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Evaluation of *in-vitro* and *in-vivo* antimicrobial potential of *Typha elephantina* leaves extracts using *Cyprinus carpio* as a research model

Bashir Ahmad[1-2]*, Ali Muhammad Yousafzai[1], Wali Khan[2], Hafeez Ur Rahman[3], Mazen Almehmadi[4], Mustafa Halawi[5], Saad Alghamdi[6], Atif Ali Khan Khalil[7] and Fazli Khuda[8]

[1]Department of Zoology, Islamia College, Peshawar Pakistan
[2]Department of Zoology, University of Malakand, Khyber Pakhtunkhwa, Pakistan
[3]Department of Zoology, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan
[4]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, Taif, Saudi Arabia
[5]Department of Medical Laboratory Technology, College of Applied Medical Sciences, Jazan University, Jazan, Saudi Arabia
[6]Laboratory Medicine Department, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia.
[7]Department of Biological Sciences National University of Medical Sciences, Rawalpindi, Pakistan
[8]Department of Pharmacy, University of Peshawar, Peshawar, Pakistan

Abstract: Present study investigate the *in-vitro* antibacterial and antifungal potential of *Typha elephantina* leaves aqueous extract (T. Eaq), ethanolic extract (T. Eeth) and methanolic extract (T. Emth) at different dosages against selected bacteria and fungi using dis diffusion method and Potato Dextrose Agar method. The study was also proceeded *in- vivo* against one strain of fungi (*Aspergillus niger*) using aqueous (T. Eaq) extract only. *In-vitro* study showed that *Citrobacter freundii* was highly sensitive while *Salmonella typhimurium* was the least among all. The antifungal activity was dose dependent and differs according to the fungal strain. *Aspergillus niger* was highly sensitive in order of aqueous extract (T. Eaq), ethanolic extract (T. Eeth) and methanolic extract (T.Emth), followed by *Alterneria solani, Candida albicans* and *Aspergillus ustus*. The *in-vivo* antifungal study was carried using *Cyprinus carpio* which were first infected with *Aspergillus niger* and then treated with (T. Eaq) at different doses. During *in-vivo* study various hematobiochemicl parameters and bio-accumulative stress of some heavy metals were assessed. Highly significant ($P<0.05$) remedial effects were observed at day 21st of treatment with extract at 100mg/ kg body weight. Differential accumulation was found i.e in skin the accumulation was highest followed by intestine gills and muscles tissues. Liver showed least accumulation.

Keywords: Antibacterial, antifungal, biomarkers, bioaccumulation, *In vitro*, metals, susceptible, zone of inhibition.

INTRODUCTION

The rise of antibiotic-resistant organisms is a principal public health problem (Ferri, Ranucci *et al*., 2017) Worldwide, there have been enormous scientific data on antibiotic resistance. The discovery of new antibiotics was considered to be helpful in diminishing the microbial ailments. But this was seemed wrong as nonstop pathogenic resistance to these antibiotics. Many factors are there in the endless occurrence of microbial resistance to therapeutic agents, such as non-selective use of antibiotics and parallel transferred of resistance gene between bacteria. Global rise in resistance to antibiotics has compelled the scholars to search for other possible potential antimicrobials (Jani *et al*., 2021). Fish is an important source of food and recreation, and is a key unit in many natural food webs (Kytinou *et al*., 2020). Rearing fish under scientific principles (Fisheries and aquaculture) both are important food production systems that has a great contributions in our food and nutrition sector. The actual contribution that fisheries can make to nutrition and food depends on the supply, distribution, and utilization of fish (Berry *et al*., 2015; Powell *et al*., 2015). In aquatic environments, almost every freshwater fish is exposed to at least one species of fungus during its lifetime (Li *et al*., 2021). Fungi can attack fishes of all the ages and it can also prevent successful hatching when it invades fish eggs. One of the virulent parasite of fish is Aspergillus species which have been reported by Firoz, Mehdi, Hamidreza (2011), (Fadaeifard *et al*., 2011). In order to improve the performance of infected animal certain natural remedy i.e. a plant derived products called Phytobiotics can also be (Cristea *et al*., 2012). The phytobiotics have a wide variety of properties such as: antioxidant, antimicrobial, anticarcinogenic, analgesic, insecticidal, antiparasitic, anticoccidial, growth promoters appetite enhancement, stimulant of secretion of bile and digestive enzyme activity (Ramezani *et al*., 2021).

Further the accumulation of heavy metals in aquatic life indicates the way of Water pollution and fishes are commonly used as bio-indicators of heavy metals (Authman *et al*., 2015). Heavy metals such as cobalt, Iron, copper, manganese, zinc and molybdenum are imprtant in trace amount for human being (Maret, 2018). Metals are considered as main environmental contaminants and are non-biodegradable which cytotoxic, carcinogenic and mutagenic effects in animals (Shivakumar *et al*., 2014).

**Corresponding author:* e-mail: basheer.icup@gmail.com

The evaluation of phytobiotics in aquaculture is a relatively new area of research showing hopeful results. Therefore present study has been aimed to investigate the antifungal efficacy of mentioned plant extract. The control of many infections caused by microbes, using synthetic bactericides and fungicides is of best option but is not an eco-friendly approach as numerous are reported to have serious health hazards and have been associated to an increased incidence of several types of cancer. Alternative approaches is to control fungal infections have been studied by using compounds extracted from herbal bases in an effort to decrease the use of synthetic fungicides (Tegegne *et al*., 2008). Several studied have been demonstrated that many plants exhibit antibacterial and antifungal potential (Pârvu *et al*., 2011). Traditionally Plants have been used for long time as a remedy for various infectious ailments and are well-thought as a best source of new antimicrobial agents (Singh *et al*., 2018). Plants can produce vital secondary metabolites such as phenolic compound with reputable potent antimicrobial and antifungal activities, Current study therefore aims to investigate the effect of *Typha elephantina* leaves aqueous, ethanolic and methanolic extracts against selected bacterial and fungal strains both *in vitro* and *in-vivo* respectively. During *in-vivo* study various hematological, biochemical and accumulation of some heavy metals were analyzed in *Cyprinus carpio* as model animal.

