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RESEARCH ARTICLE

In-vitro and *in-vivo* evaluation of the antibacterial potential of *Typha elephantina*

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ABSTRACT

The present study was aimed to evaluate the *in-vitro* and *in-vivo* antibacterial effects of the *Typha elephantina* aqueous extract (TE.AQ), ethanolic extract (TE.ET) and *T. elephantina* methanolic extract (TE.ME) against eight selected clinical pathogens. The test samples were tested for *in-vitro* analysis (by disc diffusion method) at different concentrations of 5, 15, 25, 50 and 100 mg/dL against both gram positive and gram-negative strains. The highest potential was observed in TE.ME at a concentration of 100 mg/dL against *Pseudomonas aeruginosa* exhibiting 19.67 ± 0.577 mm zone of inhibition (ZOI). The same fraction also showed good activity against *Staphylococus aureus* with ZOI of 17.50 ± 0.70 mm. The TE.ET was found most active against *P. aeruginosa and Streptococcus pyogenes* having ZOI of 18.53 ± 0.503 and 16.2 ± 1.55 mm respectively at a concentration of 100 mg/dL. The most sensitive bacteria *P. aeruginosa* was selected for *in-vivo* study (using poultry chicks) for induction of infection in chicks. The effects of TE.AQ, TE.ET and TE.ME were determined at concentrations of 300 mg/kg body weight based on hematological parameters, liver enzymes and gross pathological findings of lungs and livers. The findings of the *in-vivo* study in chick's model showed that treatment of experimental animals with TE.ME significantly restored the hematological parameters, liver enzymes and architecture of lungs and livers. Based on scientific evidence, the current study suggests that TE.ME may serve as a best and new natural antibacterial agent and can be used against infections caused by *P. aeruginosa*.

Keywords: *Typha elephantina, in-vitro* and *in-vivo,* zone of inhibition, *Pseudomonas aeruginosa,* chicks, aqueous, ethanolic and methanolic.

INTRODUCTION

Infectious diseases have been the most serious health issue in the world today. Some of the pathogenic bacterial species include *Staphylococcus aureus, Bacillus subtilis*, *Bacillus licheniformis, Escherichia coli, Pseudomonas aeruginosa*, *Salmonella enterica* and *Salmonella typhimurium* caused various infections in animals and human (Emiru *et al.*, 2019). The consistent use of synthetic antibiotics lead to microbial resistance. Besides these, the antibiotics may also cause adverse effects in the host like hypersensitivity, immune suppression, and other allergic reactions (Ahameethunisa & Hopper, 2010).

Microbial resistance depends upon their genetic ability to transmit and become resistant to various treatments, which are used as therapeutic agents (Singh, 2015). The increase of bacterial resistance to existing antibiotics has requisite the search for new antibacterial agents and thus has forced the researcher to search new antimicrobial agent from medicinal plants (Lewis, 2013). Medicinal plants have been explored for various pharmacological activities such as antimicrobial, anthelmintic, analgesic, antipyretic and insecticidal activities (Saleem *et al.*, 2020). Plant constituents can be used directly as healing agent as well as act as preliminary materials for the synthesis of drugs or as models of biologically active compounds (Al-Salt, 2012). Plant base antimicrobial agents have least side effects and have higher medicinal effect to heal various diseases (Inglin *et al.*, 2015). Certain bioactive compounds of plants origin like alkaloids, tannin, flavonoid and phenolic, are very important due to its physiological effect on the human body, especially antioxidant activity (Zeb, 2020). So, the analysis of such medicinal herbs are very essential to isolate and employ their active ingredients in medicines for the development of new drugs against the drug-resistant microorganisms (Fankam *et al.*, 2014).

Typha elephantina Roxb is a member of family *Typhaceae,* locally known as (Barr" in Pakistan). The family *Typhaceae* consists of one genus (Typha) and several important medicinal plants species (*Typha, angustifolia, Typha angustifolia,*

Typha xglauca, Typha latfolia) (Shukla & Mishra, 2015; Ha *et al.,* 2019). Hence, the aim of the current study was to evaluate *in-vitro* and *in-vivo* antibacterial potential of TE.AQ, TE.ET and TE.ME extract of *Typha elephantina* leaves against different pathogenic bacterial strains.

MATERIALS AND METHODS

Plant materials and extraction

Typha .elephantina (*T. elepentenia*) was collected from different regions of District Swat and was identified and authenticated by Dr. Muhammad Nisar Ahmad (Professor, Department of Botany, University of Malakand, Khyber Pakhtunkhwa, Pakistan) for further process. From fresh leaves of *T. elephantina*, extract in water (TE.AQ), ethanol (TE.ET) and methanol (TE.ME) was prepared following the previously reported techniques (Rockwood *et al*., 2013). The whole experiment was carried in the microbiology laboratory at the department of Biotechnology, University of Malakand, and Khyber Pakhtunkhwa, Pakistan.

