INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
Volume-7, Issue-1 Jan-Mar-2017 Coden: IJPAJX-CAS-USA, Copyrights@2015ISSN-2231-4490
Received: 4th Nov-2016 Revised: 24th Dec-2016 Accepted: 25th Jan-2017
DOI: 10.21276/Ijpaes http://dx.doi.org/10.21276/ijpaes Research Article

EVALUATION OF HEMOSTATIC AND ANTIMICROBIAL ABILITY OF DIFFERENT PARTS OF PLANT *TRIDAX PROCUMBENS* WITH PHYTOCHEMICAL SCREENING.

Ashwini Kedari[1] and Dr. Rekha Gupta[2]

Department of Biotechnology, Modern College of Arts, Science and Commerce, Pune-411016.

ABSTRACT: The plant *Tridax procumbens* has been used in traditional medicine to cure several ailments. The plant extract is routinely used in Central India by herbalists and rural dwellers to stop bleeding from nose, gum and skin. Keeping this view in mind, the hemostatic ability was studied using different parts (leaf, stem and flower) of plant *T. procumbens* using methanol, chloroform, n-hexane and water solvent extracts with the goal of reducing the blood clotting time(CT) of human blood. Out of the tested solvent extracts, the methanolic flower extract showed the highest ability with reduced CT of 4:40± 0.08 min. The antimicrobial activity of the leaves, stem and flower of *T. procumbens* was tested by agar well diffusion method against two bacterial pathogens i.e. gram negative *Escherichia coli* and gram positive *Bacillus subtilis*. The flower extracts showed maximum antimicrobial activity against the tested strains i.e. 15.5±0.8mm and 16.8±1.0mm respectively. For more efficient results of the plant extracts as an antimicrobial source, molecular studies of DNA damage ability of bacterial genomic DNA was studied which showed the results of the two bacterial DNA i.e. *Escherichia coli* and *Bacillus subtilis* was effectively damaged by the plant extracts. With an naddition to this phytochemical screening of phenol and flavonoids were checked which showed comparative presence in the different plant extracts.

Key words: Hemostatic ability, antimicrobial, DNA damage, phytochemicals.

*Corresponding author: Ashwini Kedari. Department of Biotechnology, Modern College of Arts, Science and Commerce, Pune-411016., India E-mail: ashwinikedari777@gmail.com

INTRODUCTION

Tridax procumbens is a common medicinal herb of Family Asteraceae is found perennially throughout India. It has been commonly known as ghamara and coat buttons in English, is a native of tropical America. It was reported that in some tribal area of India, the leaf juice can be used to cure fresh wounds, stop bleeding and also as a hair tonic (Manjusha Borde et al., 2014). The demand of healthcare need has increased worldwide due to the emergence of various diseases and failure in the irradiation of the existing ailments. India has been gifted with rich medicinal plants which form the backbone of several ailments ranging from cough and cold to life threatening diseases. The World Health Organization (WHO) has also recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines (S. Ramya, et al., 2008) [27].

The plant T.procumbens is reported for various pharmacological activities like wound healing, antidiabetic activity, antimicrobial activity, immunomodulatory property (Jain Ankita and Amita Jain, 2012) [10]. A major stumbling block to the successful management of infectious diseases has been the propensity with which microorganisms are able to develop resistance to routinely used antibiotics. The emergence of pathogenic microorganisms that are resistant or multi resistant to a major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs (Peterson and Dalhoff, 2004) [19]. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics (such as hypersensitivity, allergic reactions, immunosupression etc.) and are major burning global issues in treating infectious diseases.

The antimicrobial compound of plant origin have enormous therapeutic potential and have been used since time immemorial. They have been proved effective in the treatment of infectious diseases simultaneously mitigating many of the side effects which are often associated with synthetic antibiotics (Awe and Omojasola, 2003) [24]. The study reveals that medicinal value of plant depends up on their phytochemical component including alkaloids, tannins, flavonoids and other phenolic compounds that produce and propagates physiological action in a human body (Mayura Kale et al., 2008) [14]. In the present scenario, there is an urgent and continuous need for exploration and development of cheaper, effective new plant based drugs with better bioactive potential and least side effects. Therefore, the present study aims to open new avenue for the improvement of medicinal uses of *Tridax procumbens* for hemostatic and antimicrobial activity. Hence, recent attention has been paid for studying the effect of T.procumbens on blood clotting time of normal human beings and to establish the antimicrobial activity of T.procumbens.

MATERIAL AND METHODS

Plant material

Tridax procumbens plant was procured from Botanical garden of Savitribai Phule Pune University, Pune. These were grown and established in well insulated plastic pots and maintained under controlled environmental conditions in the Department of Biotechnology, Modern College, Ganeshkhind, Pune, India. The plants samples were then used for fresh preparation of various extracts. The fresh plant material leaf, stem and flower were taken separately for preparation of extracts. These samples were washed with tap water, air dried and homogenised to a fine powder and stored in air tight bottles under low temperature in a refrigerator for further use.

Extraction

Extracts were prepared by polarity extraction method described by Verma *et al.* (2012) [30].

For present studies four different solvents (Hexane, Chloroform, Methanol and Water) were taken for preparation of extracts from three different parts of *Tridax procumbens* plant (leaves, stems and flowers separately). Out of this methanol, chloroform and water are polar solvents whereas hexane is a non-polar solvent. Hence a total of 12 different extracts prepared from 4 types of solvents. The extraction was carried out with 1g of fine powder of each part using 20ml of each solvent-Methanol, Chloroform and Hexane in a soxhlet apparatus separately for 24hours. The extracts were then filtered and concentrated in rotary evaporator. Dried extracts thus obtained were weighed and dissolved with appropriate amount of Dimethyl Sulfoxide(DMSO) and these dissolved extracts were used for further studies. For aqueous extraction, the fine powder of each part was soaked in 20ml distilled water for 24h and then filtered using Whatman filter paper no. 1. Extract collected were used for experimentation.

