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## A Comparative antimicrobial analysis of *Tridax procumbens* L. various extracts on waterborne bacterial pathogens

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### ABSTRACT

The present study focussed on the bactericidal effect of *Tridax procumbens* L. against water borne bacterial pathogens. The bacterial species used in present study were *Escherichia coli*, *Vibrio cholerae*, *Salmonella typhimurium* and *Klebsiella pneumoniae*, which cause serious diseases like Diarrhoea, Cholera, Salmonellosis, Pneumonia, etc. CLSI recommended broth microdilution method was used in this study for assessing the antibacterial efficacy of the candidate plant extract. Results were depicted in the form of IC<sub>50</sub> (mg/ml) and MIC (mg/ml) values. On the basis of this study it can be interpreted that *Tridax procumbens* L. proved to be a very potential source of antibacterial agent against some water borne bacterial.

**Key Words:** Medicinal plants, CLSI, antibacterial susceptibility, diarrhoea, McFarland, microdilution.

### INTRODUCTION

Plants play a very prospective role, as it is a one of the most important component of the biodiversity. They act as a centralised key in maintaining the balance and stability of the earth's environment. Our universe is composed of about 5,00,000 species of plants (Sawant and Godghate, 2013). Herbal treatment has been originated from Greek very anciently, i.e., around 1600BC (Baker, 1970). Medicinal herbs in a treatment of various illnesses are performing an excellent role in all over the world. Several immeasurable diseases in human beings are cured by medicinal plants gifted by nature (Bushra et al., 2003). Now a day investigation of various extracts prepared from different medicinal plants by using various polar and non-polar solvents are being used as a potential source of antimicrobial agents and can be used for the development of formulation or drug (Bonjar and Farrokhi, 2004).

On the other part human is surrounded by various microorganisms such as bacteria, virus, fungi and many more which are highly pathogenic. As the time passes on bacterial microorganism become resistant to several antibiotics, at this stage several plants possessing antimicrobial potential becomes effective in the treatment of diseases caused by bacteria (Aniel and Naidu, 2011). Many reports have been developed on the antibacterial activity of the crude extract of the plant material inhibiting the growth and activity of various pathogens, different types of report have been studied but only fewer are in process of *in-vitro* study. Effect of several herbal plant based medicine are in practice for the development of many herbal drug in a market for consumption purposes (Greensfelder, 2000; Patwardhan et al., 2003, 2005). With the range of these views and several properties of the *Tridax procumbens* L. which shows the antimicrobial activity against both gram-positive and gram-negative bacteria (Mahato and Chaudhary, 2005), Anti-coagulant property (Mohammed et al., 2001), anti-

inflammatory (Nia et al., 2003) Anti-septic, insecticidal, Parasiticidal, to check haemorrhage from cuts, bruises, dysentery, diarrhoea and wounds (Saxena and Sosana, 2005), hepatoprotective (Vilwanathan et al., 2005), also used in wound healing (Raina et al., 2008) antidiabetic activity (Bhagwat et al., 2008) hypotensive activity (Salahdeen et al., 2004). *Tridax procumbens* L. shows the immunomodulating property (Tiwari et al., 2004; Oladunmoye et al., 2006) it also bears the insect repellent property (Rajkumar and Jebanesan, 2007).

On the basis of these activities of *Tridax procumbens* L. various extract such as acetone, 50% ethanolic, methanolic; of the whole plant was selected to interpret the antimicrobial action on water borne pathogens. In the present study comparative antibacterial effect of *Tridax procumbens* L. various extracts was seen against some water borne bacteria such as *Escherichia coli*, *Vibrio cholerae*, *Salmonella typhimurium* and *Klebsiella pneumoniae* which are the causal organism of diseases like Diarrhoea, Cholera, Salmonellosis and Pneumonia etc.

### MATERIALS AND METHODS

#### Collection of plant material and its Characteristic features

Plant material of *Tridax procumbens* L. belonging to family Asteraceae was collected from the Science Faculty, Department of Botany, University of Allahabad, Allahabad and identified on the basis of its morphological characters. Plant specimen was submitted to the Duthie herbarium, Department of Botany, University of Allahabad, Allahabad with voucher specimen number AUBD-2. *Tridax procumbens* L. also known as Ghamra in English it is popularly known as Coat buttons due to the presence of special type of flower. It is present all over the India as indigenous medicinal plant. In nature it is perennial herb having short, hairy blade like leaves. Corolla of the plant is yellow in colour. It is commonly grown in open places, crude textured soils of tropical regions, dry localities. It grows prostrate, annual, creeper herb (Jain and Jain, 2012).

