Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 13, No. 4, p. 131-140, 2018
http://www.innspub.net

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RESEARCH PAPER
OPEN ACCESS

Antibacterial and teratogenic activity of *Eleusine indica* leaf extracts

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Article published October 06, 2018

Key words: Antibacterial, Phytochemical, Teratogenic.

Abstract

The present study determined the antibacterial activity of *E. indica* against *E. coli* and *S. aureus* at 12, 24 and 36 hrs of incubation. Also, its teratogenic potential using zebra fish as model animal was made. Malformations which include stunted-tail, growth retardation, yolk deformity and delayed development were observed. Similarly, mortality rate, hatchability and heartbeat of zebrafish embryo were also noted. Results showed bacterial inhibition against *E. coli* at 12, 24 and 36 hrs of incubation. For the teratogenicity of *E. indica*, LC_{50} of ethanol and hot water extracts was computed at 2200 ppm and 12890 ppm, respectively. Also, exposure of zebra fish embryo to ethanol extract of 10000 to 5000 ppm caused 100%mortality at 24 hours of incubation and reduced heartrates for the surviving embryos. Furthermore, malformations were also recorded which include stunted-tail, growth retardation, yolk deformity and delayed development. Thus, the potential antibacterial activity against *E. coli* and teratogenic potential of *E. indica* ethanol extracts.

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Introduction

Plants are rich in phytochemicals and are sources of alternative drugs throughout the world (Srivastava *et al.,* 1996) (WHO, 2002). In addition, due to occurrence of infectious diseases there is an increasing attention on the discovery of plant based antimicrobial drugs. (Anderson, 2003). Plants contain numerous biologically active compounds, many of which have antimicrobial activity (Cowan, 1999).However, it is also vital to assess their pharmacological potentials and properties, their benefits and side effects (Firenzuoli and Gori , 2007). These plant metabolites could also pose danger or even death to developing embryo. The plants or their toxins capable of causing fetal death or abnormalities are referred to as teratogens which crosses the placenta during gestation period causing birth defects, mortality and other forms of malformations (Lather *et al*., 2011).

In this study, the antibacterial and the teratogenic potential of *E. indica* was explored.

Materials and methods

Collection and extraction E. indica leaves

The leaves of *E. indica* without insect bites and plant diseases were collected at San Jose City, Nueva Ecija and disinfected with sodium hypochlorite then rinsed with distilled water three times. The leaves were then cut into small pieces, air dried and were pulverized using pulverizing blender. The leaves were extracted using hot water and ethanol as solvents.

For ethanol extraction, 50 grams of pulverized leaves of *E. indica* were soaked for 72 hours in 200 ml of 80 % ethanol and the extract was filtered with sterilized filter paper disk (10 mm) and the filtrate was separated using a rotary evaporator set at 60 rev/min at 45°C. Meanwhile for the hot water extract, 50 grams of the pulverized leaves of *E. indica* was boiled in 200 ml of distilled water for 30 minutes. The extracts were filtered using a sterile filter paper disc (10 mm) and stored in a clean and sterilized amber bottle.

Sub-study 1: Antibacterial Determination of E. indica

Preparation of Bacterial Culture of Bacteria

E. coli (ATCC 2592; AN 1964) and *S. aureus* (ATCC 6538; AN 1823) were used as test organisms. Nutrient agar slants and Nutrient broth were used to maintain the two organisms, and Mueller-Hinton Agar was used for the bio assay. *E. coli* and *S. aureus* were grown separately in nutrient broth for 24 hours at ambient room temperature. The growth of the bacteria was adjusted until its turbidity was the same as McFarland No.0.5. The comparable bacterial density is 1.5 x 10^8 cells/ml.

Disc Diffusion Assay

Disc diffusion assay was carried out in three replicates were adopted following the protocol of Dulay *et al.* (2014) with minor modifications. The sterilized paper discs were soaked in the dissolved crude extract of *E. indica* and allowed to dry inside the chamber. Streptomycin sulphate was used as the positive control and 80% ethanol for the negative control. For the bacterial inoculum, 0.2 ml of the inoculum was spread evenly in the assay plates using sterile cotton swab. The discs treated with the extract was seeded equidistantly using sterile needles. Several forceps were used in every treatment. The plates were properly labelled and incubated at room temperature for 24 hours in an inverted position. The zone of inhibition was measured using a digital vernier calliper. The zone of inhibition indicated the antibacterial property of the extract.

