ISSN- 2394-5125 VOL 07, ISSUE 09, 2020

IN VITRO ANTIBACTERIAL EFFECT OF *TRIDAX PROCUMBENS* L. DIETHYL ETHER LEAF EXTRACT AGAINST *AEROMONAS HYDROPHILA* AFFECTING GOLD FISH (*CARASSIUS AURATUS)*

S. Jeba Sheeba, Research Scholar, Department of Zoology, S.T.Hindu College, Nagercoil-629002, Affliated to Manonmaniam Sundaranar University, Tirunelveli

R. Raja Jeya Sekar, Assistant Professor, Department of Zoology, S.T.Hindu College, Nagercoil-629002, Affliated to Manonmaniam Sundaranar University, Tirunelveli

S.M.Vijila, Assistant Professor, Department of Zoology, Pioneer Kumaraswamy College, Nagercoil, Affliated to Manonmaniam Sundaranar University, Tirunelveli

ABSTRACT

In the present study, *Aeromonas hydrophila*, a bacteria isolated from infected goldfish (*Carassius auratus*), was tested for its antibacterial efficacy against *Tridax procumbens* leaf – diethyl ether extract. Diffusion disc plates on agar were used to measure the antibacterial activity. Different concentrations 25 % (0.25 µl), 50 % (0.5 µl), 75 % (0.75 µl) and 100 % (1.0 µl) of *T. procumbens* leaf - diethyl ether extract was tested against *A. hydrophila.* The *T. procumbens* leaf – diethyl ether extract was exhibited a significant level of inhibition against *A. hydrophila.* Maximum zone of clearance was measured after 24 hours of incubation at 37°C. The result of the present study revealed that the fish pathogen *A. hydrophila* was exhibited maximum zone of clearence (24 mm) at 100 % concentration of diethyl ether- leaf extract, where as the minimum zone of clearance 10 mm was recorded at 25 % of concentration. Moreover the current analysis was carried out to identify the bioactive constituents. The phytochemical analysis of the *T. procumbens* leaf –diethyl ether extract showed the presence of tannins, terpenoids, steroids and coumarins. These compounds were exhibited antibacterial activity against the fish pathogen *A. hydrophila.* As per the result of the present study, it can be concluded that phytochemicals were identified in *T. procumbens* leaf – diethyl ether extract was effective against *A. hydrophila,* a microbial pathogen that has infected goldfish (*C. auratus*).

Keywords: *A. hydrophila*, *T. procumbens*, diethyl ether, phytochemicals, antibacterial activity.

1. INTRODUCTION

The fish pathogen *A. hydrophila* is one of the most prevalent bacteria in fresh water environments globally [1]. *Aeromonas* sp. is a Gram-negative, Aeromonadaceae - family bacteria

that are facultative pathogens. Diverse bacterial species of *Aeromonas* sp. is responsible for the significant mortality of infected wild and farmed fishes, inflicting enormous economic damage [2]. It causes several zoonotic illnesses in human, fish and also linked to cellulitis, an infection that produces tissue inflammation [3, 4]. *Aeromonas* sp. causes diseases called gastroenteritis [5], an inflammation of the respiratory system, and diarrhoea followed by fever [6]. Sepsis is a deadly consequence of infections caused by *Aeromonas* sp. It has been identified as the most prevalent pathogenic bacterial species in aquaculture and is often responsible for lesions on fish farms and high death rates, which have provoked a global economic catastrophe in the aquaculture sector [7, 8].

In addition to affecting the microbiota of aquatic habitats, the unregulated use of antibiotics to suppress pathogenic microbes also resulted inappropriate changes to the microbiota of these systems [9,10]. Humans who routinely consume antibiotic-treated fish have developed serious health issues [11]. Additionally, fishes were produced immune system with damaged antibodies which works against specific, adaptive pathogenic bacteria [11, 12]. At present, numerous fish diseases are treated by using plant-based extracts in order to increase innate behaviour and immunity, as well as to prevent the spread of infection [13, 14,15]. Attempts have been made to use plant-based extracts to combat fish infections as a substitute for commercial medicines that protect certain pathogenic bacteria from resistance [16, 17]. In industrialized nations, 80 % of the population uses traditional medicines derived from plants, which act an effective therapeutic agent in both contemporary and traditional medical systems [18].