MATERIALS AND METHODS

Plant collection and Extraction

Typha elephantina (T.E) was collected from local area of district, swat and was authenticated by Prof Dr. Muhammad Nisar for further the study and the voucher (Ref: Typ.3455) has been deposited in the department of Botany university of Malakand for record. The leaves of *Typha elephantina* (T.E) were extracted in water ethanol and methanol as *Typha elephantina* aqueous extract (T. Eaq), *Typha elephantina* ethanolic extract (T.Eeth) and *Typha elephantina* methanolic extract (T.Emth) following the procedure of (Ara and Nur, 2009) .

Antibacterial activity

Citrobacter freundii, Enterobacter aeruginosa, Salmonella typhimurium, Shigella sonnei and *Vibrio cholera* species were selected for this study. Bacteria were cultured on nutrient agar Slant at 4°C temperature maintenance. Incubate the culture for 24 h at 37°C to yield active colony for the experiment. Antibacterial activity of the extracts was determined by disc diffusion method, described by (Bauer *et al*., 1966). Five different concentrations of extract namely, 5mg/dL, 15mg/dL, 25 mg/dL, 50 mg/dL and 100 mg/dL were used along with a standard of ciprofloxacin (50 mg/dL) and DMSO (Dimethyle Sulfoxide) as negative control.

Antifungal Activity

The antifungal activity of *Typha elephantina* leaves aqueous extract (T. Eaq), ethanolic extract (T. Eeth) and methanolic extract (T. Emth) was evaluated against *Candida albicans, Candida tropicalis, Aspergillus niger, Aspergillus ustus and Alterneria solani* by using method of (Pundir and Chauhan, 2012). All the test organism were separately cultured on Potato Dextrose Agar (PDA) plates that contains supplement of chloraphenicol to overwhelm bacterial growth and incubated for 48 hours at room temperature (24C), to get fresh, active colonies of molds for experiment. 2ml of each extract i.e. aqueous, ethanol and methanol was mixed with 20ml of molten PDA medium and keep it for 30 minutes at room temperature to become solidify.

Now the test organism was aseptically inoculated onto the agar plate containing the three types of extract. Ampicillin (Amp)100 mg/dL) were used as positive control and 1ml of distilled water (DW) were used as negative control (Georgii and Korting, 1991; McCutcheon *et al*., 1994). The inoculated plates were done in triplicates and incubated at room temperature. Colony diameter was measured and recorded after 4 days and the mean of three readings is listed (Pundir *et al*., 2012). The Percent growth inhibition was calculated as % growth inhibition = Ia – Ib ÷ Ia x 100: where Ia represents control colony diameter and Ib represents treated colony diameter. The colony diameter is in millimeters.

In-vivo antifungal study

Fish and experimental design

To evaluate the *in-vivo* antifungal potential of *Typha elephantina* leaves aqueous extract (T. Eaq). A total number of 48 Juvenile of *Cyprinus carpio* (common carp) were received from Carp pond at university of Malakand Department of Zoology and were randomly distributed in to four groups.12 fish in each aquaria were kept for a period of 21 days.

Group (C): served as control group
Group (I-1): infected with *Aspergillus niger* and then treated with T.E.aq extract at dose rate 50mg/kg body weight.
Group (I-2): infected with *Aspergillus niger* and then treated with T.E.aq extract at dose rate 100mg/kg body weight.
Group (I-3): infection with *Aspergillus niger* and then treated with Clotrimazole at dose rate 30mg/kg body weight.

The infection was induced with 0.2 ml of *Aspergillus niger* suspended in phosphate buffered saline at concentration 1.5x103 colony forming units (CFU)/ml/ fish intraperitoneally according to (Zaki and Fawazi, 2015) method.

The Microbes

Aspergillus niger strains were obtained from Department of Biotechnology University of Malakand, Khyber pakhtoonkwa Pakistan.

Hematological parameters

Blood samples were collected from the caudal vein of *Cyprinius carpio* of all experimental groups at day 7th and 15th of the experiment for the determination of erythrocytes (RBCs), hemoglobin (Hb) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets(PLT) were analyzed by the method of (George *et al*., 2010).

Biochemical Studies

For the biochemical study collected blood samples were then centrifuged at 3000 rpm for 10 minutes. The serum isolated was used for biochemical analysis. Serum activities of aspartate amino transaminase (AST) and alanine amino transaminase (ALT) beside alkaline phosphatase (ALP) were estimated using commercial kits. Moreover, serum total protein, serum creatinine level and serum urea concentration was

Assessment of heavy metals

Fish selection and experimental design

Cyprinus carpio (1-year-old, average total length 21.22 ±1.13cm, average total weight 201.12±5.04g) were obtained from a fish farm of University of Malakand department of Zoology. Fish were shifted alive in water tank supplied by oxygen using air pumps. Before experiment all fish were acclimatized using glass aquaria (92 ×51 ×35 cm) containing 40-50 litre dechlorinated tap water. For consistent aeration purpose an electric air pump was used in each aquarium. Approximately 26±1 0C water temperature was maintained during the experiment. Fish were fed daily on a commercial fish diet having 26-30% crude protein. The experiment was continued for 21 days. A total number of 48 of apparently healthy *Cyprinus carpio* of the same length and size were arranged into various groups under control environment to assess the bioaccumulation of heavy metals.

Group (C): served as control group, group
Group (I-1): infected with *Aspergillus niger* and then treated with T. Eaq extract at dose rate 50mg/kg body weight.
Group (I-2): infected with *Aspergillus niger* and then treated with T. Eaq extract at dose rate 100mg/kg body weight and
Group (I-3): infection with *Aspergillus niger* and then treated with Clotrimazole at dose rate 30mg/kg body weight.