Blood Coagulation study

To study the hemostatic activity of the plant T.procumbens, clotting time (CT) of the various solvent extracts was determined in vitro by employing Lee-White's method. For the same, venous blood was collected in a sterile and dry test tube without the addition of an anticoagulant and the time required for clotting was noted. Venous blood was collected and stop-watch was started as soon as the blood entered the syringe. The test tubes were filled each with blood up to 1 ml mark; one test tube was labelled for normal CT. In the other set of test tubes, 0.5 ml of solvents were added to make the respective blanks and in the rest, 0.5 ml of extracts - methanolic extract, chloroform extract, hexane extract and water extract of leaf, stem and flower were added respectively. All these test tubes were then placed in water-bath at 37°C. Each of the test tubes was removed after every 45sec to check whether clotting had taken place. The watch was immediately stopped when there was clotting in a particular test tube and the time was noted in minutes.

Phenols

The total phenolic content of the different samples was estimated using method described by Wolfe at al. (2003) [11]. Folin-Ciocalteau reagent (0.125ml) in 0.125ml of each extract diluted in deionised distilled water. The reaction mixture was incubated for 5mins at room temperature after which 1.25ml of 7% sodium carbonate (Na_2CO_3) solution was added and the volume was adjusted to 3ml using distilled water. The mixture was incubated for 90min and absorbance was taken at 760nm by spectrophotometer. The measurement was then compared with Gallic Acid Equivalent (GAE) per gram of plant extract.

Flavonoids

Flavonoid content of *T. procumbens* was determined by the technique given in 2012 by Singh et al [26]. The reaction mixture contained plant extract (400 µl) + distilled water (800 µl) and 60 µl of 5% sodium nitrate. The reagents were allowed to react for 5min after adding 10% of aluminium chloride to it. Later 400µl of 1M NaOH was added to the mixture and the total volume was made up to 2ml with distilled water. The absorbance of the pink colour developed was determined at 510nm. The amount was calculated from the standard graph of Quercetin dehydrate and the results were expressed as mg of Quercetin dehydrate equivalent (QE) per gram of the extract.

Agar Well Diffusion method

The antimicrobial activity was tested by the method of Perez *et al.* (1990) [18] using fresh juice extract of leaves, stem and flower of *Tridax procumbens*. The bacteria used for the test included gram-positive organism *Bacillus subtilis (B. subtilis),* and gram-negative organism *Escherichia coli (E.coli JM 109).* These bacteria were cultured in Luria broth medium and incubated at 37°C for 24 hrs. The inoculations of microorganisms were prepared from the bacterial culture. The inoculums suspension was spread uniformly over the agar plates using spreader, for uniform distribution of bacteria. Subsequently using a sterile borer, well of 0.5cm diameter was made in the inoculated media in addition to 0.1ml of each extract and control Ciprofloxacin (10µg) was filled into the well. Later the plates were placed at room temperature for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24 hour at 37°C for room temperature. The result was recorded by measuring the diameter of inhibition zone at the end of 24-72 hour. Zone of inhibition surrounding the disc was measured using a transparent ruler and the diameter was recorder in mm.

DNA damage assay in bacteria

DNA isolation - The genomic DNA was isolated by the method of Hassan A.M. El. Demerdash, 2012[8] with some modifications. The microorganisms *E.coli JM 109* and *Bacillus subtilis* were cultured in Luria agar medium at 37°C for 24hrs. After confirmation of the bacterial growth by gram staining, 50µl of the fresh leaves, stem and flower extracts of the plant was added in the previous three different culture flasks. Then, 1.5ml of the extract added bacterial culture was taken in the eppendorfs and subsequently was spin at 5000rpm for 5min. The bacterial pellet was then collected and 10µl Proteinase K, 10µl lysozyme, 40µl of 10% SDS, and 150µl of PBS (Phosphate buffer saline) was added to it which was then incubated at 40°C for 1 hr. After the incubation equal volume of PCI was added, mixed well and was vortex properly. Additionally, there was spin at 10,000 RPM for 10min for phase separation – aqueous phase (upper phase), inter phase and organic phase. The DNA is mostly present in the aqueous phase. Then, the aqueous phase was collected in a new tube. And equal volume of chilled absolute ethanol was added. Mixing has to be done by inverting the tube several times. Later, a spin at 10,000 rpm for 10min has to be given to the tube for the DNA pellet to be visual. The finally obtained DNA pellet was dissolved in nuclease free water.

Agarose gel electrophoresis: 1% agarose gel was prepared containing 3µg/ml in 1X TAE buffer. The gel was poured in the gel cassette and the comb was arranged to prepare wells and allowed to solidify. 6µl gel loading dye was added to the reaction mixture and electrophoreses in 1% agarose gel at 100V till the dye front travels 1/3rd distance. The gel was viewed under transilluminating UV light.

RESULTS AND DISCUSSION

Blood Coagulation study

Clotting time determination is a routine laboratory test for coagulation factor deficiency and increase in normal clotting time thus signifies these deficiencies in coagulation. The process of hemostasis which involves three processes: vasoconstriction, platelet plug formation and clot formation. In the last process, coagulation occurs in the blood which has come out of the blood vessel (extrinsic clotting) as well as within the occluded vessel by vasospasm (intrinsic clotting), and the plugs are formed due to extra vascular as well as intravascular clots, respectively and to relate between clotting time and hemostatic activity this process was considered(Godkar, 1994)[6]. The effect of various extracts (methanol, chloroform, n-hexane and water) of leaves, stem and flower of Tridax procumbens on human clotting time was observed in vitro by Lee White Method.

