

Original Article**DOI - 10.26479/2015.0101.05**

PHYTOCHEMICAL SCREENING AND IN VITRO ANTIMICROBIAL ACTIVITY OF *TRIDAX PROCUMBENS* L.

S. B. Jhample¹, S. B. Gajdhane², P. J. Kasabe¹, P. K. Bhagwat², P. B. Dandge^{1*}

1. Department of Biochemistry, Shivaji University, Kolhapur 416004, Maharashtra, India

2. Department of Microbiology, Shivaji University, Kolhapur 416004, Maharashtra, India

ABSTRACT: Background: *Tridax procumbens* has an eminent identity as medicine in traditional Ayurveda practices for variety of disorders. Several pharmacological activities like anti-diabetic, anti-inflammatory, anti-cancer, hepatoprotective, bloods clotting etc. are associated with it. Objective: Phytochemical screening and antimicrobial activity of acetone and methanolic extracts of *Tridax procumbens* L. and its blood clotting activity. Method: The phytochemical screening and antibacterial activity of the acetone and methanolic extracts of plant parts was determined by using agar well diffusion assay on both gram positive and gram negative bacteria. Results: Extracts showed considerably good antibacterial activity against gram positive and gram negative bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *E. coli* and *serettia marsescens*. The qualitative phytochemical analysis of powdered extracts show presence of Phytoconstituents as tannins, alkaloids, saponins, flavonoids, phenols steroids, anthocyanins, proteins, amino acids and carbohydrate. Aqueous extract of leaves showed the enhanced blood clotting activity in its presence. Conclusion: Crude extracts showed significant antimicrobial activity and aqueous leaves extract showed enhanced blood clotting activity which can be used for therapeutic purpose which is need of the hour.

Keywords: *Tridax procumbens* L., Antibacterial activity, Bioactive, Phytoconstituents.

*** Corresponding Author: Dr. P. B. Dandge Ph D**

Department of Biochemistry, Shivaji University, Kolhapur 416004, Maharashtra, India

Email ID: pbd_biochem@unishivaji.ac.in

© 2015 Life Science Informatics Publication All rights reserved

Peer review under responsibility of Life Science Informatics Publications

2015 May-June RJLBPCS 1(1) Page No.44

INTRODUCTION

Indian traditional medicinal system is fundamentally based on Ayurveda and there is an emerging interest of the world to study and to evaluate the rich heritage of traditional medicinal system and exploit the potential of natural bioactive components. From thousands of years ago the mankind has acquaintance about the benefits of different bioactive components with therapeutic potential. According to Ayurveda different plant extracts had significantly contributed for remedial effects on mankind. Even till today plant materials serves as the potential sources of drugs [1] and has a major role in combating illness. Presently science had already accepted the several plant derived drugs which are having either identified or unidentified chemical structures that are found to be clinically beneficial in various diseases [2]. Almost several drugs used today are derived from natural sources. Many of the plant extracts are principally used in traditional medicines because they are readily accessible in rural areas and are relatively cheapest than modern medicines. Several plants and the plant parts are extensively exploited for the treatment of various diseases in diverse parts of the world [3]. Significantly the therapeutic potential of these plants is studied. They are screened for the presence of antimicrobial activities, phytochemical constituents and antioxidant properties etc. [4-7, 20,] and the consequences obtained from these scientific studies have aided in the rationalization of medicinal use of these plants [8, 9]. *Tridax procumbens* L. is frequently occurring grass in all parts of the world. Conventionally, it is used in the treatment of bronchial catarrh, dysentery, malaria, stomach ache, diarrhoea, high blood pressure and to ensure haemorrhage from cuts, bruises and wounds and also to avert hair fall. It possesses insecticidal, antiseptic, parasitocidal and hepatoprotective properties and has marked depressant action on respiration [10-13]. Although this plant is studied very well from pharmacological and biochemical point of views, geographical locality and environmental conditions play an important role in the growth of plants and so in its phytochemical constituents. Hence the present study deals with the exploration of bioactive Phytoconstituents and antibacterial potential of *Tridax procumbens* L. from local area of Kolhapur district (MS) INDIA.