Bacterial strains culturing

The bacterial strains were cultured in nutrient broth (100 mL) i.e. peptone, 1.5 gram, NaCl, 0.6 gram, Yeast extract, 0.3 gram were dissolved in a flask having 30 mL distilled water and again added more water to raise its volume to 100 mL. The dissolved media was sterilized at 121°C for 20 minutes in an autoclave, to culture the bacteria. Nutrient agar (Sigma Aldrich) was made in flask, sterilized in an autoclave and poured in petri plates for solidification (Ali *et al*., 2012; Singh *et al*., 2017).

These bacterial strains include both gram positive and gram negative bacteria such as *Staphylococcus aureus (S. aureus), Staphylococcus epidermidis (S. epidermidis), Streptococcus pyogen* (*S. pyogenes*), *Escherichia coli (E. coli), Klebsiella pneumonia (K. pneumonia), Proteus vulgaris (P. vulgaris), Pseudomonas aeruginosa (P. aeruginosa*) *and Salmonella typhi (S. Typhi)*, respectively.

The bacterial strains were placed into a vessel of specific nutrient medium, and the vessel was vortexed to make a homogeneous bacterial suspension. The resulting inoculum suspension comprises 5 x 10^7 to 5 x 10^8 CFU/ml for gram-negative and gram-positive and can be used instantly. This range is same to the 0.5 McFarland standard (Maksoud *et al*., 2018).

***In-vitro* antibacterial activity of TE.AQ, TE.ET and TE.ME**

Disc diffusion method was used to evaluate the antibacterial effect of TE.AQ, TE.ET and TE.ME at a concentration of 5, 15, 25, 50 and 100 mg/dL. After solidification of nutrient agar in petri plates, individual plates was inoculated with its respective bacterium (cultured in nutrient broth), through cotton swab, bacterium was uniformly distributed on the surface of plate. About 6 mm diameter discs were placed on the petri plates after inoculation of bacteria. Then 10 µL amounts of different concentrations of extracts were applied to each disc pipette along with a standard antibiotic (Norfloxacine). The antibacterial activities of the extracts were observed after 24 hours of the incubation (35°C) temperature. The zones of inhibition produced by different concentrations of extracts were measured in triplicates to determine the efficacy of the extracts against various strains.

***In-vivo* antibacterial activity of TE.AQ, TE.ET and TE.ME**

A total of sixty poultry one day old chicks were reared in Bio Park, University of Malakand. These chicks were vaccinated for different diseases according to its schedule. *Pseudomonas aeruginosa* was selected for *in-vivo* study. Ethical Committee of the Department of Biotechnology, University of Malakand approved the experimental protocols and ensured its compliance with provisions of the "Animal Bye-Laws 2008, Scientific Procedures Issue-I of the University of Malakand". As the extract was highly effective against *P. aeruginosa*. The bacteria was administered intra peritonealy at the rate of 3 x 10^8/0.2 mL, to all chicks except group (NC), according to the previously reported procedure Singh *et al*. (2017). After the occurrence of clinical signs and symptoms, all the animals were treated orally with TE.AQ, TE.ET and TE.ME for 15 days. At the age of 28 days, '36' chicks were selected and divided into seven groups, each group had six chicks.

Group NC: served as negative control.

Group PC: served as positive control.

Group NF: treated with Norfloxacine (NF) standard drug at a dose of 100 mg/kg body weight for 15 days.

Group TE.AQ: treated with TE.AQ at a dose of 300 mg/kg body weight for 15 days

Group TE.ET: fed with TE.ET at a dose of 300 mg/kg body weight for 15 days.

Group TE.ME: treated with TE.ME at a dose of 300 mg/kg body weight for 15 days.

Blood collection and analysis

Blood samples were taken from experimental animals at different intervals (day 1, 8 and 15) and were analyzed for hematological parameters such as total red blood cell count (TRBC), hemoglobin concentration (Hb), mean hemoglobin concentration (MHC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), total leukocyte count (TLC), neutrophils (N) and lymphocyte (L) count, according to previously described techniques (Ahmad & Zeb, 2019). The activities of liver enzymes such as alkaline phosphatase (ALP), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were also analyzed according to the enzymatic colorimetric method (Adorian *et al*., 2019).

Statistical analysis

All the results obtained were analyzed statistically. Mean and standard deviation of the values were calculated using Graph Pad Prism 5 software.