Sub-study 2. Teratogenic Activity of E. indica

The methodology for teratogenicity of *E. indica* was adopted from the previous study of Dulay el at. (2014).

Zebrafish Maintenanc

A glass aquarium approximately 21 cm (height), 15 cm (width) and 31 cm (length) containing untreated and clean tap water with continuous flow of oxygen via aerator was used to maintain adult female and male zebrafish at a ratio of 1 female : 2 male.

Acclimatization was carried out for one week at 26 ± 1°C (room temperature) condition prior to spawning and fertilization.

Zebrafish Spawning and Fertilization

Matured zebrafish were placed first in a coarse plastic mesh submerged into the aquarium water to protect the eggs from being devoured by the matured zebrafish once fertilized.

To stimulate spawning, dark condition was maintained for 12 hours by covering the aquarium with black bag. Fertilized eggs were seen in the bottom of the aquarium with the aid of the light.

Embryo Harvesting and Uniformity Assessment

To collect fertilized eggs, hose tubes were used. After 12 hours post fertilization it was transfer in a beaker. Morphological uniformity assessment was done before teratogenicity assay, unfertilized coagulated and ruptured eggs were discarded.

E. indica extract was diluted using embryo water (Table 2) Hank's solution by Westerfield, 2000. Different concentrations (10000ppm, 5000ppm, 1000ppm, 500ppm) were prepared in which three milliliters of each solution will be used.

Zebrafish (Danio rerio) teratogenicity and toxicity assay

Three embryos were placed in each well of ELISA plates containing three mililiters of each concentration of *E. indica* extract together with three fertilized eggs. Mortality, hatchability and morphological abnormalities were examine and was determined using a compound microscope for 12, 24, and 48 hours post treatment application and photos was taken using a camera. Number of heart rates and the LC 50 were also noted.

Results and discussion

Antibacterial Activity of E. indica

Results as presented in Table 1 and Table 2 revealed the potential of *E. indica* ethanol extract to inhibit the growth of *E. coli* at all incubation period while no inhibitory action against *S. aureus* was also revealed.

Against *E. coli*, zone of inhibitions were observed in discs treated with ethanol extracts with 15.08 mm at 12 hrs of incubation, 14.04 mm at 24 hrs, 10.05 mm at 36 hrs which was reduced to 2.78 mm at 48 hrs of incubation (Table 1).

Table 1. Antibacterial activity of *E. indica* leaf extracts against *E. coli.*

*E.indic*a extracts	Diameter zone of inhibition			
	12hrs	24hrs	36hrs	48hrs
Hot Water Extract	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}
Ethanol Extract	15.08^{a}	14.04^{b}	10.04^{ab}	2.78^{b}
(-) Control	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}
(+) Control	11.83^{a}	43.85^{a}	22.83^{a}	11.61^{a}

In the mean column, means having the same letter of superscript in the same column were not significantly different from each other at 5% level of significance using DMRT.

Table 2. Antibacterial activity of *E. indica* leaf extracts against *S. aureus.*

E. indica extracts	Diameter zone of inhibition			
	12hrs	24hrs	36hrs	48hrs
Hot Water Extract	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
Ethanol Extract	5.14^{b}	0. 00^{b}	0.00^{b}	0.00^{b}
(-)Control	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
(+) Control	25.26^{a}	51.93^{a}	46.40^{a}	30.33^{a}

In the mean column, means having the same letter of superscript in the same column were not significantly different from each other at 5% level of significance using DMRT.