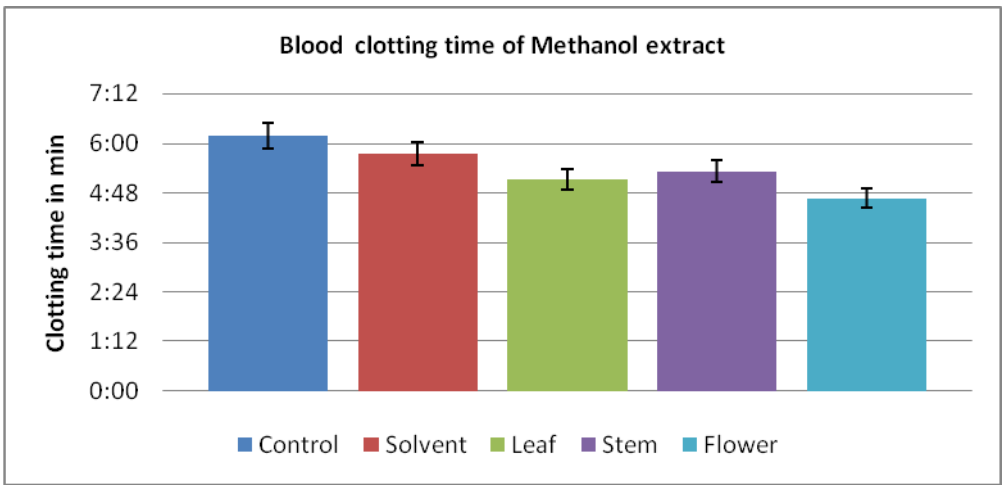

Fig -1 Graphical representation of blood clotting time of leaf, stem and flower in methanolic extract.

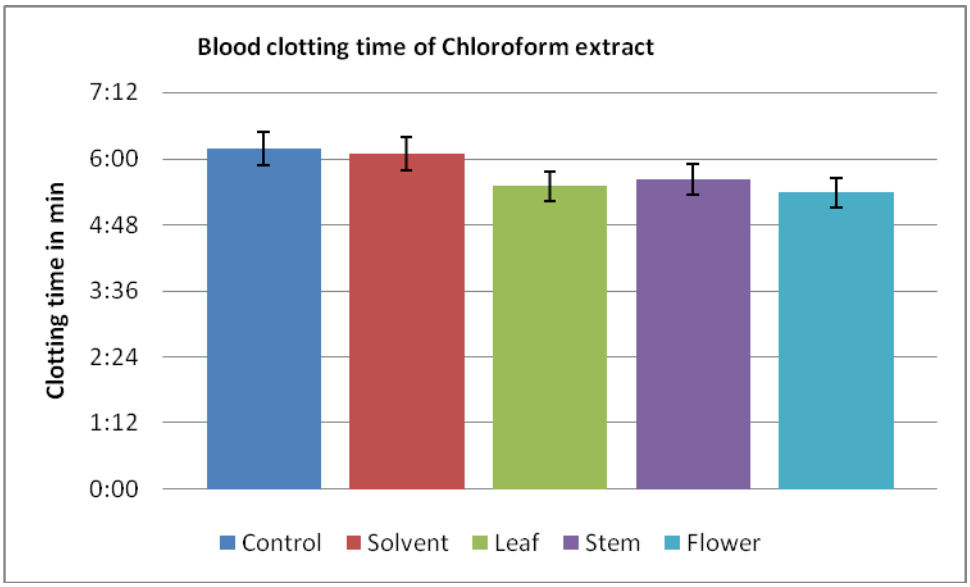

Fig -2 Graphical representation of blood clotting time of leaf, stem and flower in chloroform extract.

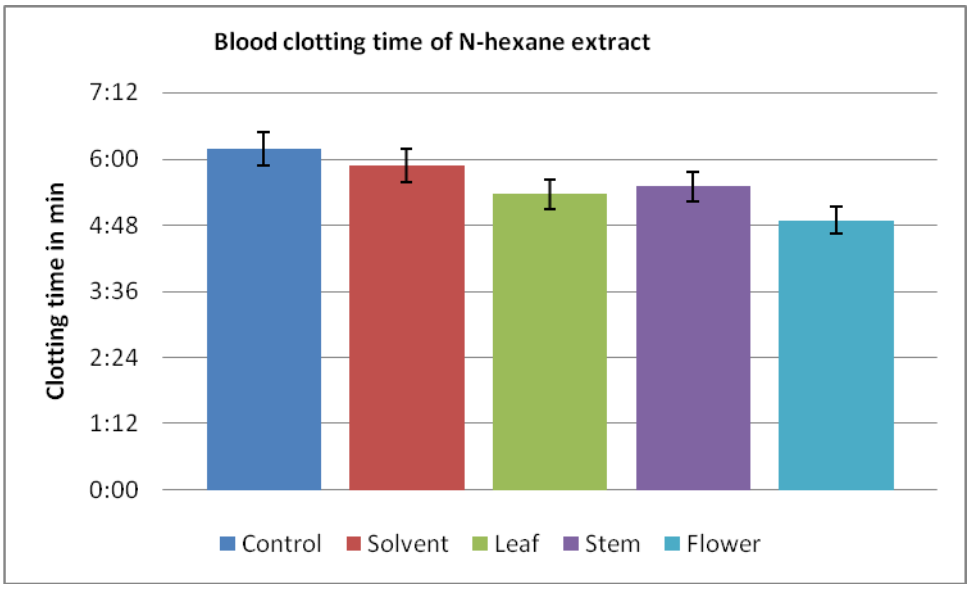

Fig -3 Graphical representation of blood clotting time of leaf, stem and flower in n-hexane extract.