2. MATERIAL AND METHODS

Plant material and extract preparation

The plants are collected randomly from local area of Shivaji University campus, Kolhapur, Maharashtra (India). It was authenticated from Botany Department, Shivaji University, Kolhapur. The plant parts such as roots, leaves, and flowers were separated from plant and washed thoroughly

with distilled water. Further these cleaned plant materials were shade dried. After complete drying, the plant materials were subjected to mechanical grinder, in order to get a fine powder and the powdered samples were stored in air tight containers. The aqueous, methanolic and acetone extracts were prepared by dissolving 10% (w/v) of powder in respective solvents [14]. The extraction was carried out for 48 hours and the solvents were evaporated by using rotary evaporator. The residual extracts were redissolved in dimethyl sulphoxide (DMSO) and utilized for further studies.

Evaluation of Antimicrobial activity

To investigate antimicrobial activity of *Tridax procumbens* L. both the gram positive and gram negative test microorganisms such as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Serratia marsescens* were used. All the microorganisms are cultured on nutrient agar slants and cultures were maintained at 4°C. The organic solvent extracts of plant parts with extract concentration of 1mg/1ml were used to evaluate the antimicrobial activity by using agar well diffusion method [14] 3 Wells of 10 mm were bored on previously spread inoculated nutrient agar plates, 100 µl of the respective extracts were directly poured in to the wells and allowed for diffusion further it was incubated at 37°C for 24 hours. The antimicrobial activity was determined by measuring zones of inhibition (in mm) after completion of particular incubation tenure.

Phytochemicals screening

For the qualitative Phytochemical analysis, acetone and methanolic extracts of leaves of *Tridax procumbens* plant were tested by using standard protocols [11, 15-18].

Detection of Steroids

For detection of steroids 0.5 ml of extract was dissolved in 5 ml of chloroform to this mixture concentrated sulphuric acid was added from the side of the test tubes upper layer at surface appeared red and acidic layer showed yellow with green fluorescence indicating the presence of steroids.

Detection of carbohydrates

Molisch's test:

1 ml of extract was treated with few drops of Molisch's reagent and few drops of concentrated H₂SO₄ from the side of the test tube; formation of violet ring at the junction of two layers indicates the presence of carbohydrate.

Benedict's test:

1 ml of extract was treated with Benedict's reagent and Boiled for few minutes and observed for the formation of red precipitate indicating the presence of carbohydrates.

Detection of Proteins**Xanthoproteic test**

3 ml of extract was treated with few drops of concentrated nitric acid resulting in to the appearance of yellow color indicated the presence of proteins.

Ninhydrin test

3 ml of extract was treated with 3 ml of ninhydrin reagent and allowed to boil for a few minutes resulting in to formation of blue color indicates the presence of amino acids.

Detection of Anthocyanins

For detection of Anthocyanins 3 ml of extract was treated with 3 ml of 2N HCl & NH₃ resulting in to the appearance of pink red colour which turns in to blue violet indicating the presence of Anthocyanins.

Detection of Phenols

For the detection of Phenols 3 ml of extract was treated with few drops of alcoholic FeCl₃ solution appearance of bluish black color indicates the presence of phenols.

Detection of Tannins

For detection of tannins 3 ml of extract was added to 1% of lead acetate, formation of yellow precipitate indicates the presence of tannins and in the same way 3 ml of extract was treated with 3 ml of FeCl₃ appearance of green color indicates the presence of condensed tannins.

Detection of Alkaloids

For detection of alkaloids concentrated extract was treated with 2 ml of diluted HCl and the mixture was gently heated for 20 minutes allowed to cool and filtered. The filtrate was used for the Hager's and Wagner's test. Hager's test – when the filtrate was treated with Hager's reagent appearance of yellow colored precipitate indicates the presence of alkaloids. Wagner's test – when the filtrate was treated with Wagner's reagent formation of reddish brown precipitate indicates the presence of alkaloids.

Detection of Saponins

For detection of Saponins the plant extract was subjected for frothing test. 5 ml of warm aqueous extract was subjected for vigorous shaking and observed for the formation stable foam which indicates the presence of Saponins.