Statistically, the zones of inhibition observed in plates treated with *E. indica* ethanol extracts were comparable of those of streptomycin sulfate at 12, 24 and 36 hrs of incubation. Meanwhile against *S. aureus* (Table 2), zone of inhibition was only observed in ethanol extract at 12 hrs of incubation with 5.14mm. Results showed the efficacy of ethanol extract as antibacterial agent against the *E.coli* while lack of antibacterial activity against *S. aureus* was also exhibited. This could be due to high resistance of *S. aureus* to antibacterial drugs (Nair *et al.*, 2005).This coincides with the study of Al-Zubairi *et al.*(2011) and Iberahim *et al.* (2015) wherein the antimicrobial potential of *E. indica* were tested .Antibacterial property of *E. indica* ethanol extract can be attributed to the presence of several phytochemical such as triterpenes, steroids, phenols, fatty acids, essential oils , anthraquinones, anthrones, tannins, flavonoids, alkaloids, and coumarins (Valentino *et al.* 2018).

Table 3. Mortality rate of *D. rerio* embryo.

Extract	Concentration (ppm)	Mortality rate (%)			LC 50	
		12hpa	24hpta	36hpta	48hpta	
Ethanol	10000	100^{a}	100^{a}	100^{a}	100^{a}	
	5000	100^{a}	100^{a}	100^{a}	100^{a}	
	1000	0.00^{b}	0.00^{b}	0.00^{a}	100^{a}	2200 ppm
	500	0.00^{b}	0.00^{b}	66.66^{b}	100^{a}	
	0.00	0.00^{b}	0.00 ^{b}	0.00 ^{c}	0.00	
Hot water						
	10000	0.00^{b}	0.00^{b}	0.00^{c}	33.33^{b}	
	1000	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{b}	
	5000	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{b}	12890 ppm
	500	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{b}	
	0.00	0.00^{b}	0.00^{b}	0.00 ^{c}	0.00 ^{b}	

In the mean column, means having the same letter of superscript in the same column were not significantly different from each other at 5% level of significance using DMRT.

Table 4. Hatchability of zebrafish embryos after 48 hours of exposure in various HWE and Ethanol extract concentrations of *E. indica* leaves.

Extract	Concentration (PPM)	Hatchability (%)
Ethanol Extract	10000	0.00^{b}
	5000	0.00^{b}
	1000	0.00^{b}
	500	0.00^{b}
	0.00	100^{a}
Hot Water Extract	10000	66.67^{a}
	5000	100^{a}
	1000	100^{a}
	500	100^{a}
	0.00	100^{a}

In the mean column, means having the same letter of superscript in the same column were not significantly different from each other at 5% level of significance using DMRT.

These phytochemicals were proven as sources of antibacterial efficacy (Krishnaraju, 2005). Flavanoids cause inhibiting action to nucleic acid, cytoplasmic membrane function, energy metabolism, attachment and biofilm formation, porin on the cell membrane, alteration of the membrane permeability, and attenuation of the pathogenicity (Xie *et al.*2015). Similarly, triterpenoids were active antibiotics both for gram negative and positive bacteria (Awanchiri *et al.* 2008).

Table 5. Mean percentage heartbeat rate of zebrafish embryos after 36 hours of exposure in various concentrations of HWE and Ethanol Extract of *E. indica* leaf.

Extract	Concentration (ppm)	Heartbeat rate (per minute)
Ethanol Extract	10000	N.H
	5000	N.H
	1000	54.00^{b}
	500	20.66^{bc}
	0.0	148.00^{a}
Hot Water Extract	10000	30.00^{de}
	5000	91.33^{bc}
	1000	84.66^{bc}
	500	121.33^{ab}
	0.0	148.00^{a}

Means in the same column having the same letter of superscript were not significantly different from each other at 5% level of significance using DMRT. The N.H. means no heartbeat.

Teratogenic activity of E. indica

Mortality rate of *D. rerio* embryos: The mortality rate of the embryos in different hours of exposure is shown in Table 3. Mortality is indicated by the coagulation of the embryo. In this study, 10000 ppm to 5000 ppm of ethanol extract showed 100% mortality as early as 12 hours while 100% mortality was recorded in 1000ppm and 500ppm after 48 hrs of incubation. For hot water extract, mortality was only observed at 10000ppm concentration after 48 hrs of incubation with 33.33% mortality. At 24 hrs of incubation the Lethality Concentration 50 of *E. indica* ethanol extract was computed at 2200pmm and 12890ppm for hot water extracts.

Table 6. Teratogenicity of various concentrations of *E. indica* leaves extracts at 12, 24, 36, and 48 hours of exposure.