RESULTS

***In-vitro* antibacterial activity of TE.AQ, TE.ET and TE.ME**

The *in-vitro* antibacterial potential of TE.AQ, TE.ET and TE.ME was evaluated at different concentration against clinically important gram negative and gram positive bacterial strains and was compared with Norfloxacine. The results showed that TE.ET and TE.ME extracts had strong inhibitory effect while TE.AQ was found less effective against various bacterial strains. The maximum antibacterial potential was found in TE.ME at a concentration of 100 mg/dL against *P. aeruginosa* with zone of inhibition (ZOI) of 19.67 ± 0.057 mm as shown in Table 1. The *S. aureus* was observed susceptible to the same fraction with ZOI of 17.50 ± 0.70 mm followed by *S. pyogenes* with ZOI of 15.9 ± 0.10 mm at a concentration of 100 mg/dL. The TE.ET had varied degrees of antibacterial effects against both gram negative and gram positive strains. However, the maximum activity against *P. aeruginosa* was observed at a concentration of 100 and 50 mg/dL with ZOI of 18.53 ± 0.50 and 17.67 ± 0.57 mm, respectively. It was also found active against *S. pyogenes* exhibiting ZOI of 16.2 ± 1.55 mm at a concentration of 100 mg/dL. The TE.AQ showed little

Table 1. *In-vitro* antibacterial activity TE.AQ, TE.ET and TE.ME against various bacterial strains

Solvent	Conc (mg/dL)	Zone of Inhibition (mm)							
		S. aureus	*S. epidermidis*	*S. pyogenes*	*E. coli*	*K. pneumonia*	*P. aeruginosa*	*P. vulgaris*	*S. Typhi*
TE.AQ	5	3.66±0.47	0.0±0.0	0.0±0.0	6.133±0.23	0.0±0.0	0.0±0.0	5.03±0.057	0.0±0.0
	15	5.86±0.057	0.0±0.0	0.0±0.0	9.833±0.20	0.0±0.0	0.0±0.0	6.96±0.057	0.0±0.0
	25	8.50±0.20	9.9±0.05	0.0±0.0	7.167±0.15	1.63±0.057	0.0 ±0.0	9.23±0.49	1.23±0.20
	50	9.13±0.13	9.16±0.23	0.0±0.0	8.867±0.25	1.90±0.10	0.0±0.0	10.6±0.57	2.80±0.10
	100	13.0±0.50	10.3±0.57	11.33±0.57	5.333±0.25	3.73±0.25	0.0±0.0	12.9±0.10	5.10±0.17
TE.ET	5	5.33±0.05	7.06±0.05	0.00±0.0	4.27±0.15	0.0±0.00	11.3±1.528	4.06±0.11	0.0±0.0
	15	5.96±0.057	5.90±0.10	10.0±0.1	4.86±0.057	0.0±0.0	12.9±0.75	6.06±0.11	2.20±0.26
	25	7.96±0.11	7.30±0.10	10.3±0.60	7.36±0.208	4.03±0.11	14.87±0.20	9.96±0.05	2.03±0.11
	50	11.9±0.79	9.00±1.30	12.0±0.28	9.96±0.057	4.33±0.15	17.67±0.57	10.6±0.10	3.03±0.15
	100	14.4±0.43	10.6±0.57	16.2±1.55	12.97±0.05	5.33±0.30	18.53±0.50	15.0±0.36	8.30±0.60
TE.ME	5	7.33±0.10	7.10±0.26	4.96±0.20	5.93±0.11	0.0±0.0	8.20±0.60	6.86±0.15	1.90±0.10
	15	8.00±0.10	7.66±0.57	6.30±0.20	6.90±0.10	0.0±0.0	9.06±1.52	7.03±0.05	1.80±0.10
	25	8.03±0.15	8.00±0.10	7.50±0.20	8.26±0.37	1.76±0.15	14.00±1.00	7.96±0.05	1.40±0.17
	50	13.70±0.65	9.93±0.05	9.96±0.05	9.83±0.64	2.50±0.17	14.97±0.11	9.96±0.05	4.33±0.2
	100	17.50±0.70	11.6±0.5	15.9±0.1	13.8±0.49	3.80±0.36	19.67±5.77	13.3±0.57	6.667±0.57
Norfloxacine (NF)	50	22.33±0.57	23.67±0.57	21.33±0.57	19.37±0.32	20.77±0.68	21.50±0.62	19.87±0.35	18.77±0.90

NC: negative control, PC: positive control, NF: Norfloxacine treated group at a dose of 100 mg/kg, TE.AQ: group treated with TE.AQ at a dose of 300 mg/kg, TE.ET: group fed with TE.ET at a dose of 300 mg/kg, TE.ME: group administered with TE.ME at a dose of 300 mg/kg

Table 2. Weight (grams) of the chicks at different time periods

Groups	Day zero	Day 8th	Day 15
Negative control	39 6 ± 1.0	467 ± 1.0	491 ± 1.09
Positive control	352 ± 1.0	345 ± 1.0	340 ± 1.06
NF (100 mg/kg)	420 ± 1.0	410 ± 1.0	480 ± 1.07
TE.AQ (300 mg/kg)	423 ± 1.0	414 ± 1.0	427 ± 1.01
TE.ET (300 mg/kg)	431 ± 1.0	429 ± 1.0	472 ±1.04
TE.ME (300 mg/kg)	437 ± 1.0	425 ± 1.0	481 ± 1.05