Detection of Flavonoids

For detection of flavonoids, alkaline reagent test was performed. The extract was treated with 10% of NaOH solution resulting in to the formation of deep yellow color indicated the presence of flavonoids.

Blood coagulation activity

To detect the blood coagulation activity 1 drop of blood was mixed with the 1 drop of aqueous leaves extract with the concentration of 10% (w/v) prepared by fresh weight basis. The time required for blood clotting was observed and compared with time required for normal blood clotting [19].

3. RESULTS AND DISCUSSION

Anti-microbial activity

Antimicrobial activity was explored by using acetone and methanolic extracts of plant parts as root, leaves and flowers respectively. The results are compared with standard antibiotic streptomycin. The results are represented in the figure 1, 2 and 3.

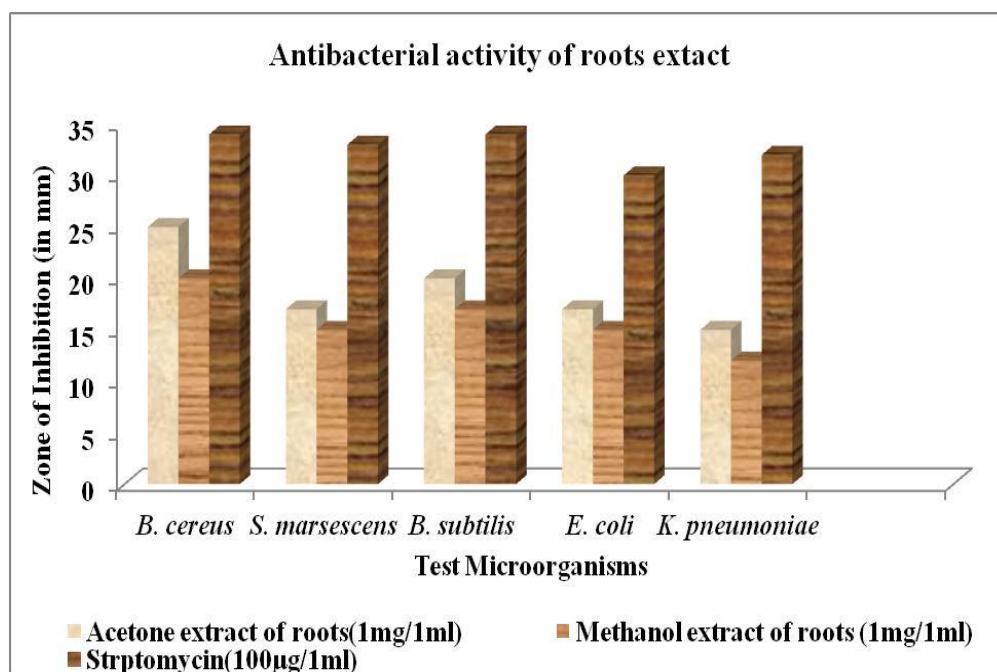


Figure1: Antibacterial activity of roots extracts from *Tridax Procumbens* L.