Since ancient times, *Tridax procumbens* L, a member of the daisy family Asteraceae is initiate perennially in various tropical and subtropical region as well as tenderly temperate regions worldwide [19]. *T. procumbens* L, frequently known as “coat buttons”. The leaves are zig zag and arrowhead-shaped [20]. The remedial properties of medicinal plants,search of no antimicrobial compounds is an urgent needs because of disclosure of drug resistance of bacteria [21]. *T. procumbens* has enormous antibacterial and antifungal potential [22]. The medicinal plant is globally distributed and available almost all seasons in almost all part of the country. *T. procumbens* has been used to treat typhoid fever, cough, epilepsy, asthma and diarrhea [23]. *T. procumbens* has various medicative properties including to immunomodulatory,anti oxidant, antihepatotoxic, analgesic, antidiabetic, anti inflammatory, antifungal and antimicrobial activities

[24]. This study based on the antibacterial activity of *T. procumbens* leaf –diethyl ether extract against *A. hydrophila* infected goldfish (*C. auratus*).

2. MATERIALS AND METHODS

2. 1 Collection of *T. procumbens* plant leaves

Fresh leaves of *T. procumbens* were collected from Kanyakumari District of Tamil Nadu, India and transported to laboratory. The leaves were rinsed with distilled water before being dried under the shade of sunlight and crushed finely. The sample were adequately crushed using a mortar and pestle to obtained a fine, homogenous powder, which was then stored in paper bags free from moisture [25].

2.2 Preparation of plant extracts

T. procumbens leaf extracts were obtained utilize a continuous extraction system (Soxhlet extractor) using the method given by Wang (2006) [26] for extracting plant leaves with organic solvents such as diethyl ether. In addition to 300 mL of diethyl ether, 30 g of plant powder was added to the thimble holder of the Soxhlet device (rate 1:10 w: v). In the thimble-holder of Soxhlet apparatus, a 75 % diethyl ether extraction solvent was utilized. Four hours were spent extracting until the solvent that emerged from the thimble turned colourless. Subsequently, in order to concentrate the extracts, they were dried using a rotating vacuum evaporator at temperatures below 40 ° C until the moisture content reached around 8 % (Dry basis). The crude extracts were filtered to obtain by using Whatman No. 1 filter paper. Samples were transferred in sterile vials and refrigerated at 4 ° C for future studies.

2.3 Phytochemical analysis of *T. procumbens* leaf-diethyl ether extract

The phytochemical analysis of crude *T. procumbens* leaf-diethyl ether extracts was conducted to determine the presence or absence of various bioactive constituents or secondary metabolites such as carbohydrates, coumarins, tannins, saponins, flavonoids, glycosides, phenols, proteins, steroids, and terpenoids using standard protocols [27].

2.4 Chromatographic purification of *T. procumbens* leaf extract

2.4.1 Thin Layer Chromatography of *T. procumbens* leaf extract

Thin-Layer Chromatography (TLC) using petroleum ether : acetone (3:1) solvent systems were used to identify the primary constituents contained in the *T. procumbens* leaf – diethyl ether extract.

ISSN- 2394-5125 VOL 07, ISSUE 09, 2020

The chamber is composed of a petroleum ether: acetone solvent, and the extract is delivered via capillary tubes on a TLC plate that has been pre-coated. To determine the spot of each applied leaves extract on the plate, a thin line and dots are drawn on the plate. After each mobile phase was applied to the TLC plate, it was air-dried and examined under ultraviolet light. The development of the separated bands as reflected by their retention factor (R_f) values was determined [28].

2.5 Collection of naturally infected goldfish - *C. auratus*

The infected gold fish sample was gathered from an aqua farm at Kanyakumari District of Tamil Nadu, India. Various kinds of clinical signs and behavioral alterations have been discovered and characterized in these infected gold fish. Under a microscope, scrapings from the body surface and fins of diseased gold fish were examined to detect and characterize the presence of moulds and other parasites [29].

The samples were placed in sterile tubes with strap closures, sample name, collection location, and collection date were recorded on the card number.

2.6 Isolation of bacterial strains from infected goldfish (*C. auratus)*

Naturally infected gold fish were dissected and the infected epidermis was homogenized with sterile PBS (Phosphate Buffer Saline). The sample was serially diluted to reduce bacterial proliferation [30]. The sample was then inoculated into nutrient agar using the spread plate method [31]. After examining and counting the colonies, the plates were incubated for 24 hours at 37^0C. On the basis of physical similarities, colonies were chosen and streaked on nutrient agar media until a pure culture was obtained. Pure colonies were picked up and streaked on nutrient agar slants and stored at 4°C for subsequent identification.