NC: negative control, PC: positive control, NF: Norfloxacine treated group at a dose of 100 mg/kg, TE.AQ: group treated with TE.AQ at a dose of 300 mg/kg, TE.ET: group fed with TE.ET at a dose of 300 mg/kg, TE.ME: group administered with TE.ME at a dose of 300.

inhibitory effect against few strains but was not significant in comparison with the standard drug (norfloxacine). The TE.ME was found active against seven of eight tested bacterial strains which confirmed the antibacterial efficacy of *Typha elephantina* leaves extract. The most sensitive bacteria, *P. aeruginosa* was selected for *in-vivo* studies and the effect of tested samples was evaluated.

***In-vivo* study**

The *in-vivo* antibacterial study of TE.AQ, TE.ET and TE.ME against *P. aeruginosa* was carried out in chicks model. All chicks were weighted before, during and after treatment. There was a significant decrease in weight of the infected chicks as compared to normal control, shown in Table 2. However, when treatment was completed, significant normal body weight was regained in animals fed with norfloxacine (NF) and TE.ME, while the TE.AQ and TE.ET showed no weight gain effects. Complete blood profile of all the groups were studied before experiment and during infection at day zero and during infection, at day 8th of the treatment, shown in Table 3. From the results, it is clear that all the blood parameters in all the experimental groups were significantly ($p<0.05$) impaired during infection when compared to the normal control group. After completion of treatment, at day 15th, animals in group that were infected with *P. aeruginosa*, but not treated, showed significantly ($P<0.05$) abnormal hematological parameters, revealed toxicity. The animals administered with TE.ET and TE.ME at a dose rate of 300 mg/kg body weight, showed remedial effects on hematological variables when compared to toxic control animals. However, a significant ameliorative effect was shown by TE.ME which confirmed its effectiveness against *P. aeruginosa* infection (Table 3). The significant elevation in the serum ALT, AST and ALP were observed in all groups during infection at day 8th (Table 4). However, a significant reduction in serum enzymes activities was observed in animals administered with TE.ME, at day 15th of treatment, when compared to normal control. Meanwhile, norfloxacine (NF) feed animals showed the results which were in normal reference range when compared to positive control and normal control groups.

Gross pathological examination

All the chicks were slaughtered at the end of the experiment on day 15th and gross pathological findings were studied as shown in Figure A1, A2, A3, A4, A5 and A6. In Figure A1, there is a normal liver having no lesion with no alteration as compared to the positive control. The group that was infected but not treated had a severe lesion on the liver as shown in Figure A2. The TE.AQ group had almost the same lesions to that of the positive control. The liver of animals in TE.ET was found with reduced lesions, while the livers of the animals treated with TE.ME had normal liver histology. In the Figure B1, B2, B3, B4, B5, and B6, the lungs of the different groups have been shown. In Figure B1, there was normal lung having no lesions as compared to the positive control group. The group that was infected but not treated (B2) had haemomoraghic lungs. The Norfloxacine treated group (B3) had a normal lung. It can be concluded that TE.ME has better potential against *P. aeruginosa* as compared to other groups.