Extracts	Toxicological endpoints	Time of exposure (hour)	Concentration (%)				
			0	0.05	0.10	0.50	1
E. indica ethanol extract	Lethal						
	Coagulation	12	-	-	-	+	+
		24	-	-	-	+	+
		36	-	-	-	+	+
		48	-	-	-	+	+
	No somites	12	-	-	-	-	-
		24	-	-	-	-	-
		36	-	-	-	-	-
		48	-	-	-	-	-
	No heartbeat	12	-	-	-	+	+
		24	-	-	-	+	+
		36	-	-	-	+	+
		48	-	-	-	+	+
	Tail not detached	12	-	-	-	-	-
		24	-	-	-	-	-
		36	-	-	-	-	-

		48	-	-	-	-	-
	Teratogenic	12	-	-	+	-	-
	Malformation of head	24	-	-	+	-	-
		36	-	-	+	-	-
	Malformation of tail	48	-	-	+	-	-
		12	-	-	-	+	+
		24	-	-	-	+	+
		36	-	-	-	+	+
	Scoliosis	48	-	-	+	+	+
		12	-	-	-	+	+
		24	-	-	-	+	+
		36	-	-	-	+	+
	Stunned Tail	48	-	-	-	+	+
		12	-	-	-	-	-
		24	-	-	-	-	-
		36	-	-	-	-	-
	Growth retardation	48	-	-	-	-	+
		12	-	-	-	+	+
		24	-	-	-	+	+
		36	-	-	-	+	+
	Limited Movement	48	-	-	-	+	+
		12	-	-	-	-	-
		24	-	-	-	-	-
		36	-	-	-	-	-
		48	-	-	-	-	-

Note: (+) = presence, (-) =absence.

This suggest that at 2200ppm (ethanol extracts) and 12890ppm (hot water extracts), 50% of embryo will die after 24 hours of exposure. Teratogenecity of *E. indica* can also be attributed to the phytochemicals, though it ehibit various functional activities such as antibacterial, anti-oxidant it can also pose toxic actions to the developing embryo (Jahfar, 2003).

Table 7. Teratogenicity of various concentrations of *E. indica* hot water extracts at 12, 24, 36, and 48 hours of exposure.

E. indica	Toxicological endpoints	Time of exposure (hour)	Concentration (%)				
Hot water Extract	Lethal						
	Coagulation	12					
		24	-	-	-	-	-
		36	-	-	-	-	-
	No somites	48	-	-	-	-	-
		12	-	-	-	-	-
		24	-	-	-	-	-
		36	-	-	-	-	-
	No heartbeat	48	-	-	-	-	-
		12	-	-	-	-	-
		24	-	-	-	-	-
		36	-	-	-	+	+
	Tail not detached	48	-	-	-	+	+
		12	-	-	-	-	-

	24	-	-	-	-	-
	36	-	-	-	-	-
	48	-	-	-	-	-
Teratogenic	12	-	-	-	-	-
Malformation of head	24	-	-	-	-	-
	36	-	-	-	-	-
Malformaton of tail	48	-	-	-	-	-
	12	-	-	-	-	+
	24	-	-	-	-	+
	36	-	-	-	-	+
Scoliosis	48	-	-	-	-	+
	12	-	-	-	-	-
	24	-	-	-	-	-
	36	-	-	-	-	-
Stunned tail	48	-	-	-	-	-
	12	-	-	-	-	+
	24	-	+	-	-	+
	36	-	+	-	-	+
Growth retardation	48	-	+	-	-	+
	12	-	-	-	-	-
	24	-	-	-	-	-
	36	-	-	-	-	-
Limited movement	48	-	-	-	-	-
	12	-	-	-	-	-
	24	-	-	-	-	-
	36	-	-	-	-	-
	48	-	-	-	-	-

Note: (+) = presence, (-) =absence.

Hatchability rate of *D. rerio* embryos: Effective hatching process is anticipated in normal embryos. The observed hatchability of the embryos exposed in different treatment concentrations of *E. indica* plant is presented in Table 4. As recorded, 100% of zebra fish hatched only at different concentrations of hot water extract of *E. indica*.