Digestion of fish tissue and metals assessment

After completion of the experiment tissues samples from all the fish were taken and weighted for the analysis of heavy metals. These samples were digested in perchloric acid (70%) and nitric acid (55%) and marked with blotting papers and then shifted to 100ml volumetric bottles. following procedures (Yousafzai *et al*., 2010) with little change. Until a clear and transparent solution was prepared the flasks were kept on warm plates and permitted to absorb at 200 to 250°C. The thick white fume from the flask after brown fumes was a sign of digestion process ending. All digested samples were subjected to assess heavy metals such as Zn^{+2} , Ni^{+2}, Cr^{3+}, Cd^{2+} in tissue sample of each fish was determined through the atomic absorption spectrophotometer (Spectra AA-700) in the CRL (Centralized Resource Laboratory) University of Peshawar. To identify the concentration of heavy metals present the ODs (Optical Densities) found were adjusted against the standard curvatures and Standard curves were organized.

Ethical approval

This is certified that the department of research and ethical committee (DRIC) of University of Malakand reviewed aims objectives and methodology of research entitle *in-vitro* and *in-vivo* Antimicrobial Potential of *Typha elephantina* leaves extracts using *Cyprinus carpio* as a Research Model The committee expect no adverse consequences on the subject/patient. Furthermore the study is safe for human population and provides benefits for the human being.

STATISTICAL ANALYSIS

Results obtained were analyzed statistically, mean, standard deviation, one-way ANOVA and Tukey test were conducted using the Graph pad Prism, Demo Version 05 (www.graphpad.com).

RESULTS

In-vitro antibacterial analysis

The *in-vitro* antibacterial activity of different extracts such (T. Eaq), (T. Eeth) and (T. Emth) are shown in (table 1). The three types of extracts at all concentrations displayed good efficacy against all tested bacterial strains. The *Citrobacter freundii* showed its maximum zone of inhibitions as 18.00±1.00, 17.43±0.60 and 17.73±0.68 mm in response to (T. Eeth), (T. Emth) and (T. Eaq) extracts at 100mg/dL in comparison of ciprofloxacin respectively. Similarly the three types of extracts at all concentration from 5mg/dL to 100mg/dL were found effective and dose dependent increased was observed in zone of inhibitions against *Vibrio cholera* (9.967± 0.057mm, 12.78±0.48 mm and 13.63±0.55mm), *Shigella sonnei* (9.850±0.18 mm, 12.7±0.472mm and 12.80±0.36 mm) and *Enterobacter aerogenes* (8.83±0.850 mm,11.43 ±0..35 and 12 .50±1.29) at 100 mg/dL of concentration in response to (T. Eaq), (T. Eeth) and (T. Emth) when compared to Ciprofloxacin. *Salmonella typhimurium* was

found least sensitive strain among all tested microbes showed 3.30±0.12 mm, 4.93 ± 0.55 and 10.36 ±0.63mm zone of inhibition when (T. Eaq), (T. Eeth) and (T. Emth) extracts were used at 100mg/dL of concentration (table 1).

In-vitro antifungal analysis

In-vitro antifungal activity of (T.Eaq) and (T.Eeth) and (T.Emth) extracts of (T.E) leaves extracts showed dose dependent improvement in growth inhibition against all the tested fungi. Thus (T.Eaq) extract inhibited the growth of *Aspergillus niger* (*A. niger*) by 88.46% at a concentration of 300mg/dL. This is followed by *Alterneria solani (A. solani), Candida albicans* (*C. albicans*), *Alterneria ustus* (*A. ustus*) and *Candida tropicalis* (*C. tropicalis*) showed 83.26% and 80.45%, 74.31% and 66.04% growth of inhibition at concentration of 300 mg/dL respectively. The aqueous extract inhibits the *Candida tropicalis* (*C. tropicalis*) by 56.02% and 51.46% at 200mg/dL and 75m/dL, while ethanol showed 63.43% and 49.71% inhibition at 200mg/dL and 75 mg/dL fig. 1 (A). In other hand (T. Eeth) extract at 300 mg/dL, inhibit the *C. albicans* most potently by 77.46%, followed by *A. solani, A. ustus* and *A. niger* showed percent growth inhibition as 76.45%, 74.01% and 70.74% respectively and *C. tropicalis* was inhibited by 66.24% respectively. Since (T. Emth) extract revealed its maximum efficacy at 300mg/dL of concentration against *C. albicans, A. ustus*, *A. niger* and *A. solani* which inhibiting these organisms by 78.36%, 75.38%, 73.21% and 73.21% respectively. The (T. Emth) extract at high concentration moderately inhibited the growth of C. *tropicalis* by 61.95% shown in (fig. 1 (B). The least inhibiting fungi were *C. tropicalis* at all concentration in response to all three types of extracts. The methanol extract (T. Emth) displayed 53.00% and 52.39% of growth inhibition against C. *tropicalis* respectively. While showed maximum growth inhibition against C.albicans (fig. 1 (C). From the results it is clear that extracts showed dose dependent activity against all tested organism. However A.niger found most susceptible in response to aqueous extract so it was selected for *in-vivo* study in fish sample.