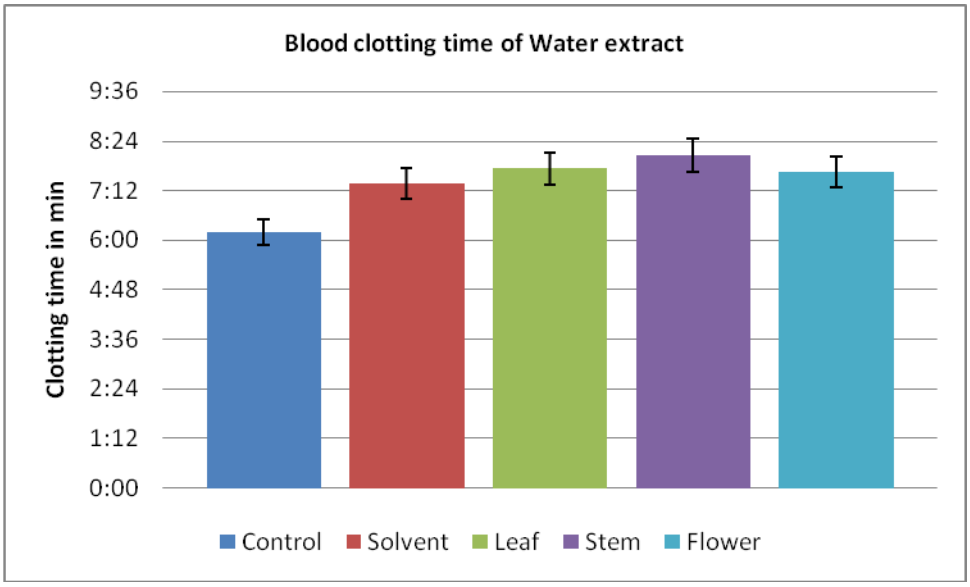

Fig-4 Graphical representation of blood clotting time of leaf, stem and flower in water extract.

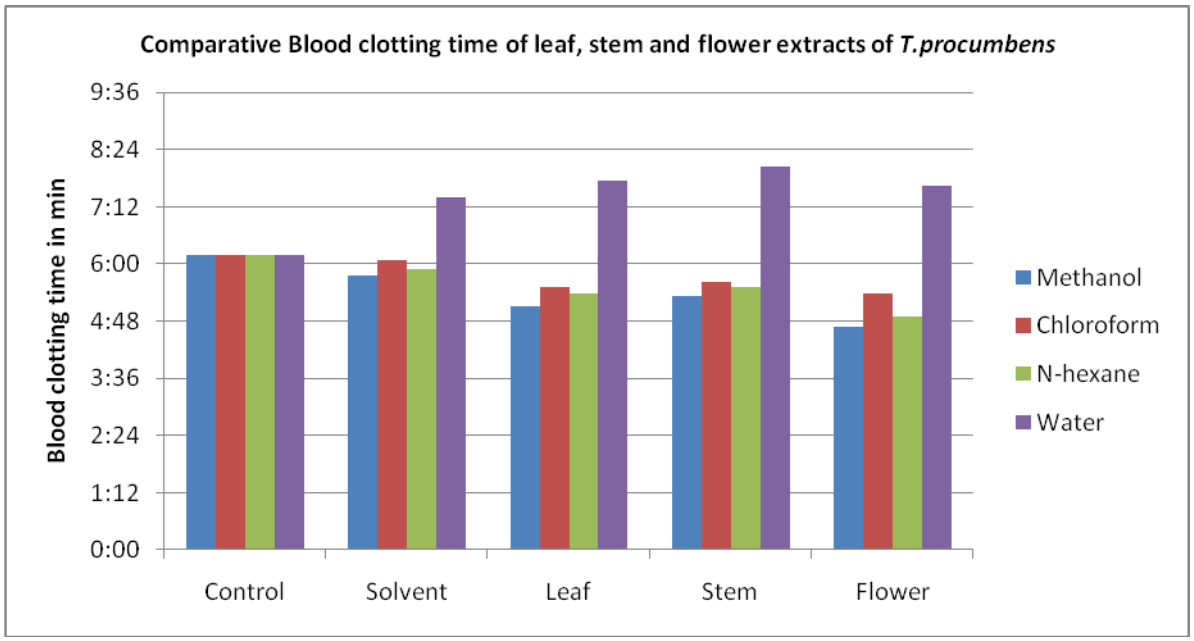

Fig -5 Graphical representation of comparative blood clotting time of leaf, stem and flower in methanol, chloroform, n-hexane and water extract.

The control which was only normal human blood without anticoagulant and extract showed the CT(clotting time) as 6:11 ± 0.01min whereas the CT of solvents were 5:45± 0.02min, 6:05± 0.02min, 5:53± 0.02min, 7:23±0.02min for methanol, chloroform, n-hexane and water which is depicted in image no. 1,2,3 and 4 respectively. The lowest blood clotting time was observed in the methanolic extract of flower i.e. 4:40± 0.08 min whereas the methanolic extract of leaf and stem also showed reduced clotting time i.e. 5:07± 0.02min and 5:19 ± 0.01min respectively compared to other extracts. The chloroform extract of leaf and stem showed CT 5:30±0.02min, 5:37 ±0.02min respectively but flower showed relatively less clotting time i.e. 5:23±0.02min. The n-Hexane extract showed lower clotting time compared to chloroform extracts in which the flower showed the least clotting time of 4:53± 0.02min whereas leaf extract showed 5:22± 0.02min and stem extract showed 5:30± 0.02min. The water extract showed increased clotting time i.e. 7:44± 0.01min, 8:03±0.02min, 7:39± 0.01 of leaf, stem and flower respectively compared to the control, solvent and other extracts portrayed in fig-5 which indicates that there is decrease or no solubility of the bioactive compound in the water extracts responsible for blood clotting. The difference in activity between the water and other solvent extracts can be explained by the fact that different solvents have varying capacities to extract phytoconstituents based on their solubility and polarity. Thus, it indicates that the methanol, n-hexane and chloroform extracts with could have led to better extraction of these bioactive principles with enhanced hemostatic activity of the plant in the present study.