#### Preparation of acetone, 50% ethanolic, methanolic extract of *Tridax procumbens* L.

Collected plant materials were washed thoroughly by tap water and kept for shade dry. Dried plant material was

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chopped into small pieces for the preparation of acetone, 50%ethanolic and methanolic extracts by using rotary evaporator apparatus.

### Procurement and maintenance of culture

Bacterial cultures were procured from MTCC, Chandigarh, India. Procured cultures were *Escherichia coli* MTCC No. 8936, *Vibrio cholerae* MTCC No. 3906, *Salmonella typhimurium* MTCC No. 3231, and *Klebsiella pneumoniae* MTCC No. 4032. The cultures of these water borne bacteria were maintained in Muller-Hinton broth, Nutrient Agar plates and slants (figure 1). Revival and sub-culturing of pathogens was done within every 15 days. Mother/stock culture were kept at 4°C for future culturing and studies. Prior to antibacterial testing, fresh re-cultures was done a week before.

### Antibacterial test

Antibacterial test was performed in 96 well micro titre plates in duplicate and minimum inhibitory concentration (MIC) of various extracts of *Tridax procumbens* L. was determined by Clinical and Laboratory Standards Institute (CLSI) recommended broth micro-dilution method (CLSI, 2008). Stock solution (50mg/ml) of extracts was prepared in DMSO. In brief, the initial bacterial inoculum suspension was prepared as per 0.5 McFarland standards (corresponding to a CFU of  $1.5 \times 10^7$  cell/ml). The MIC and IC<sub>50</sub> mg/ml values were obtained spectrophotometrically by (SpectraMax Plus384, Molecular Devices Corporation, USA) at 480 nm, after an incubation of 24 hrs at 35±2°C. 96 well plate was taken and setup was arranged as shown in figure 2.

## RESULTS AND DISCUSSION

Result was assayed on the basis of optical density in the form of turbidity; IC<sub>50</sub> and MIC Values by using spectrophotometer model no. - Spectramax plus 384 Molecular Devices, USA. Effects of *Tridax procumbens* L. various extracts on water borne bacterial pathogen, i.e. *Escherichia coli*, *Salmonella typhimurium*, *Vibrio cholerae*, *Klebsiella pneumoniae* was depicted in the form of IC<sub>50</sub> and MIC values (table 1).

Acetone extract was found effective against *E. coli* and *V. cholerae* whereas 50% ethanolic extract was found effective against *Klebsiella pneumoniae* and methanolic extract was effective only on *V. cholerae* (figure 3-5). In the present experiment, MIC and IC<sub>50</sub> value predict that tested water borne bacterial pathogens are sensitive to the acetone extract of *Tridax procumbens* L. whereas pathogens such as *Salmonella typhimurium* and *Klebsiella pneumoniae* are prevailing to be resistant from this *Tridax procumbens* L. acetone extract. Methanolic extract was found to be a good inhibitor of *V. cholerae* and ethanolic extract was found to inhibit the growth of *K. pneumoniae*. According to the study it can be presumed that plant based antibacterial drug have huge curative potential so they can serve for this purpose because it has lesser side effects on human health. The present result shows that *Tridax procumbens* L. is effective against gram negative water borne bacteria. Inhibitory effect of *Tridax procumbens* L. can be seen due to the presence of compounds like alkaloids, tannins, flavonoids and saponins etc. The present result proves that acetone extract can be employed for the development of formulation for the purification of water from bacteria like *Escherichia coli*, *Vibrio cholerae* from diseases like cholera and diarrhoea (Aniel and Naidu, 2011). As it is cleared from the previous literature that extract of organic

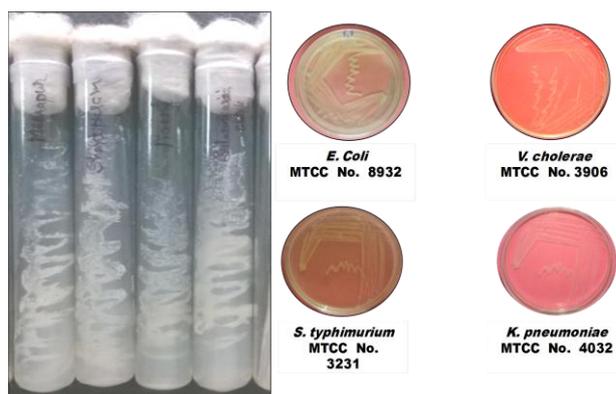


Figure 1: Cultures of *E. coli*, *V. cholerae*, *S. typhimurium* and *K. pneumoniae* procured from MTCC in slants and plates.