Table 3. Hematology of experimental groups

	Normal control	Positive control	Norfloxacine	TE.AQ	TE.ET	TE.ME
Day zero						
TRBC $x10^3/mm^3$	3.23 ± 0.15	3.33 ± 0.208	3.37 ± 0.15	3.35 ± 0.01	3.36 ± 0.15	3.25 ± 0.015
Hb g/dL	11.4 ± 0.13	12.0 ± 1.0	12.70 ± 0.10	12.4 ± 0.15	12.61 ± 0.10	11.75 ± 0.15
MCH (Fl)	46.00 ± 1.0	41.00 ± 1.04	44.00 ± 1.0	38.56 ± 1.0	35.46 ± 1.0	47.34 ± 1.0
MCHC (%)	30.00 ± 1.04	31.00 ± 1.0	35.00 ± 1.0	33.34 ± 1.0	27.33 ± 0.57	28.53 ± 1.02
MCV (µ3)	114 ± 1.03	102.00 ± 1.03	139.00 ± 1.0	136.23 ± 1.0	104.64 ± 1.0	117.55 ± 1.01
TLC $x10^6/mm^3$	19.2 ± 0.1	19.50 ± 0.05	19.90 ± 0.01	19.47 ± 0.1	19.73 ± 0.1	19.16 ± 0.01
N (%)	34.00 ± 1.01	36.00 ± 1.07	37.00 ± 1.0	33.64 ± 1.0	38.56 ± 1.0	38.67 ± 1.06
L (%)	53.00 ± 1.0	53.00 ± 1.02	51.00 ± 1.0	54.25 ± 1.0	54.45 ± 1.0	55.23 ± 1.07
Day 8th						
TRBC $x10^3/mm^3$	3.24 ± 0.1a	2.67 ± 0.01b	2.7 ± 0.015 c	2.69 ± 0.020b	2.75 ± 0.010c	2.77 ± 0.020c
Hb g/dL	11.33 ± 0.1a	7.53 ± 0.05b	8.74 ± 0.066d	7.98 ± 0.0057c	8.40 ± 0.100d	8.467 ± 0.251d
MCH (Fl)	45.37 ± 0.57a	26.93 ± 0.86b	34.28 ± 0.59c	29.10 ± 0.19d	30.76 ± 0.78d	30.92 ± 1.00d
MCHC (%)	35.37 ± 1.00a	23.51 ± 0.56 b	28.96 ± 0.50c	25.14 ± 0.52d	26.93 ± 0.44e	26.56 ± 0.50e
MCV (µ3)	116.63 ± 1.0a	93.44 ± 1.34b	108.74 ± 2.08c	99.33 ± 0.57d	104.05 ± 1.00e	104.70 ± 0.57e
TLC $x10^6/mm^3$	19.52 ± 0.1a	24.00 ± 1.00b	19.67 ± 0.57c	22.13 ± 0.47d	21.67 ± 0.57d	20.67 ± .57cd
N (%)	35.76 ± 1.00a	50.56 ± 1.0b	40.67 ± 0.57c	48.33 ± 1.52bd	44.33 ± 1.15d	45.33 ± 1.52d
L (%)	52.35 ± 1.00a	39.22 ± 0.37b	47.00 ± 0.50c	41.33 ± 1.57bd	45.33 ± 1.52e	44.00 ± 0.51e
Day 15th						
TRBC $x10^3/mm^3$	3.32 ± 0.1a	2.81 ± 0.1b	3.31 ± 0.04a	2.99 ± 0.01bc	3.20 ± 0.05a	3.22 ± 0.09a
Hb g/dL	11.48 ± 0.1a	7.53 ± 0.01b	11.41 ± 0.64a	8.98 ± 0.005b	10.40 ± 0.10c	11.47 ± 0.25a
MCH (Fl)	47.34 ± 1.0	29.60 ± 0.66b	45.95 ± 1.00a	32.76 ± 1.34bc	40.43 ± 1.06d	43.92 ± 0.13a
MCHC (%)	35.49 ± 1.00	25.51 ± 0.79b	36.63 ± 1.73a	30.81 ± 0.46c	33.60 ± 0.75d	36.90 ± 0.69a
MCV (µ3)	116.34 ± 1.0a	94.47 ± 1.3b	114.78 ± 2.0a	102.73 ± 2.5c	108.37 ± 1.1d	114.76 ± 1.5a
TLC $x10^6/mm^3$	19.56 ± 0.1a	24.74 ± 0.1b	19.54 ± 0.1a	22.86 ± 0.1c	21.14 ± 0.1c	20.03 ± 0.1a
N (%)	39.89 ± 1.00a	55.34 ± 1.0b	40.48 ± 1.0a	46.49 ± 1.0c	43.51 ± 1.0d	40.97 ± 0.5a
L (%)	48.34 ± 1.00a	36.56 ± 1.0b	47.49 ± 1.0a	39.28 ± 1.0bc	44.74 ± 1.0d	46.68 ± 1.0a

Mean values in the same row having different superscripts (a-e) are significantly different (P<0.05). NC: negative control, PC: positive control, NF: Norfloxacine treated group at a dose of 100 mg/kg, TE.AQ: group treated with TE.AQ at a dose of 300 mg/kg, TE.ET: group fed with TE.ET at a dose of 300 mg/kg, TE.ME: group administered with TE.ME at a dose of 300 mg/kg.

Table 4. Liver biomarkers of experimental groups

	Normal control	Positive control	Norfloxacine	TE.AQ	TE.ET	TE.ME
Day zero						
ALT U/L	65.33 ± 1.52	66.00 ± 1.73	68.67 ± 2.06	65.00 ± 1.00	65.00 ± 2.0	62.67 ± 1.53
ALP U/L	35.67 ± 1.52	33.00 ± 2.64	37.33 ± 2.51	38.00 ± 1.00	39.33 ± 0.57	38.67 ± 1.15
AST U/L	137.32 ± 2.08	139.3 ± 1.52	139.0 ± 2.00	137.0 ± 1.00	135.73 ± 1.52	136.33 ± 1.52
Day 8th						
ALT U/L	60.00 ± 1.00a	140.0 ± 2.64b	121.0 ± 1.00c	135.0± 1.73d	129.35 ± 0.57c	125.75 ± 2.51c
ALP U/L	42.00 ± 2.64a	96.33 ± 2.08b	88.00 ± 1.44c	81.33± 0.74d	75.67 ± 1.52e	76.33 ± 1.52e
AST/ U/L	137.04 ± 1.00a	233.32 ± 2.08b	212.71± 2.08c	222.77± 0.28d	218.03 ± 1.00e	213.38 ± 2.02c
Day 15th						
ALT U/L	60.00 ± 2.00a	98.33 ± 1.15b	59.67 ± 2.30a	78.67 ± 0.57c	67.33 ± 1.52d	62.33 ± 2.08a
ALP U/L	34.67± 2.08a	85.33 ± 1.52b	35.00 ± 1.00a	69.33 ± 1.52c	53.00 ± 2.64d	35.33 ± 1.52a
AST U/L	138.09 ±1.00a	231.02 ± 2.00b	134.04 ± 1.00a	198.79 ± 1.52c	166.74 ± 2.51d	137.30 ± 2.64a