In-vivo antifungal analysis

A significant changes were observed in the hematological and serum parameters of (*Cyprinus carpio*) of group= I-1, group= I-2 & group= I-3 (i.e., infected and treated) when compared to control uninfected fish i.e. Group =C). The hematological parameters such as total erythrocyte count (TEC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) along with total leucocytes count (TLC) were observed to be signinificanly ($P<0.05$) altered at day 7^{th} in all experimental groups shown in (table 2). However all the experiment animals groups were regularly treated with different doses of extracts and chemical up to twenty one days after infection at day first. Results were obtained at day 21^{st} of the experiment revealed that T. Eaq extract at dose rate 50 mg/ kg body weight (group= I-1) showed no significant ameliorative effects on all hematological indices when compared to control fish (group=C) respectively. While T. Eaq extract at dose rate 100 mg/ kg body weight (group= I-2) showed highly significant ($P<0.05$) curative effects on the hematological parameters i.e. reverting them toward their normal levels when compared to control fish (group= I-1). As results of (group= I-2) were comparable with results of (group= I-3) fish which were nourish with Clotrimazole at dose rate 30 mg/ kg body weight. Clotrimazole was used as standard drugs for comparison. Similarly the serum parameters like alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were found significantly ($P < 0.05$) in higher level in fish of (group= I-1), (group= I-2) and (group= I-3) respectively. This highly significant ($P< 0.05$) rise in the above mentioned serum parameters was observed at day 7^{th} of post infection with *A. niger* when compared to control fish (group=C). Treatment with T.E.aq extracts at dose rate 50mg/kg body weight (group= I-1) up to twenty one days of experiment showed no curative effects on the above mention parameters. While administration of T. Eaq extract for twenty one days at high dose i.e.100mg/ kg body weight (group= I-2) significantly ($P<0.05$) reduced the increase levels of serum ALT, AST and ALP toward normal range when compare to control fish (group= I-1) shown in table 3.

The elevated levels of kidney related serum parameters such as urea uric acid and creatinine showed infection caused by *A. niger* at day 1^{st} of the experiment in fish groups such as group= I-1, group= I-2 & group= I-3 respectively. All these groups were treated regularly for twenty one days from first day of infection with different dose of T.Eaq extracts and standard drug. Results obtained at twenty first (21) day of post treatment revealed that animals of group= I-1 which were administered at dose 50mg/kg body weight, showed no significant reduction in the serum urea uric acid and creatinine levels. While statistically significant ($P<0.05$) decrease was observed in levels of urea uric acid and creatinine of group= I-2 animals feed with (100mg/kg body weight), when compared with control animal (group=C). Changes in the lipid profile activity like total cholesterol, triglycerides, LDL and HDL of challenged groups were observed at day 7^{th} of post infection when compared to control group animal. However these parameters were found to be normalized with treatment of T. Eaq extracts extract for 21 days regularly. But significant ($P<0.05$) normalization was brought by high dose of T. Eaq extract (100mg/kg body weight) (group= I-2), when compared to normal control group animals. While the low dose (50mg/ kg body weight) (group= I-1), effect was non-significant (table 3).

Table 1: Antibacterial activity of *Typha elephantina* leaves aqueous (T. Eaq) ethanolic (T. Eeth) and methanolic (T. Emth) extracts at different concentrations against selected bacteria strains at various concentrations.

Bacteria	Zone of inhibition in (mm) Mean ± SD by (T. Eaq) extract at different concentrations in mg/dL					Ciprofloxacin 50 mg/dL	DMSO 0.5ml
	5	15	25	50	100		
Citrobacter freundii	7.40±0.52	12.07±0.15	16.10±0.26	16.20±0.68	17.73±0.17	27.13±0.67	0.0 ± 0.0
Enterobacter	2.70±0.519	4.40±1.082	4.40±1.153	6..50±0.26	8.83±0.85	25.67±0.577	0.0 ± 0.0
aeruginosa Salmonella typhimurium	1.23±0.20	2.80±0.10	2.90±0.10	2.22 ±0.19	3.30 ±0.12	28.33±0.57	0.0 ± 0.0
Shigella sonnei	3.383± 0.35	6.600±1.17	7.047±0.63	6.930±0.50	9.967±0.05	21.37±0.32	0.0 ± 0.0
Vibrio cholera	4.100± 0.75	7.63 ± 1.03	9.933±0.05	6.433±0.45	9.850±0.18	24.77±0.68	0.0 ± 0.0
	Zone of inhibition in (mm) Mean ± SD by (T. Eeth) extract at different concentrations in mg/dL					Ciprofloxacin 50 mg/dL	DMSO 0.5ml
	5	15	25	50	100		
Citrobacter freundii	6.100±0.10	13.67±0.57	17.43±0.60	16.53±0.46	17.19±0.17	27.13±0.67	0.0 ± 0.0
Enterobacter	4.163±1.88	9.147±0.26	10.30±0.00	11.00±0.00	11.43±0..35	25.67±0.577	0.0 ± 0.0
aeruginosa Salmonella typhimurium	1.01±00	1.60±0.54	2.747±0.45	4.700±1.04	4.93 ± 0.55	23.33±0.57	0.0 ± 0.0
Shigella sonnei	5.000±0.10	7.667±0.57	9.933±0.05	7.100±0.26	13.63±0.55	21.37±0.32	0.0 ± 0.0
Vibrio cholera	5.000 ±0.72	9.000±0.20	10.07±0.47	11.82±1.48	12.80± 0.36	24.77±0.68	0.0 ± 0.0
	Zone of inhibition in (mm) Mean ± SD by (T. Emth) extract at different concentrations in mg/dL					Ciprofloxacin 50 mg/dL	DMSO 0.5ml
	5	15	25	50	100		
Citrobacter freundii	9.90±0.100	12.17±0.28	14.00±1.00	17.33±0.577	18.87±0.96	27.13±0.67	0.0 ± 0.0
Enterobacter	4.900 ±0.10	6.730±1.24	7.12±0.55	8.133±0.20	10.36±0.63	25.67±0.577	0.0 ± 0.0
aeruginosa Salmonella typhimurium	4.22±0.01	6.34±0.11	7.62±0.63	9.60±1.442	12.50±1.29	28.33±0.57	0.0 ± 0.0
Shigella sonnei	5.217± 0.12	8.450±0.77	9.467±0.58	9.747±0.54	12.78 ±0.48	21.37±0.32	0.0 ± 0.0
Vibrio cholera	6.00 ±0.300	9.13 ±0.550	9.60±1.442	11±0.23	12.70±0.472	24.77±0.68	0.0 ± 0.0

Note. (T. Eaq) = *Typha elephantina* aqueous extract, (T. Eeth) = *Typha elephantina* ethanolic extract and (T. Emth) = *Typha elephantina* methanolic extract

Table 2: Hematological parameters of various groups, feed with different concentrations of *Typha elephantina* leaves aqueous extract (T.Eaq) and standard drugs.