Phytochemical screening

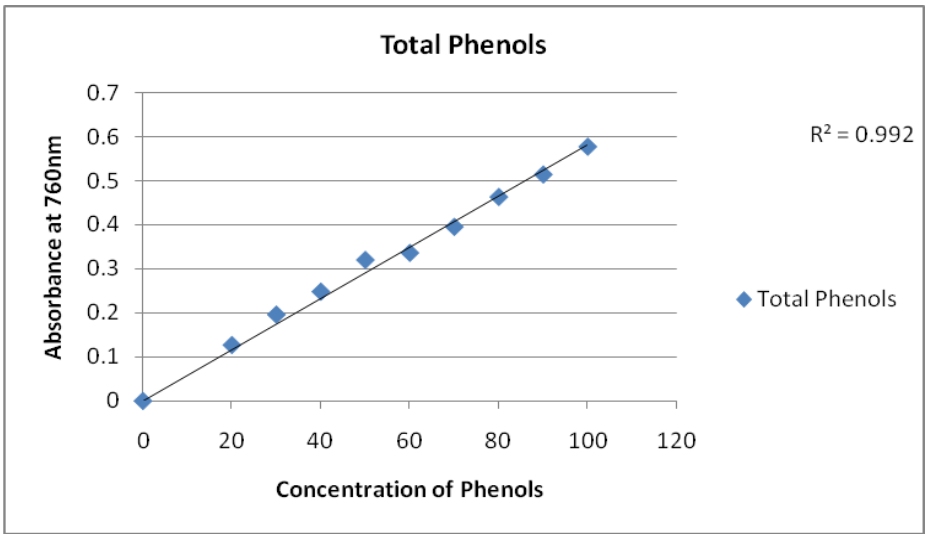

Fig -6 Calibration curve of phenols expressed in Gallic Acid Equivalent (GAE)

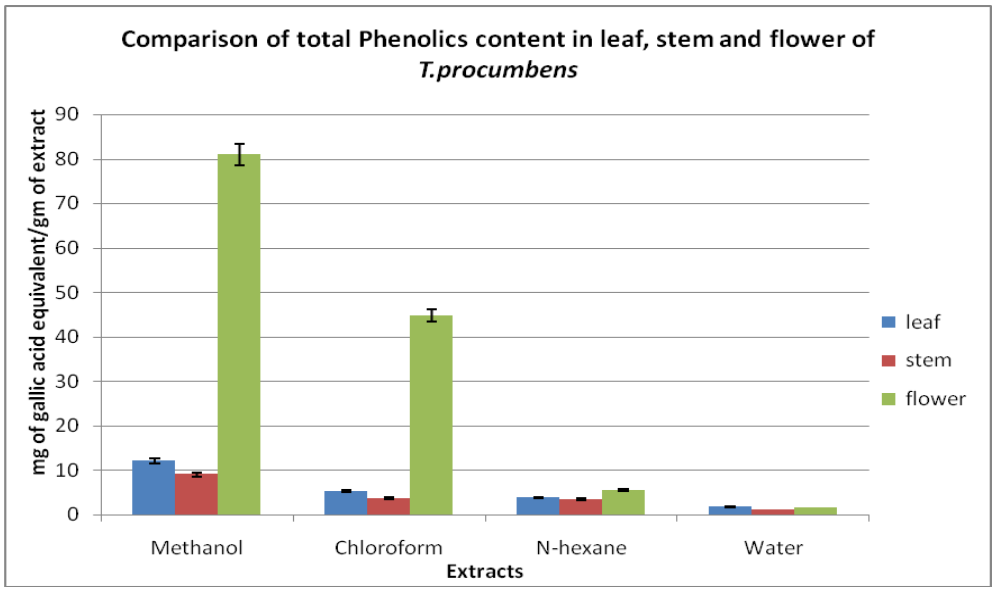

Fig -7 Total Phenols expressed in Gallic Acid Equivalent (GAE) per gram of plant extract.

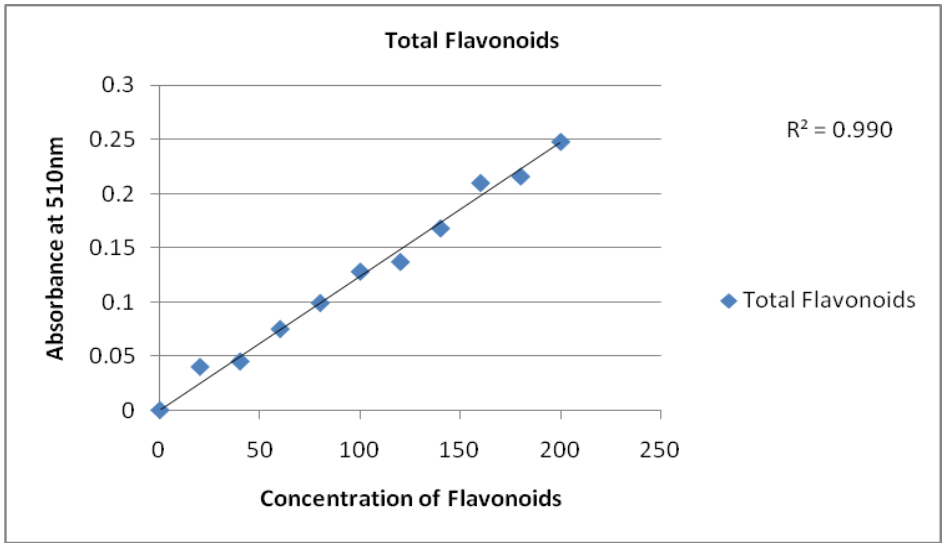

Fig -8 Calibration curve of flavonoids expressed in Quercetin dehydrate equivalent (QE)