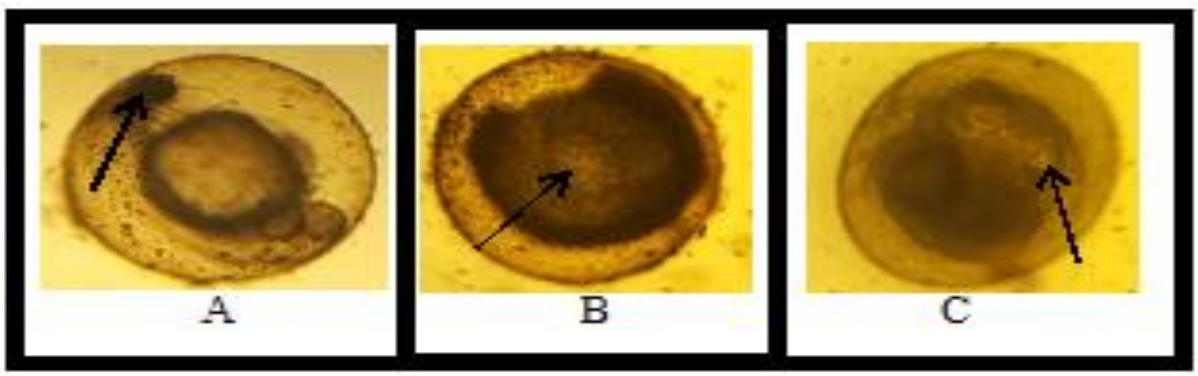

Fig. 1. The different morphological abnormalities of embryos treated with HWE and EOH extract of *E. indica* (A) stunted-tail at 1% HWE of *E. indica* in 12 hpta. (B) growth retardation in 1% EOH extract in 12 hpta. (C) yolk deformity in 1% EOH. extract in 12 hpta.

Heartbeat rate of *D. rerio* embryo: In this study, heartbeat rates were observed at pharyngula stage of *D. rerio* embryonic development. Heartbeat rates of *D. rerio* embryos exposed in different treatment

concentrations of *E. indica* extracts are presented in Table 5.

No visual heartbeat was observed in embryos after 36 hpta exposure in 10000 ppm, and 5000 ppm of *E. indica* ethanol extract due to coagulation as early as 12 hpta. These results clearly states that the higher the concentration on ethanol extract of *E. indica*, the more toxic it gets to *D. rerio* embryos. However, a mean heartbeat rate was recorded in hot water extract with 30/min, 91/min, 84.66/min and 121.33/min at 10000, 5000, 1000 and 500 ppm whereas in ethanol extract a mean heartbeat rate of 54/min and 20.66/min were recorded at 1000 ppm and 500 ppm. All of which are significantly lower than control with a heart rate of 148 beats/min. Therefore, exposure of zebrafish to different concentrations of *E. indica* extracts resulted to decreasing heartrate.