Parameters	Day 7th (During infection)				Day 21st (Post treatment)		
	Group=(C)	Group= I-1	Group= I-2	Group= I-3	Group= I-1	Group= I-2	Group= I-3
RBCsx 10^6/ mm^3	1.94±0.05	1.14± 0.02***	1.23 ± 0.01**	1.25±0.02**	1.63±0.04**	1.83 ± 0.05*	1.89± 0.10*
Hb (g/dL)	8.81±0.73	4.96±0.17***	5.63±0.09***	5.88±0.10***	6.13± 0.34**	8.13± 0.76*	8.57± 0.65*
MCV (mm^3)	186.7±2.51	162.3±3.21***	167.0±2.00***	177.0±1.00**	165.5±3.97**	175.7±0.68*	180.0±1.64*
MCH (pg)	65.55±1.47	36.81±1.72***	42.71±1.22***	46.89±1.30**	41.67±2.51***	58.67±1.15*	61.33±1.52*
MCHC (g/dL)	27.27±1.00	18.23±1.05***	22.13±0.99**	21.90±1.52**	20.86±0.64**	25.19±1.05*	26.30±0.94*
HCT (%)	28.57±1.08	20.31±0.95***	21.22±1.04***	23.85±1.50**	22.31±0.95**	25.45±1.52*	26.01±1.25*
WBCx10^3/ mm^3	3.81±0.09	7.36±0.51***	5.56±0.19**	5.10±0.28**	5.78±0.21**	4.31±0.53*	4.13±0.45*
Neutrophils %	17.69±0.75	29.49±0.71***	27.08±0.61***	24.14±0.53***	25.86±0.97***	19.20±0.39*	18.29±0.37*
Eosinophils%	5.77±0.09	8.89 ±0.57***	7.11 ±0.35**	7.02 ±0.57**	6.40±0.15**	5.75±0.13*	5.37±0.15*
Monocyte %	4.33±0.09	1.74±0.06***	1.94±0.12**	2.10±0.29**	2.47±0.05**	4.13±0.46*	4.46±0.11*
Lymphocyte %	71.38±0.85	48.67±0.84***	52.12±2.18***	63.97±2.56**	54.97±1.57***	66.83±1.99** *	69.38±1.00*

(Note). *=significantly equal to group=(c) while ** and ***=significantly different from group=(c), according to Dunnett's Multiple Comparison Test. Group, (C): served as control group, Group, (I-1): infected with *Aspergillus niger* and then treated with T. E.aq extract at 50mg/ kg body weight, Group, (I- 2): infected with *Aspergillus niger* and Then treated with T. E.aq extract at 100mg/ kg body weight, Group, (I-3): infection with *Aspergillus. niger* and then treated with Clotrimazole at dose rate 30mg/ kg body weight.

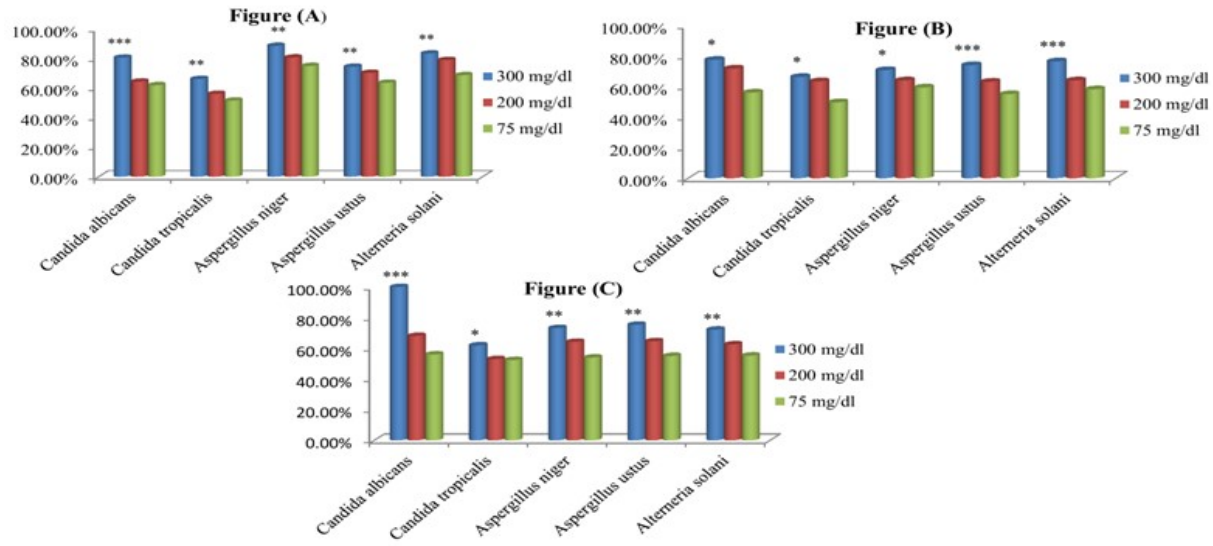
Table 3: Serum related parameters of various groups feed with different concentrations of *Typha elephantina* leaves aqueous extract (T.Eaq) and standard drugs.