2.7 Characterization and identification of pathogens

A. hydrophila was identified by its colony form, gram nature, shape and motility. In addition, the isolates were subjected to the following biochemical tests to identify the bacteria based on their reactions: indole, methyl red, voges-proskauer, citrate utilization, nitrate reduction, hydrogen sulphide production, urease, catalase and oxidase. The outcomes of biochemical characterization were compared to those published in earlier studies [31].

2.8 Antibacterial activity of *T. procumbens* leaf – diethyl ether extract

Different concentrations of the extracts 25 % (0.25 µl), 50 % (0.5 µl), 75 % (0.75 µl) and 100 % (1.0 µl) were tested against *A. hydrophila*. In order to cultivate the bacterial strain,

ISSN- 2394-5125 VOL 07, ISSUE 09, 2020

nutrient agar was used. Agar-based diffusion disc plates were utilized to assess the antibacterial activity. The antibiotic disc was commercially available. The antibiotic disc were used as the positive control and placed on the centre of the agar plates.

Added different concentrations of leaf extracts of *T. procumbens* (25 %, 50 %, 75 % and 100 %) on each well. On the agar plate, the zones of inhibition (diameter in mm) were examined after each plate was incubated at 37°C for 24 hours. The antibacterial activity of solvent blanks produced in the same manner was examined [32].

3 RESULTS

3.1 Identification of pathogens and biochemical analysis

The isolated bacteria from infected goldfish (*C. auratus*) were identified as *A. hydrophila* based on their biochemical and morphological characterization.

3.2 Qualitative analysis of phytochemical analysis of *T. procumbens* leaf - diethyl ether extract

The phytochemical analysis indicated the presence of bioactive chemical compounds such as coumarins, tannins, saponins, flavonoids, glycosides, phenols, proteins, steroids, terpenoids, as well as carbohydrates. Table 1 showed the results of a qualitative phytochemical analysis of *T. procumbens* leaf – diethyl ether extract. The phytochemical analysis showed the *T. procumbens* leaf – diethyl ether extract recorded the presence of tannins,terpenoids, steroids and coumarins (Plate.1).

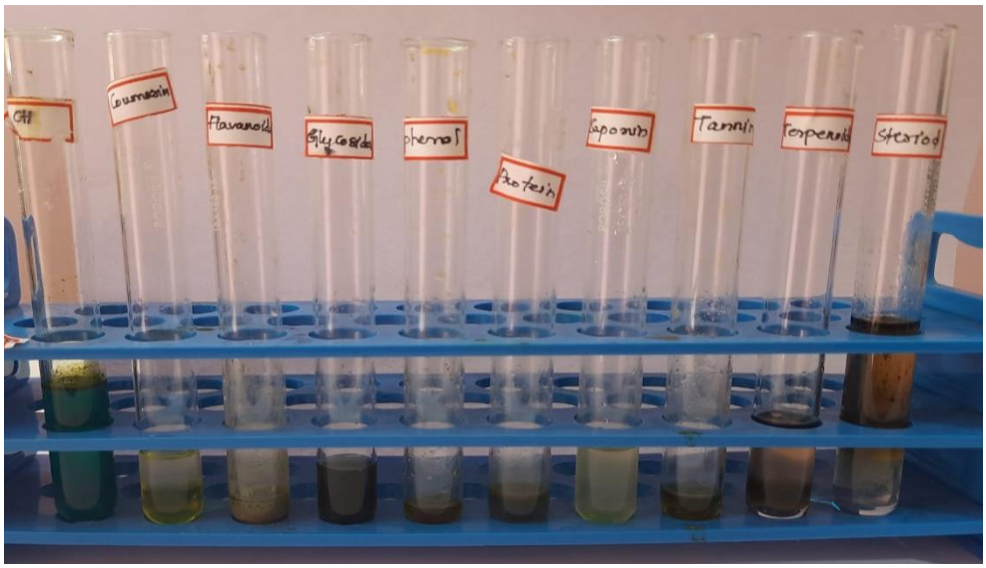

Plate 1: Phytochemical analysis of *T. procumbens* leaf – diethyl ether extract

Sl. No.	Phytochemical screening	Diethyl ether
1	Tannins	Positive
2	Flavonoids	Negative
3	Carbohydrates	Negative
4	Terpenoids	Positive
5	Phenols	Negative
6	Proteins	Negative
7	Steroids	Positive
8	Saponins	Negative
9	Coumarins	Positive
10	Glycosides	Negative

