based herbal supplement on production and egg quality in Nicobari fowl. J. Medici. Plant Res.7 (40): 2999-3002.

Sunder, J., Jeyakumar, S., Sujatha, T. & Kundu, A. (2014). Grommune: Morindacitrifolia-based herbal tonic for growth and immunity for commercial broilers. Ind.J.Anim.Sci. http://dx.doi.org/10.1080/09712119 .2014.928628.

Sunder, J., Sujatha, T., Raja, A. & Kundu, A. (2016). Immunomodulatory effect of Morindacitrifolia&& Andrographispanniculata on expression of toll like receptors in Nicobari fowl. Ind.J.Anim.Sci. 86: 1006-1008.

Sunwoo,H., Nakano,H.T., Dixon,W.T. & SIM,J.S., (1996). Immune Responses in Chickens Against Lipopolysaccharide of *Escherichia coli* &*Salmonella typhimurium*.Poult Sci. 75:342-345.

Tareq, S. M., Ibrahim, M., Shahadat, S., Chowdhury, M. U. &Jakaria, M. (2017). Comparative anti-dirrhoeal& antimicrobial activities of methanol extract of Leeaindica (Burm F) Merr&LeeamacrophyllaRoxb Ex Hornem (FamVitaceae) & four Bangladeshi market preparations. Der. Pharmaceutical Chemistry. 9: 27-34.

Wiart, C., Mogana, S., Khalifah, S., Mahan, M., Ismail, S., Buckle, M.., Narayana, A. K. &Sulaiman, M. (2004). Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia. Fitoterapia.75:68-73.

Yousefi, M., H, Jonaidi.,& B, Sadeghi. (2021). Influence of peripheral lipopolysaccharide (LPS) on feed intake body temperature & hypothalamic expression of neuropeptides involved in appetite regulation in broilers & layer chicks. Br. Poult. Sci. 62:110–117.

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