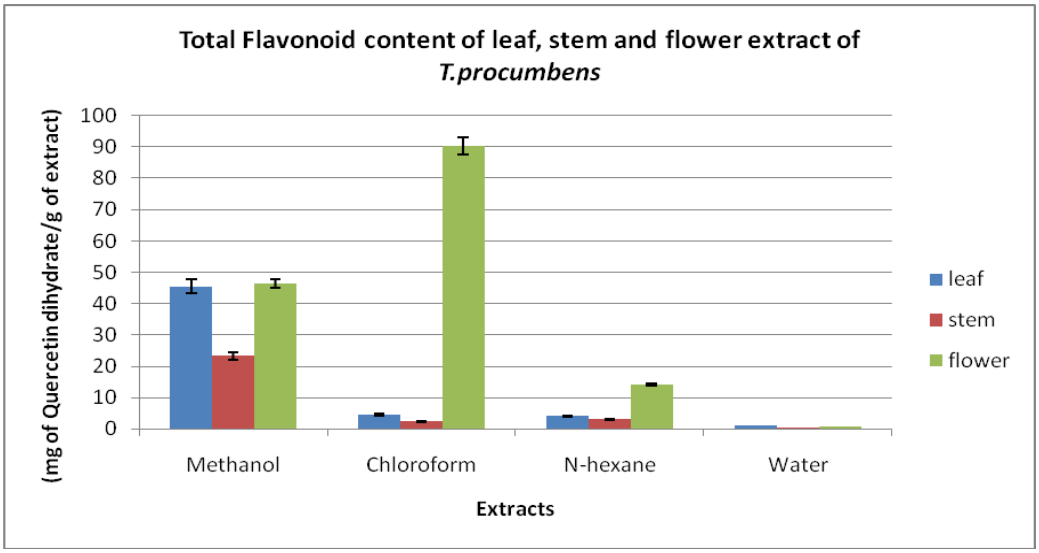

Fig -9 Total flavonoids expressed in mg of Quercetin dehydrate equivalent (QE) per gram of the extract.

The amount of various phytochemicals estimated during present studies showed overall much higher quantity of all these secondary metabolite found in the flower extracts all the solvents studied. Analysis of the present studies revealed a relatively high phenolic content in flower as compared to leaf and stem samples of the plant (Fig -7). The flower samples also estimated to have relatively much higher quantity of phenols of all the four solvents used for extractions. The maximum amount of phenols was observed from methanolic extract followed by chloroform, hexane and very less in water. Similarly same pattern has been observed in the total flavonoid content estimated from leaf, stem and flower in different solvents. The flower samples are again showing more amount of flavonoid content in all the solvents. However, more amounts of flavonoids were estimated in chloroform extract (Fig -9). The result shows that the plant is a rich source of total phenols and flavonoids which are highly effective in blood coagulation. Similarly results on quantity of phenols and flavonoids were observed by Khan et al. in 2012 [20] who studied antioxidant activity of various solvent extracts in Sonchus asper. They also observed more amount of phenols and flavonids compounds in methanol extract followed by chloroform>ethyl acetate>nhexane.

Antimicrobial activity

Table 1 – Antimicrobial inhibition zone

	Diameter of inhibition zone in mm			
Organisms	Control	Leaf	Stem	Flower
E.coli	14.2±0.5	14.9±0.5	14.2±0.6	15.5±0.8
B.subtilis	14.5±0.5	16.2±0.7	15.6±0.4	16.8±1.0

The antimicrobial activity of different parts of plant *Tridax procumbens* was studied in the present investigation which revealed that the flower of the specimen plant has the highest antimicrobial activity compared to leaf and stem. The flower extract showed maximum antimicrobial activity against *B.subtilis*(16.8±1.0) and E.coli (15.5±0.8) whereas the leaf extract showed higher antimicrobial activity against *B.subtilis* (16.2±0.7) and lower activity against *E.coli* (14.9±0.5). The stem extract showed low antimicrobial activity against *Bacillus subtilis* (15.6±0.4) and E.coli (14.2±0.6) compared to flower and stem showed in table no. 1. While work done by (Anjana Sharma et al., 2009) [1] using aqueous extract of plant *Terminalia chebula* and *Zinziber officinale* showed the zone of inhibition against *E.coli* (9mm,0mm) which manifest that the present study of the plant T.procumbens is more utile and potential as a new source of antimicrobial agent. Sharma et al. (2008) [25] found no activity of extract of leaves against *E. coli* and *S. aureus* while the flower extract showed antimicrobial activity.

Tridax procumbens can be attributed to the presence of flavonoids and phenols which are substances known to have several mechanisms of action such as inhibition of DNA gyrase, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, etc. (Cushnie and Lamb, 2005) [4]. Flavonoids obtained from tea are known to be inhibitory to a number of microbes including phytopathogens such as *Pseudomonas* (Friedman, 2007*)* [5]. The finding of phytochemicals such as flavonoids and phenols in the extract of *T. procumbens* along with the ability to inhibit microbes reiterates the fact of being a potential antimicrobial source and a new drug formulation.

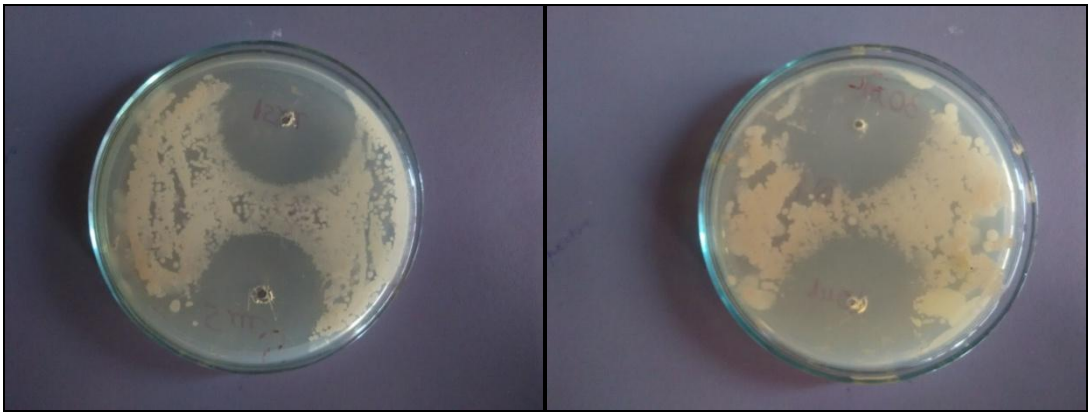

Fig -10 Inhibition zone of *B.subtilis*. **Fig -11. Inhibition zone of *E.coli*.**