Table 1: Phytochemical constituents of *T. procumbens* leaf – diethyl ether extract

3.3 Thin Layer Chromatography of *T. procumbens* leaf –diethyl ether extract

Thin Layer Chromatography (TLC) was utilized using petroleum ether: acetone (3:1) solvent systems in order to identify the primary components that were found in the most effective extracts of *T. procumbens* leaves. Retention factor (R_f) values were obtained to express the movement of the separated bands (Plate .2).

$$RF = \frac{\text{Distance Traveled by the Compound}}{\text{Distance Traveled by the Solvent Front}}$$

ISSN- 2394-5125 VOL 07, ISSUE 09, 2020

Plate 2: Thin Layer Chromatography analysis of *T. procumbens* leaf –diethyl ether extract

Sl. No.	Colour of spot	R_f value of sample	Pigment
1	Greenish yellow	0.10	Violaxanthin
2	Grey	0.83	Pheophytin
3	Blue green	0.42	Chlorophyll b
4	Green	0.49	Chlorophyll a
5	Dark yellow	0.92	B – carotene
6	Yellow	0.40	Leutin

Table 2: Identifying the pigment and R_f value of sample

Thin-layer chromatography can be used to separate and distinguish the pigments in leaf extract (Table.2).

3.4 Antibacterial Activity of *T. procumbens* leaf – diethyl ether extract against *A. hydrophila*

The antibacterial activity of samples was evaluated against the identified strains of bacteria. The inhibition zone of sample extract was varying depending on the microorganism and

the solvent used for the extraction. The zone of inhibition indicated that the action of these samples against the bacteria. The antimicrobial activity of the *T. procumbens* leaf – diethyl ether extract samples was initially evaluated by agar plate diffusion method using *A. hydrophila* isolated from infected goldfish (*C. auratus).*

Sample	Solvent used	ZONE OF INHIBITION					
		Concentration leaves extract				Positive control (mm)	Negative Control DMSO (mm)
		0.25µl	0.5 µl	0.75 µl	1.0 µl		
T. procumbens	Diethyl ether	10 mm	13 mm	18 mm	24 mm	18 mm Tetracycline	No Zone

Table 3: Antibacterial activity of different concentrations of *T. procumbens* leaf – diethyl ether extract

.

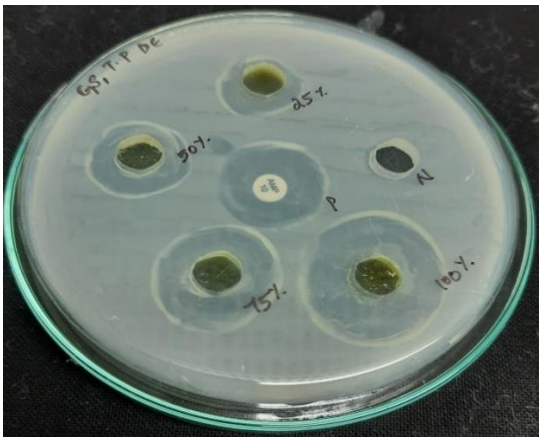

Plate 3: Antibacterial activity of different concentrations of *T. procumbens* leaf – diethyl ether extract

The efficacy of diethyl ether extract to inhibit the in vitro growth of *A. hydrophila* was showed in Table 3. The diethyl ether extracts of *T. procumbens* leaves were tested with four different concentrations of 25 %, 50 %, 75 % and 100 % respectively, against the fish pathogens of *A. hydrophila* (Plate 3). In the present study, *T. procumbens leaf* – diethyl ether extract leaves showed maximum zone of inhibition at the concentration of 100 % (24 mm), whereas the 75 %,

ISSN- 2394-5125 VOL 07, ISSUE 09, 2020

50 % and 25 % concentration of *T. procumbens* leaf – diethyl ether extract resulted the moderate zone of inhibition of 18 mm, 13 mm and 10 mm respectively.