Parameters	Day 7th (During infection)				Day 21st (Post treatment)		
	Group=(C)	Group= I-1	Group= I-2	Group= I-3	Group= I-1	Group= I-2	Group= I-3
ALT (U/l)	48.00±1.000	79.00±6.24***	70.00±1.00***	61.33±1.52**	65.33±1.52**	46.14±1.52*	45.34±1.53*
AST (U/l)	69.03±1.20*	196.3±2.499***	145.7±1.489***	124.0±1.00**	119.0±1.00**	57.00±2.0*	58.00±0.53*
ALP (U/l)	26.59±0.79*	69.77±0.99 **	59.40±0.54***	56.34±0.49***	41.51±1.01**	25.99±0.49*	26.80±1.00*
Creatinine mg/dL	0.60±0.05*	1.40±0.1***	1.18±0.05**	1.02±0.01***	0.88±0.003**	0.58±0.73*	0.60±0.01*
Urea mg/dL	18.45±1.00*	31.01±1.63***	28.26±0.76***	27.12±0.91***	27.63±0.74***	20.54±0.77*	19.26±0.63*
CK(U/L	354.0±16.46*	517.0±15.39***	512.7±6.80**	510.7±18.50**	510.0±17.09**	403.0±10.58*	399.0±6.00*
Total CHOL mmol/dL	3.20±0.15	4.66±0.23**	4.22±0.055**	4.15±0.06**	4.41±0.19**	3.450±0.22*	3.267±0.28*
HDL mmol/dL	1.69±0.16	1.13±0.1***	1.20±0.07**	1.27±0.20**	1.26±0.06**	1.59±0.06*	1.61±0.02*
LDL mmol/dL	1.65±0.05	2.00±0.017**	1.97±0.005**	1.91±0.046**	1.91±0.01**	1.73±0.03*	1.66±0.02*
T.G mg/dl	1.03±0.16	1.84±0.058***	1.56±0.18**	1.48±0.1**	1.39±0.12**	0.96±0.064*	0.92±0.057*

Table 4: Showing bioaccumulation of various heavy metals Mean ± SEM (um/dL) in muscles, skin, gills and intestine of *Cyprinus carpio* of all experimental groups feed with different concentrations of *Typha elephantina* leaves aqueous extract (T.Eaq) and standard drug.

Muscles				
Groups	Zn	Ni	Cd	Cr
Group C	0.047±0.016 *	0.0034±0.007*	0.0043 ±0.002*	0.0035±0.0008*
Group= I-1	0.59±0.036***	0.0059±0.010***	0.0057 ±0.002***	0.0055±0.0004***
Group= I-2	0.0049±0.003*	0.0039±0.010*	0.0047 ±0.002**	0.0036±0.0002*
Group= I-3	0.0048±0.002*	0.0038±0.003*	0.0048±0.001*	0.0034±0.002*
Skin				
Groups	Zn	Ni	Cd	Cr
Group C	1.31±0.5031 *	0.045±0.0110*	0.018±0.007 *	0.050±0.0018*
Group= I-1	1.57 ±0.131 b***	0. 069 ±0.050***	0.038 ±0.0029***	0.092±0.0013***
Group= I-2	1.35±0.0043*	0.049±0.005**	0.019±0.0033*	0.077±0.0047**
Group= I-3	1.34±0.0036*	0.047±0.08*	0.018±0.0056*	0.079±0.0093**
Gills				
Groups	Zn	Ni	Cd	Cr
Group C	0.49±0.0061 *	0.041±0.004*	0.020±0. 0011*	0.053±0.008*
Group= I-1	0.79±0.0017***	0.071±0.009***	0.029±0.0015**	0.060±0.034**
Group= I-2	0.53±0.005*	0.049± 0.004**	0.026±0.001**	0.054±0.032*
Group= I-3	0.51±0.002*	0.042± 0.001*	0.024±0.004*	0.052±0.025*
Liver				
Groups	Zn	Ni	Cd	Cr
Group C	0.052±0.008 *	0.0032±0.002*	0.0061±0.005*	0.0043 ±0.004*
Group= I-1	0.082±0.004 b***	0.0069±0.009***	0.0089±0.009***	0.0057 ±0.005**
Group= I-2	0.0061±0.009**	0.00400±0.01**	0.0077±0.001**	0.0049±0.006*
Group= I-3	0.0051±0.003*	0.003700±0.05*	0.0064±0.003*	0.0045±0.006*
Intestine				
Groups	Zn	Ni	Cd	Cr
Group C	0.54±0.0441 *	0.036±0.0056*	0.037±0.01266*	0.048±0.01242*
Group= I-1	0.72±0.055 b***	0.056±0.0070***	0.060 ±0.0141***	0.079±0.066 ***
Group= I-2	0.57±0.00695*	0.044±0.01835**	0.047±0.02539**	0.050±0.008*
Group= I-3	0.52±0.00223*	0.041±0.01123**	0.040±0.0212*	0.049±0.231*

(Note). *= significantly equal to group=(c) while ** and ***=significantly different from group=(c), according to Dunnett's Multiple Comparison Test. Group, (C): served as control group, Group, (I-1): infected with *Aspergillus niger* and then treated with T. E.aq extract at 50mg/ kg body weight, Group, (I- 2): infected with *Aspergillus niger* and Then treated with T. E.aq extract at 100 mg/ kg body weight, Group, (I-3): infection with *Aspergillus niger* and then treated with Clotrimazole at dose rate 30mg/ kg body weight.



(A). Fungal growth inhibition in percent by (T. Eaq) extract. (B). Fungal growth inhibition in percent by (T. Eeth) extract. (C). Fungal growth inhibition in percent by (T. Emth) extract.

Fig. 1: Percent inhibition of fungal growth by extracts of *Typha elephantina* at different concentrations.

Heavy metals analysis

The concentration of metals were highest in the gills followed by skin, intestine, liver and muscles tissues in order of Zinc (Zn) cadmium (Cd), chromium (Cr) and nickel (Ni) in fishes that were exposed to *Aspergillus niger* and then treated with *Typha elephantina* leaves aqueous extract (TE.aq) at dose 50mg/kg body weight (group=I-1) when compared to control fish (group=C). However administration of extract (100mg/kg body weight) (group = I-2) and Clotrimazole (30mg/kg body weight), (group = I-3), significantly ($P<0.05$) decreased the raised value of Zinc (Zn) cadmium (Cd), chromium (Cr) and nickel (Ni) respectively.