Estimation of DNA damage in bacterial genomic DNA

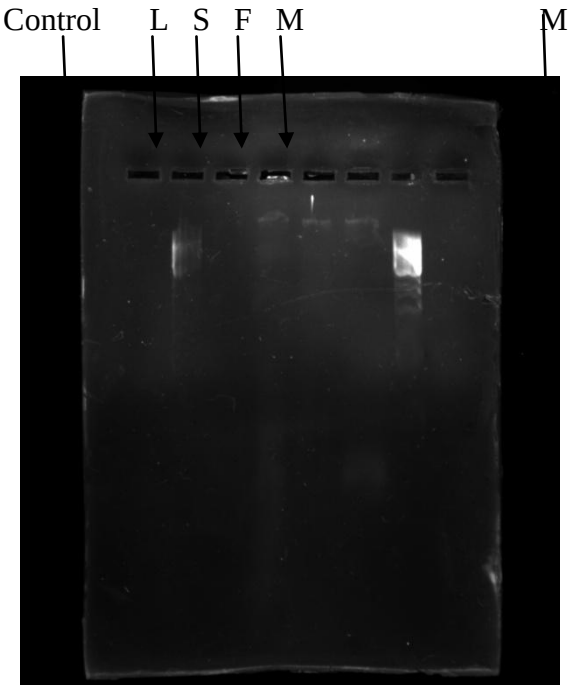

Fig -12. DNA damage of *E.coli*

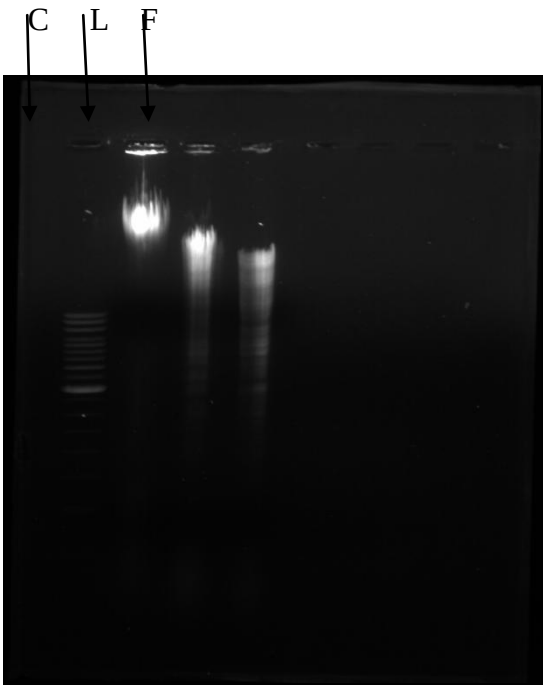

Fig -13. DNA damage of *Bacillus subtilis*

C: Control
L: leaf extract+DNA
S: stem extract+DNA
F: flower extract+DNA
M: Marker DNA

It is clear from fig 12&13 that the plant extracts effectively damage the bacterial genomic DNA indicating a clear smear of damaged DNA and smear indicates extensive DNA fragmentation(Sreemanti Das et al.,2012) [28] whereas the control shows nearly no smear of DNA. The DNA fragmentation is the separation or breaking of DNA strands into pieces which is a biochemical hallmark of apoptosis (Michael A Kohanski et al., 2010) [16] indicating that the plant acting as an efficient antimicrobial source. Along with the results of antimicrobial assay and DNA damage assay, this study shows novel approach of fortifying a plant as an extremely utile antimicrobial source. The high concentration of total phenols and flavonoids in the plant is also responsible for defence mechanism against pathogens which cause various physiological conditions (Mayura A. Kale et al., 2008) [14].

CONCLUSION

The lowest blood clotting time was observed in the methanolic extract of flower. Thus, it indicates that there is a presence of bioactive compound in the methanol, n-hexane and chloroform extracts with increasing solubility of the responsible bioactive compound respectively which enhances the clot formation earlier evaluating hemostatic ability of the plant *Tridax procumbens* and opening a gateway for search and formulation of novel bioactive compounds for hemostatic drug formation. The maximum antimicrobial activity of flower extract of *Tridax procumbens* can be attributed to the presence of flavonoids and phenols which are substances known to have several mechanisms of action such as inhibition of DNA gyrase, inhibition of energy metabolism, etc. With these, there is need for the preparation of different formulations towards ensuring acceptable dosing to field trials. Therefore, we have to exploit the potent possibilities of this plant which possess high therapeutic value and many other uses.

REFERENCES

[1] Anjana, Sharma; Rani, Verma and Padmini, Ramteke. 2009. Antibacterial Activity of Some Medicinal Plants Used by Tribals against UTI Causing Pathogens. World Appl Sci J, 7 (3), pp.332-339.

[2] Chitra Pai, Ujjwala Kulkarni, Manjusha Borde et al. 2011. Antimicrobial Activity of *Tridax procumbens* with Special Reference to Nosocomial Pathogens, British Journal of Pharmaceutical Research 1(4), pp.164-173.

[3] Ikewuchi JudeC., C. Ikewuchi Catherine and M. Igboh Ngozi. 2009. Chemical Profile of *Tridax procumbens* Linn, Pakistan Journal of Nutrition 8 (5), pp.548-550.

[4] Cushnie, T., Lamb A.J. 2005. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents, 26, pp.343–356.

[5] Friedman. 2007. M. Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. Mol. Nutr. Food Res., 51, pp.116–134.

[6] Godkar PB. 1994. Textbook of Medical Laboratory Technology, Edn 3,Bhalani Publishing House, Mumbai, pp.477-490.