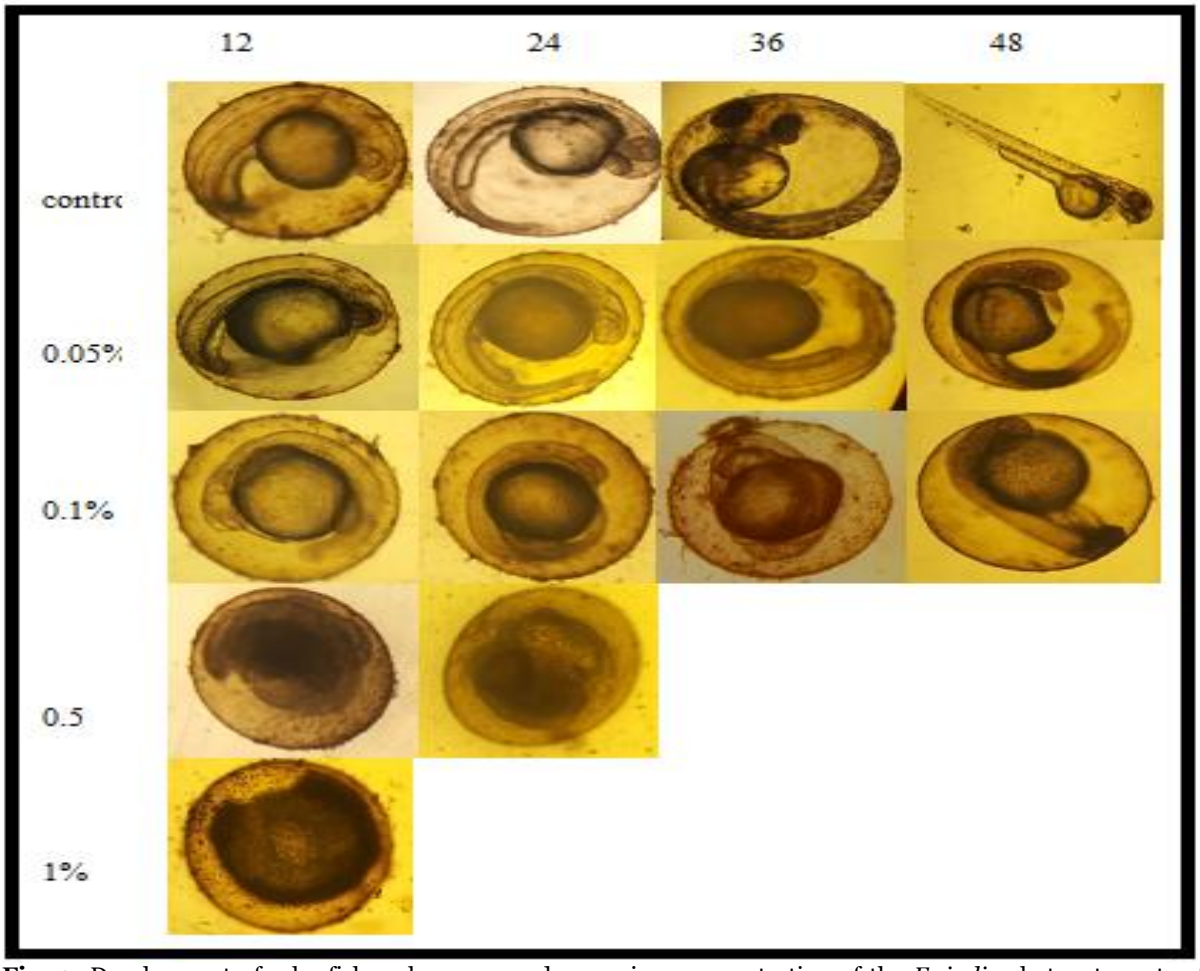

Fig. 3. Development of zebrafish embryo exposed on various concentration of the *E. indica* hot water extract after 12, 24, 36 and 48 hpta.

Malformation of the zebra fish embryo as influenced by E. indica extracts

Teratogenic effects were detected in different developmental stages of embryos exposed with various concentrations of leaves of *E. indica* (Fig. 1). Embryo with tail malformations of the embryos were mostly bent tail at 1000 ppm of *E. indica* leaves ethanol extract at 48 hpta and head malformation at 12 hpta. Moreover, growth retardation and curvature of scoliosis was observed at 5000 ppm concentration of *E. indica* ethanol extract (Table 6; Fig. 2).

Meanwhile, on hot water extract of *E. indica*(Table 7; Fig. 3), malformation of the tail was observed at 10000 ppm at 12hpta whereas, stunned tail was observed oat 24hpta. Lastly, no growth retardation was observed in zebra fish treated with hot water

extract of *E. indica*. Similar observations were made that some medicinal plants may cause abnormalities in specific parts of the developing embryo, it may not affect the mother but is harmful to the developing young (Baradaran. 2014).

The chemicals in medicinal plants may be natural to the plant, but foreign to the human body and may be harmful resulting in issues of safety and efficacy (Arsad *et al*., 2012). Additionally, flavonoids regulate growth and development which could be a protective mechanism against pathogens and solar radiation which could be harmful or beneficial(Talhi and Silva 2012; Agati, 2012).

Conclusion

Based from the results of the study it can be concluded that ethanolic extract of E. indica leaves is a potent antibacterial remedy against E. coli. In addition, teratogenicity of E. indica caused mortality with LC50 of ethanol and hot water extracts at 2200 ppm and 12890 ppm, respectively, and malformations of the zebra fish.