Higher concentration of metals (group=I-1), showed that aqueous extract of T.E at 50 mg/kg body weight had no effect. While the administration of extract (100mg/kg body weight) and Clotrimazole (30mg/kg body weight). After the exposure to *Aspergillus niger* significantly ($P<0.05$) decline the levels of metals when compared to control fish (table 4). This revealed that *Typha elephantina* leaves have potent therapeutic role by decreasing the accumulation of heavy metals in various organs either increasing metabolic rate or excretory process of liver and other homeostatic organs.

DISCUSSION

Antimicrobial resistance (AMR) is a main problem to the health of human and animal and food safety. Creating fundamental questions about the role plays by environment in the selection and spread of resistant organisms. Several environmental agencies are involve in the dispersal of resistance organism such as industrial discharges (mainly from pharmaceutical industries), agricultural activities, and human wastes infecting the environment (Tacconelli, Carrara *et al*., 2018). Microbial resistance is increasing progressively challenging the scientists to minimize and control the problem. For this purpose highly sophisticated research is necessary to develop better understanding about the mechanisms of inherent resistance and to develop new drugs of natural origin.

Present study reveals that all the three extract of *T.elephantina* leaves namely aqueous extract (T. Eaq), ethanol (T. Eeth) and methanol (T. Emth) extract at all concentrations i.e. 5mg/dL, 15mg/dL 50mg/dL, 75mg/dL and 100mg/dL had wide antibacterial spectrum and being highly active against *Citrobacter freundii* (*C. freundii*) and *Shigella sonnei* (*S. soni*) sps. The aqueous extract at 50mg/dL showed highest zone of inhibition against *C. freundii* and at 100mg/dL against *S.soni* and *Vibrio cholera* followed by *Enterobacter aerogenes* and Salmonella *typhimurium*. Results of present study are agreed with Chaudhry, *et al*., (Chaudhry and Tariq, 2006), whom presented parallel results, investigating the anti-microbial efficacy of *Cinnamomum cassia* against varied microbial flora with its nutritive and therapeutic influences. Ethanolic extract (T. Eeth) showed the same antibacterial activity against all test microbes at 100mg/dL except *C. freundii*, higher inhibition zone was given as compared to other respectively. Results of (T. Eeth) extract are comparable to those of Dhiman, R., *et al*. (Dhiman *et al*., 2016), who studied the antibacterial activity of five medicinal plant extracts against some human pathogenic bacteria. Their results signified that highest inhibition was noticed with ethanol extract of *Sesamum indicum* against selected microbes. The results of methanol (T.Emth) extract showed that the maximal

inhibition zones was showed by *C. freundii* at 25 mg/dL, followed by *S. sonnei*, then *V. cholera*, *Salmonella typhimurium* and in the last *Enterobacter aerogenes*. The *Enterobacter aerogenes* showed maximum zone of inhibition at 5mg/dL of methanol extract, but showed resistance when concentration was increased from 15mg/dL to 100mg/dL. Similarly *Salmonella typhimurium* was found resistance at highest concentration of (T. Emth) extract. These results are equivalent to the study of Ahmed *et al*. showed the methanolic extract of *Sesame* displayed a mild antimicrobial action against targeted microbe (Ahmed *et al*., 2009). The efficacy of the plant extracts differs according to the fungus types. In fact, the data shows that extracts of all types exhibit dose dependent increased in antifungal activity. All extract were found to be highly potent at highest concentration against all tested fungi. Among all fungi *Aspergillus niger* is more sensitive followed by *A. solani* then *C. albicans* and *A. ustus*, showed such decreasing order level of sensitivity using (T.Eaq), (T.Eeth) and (T.Emth) extracts. *C. tropicalis* was found to be the least susceptible in response to the three types of extracts used. These results are in line with those described by Adekunle and Ikumapayi which presented that all the test microbes including *Aspergillus flavus, Candida albicans,* and some other offer different levels of susceptibility.

The overall results indicate that the (T. Eaq) extract has strong antibacterial and antifungal activity over all selected microbes as compared to ethanol and methanol extracts. Among the all test organisms the *A. niger* is most sensitive at all concentration while *C. albicans* was found to be highly sensitive against (T. Eeth) and (T. Emth) extract at all doses. In other hand *C.tropicalis* was least susceptible and all other test fungi showed mild to moderate level of growth inhibition against the three types extracts respectively.

In-vivo antifungal analysis

It has been proof experimentally by various investigators that mycotic infections are common in all animals of diverse habitat. A fresh water fish was found highly sensitive to fungal infection especially to *Aspergillus niger* (*A. niger*) considered extremely pathogenic. (Chauhan *et al*., 2014).

The pathogenesis of *A. niger* in fish lead to alteration in hematological and serum biochemical markers and cause damage to tissue which leads to mortality of fish. In the present study infection of *cyprinus carpo* with *A. nigr* cause changes in the levels of blood parameters. The level of red blood cells (RBCs) and related parameters such as hemoglobin (Hb), MCV, MCH and MCHC of the experimental group I-1, I-2& I-3 (i.e., infected) found reduced when compared to control uninfected fishes. The decrease was highly significant at day 7th of post infection in all the test groups. While WBCs, neutrophils and eosinophils concentration was statistically ($P<0.05$) raised and that of monocyte and lymphocyte was decreased significantly compared to control fish (group=C). Treatment with T. Eaq extract at different doses positively ameliorates the altered hematological parameters towards their normal levels. A highly statistically significant statistically ($P<0.05$) significant curative effect was observed in group = I-2, which was exposed to high dose of T. Eaq, leaves extract. While the group= I-1 fishes showed non-significant effect on hematological parameters. Results of high dose extract were comparable with a standard drug administered fishes (group= I-3).