[7] Gunjan Guha, Venkatadri Rajkumar, Lazar Mathew. 2011. The antioxidant and DNA protection potential of Indian tribal medicinal plants, Turk J Biol 35, pp.233-242.

[8] Hassan A.M. El. Demerdash. 2012. A Simple and Inexpensive Procedure for Chromosomal DNA Extraction from *Streptococcus thermophilus* Strain. Middle-East Journal of Scientific Research 11 (1), pp. 13-18.

[9] Habila J. D., I. A. Bello, A. A. Dzikwi. 2010. Total phenolics and antioxidant activity of *Tridax procumbens* Linn., African Journal of Pharmacy and Pharmacology Vol. 4(3), pp.123-126.

[10] Jain Ankita and Amita Jain, *Tridax procumbens (L.)*: A Weed With Immense Medicinal Importance: A Review. 2012. International Journal of Pharma and Bio Sciences, Vol 3(1), pp.544- 552.

[11] Kelly Wolfe, Xianzhong Wu, Rui Hai Liu. 2003. Antioxidant Activity of Apple Peels, J. Agric. Food Chem. 51, pp.609−614.

[12] Dube K.G., S.V. Ghude, S.M. Raikwar. 2013. Studies on the haemostatic activity in *Tridax procumbens*, Asiatic J. Biotechnology Resources, Vol.04 (1), pp 35-39.

[13] Lachman L, Lieberman HA, Kanig JL. 1991. The Theory and Practice of Industrial Pharmacy, Edn 3, Varghese Publishing House, Mumbai, pp.254.

[14] Mayura A. Kale, Sadhana R. Shahi, Vijay G. Somani. 2008. Hemostatic activity of the leaves of *Tridax procumbens* Linn, International Journal of Green Pharmacy, Vol2 (1) pp. 54-55.

[15] Manjusha Borde, Ujjwala Kulkarni, Harish Lalan. 2014. Effect of various extracts of leaves of *Tridax procumbens* on human blood clotting time: A comparative *in vitro* study, Scholars Research Library J. Nat. Prod. Plant Resource, 4 (6), pp.9-14.

[16] Michael A Kohanski, Daniel J Dwyer and James J Collins. 2010. How antibiotics kill bacteria: from targets to networks.Nat Rev Microbiol. 8(6), pp.423–435.

[17] Parihar, P., Parihar, L. and Bohra, A. 2006. Antibacterial activity of Athyrium pectinatum (Wall.) Presl. Natural Product Radiance, 5(4), pp.262-265.

[18] Perez C., Pauli M. and Bazerque P. 1990. An antibiotic assay by agar-well diffusion method. Acta Biologiae et Medecine Experimentaalis, 15, pp.113-115.

[19] 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.Anti.Chemo,53, pp.902-905.

[20] Rahmat Ali Khan, Muhammad Rashid Khan, Sumaira Sahreen, Mushtaq Ahmed. 2012. Evaluation of phenolic contents and antioxidant activity of various solvent extracts of Sonchus asper (L.) Hill; Chemistry Central Journal, 6, pp.12.

[21] Dhanabalan R., A. Doss, M. Jagadeeswari, S. Balachandar. 2008. In vitro Phytochemical Screening and Antimicrobial Activity of Aqueous and Methanolic Leaf Extracts of *Tridax procumbens* against Bovine Mastitis Isolated *Staphylococcus aureus.* Ethnobotanical Leaflets, 12, pp.1090-95.

[22] Regina R.Mary and Syed Mohammed Imthiyaz Begum. 2014. Antimicrobial activity of different solvent extracts of *Tridax procumbens* (Asteraceae) from leaf and stem against Human pathogens, International Journal of Scientific Research, Vol 3(8), pp.487-489.

[23] Rita Mahajan and Dhananjay More. 2012. Evaluation of Anticoagulant activity aqueous and ethanolic extracts and their isolated phytochemicals of some medicinal plants. International Journal of Pharmacy and Pharmaceutical Sciences, Vol 4(4), pp. 498-500.

[24] Awe S. and P.F. Omojasola. 2009. A Comparative Study of the Antibacterial Activity of Piliostigma reticulatum Bark Extract with Some Antibiotics, Ethnobotanical Leaflets, 13, pp.1197-1204.

[25] Sharma, B., Kumar, P. 2008. Extraction and Pharmacological Evaluation of Some Extracts of *Tridax procumbens* and *Capparis decidua.* International Journal of Applied Research in Natural Products, 4, pp.5-12.

[26] Singh V, Guizani N, Essa MM, Hakkim FL, Rahman MS. 2012. Comparative analysis of total phenolics, flavonoid content and antioxidant profile of different date varieties (Phoenix dactlyferia L.) from Sultanate of Oman. International Food Research Journal, 19(3), pp.063-1070.

[27] Ramya S., V.Govindaraji, K. Navaneetha Kannan, R. Jayakumararaj. 2008. In Vitro Evaluation of Antibacterial Activity Using Crude Extracts of Catharanthus Roseus L. (G.) Don;Ethnobotanical Leaflets, 12,pp.1067-72.

[28] Sreemanti Das, Santu Kumar Saha, Arnab De. 2012. Potential of the homeopathic remedy, Arnica Montana 30 C, to reduce DNA damage in E.coli exposed to UV radiation through up-regulation of nucleotide excision repair genes, Journal of Chinese Integrative Medicine, Vol.10, No.3, pp. 337-346.

[29] Sunil Christudas, Kulathivel TM, Agastian. 2012. Phytochemical and antimicrobial studies of leaves of *Tridax procumbens L*. Asian Pacific Journal of Tropical Biomedicine, pp.159-16.

[30] Verma SC, Jain CL, Rani R, Pant P, Singh R, Padhi MM. 2012. Simple and rapid method for identification of curcuma longa rhizomes by physiochemical and HPTLC fingerprint analysis. Chemical Science transactions, 1(3), pp.709-715.

INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

ISSN 2231-4490

International Journal of Plant, Animal and Environmental Sciences