NC: negative control, PC: positive control, NF: Norfloxacine treated group at a dose of 100 mg/kg, TE.AQ: group treated with TE.AQ at a dose of 300 mg/kg, TE.ET: group fed with TE.ET at a dose of 300 mg/kg, TE.ME: group administered with TE.ME at a dose of 300 mg/kg.

DISCUSSION

The incidence of life-threatening disease caused by pathogens has increased globally and is one of the causes of morbidity and mortality in the developed nation (Rather *et al*., 2017). Gram-positive bacteria are the leading cause of a diverse range of lethal infections such as skin and soft-tissue infections and urinary tract infections (UTI), however, clinical microbiologists thought that gram-negative bacteria pose a big hazard to public health (Wright *et al*., 2017). A diverse array of human infections including pneumonia, necrosis, inflammation and hemorrhage in lungs and other tissues caused by *P. aeruginosa* and *K. pneumonia*. Similarly, *E. coli* caused chronic urinary tract infections and has been a cause of transferring antibiotic-resistant genes from infected food animals to human (Foxman, 2010). It is necessary to control microbial infections and its resistance to various drugs by developing' new antibiotic. Herbal medicines are very potent and have minimum side-effects. This project was done to determine the effect of leaf extract of *Typha elephantina* against eight selected bacterial strains. Highly significantly antibacterial potential was found in TE.ME at a concentration