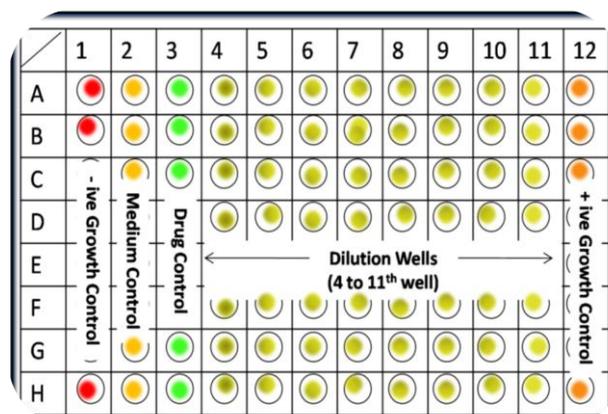
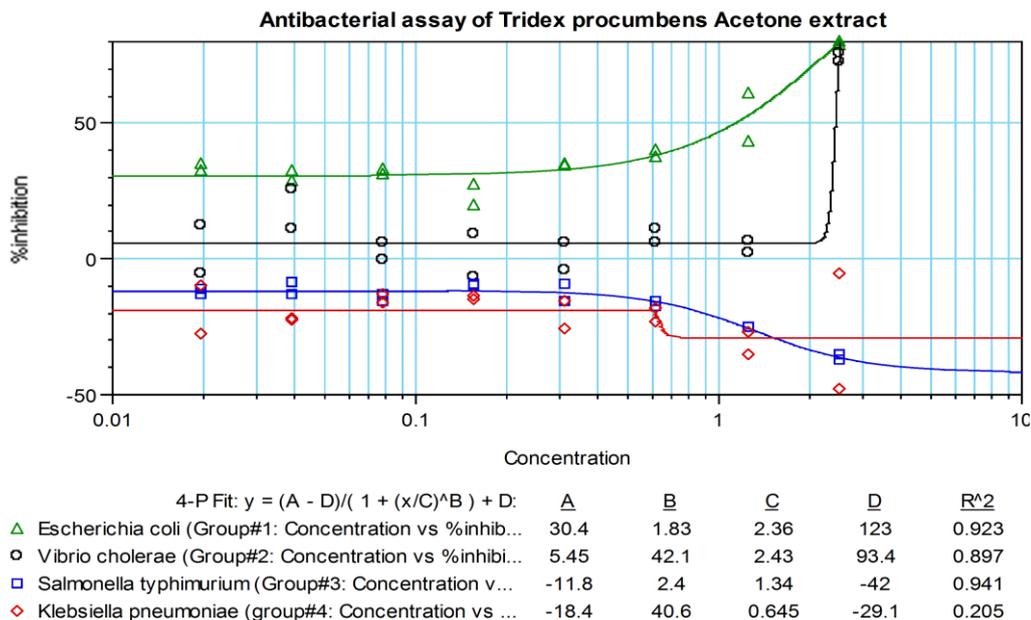


Figure 2: Pictorial representation of CLSI recommended broth micro-dilution protocol.

Table 1: Antibacterial assay of *Tridax procumbens* L. extracts against water- borne bacterial pathogens.