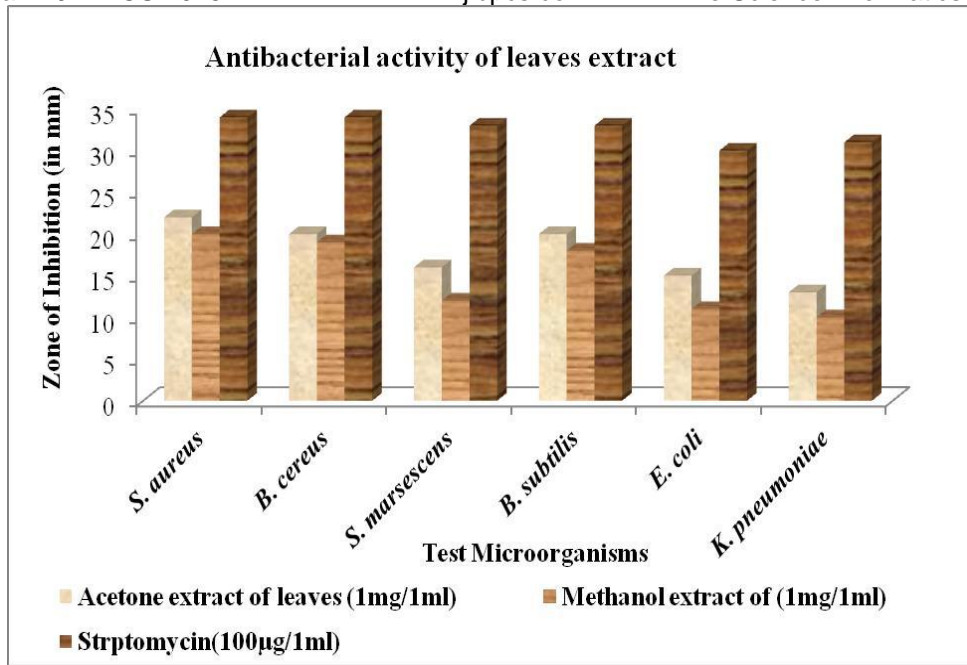


Figure2: Antibacterial activity of leaves extract of *Tridax Procumbens L*

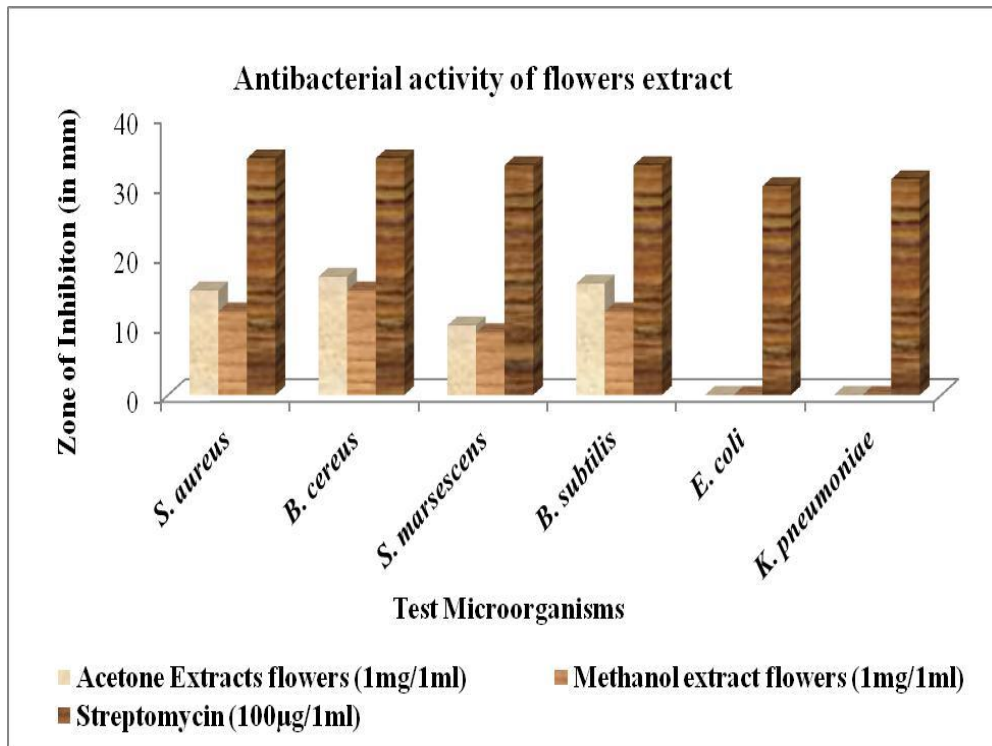
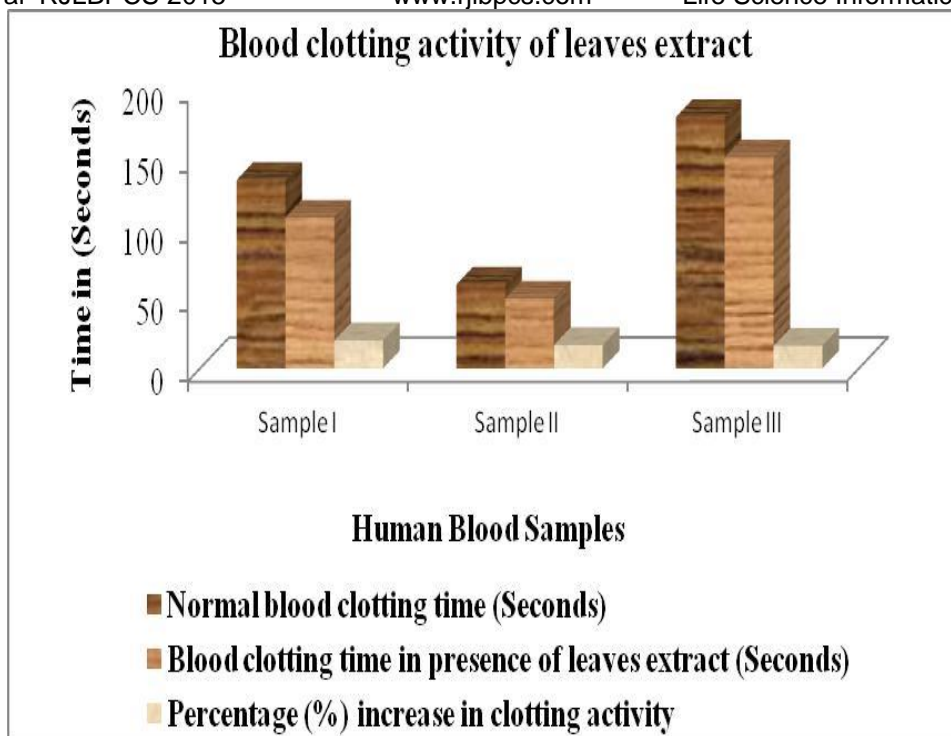