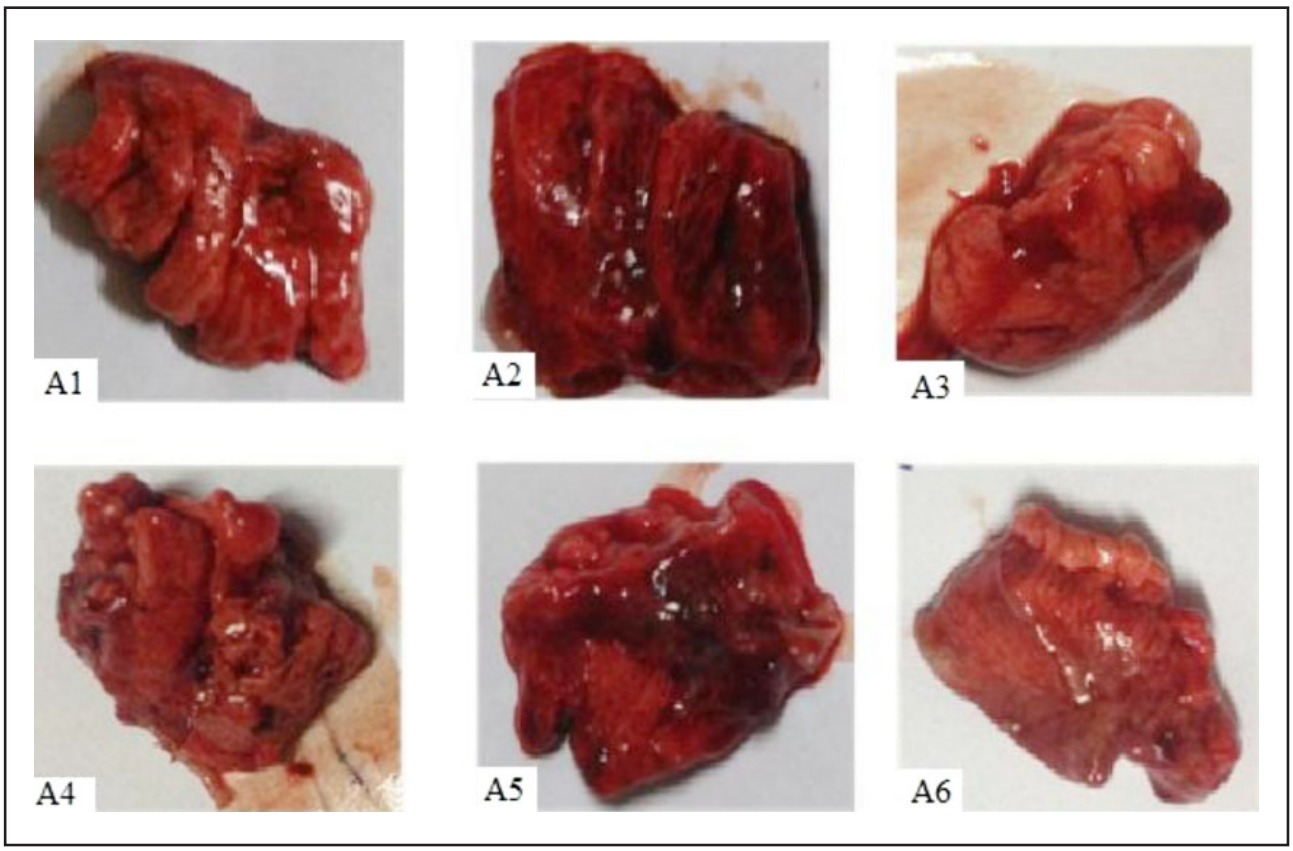

Figure 1. Gross pathological findings of lungs of all the chicks.

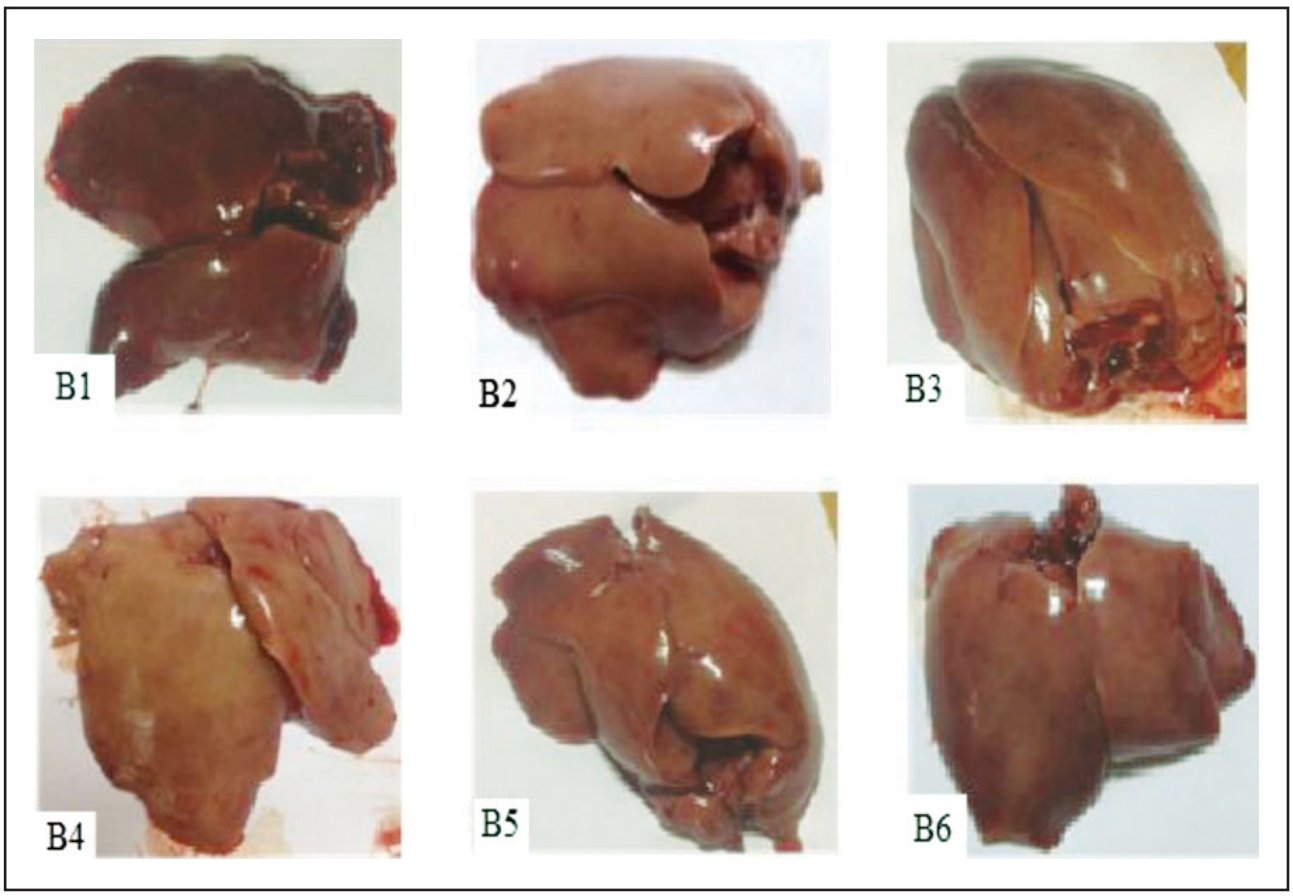

Figure 2. Gross pathological findings of livers of all the chicks.