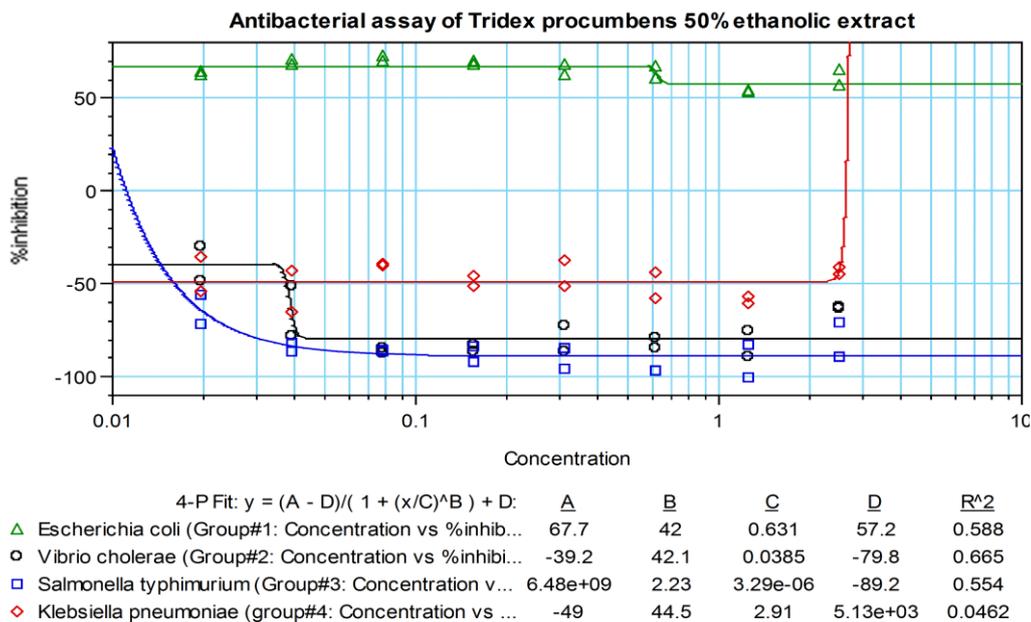
Selected Pathogens	Acetone extract		50% ethanolic extract		Methanolic extract	
	IC <sub>50</sub>	MIC	IC <sub>50</sub>	MIC	IC <sub>50</sub>	MIC
<i>E. coli</i>	1.151	2.544	-	-	-	-
<i>V. cholerae</i>	2.428	2.528	-	-	2.586	2.627
<i>S. typhimurium</i>	-	-	-	-	-	-
<i>K. pneumoniae</i>	-	-	2.667	2.684	-	-

IC<sub>50</sub> and MIC units are mg/ml.



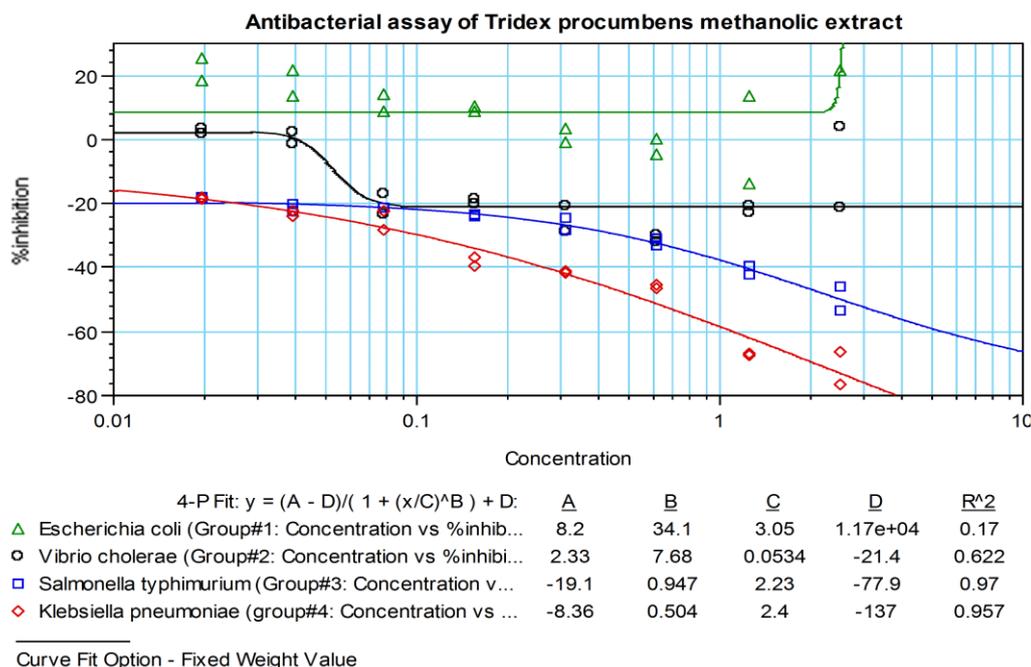
Curve Fit Option - Fixed Weight Value

**Figure 3:** Percent growth inhibition curve of *Tridax procumbens* L. acetone extract against *E. coli*, *V. Cholerae*, *S. typhimurium* and *K. pneumoniae*.