4 DISCUSSION

Aeromonas hydrophila is an important bacterial pathogen as it infects both fish and humans [33]. *A. hydophila* infection in fish culture result in decrease in production and economic losses. Use of antibiotics or chemicals to control *A. hydrophila* infections have an adverse effect on the environment and also to the host by developing resistant strains of bacteria [34,35].

In the present study the antibacterial activities of *T. procumbens* leaf-diethyl ether extracts were tested against the bacteria *A. hydrophila*. The zone of inhibitions at 100 % concentration was 24 mm followed by 75 % (18 mm), 50 % (13 mm) and 25 % (10 mm) respectively. This present study reveals that the *T. procumbens* leaf diethyl ether extract was an effective in vitro against. *A. hydrophila*. Similar findings reported the methonolic extracts of *T. procumbens* leaf showed considerably high activity against *Bacillus subtilis* [36]. *T. procumbens* was inhibiting the growth of *B. subtilis, E. coli, F. oxysporium and T. reesei* [37]. The *T. procumbens* ethyl alcohol extract was to be most effective as an antimicrobial agent against *Pseudomonas vulgaris* [38]. The ethyl acetate extracts of *T. procumbens* leaf inhibited the growth of *Staphylococcus aureus* [39].

Phytochemicals are defined as bioactive chemical compounds produced by plants. In the present study the phytochemical analysis of *T. procumbens* leaf-diethyl ether indicated the presence of various compounds including Tannins, Terpenoids, Steroids and Coumarins. Similar findings were reported the great variety of secondary metabolities present in the plant are tannins, alkaloids, saponins, flavonoids and phenols have been a great source of important pharmaceutical compounds [40]. The phytochemical screening of the ethanol leaf extract of *T. procumbens* revealed the presence of saponins, tannins, terpenoids, flavonoids and glycosides [41]. The phytochemical screening suggest the presence of following phytochemicals such as tannins, alkaloids, saponins, flavonoids, phenols, steroids and anthocyanins were confirmed by phytochemical analysis of *T. procumbens* leaves [42,43].

The diethyl ether extract from *T. procumbens* leaves can be used as a substitute treatment for bacterial strains that cause fish disease. This study revealed that the *T. procumbens* leaf diethyl ether extract is effective in vitro against *A. hydrophila*. Treatment

with an extract of *T. procumbens* leaves seems most promising effect for the treatment of *A. hydrophila* infection in *C. auratus* gold fish.

5 CONCLUSION

The medicinal plants continue to be important therapeutic agents in both conventional and modern healthcare systems. The plant derived extracts are utilized to treat a variety of fish ailments in order to promote natural behavior and immunity as well as to prevent the transmission of infection. *T. procumbens* is a remarkable medicinal herb with a wide range of therapeutic characteristics, according to phytochemical analysis of its leaves. In this study, *A. hydrophila*, a bacterium isolated from infected goldfish (*C. auratus*) was resistant to *T. procumbens* leaf = diethyl ether extract, which exhibited promising antibacterial activity.

REFERENCES

1. Cipriano, R. C. 2001. *Aeromonas hydrophila* and motile aeromonad septicemias of fish. Fish disease leaflet 68 United States department of the interior fish and wildlife service division of fishery research, Washington, DC 20240.
2. Austin, B and Austin, D. A. 2012. Aeromonadaceae representatives (motile aeromonads). In *Bacterial Fish Pathogens*, pp. 119-146.
3. Seshadri, R., Joseph, S. W., Chopra, A. K., Sha, J., Shaw, J., Graf, J and Heidelberg, J. F. 2006. Genome sequence of *Aeromonas hydrophila* ATCC 7966T: Jack of all trades. *Journal of bacteriology*, *188*(23), 8272-8282.
4. Swann, L and White, M. R. 1991. Diagnosis and treatment of *" Aeromonas Hydrophila"* infection of fish. *Aquaculture Extension, Illinois-Indiana Sea Grant Program*. 91-2
5. Kobayashi, H. 2011. Structural and functional analysis of the serine protease from *Aeromonas sobria*. Yakugaku Zasshi Journal of the Pharmaceutical Society of Japan, *131*(12), 1781-1786.
6. Bravo, L., Morier, L., Castañeda, N., Ramírez, M., Silva, M and Castro-Escarpulli, G. 2003. *Aeromonas:* an emerging pathogen associated with extra intestinal infection in Cuba. *Revista Cubana de Medicina Tropical*, *55*(3), 208-209.