of 100 mg/dL against *P. aeruginosa.* While TE.ME showed better effect at a concentration of 100 mg/dL against *P. aeruginosa* with zone of inhibition (ZOI) of 19.67 ± 0.057 mm. In another hand TE.AQ showed little inhibitory effect against few strains but was not significant. In comparison with the standard drug (Norfloxacin) the effects of the extracts were non-significant. This potent antimicrobial action of TE.ME against *S. aureus*is in agreement with the results of other researcher which showed the same effect in *Cannabis sativa* against multiple strains such as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* (Forouzanfar *et al*., 2014). The screening of TE.ME and TE.ET against *S. pyogenes* exhibit significant inhibition activity at all concentration, while the TE.AQ was active only at 100 mg/dL. These findings are similar to antibacterial effects of kalonji oil and its comparison with methanolic and aqueous extracts of *Nigella sativa* seeds. *E. coli* was sensitive to all extracts at all concentrations, but good activity was found by the TE.ME as compared to TE.ET and TE.AQ, respectively (Singh *et al*., 2017). *K. pneumonia* showed resistance to both TE.AQ and TE.ME at concentration of 50 and 100 mg/dL, however good antimicrobial activity was exhibited by TE.ET at a concentration of 25 and 50 mg/dL.

P. aeruginosa displayed the highest sensitivity to TE.ME and TE.ET but showed resistance to TE.AQ for 25 and 50 mg/dL of concentrations. The *P. aeruginosa* was found to be the most sensitive among all studied bacteria strains. These findings are in correlation with the finding of Adeosun *et al*. (2016). Maximum antibacterial activity was given by the TE.ET against *P. vulgaris* as compared to TE.AQ and TE.ME, the bacteria showed no resistance at any concentration*. S. Typhi* was found to be the most resistant among all having the lowest zone of inhibition in response to TE.ME, TE.ET and TE.AQ for all concentrations. From the result of this study, the TE.ME revealed high inhibitory effects against most of the tested bacteria. The diverse antibacterial activity recorded for different extracts of T.E could be attributed to various metabolites found in the plant, ensuring the fact that these phytochemicals are the major antibacterial agents constituted in plant (Asong *et al*., 2019). *P. aeruginosa* is an opportunistic pathogen entering and populated in embryonated eggs causing the in-shell death of embryos and newly hatched chicks (Umar *et al*., 2017). *P. aeruginosa* is, a nonspore forming and noncapsulated gram-negative bacillus, causing lower respiratory tract infections in chicken, also responsible for 30% of pneumonia, 19% of urinary tract infections, and 10% of bloodstream infections. Loss in body weight was noted in the infected chicken as compared to the negative control. It has been shown that weight loss after 15 days of *P. aeruginosa* infection, may be due to the concentration of pro-inflammatory cytokine levels after inoculation of chicks with *P. aeruginosa* and also loss of skeletal muscle mass. All tested groups showed abnormal hematological parameters during *P. aeruginosa* infection at day 8th of the experiment as compared to the normal control group. Significantly reduced levels of TRBC, Hb and related parameters such as MCH, MCHC and MCV were observed in all experimental groups, specifically in positive control group. While, significantly high a level of TLC was observed in all tested groups. The parallel study has been reported by Tripathi *et al*. (2017) which showed a significant decrease in TRBC count and Hb level in infected in Japanese quail, increase in TLC was found in infected quails. It has been observed that pyocyanine, secreted by *P. aeruginosa* may cause the suppression of specific defense mechanisms and boost harmful inflammatory responses of the host during infection with *P. aeruginosa*.

CONCLUSION

Antibiotics have been used extensively in in order to treat and inhibit bacterial diseases. They have also been used in lesser amount in feed as growth promoters. Such trend has enhanced poultry performance excellently and economically but an increase in numbers of antibiotic-resistant bacterial strains, did occur which can be spread from poultry to humans via the food chain with serious consequences on public health. Therefore it has been conclude from present *in vitro* and *in vivo* study that the *T. elephantina* leave's methanolic extract, (TE.ME) is very potent against most of the bacterial strains. Followed by the ethanolic extract (TE.ET) and aqueous extract (TE.AQ) of the plant respectively. Current *in vivo* study indicated that all three types of extracts i.e (TE.ET), (TE.ME) and (TE.AQ) have significant antibacterial efficacy as revealed from histopathological findings. The extract is also beneficial for most of hematological and liver parameters. In addition, *T. elephantina* extract has potent antioxidant capacity. Therefor this plant extract can also be used scientifically for other fatal and chronic diseases.

Current research work revealed the antibacterial potential of *Typha elephantina* which may be due to the presence of certain secondary metabolites. Thus this plant may be used for treating various bacterial infections especially caused by *P. aeruginosa*. Hence this research recommends that the *Typha elephantina* possess best anti-oxidant property, which could be used as best source of advance medication. Thus further exploration of the plant is required.

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Conflict of interest

All the authors have no conflict of interest and read the manuscript properly and approved for publication.

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