Curve Fit Option - Fixed Weight Value

**Figure 4:** Percent growth inhibition curve of *Tridax procumbens* L. 50% ethanolic extract against *E. coli*, *V. cholerae*, *S. typhimurium* and *K. pneumoniae*.



**Figure 5: Percent Growth inhibition curve of *Tridax procumbens* L. methanolic extract against *E. coli*, *V. cholerae*, *S. typhimurium* and *K. pneumoniae*.**

solvent are much better than aqueous extract for formulation purpose (Nair *et al.*, 2005).

## CONCLUSION

In this study an attempt was made for the study of herbal drug against waterborne bacteria such as *Escherichia coli*, *Salmonella typhimurium*, *Vibrio cholera* and *Klebsiella pneumoniae* in order to inhibit growth and pathogenesis of bacteria. On the basis of the above results, we can lead the formulation also for the purification of water against such types of bacteria and diseases caused by them.

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## REFERENCES

- Ali, M., Ravinder, E. and Ramachandram, R. (2001) Phytochemical communication a new flavonoid from the aerial parts of *Tridax procumbens*. *Fitoterapia*, Volume 72, Pages 313-315. [\[DOI\]](#)
- Aniel, K.O. and Naidu, L. M. (2011) Antibacterial potential of *Tridax procumbens* L. against human pathogens." *Pharma science monitors an international journal of pharmaceutical sciences*. Volume 2, Issue 32, Suppl-1 ISSN: 0976-7908.
- Baker, H.G. (1970) *Plants and Civilization*. 2nd ed. Macmillan Press Limited, New York. [\[DOI\]](#)
- Bhagwat, D.A., Killedar, S. G. and Adnaik, R. S. (2008) Anti- diabetic activity of leaf extract of *Tridax procumbens*. *Intl. J. Green Pharma*. Volume 2, Pages 126- 128. [\[DOI\]](#)
- Bonjar, G.H.S. and Farrokhi, P.R. (2004) Antibacterial activity of some plants used in traditional medicine of Iran. *Niger J Nat Proc Med*. Volume 8, Pages 34-39.
- Bushra, Beegum, N.R. and Ganga, T.D. (2003) Antibacterial activity of selected sea weeds from Kovalam South West coast of India. *Asian Journal of Microbio. Biotech Env. Sci*. Volume 5 issue 3, Pages 319-322.
- CLSI, Reference method for broth dilution antifungal susceptibility testing of yeasts, approved standard, CLSI Document M27-A3, CLSI, Wayne, Pa, USA, 2008, 3rd edition.
- Didry, N., Dureuil, L., Trotin, F. and Pinkas, M. (1998) Antimicrobial activity of the aerial parts of *Drosera peltata* Smith an oral bacteria. *J Ethnopharmacol*, Volume 60, Pages 91-96. [\[DOI\]](#)
- Greensfelder, L. (2000) Alternative medicine: Herbal product linked to cancer. *Science*.280:1946-9. [PubMed:10877707](#). [\[DOI\]](#)
- Jain, A. and Jain, A. (2012) *Tridax procumbens* L. A weed with immense medicinal importance: A review research article. *International Journal of Pharma and Bio Sciences*, ISSN 0975-6299 Volume 3, Issue 1.
- Mahato, R.B. and Chaudhary, R.P. (2005) Ethnomedicinal study and antibacterial activities of selected plants of Palpa district, Nepal. *Scientific World*. Volume 3 issue 3, Pages 26-31.
- Nair, R., Kalariya, T. and Chanda, S. (2005) Antibacterial activity of some selected Indian medicinal flora. *Turk J Biol* Volume 29, Pages 41-47.
- Nia, R., Paper, D.H., Essien, E.E., Oladimeji, O.H., Iyadi, K.C. and Franz, G. (2003) Investigation into in-vitro radical scavenging and in-vivo anti-inflammatory potential of *Tridax procumbens*. *Nigerian journal of physiological science* Volume 18, Pages 39-43.
- Oladunmoye, M.K. (2006) Immunomodulatory effects of ethanolic extract of *Tridax procumbens* on swiss Albino rats' orogastrically dosed with *Pseudomonas aeruginosa* (NCIB 950). *International journal of tropical medicine*, Volume 1, Issue 4, Pages 152-155.
- Pandey, A., Mishra, R.K., Tiwari, A.K., Kumar, A., Bajaj, A.K. and Dikshit, A. (2013) Management of Cosmetic Embarrassment Caused by *Malassezia* spp. with *Fruticose Lichen* *Cladia* Using Phylogenetic Approach. *Hindawi Publishing Corporation, Bio-Med Research International*, Article ID 169794. [\[DOI\]](#)
- Patwardhan, B., Vaidya, A.D. and Chorghade, M. (2003) Ayurveda and natural product drug discovery. *Curr. Sci*. Volume 86 Pages 789-99.
- Patwardhan, B., Warude, D., Pushpangadan, P. and Bhatt, N. (2005) Ayurveda and traditional Chinese medicine: A comparative overview. *CAM*. [PMCID: PMC1297513](#) Volume 2, Pages 465-73. [\[DOI\]](#)
- Raina, R., Prawez, S., Verma, P. K. and Pankaj, N. K. (2008). Medicinal Plants and their Role in Wound Healing. *VetScan*. Volume 3, Issue 1, Pages 221-224.