7. Hassan, M. A., Noureldin, E. A., Mahmoud, M. A and Fita, N. A. 2017. Molecular identification and epizootiology of *Aeromonas veronii* infection among farmed *Oreochromis niloticus* in Eastern Province, KSA. *The Egyptian Journal of Aquatic Research*, *43*(2), 161-167.
8. Fang, H. M., Ge, R and Sin, Y. M. 2004. Cloning, characterisation and expression of *Aeromonas hydrophila* major adhesin. *Fish & Shellfish Immunology*, 16(5), 645-658.
9. Resende, J. A., Silva, V. L., Fontes, C. O., Souza-Filho, J. A., de Oliveira, T. L. R., Coelho, C. M and Diniz, C. G. 2012. Multidrug-resistance and toxic metal tolerance of medically important bacteria isolated from an aquaculture system. *Microbes and environments*, ME12049.
10. He, S., Zhou, Z., Liu, Y., Cao, Y., Meng, K., Shi, P and Ringø, E. 2012. Do dietary betaine and the antibiotic florfenicol influence the intestinal autochthonous bacterial community in hybrid tilapia (*Oreochromis niloticus*♀× *O. aureus*♂). *World Journal of Microbiology and Biotechnology*, 28(3), 785-791.
11. Cabello, F. C. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental microbiology*, 8(7), 1137-1144.
12. Romero, J., Feijoó, C. G., and Navarrete, P. 2012. Antibiotics in aquaculture–use, abuse and alternatives. *Health and environment in aquaculture*, *159*, 159-198.
13. Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B and Sasal, P. 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. *Aquaculture*, *433*, 50-61.
14. Citarasu, T. 2010. Herbal biomedicines: a new opportunity for aquaculture industry. *Aquaculture International*, 18(3), 403-414.
15. Panigrahi, A and Azad, I. S. 2007. Microbial intervention for better fish health in aquaculture: the Indian scenario. *Fish physiology and biochemistry*, 33(4), 429-440.
16. Miranda, C. D and Zemelman, R. 2002. Antimicrobial multiresistance in bacteria isolated from freshwater Chilean salmon farms. *Science of the Total Environment*, 293(1-3), 207-218.

17. Seyfried, E. E., Newton, R. J., Rubert, K. F., Pedersen, J. A and McMahon, K. D. 2010. Occurrence of tetracycline resistance genes in aquaculture facilities with varying use of oxytetracycline. *Microbial ecology*, 59(4), 799-807.
18. Santos, P. R. V. D., Oliveira, A. C. X. D and Tomassini, T. C. B. 1995. Controlemicrobiologico de produtosfitoterápicos. *Rev. farm. bioquim. Univ. Säo Paulo*, 35-8.
19. Mundala,S and Shivhare,R 2010. Phamacology of *Tridax procumbens* a level, Review. International Journal of Pharm.Tech.Research,2(2),1391-1394.
20. Kumar S, Prasad A, Tyer SV and Vaidya S 2012. Pharmacognostical, phytochemical and pharmacological review on *Tridax procumbens* L. Linn. Int.j.Pharm.biol. 3(4):747-51.
21. Peterson.L.R and Dalhoff.A 2004. Towards targeted prescribing; will the cure for antimicrobial resistance be specific, directed therapy through improved diagnostic testing.J.Antimicrob.Chemother.53, 902-905.
22. Ali,M., Ravinder,E and Ramachamdram, R 2001. A new flavonoid from the aerial parts of *Tridax procumbens* L; Fitoterpia,72,313-315.
23. Nino.J., Navaez.D.M., Mosquera, O.M and Correa,Y.M. 2006. Antibacterial, antifungal and cytotoxic activities of eight Asteraceae and two Rubiaceae plants from Colombian biodiversity. Braz.J.Microbial.37,566-570.
24. Bhagwat, D.A.,Killedar, S.G and Adnaik, R.S. 2008. Anti-diabetic activity of leaf extract of *Tridax procumbens*.International Journal of Green Pharmacy,2(2),126-128..
25. Sujit S Kale and Amol S. Deshmukh 2014, *Tridax procumbens*: A medicinal gift of nature. *Asian Journal of Research in Biological and Pharmaceutical Sciences. 2(4), 159-162*.
26. Wang, L., and Weller, C. L. 2006. Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science and Technology*, 17(6), 300-312.
27. Catherine C. Ikewuchi, Jude C. Ikewuchi, Mercy O. Ifeanacho. 2015. Phytochemical Composition of *Tridax procumbens* Linn Leaves: Potential as a Functional Food. *Food and Nutrition Sciences 6(11)* :992-1004.
28. Anil Saini and Dr. Parvesh Gupta, 2018. Phytochemical Studies of the Leaves of *Tridax procumbens.* International Journal of Research and Analytical Reviews, 503z.