Aspartate and alanine aminotransferases are usually found in the hepatocytes of the liver, kidney, heart, gill, muscles and other organs. Serum ALT, AST and ALP, examination of fish of different experimental groups infected with A.niger showed variation in parameters. These parameters were found significantly ($P<0.05$) higher at day 7th of post infection. High levels of Serum ALT, AST and ALP showed mycotic pathogenicity in fishes. As elevated levels occurrence of these enzymes in the serum may consider organ dysfunction (Wells *et al*., 1986). Treatment after infection with T.Eaq extracts at different doses revealed reductions in the serum enzymes when were analyzed at day 21st of the experiment. Highly statistically significant ($P<0.05$) reductions in serum ALT, AST and ALP levels were found in fishes of group= I-2, provided as 100mg/kg body weight of T. Eaq extract regularly for 21st days, when compared to normal control fish group. While no significant reduction was found by the low dose (50mg/kg body weight) of T. Eaq administration (group=I-1).

Several studies have confirmed that feeding fish with plant extracts that exhibit antipathogenic effect leads to a significant decrease in mortality after challenge with fish pathogens. Microbial infection can lead to renal dysfunction, marked by the rise in in the blood urea, uric acid and creatinine whose assessment in serum helps in determining glomerular Filtration Rate (GFR). However, neither urea nor creatinine is directly toxic and they are only a measure of renal function (Devi *et al*., 2009). A prominent increase in serum urea, uric acid and creatinine was noted in the present study in various challenged groups when compared to normal fishes. However a decline was noted on day 21st of post treatment with T. Eaq extract. This decrease was highly significant ($P<0.05$) in group=I-2, feed with high dose of extract, while non-significant with low dose of extract (in group=I-1). Cholesterol is the most known lipid in nature, because high levels of cholesterol in the blood is associated with the incidence of cardiovascular disease in humans. High Density Lipoprotein (HDL) carried cholesterol. The esters of cholesterol from peripheral tissues enter to the liver for its catabolism (scavenging action). Very Low Density

Lipoprotein (VLDL) transports mostly the endogenous triglycerides synthesized in hepatocytes from the hepatic cells to the extra-hepatic tissue including adipose tissue for storage. Low Density Lipoprotein (LDL) regulates cholesterol synthesis in extra-hepatic tissue. The triglycerides are the most abundant of all lipids.

Heavy metals like Cd, Cr, Ni, and Zn were analyzed for the bioaccumulation in the muscle, liver, gills and skin tissues of fresh water fish Mully. Combustion emission, Domestic manure, mining operations, industrial effluents and metallurgical activities are the sources of heavy metals such as Pb, Cd, Zn and Cr in the hydrosphere (Shah *et al*., 2021). In the current study of Zinc More concentration observed in gills, skin and intestine flowed by liver while muscles has shown the lowest accumulation. The reason is that the muscle is less active tissue metabolically that's why accumulated the least level of zinc (El-Moselhy *et al*., 2014) have also reported the lowest level of heavy metals in the muscles. Skin of the fish is in direct contact with water so the heavy metals accumulation in skin occurs due to the adsorption which is followed by the absorption through several mechanisms. In present study skin of *Cyprinus carpio* has accumulated high concentration of Zn as compared to other fish tissues. Excessive Zn increase can be toxic and has been connected to the neurodegeneration (Saibu *et al*., 2018). According to Muiruri *et al.* (2013) Zinc levels ranged between 28.00-49.50 (mg/kg DW) and 48.79 to 76.33 (mg/kg DW) in the dry and wet seasons respectively (Muiruri *et al*., 2013). In the current study the concentration of Zn is high in gills due to the close contact of blood and water. Similarly (Ali and Khan, 2018) has recorded highest Zn concentration in gills of *Clarias gariepinus* which is inline of our results (group=I-1) values.

Nickel is produces severe damage to respiratory system in fish and thus caused fish death (Pandey and Madhuri, 2014). In present study the concentration of nickel in gills>skin > intestine > liver and >muscle. According to Muiruri *et al* (2013) from attribute of Athi-Galana-Sabaki river in Kenya the concentrations of Ni ranged from 0.29-1.75 mg/kg DW and 0.12-0.87 mg/kg DW in the wet and dry seasons respectively. Chromium is a vital trace metal both for human and animals but in high level it is neurotoxic and carcinogen (Singh and Chowdhuri, 2017). In the current study Cr was detected in the different tissue in the order of Gills Skin >Liver> Muscle, more concentration in the fish tissues skin an gills revealed highest chromium concentration which is due large surface area for exposure to the surrounding water. In gills tissue the accumulation is frequently related with physical damage to the gill epithelium and osmoregulatory function. Likewise, (Dobaradaran *et al*., 2010) have recorded high level of Cr in the intestine of *Clarias gariepinus*. Cadmium is anthropogenic metal pollutant extremely toxic to aquatic animals with a long biological half- life and produce renal and hepatic injuries in land animals and fish (Vizuete *et al*., 2018). In the present study the mean value of cadmium concentration in the tissue in order Cd= Gills > Skin> Liver>Muscle. Cd is a non-essential, and element non-biodegradable which is reflected to be a main contaminant that sources antagonistic special effects on the marine environment.

CONCLUSION

From results (*in-vitro* study) it has been conclude that *Typha elephantina* leaves extracts i.e. aqueous extract (T.Eaq) ethanolic (T.Eaq) and methanolic (T.Emth) extract were effective against all tested bacteria and fungi at high concentration. I*n-vivo* study showed that *Typha elephantina* aqueous extract (T.Eaq) has strong curative effects against *Aspergillus niger* infection. The bacterial inhibition and ameliorative potential of the mention plant extracts were mainly due to their chemical components, so further studies should be carried out to t by exploring the plant up to compound level to develop new drugs for various ailments.

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