- Rajkumar, S. and Jebanesan, A. (2007) Repellent activity of selected plant essential oils against the malarial fever mosquito *Anopheles stephensi*. *Tropical Biomedicine*, Volume 24 Issue 2, Pages 71–75. PMID:18209711
- Raju, T.S. and Davidson, E.A. (1994) Structural features of water-soluble novel polysaccharide components from the leaves of *Tridax procumbens* L. *Carbohydrate Res*; 258, Pages 243-254. [\[DOI\]](#)
- Rathi, V., Rathi, J. C., Tamizharasia, S. and Pathak, A. K. (2008) Plants used for hair growth promotion: A review. *Pharmacognosy Review*, Volume 2, issue 3, Pages 185- 186.
- Salahdeen, H. M., Yemitan, O. K. and Alada, A. R. (2004) A Effect of Aqueous Leaf Extract of *Tridax procumbens* on Blood Pressure and Heart Rate in Rats. *African Journal of Biomedical Research*. Volume 7, Pages 27 – 29.
- Sawant, R. S. and Godghate, A.G.(2013)Preliminary Phytochemical analysis of leaves of *Tridax procumbens* L. *International Journal of Science, Environment and Technology*, ISSN 2278-3687, Volume 2, No 3, Pages 388 –394 .
- Saxena, V. K. and Sosana, A. (2005) b-Sitosterol-3-O-b-D- xylopyranoside from the flowers of *Tridax procumbens* Linn. *J. Chem. Sci.* Volume 117, Issue 3, Pages 263–266. [\[DOI\]](#)
- Srinivasan, D., Nathan, S., Suresh, T. and Perumalsamy, P.L. (2001) Antimicrobial activity of certain Indian Medicinal plants used in folkloric medicine. *J Ethnopharmacol*, Volume 74, Pages 217-220. [\[DOI\]](#)
- Suseel, L., Sarsvathy, A. and Brindha, P. (2002) Pharmacognostic studies on *Tridax procumbens* L. (Asteraceae). *Journal of Phytological Research*. Volume 15 Pages 141-147.
- Tiwari, A.K., Mishra, R.K., Kumar, A., Srivastava, S., Dikshit, A., Pandey, A. and Bajaj, K. (2011). Regular article, A comparative novel method of antifungal susceptibility for *Malassezia furfur* and modification of culture medium by adding lipid supplement. *Journal of Phytology*, ISSN: 2075-6240, Volume 3, Issue3 Pages 44-52.
- Tiwari, U., Rastogi, B., Singh, P., Saraf, D.K. and Vyas, S.P. (2004) Immunomodulatory effects of aqueous extract of *Tridax procumbens* in experimental animals. *Journal of Ethno pharmacology*, Volume 92, Pages 113–119. [\[DOI\]](#)
- Ved, D.K., Goraya, G.S. (2007) Demand and Supply of Medicinal Plants in India, National Medicinal Plant Board, New Delhi & FRLHT, Bangalore, India. PMID:17567413 PMCID:PMC2121243
- Vilwanathan, R., Shivashangari, K. S. and Devak, T. (2005) Hepatoprotective activity of *Tridax procumbens* against d- galactosamine/ lipopoly-saccharide-induced hepatitis in rats. *Journal of Ethnopharmacology*. Volume 101, Pages 55–60. [\[DOI\]](#)