29. Mukti Chanda, Monjit Paul, Joydev Maity, Gadadhar Dash, Supriya Sen Gupta, Bidhan Chandra Patra, 2011. Ornamental fish gold fish, *Carassius auratus* and related parasites in three districts of West Bengal, India. Chronicles of Young Scientists 2(1) : 51-54.
30. Sarkar, A., Saha, M and Roy, P. 2012. Identification and Typing of *Aeromonas hydrophila* through 16S rDNA – PCR Fingerprinting. *J Aquac Res Development, 3, 146.*
31. Surya Kanta Samal, Basanta Kumar Das and Bibhuti Bhusan Pal, 2014. Isolation, biochemical characterization, antibiotic susceptibility study of *Aeromonas hydrophila* isolated from freshwater fish. *Int.J.Curr.Microbiol.App.Sci* 3(12): 259 – 267.
32. Asad Syed, Natarajan Benit, Abdullah A. Alyousef, Abdulaziz Alquasim and Mohammed Arshad, 2019. In-vitro antibacterial, antioxidant potentials and cytotoxic activity of the leaves of *Tridax procumbens*. *Saudi J Biol Sci*, 27(2) : 757- 761.
33. Janda, J.M., S.L, Abbott, S.Khashe, G.H.Kellogg and T. Shimada, 1996. Biochemical characteristics and Serological properties of the genus Aeromonas. *Journal of Clinical Microbiology*; 34:1930–1933.
34. Shariff, M., 1998. Impact of diseases on aquaculture in the Asia – Pacific region as exemplified epizootic ulcerative syndrome. *Journal of Applied Ichthyology*; 14: 139- 144.
35. Thayumanavan. T., G.Vivekanadan., K.Savithamani.R., Subashkumar and P. Lakshmanaperumalsamy, 2003. Incidence of haemolysin positive and drug resistant *Aeromonas hydrophila* in freshly caught finfish and prawn collected from major commercial fishes of coastal South India. *FEMS Immunology and Medical Microbiology;* 36: 41-45.
36. Syed Mohd, Danish Rizvi, Mohd.Zeeshan, Salman Khan, Deboshree Biswas, Othman A.Al-Sagair and Jamal M.Arif, 2011. Evaluation and distribution of antibacterial potential in the aerial parts of wild *Tridax procumbens. J.Chem.Pharm.Res., 3(2) : 80-87.*
37. Ankita Jain, D.V. Rao, Amla Batra and Amita Jain 2015. A Study On Antimicrobial Potential Of *Tridax procumbens* (L.) Against clinical isolates. International Journal of Pharmaceutical Sciences and Research Vol.6(3):1330-1335.
38. Santhosh Kumar, S., R.John and G.Lakshmi Narayanam 2015. Antimicrobial activity of *Tridax procumbens* leaf. International Journal of Pharma Sciences and ResearchVol.6(3).
39. Bharathi,V., B.Varalakshmi, S.Gomathi, A.ShanmugaPriya, T.Karpagam, 2012. International Journal of Pharma Sciences and Research Vol.3(4).

40. Debolina Dattaray 2019, Traditional Uses and Pharmacology of Plant *Tridax procumbens:* A Review. Sys Rev Pharm 13(7): 476-482.
41. David M.Musyimi and Pascalia Neema, 2019. Phytochemicals and Antibacterial Activities of Leaf Extract of *Tridax procumbens* Linn. On Staphylococcus aureus and Escherichia coli. East African Scholars J Biotechnol Genet: Vol-1(6): 149-154.
42. Jhample.S.B., S.B.Gajdhane., P.J.Kasabe., P.K.Bhagwat and P.B. Dandge, 2015. Phytochemical Screening and In vitro antimicrobial activity of *Tridax procumbens* L. Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences.1(1): 44-53.
43. Rajaram S. Sawant and Ashvin G.Godghate 2013. Premilinary Phytochemical Analysis of Leaves Of *Tridax procumbens* Linn. International Journal of Science, Environment and Technology, Vol.2(3): 388-394.