References

Agati G, Azzarello E, Pollastri S, Tattini M. 2012. Flavonoids as antioxidants in plants: location and functional significance. Plant Science **196**, 67-76. https://doi.org/10.1016/j.plantsci.2012.07.014.

Al-Zubairi AS, Abdul AB, Abdelwahab SI, Peng CY, Mohan S, Elhassan MM. 2009. Eleucine indica possesses antioxidant, antibacterial and cytotoxic properties. Evidence- Based Complementary and Alternative Medicine. https://doi.org/10.1093/ecam/nep091

Anderson D. 2003. Persistence of antibiotic resistant bacteria. Current Microbiology **6,** 452-456. http://dx.doi.org/10.1016/j.mib.2003.09.001

Arsad SS, Esa NM, Hamzah H. 2014. Histopathologic changes in liver and kidney tissues from male Sprague Dawley rats treated with Rhaphidophora decursiva (Roxb.) Schott extract. Journal of Cytology and Histology **S4,** 001. https://doi.org/10.4172/2157-7099.

Awanchiri SS, Van Dufat HT, Chishirri J, Dongfack MDJ, Nguenang GM, Boutefnouchet S, Fomum ZT, Seguin E, Verite P, Tillequin F, Wandji J. 2009. Triterpenoids with antimicrobial activity from Drypetes inaequalis. Phytochemistry **70 (3),** 419-423. https://doi.org/10.1016/j.phytochem.2008.12.017

Jahfar M. 2003. Glycosyl Composition of Polysaccharide from Tinospora Cordifolia Acta Pharmaceutica **53**, 65.

Baradaran A, Nasri H, Nematbakhsh M, Rafieian-Kopaei M. 2014. Antioxidant activity and preventive effect of aqueous leaf extract of Aloe Vera on gentamicin-induced nephrotoxicity in male Wistar rats. Clinical Therapeutics **165 (1),** 7–11 https://doi.org/10.7471/CT.2014.1653.

Cowan MM. 1999. Plant products as antimicrobial agents. Clinical Microbiology Review **12(4),** 564-582.

Dulay RMR, Kalaw SP, Reyes RG, Alfonso N, Eguchi F. 2012.Teratogenic and Toxic effects of Lingzhi or Reishi medicinal mushroom,Ganoderma lucidum (W.Curt.:Fr.) P. Karst. (Higher Basidiomytes), on zebrafish embryo as model. International Journal of Medicinal Mushrooms **14,** 507-512.

Iberahim R, Yaacob WA, Ibrahim N. 2015. Phytochemistry, cytotoxicity and antiviral activity of Eleusine indica (sambau). AIP Conference Proceedings **1678**, 030013. https://doi.org/10.1063/1.4931234

Koduru S, Grierson DS, Afolayan AJ. 2006. Antimicrobial activity of olanum aculeastrum. Pharmaceutical Biology **44(4),** 283–286.

Krishnaraju AV, Rao TVN, Sundararaju D. 2005.. Assessment of bioactivity of Indian medicinal plants using Brine shrimp (Artemia salina) lethality assay. International Journal of Applied Science and Engineering **2,** 125-134.

Lather A, Valecha R, Sharma K, Garg M. 2011. Worldwide potential of plants causing teratogenicity. Review Article Spatula **1(2),** 102.

Nair R, Chanda S. 2004. Antibacterial activity of some medicinal plants of Saurashtra Region. Journal Tissue Residence **4,** 117-120.

Rabe T, Van Staden J. 1997. Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethno pharmacology **56(1),** 81–87.

Srivastava J, Lambert J, Vietmeyer N. 1996. Medicinal plants: An expandingrole in development. World Bank Technical Paper.

Talhi O, Silva AMS. 2012. Advances in Cglycosyl flavonoid Research. Current Organic Chemistry **16(7)**, 859-896.

WHO. 2002. Traditional Medicine: Growing needs and potential who policy perspectives on medicines, World Health Organization, Geneva.

Xie Y, Yang W, Tang F, Chen X, Ren L. 2015. Antibacterial activities of flavonoids: structure-activity relationship and mechanism. Current Medical Chemistry **22(1),** 132-49.