Figure3: Antimicrobial activity for flowers extract of *Tridax Procumbens L*.



**Figure4: Blood clotting activity in presence of aqueous leaf extract of *Tridax*.
Phytochemical screening**

Phytochemical screening of leaves extract of *Tridax Procumbens* L. showed the presence of some phytochemicals mentioned in the Table 1.

Phytoconstituents	Methanol extract of leaves	Acetone extract of leaves
Tannins	+	+
Alkaloids	+	+
Saponins	+	+
Flavonoids	+	+
Phenols	+	+
Steroids	+	+
Anthocyanins	+	+
Proteins	+	+
Amino acids	+	+
Carbohydrates	+	+

Determination of time required for blood clotting activity

3 different samples of blood were taken and normal time required for coagulation of blood is observed and recorded then the other 3 blood samples are taken and mixed with the leaf extract and observed for blood coagulation and time required for coagulation of blood is recorded while in case of roots and flowers no activity was observed. The results are displayed in table number 4.

DISCUSSION:

The therapeutic value of medicinal plants lies in the various bioactive phytochemical constituent's presents in it. Solvent extraction is the crucial step for isolation of various bioactive phytoconstituents showing antibacterial potency. The present study explores the significant extraction procedure for getting bioactive components extracted at its maxima using various solvents. The obtained results reveals that the *Tridax Procumbens* L. extract of leaves and roots showed more significant antibacterial activity as compared to that flowers extract. The acetone extracts of roots and a leaves showed superior antibacterial activity as compared to methanolic extract. Results for phytochemical screening suggests the presence of following phytochemicals such as tannins, alkaloids, saponins, flavonoids, phenols steroids, anthocyanins, proteins, amino acids and carbohydrate constituents in both acetone and methanolic leaves extract, hence it may also possess good medicinal antioxidant properties. As aqueous leaves extract also showed enhanced blood clotting activity it may be used as a potent haemostatic agent. Hence *Tridax Procumbens* L. is medicinally important herb with tremendous therapeutic potential to combat bacterial infections. It will be helpful in developing better pharmaceutical products by employing further detailed investigation of bioactive components.

REFERENCES:

1. Christudas S, Kulathivel TM, Agastian P. Phytochemical and antibacterial studies of leaves of *Tridax Procumbens* L. *Asian pacific journal of tropical medicine*, 2012; S159- S161.
2. Kasabe PJ, Patil PN, Kamble DD, Dandge PB. Nutritional elemental analysis and antioxidant activity of garden cress. *International journal of pharmacy and pharmaceutical sciences*, 2012; 4(3): 392-395.
3. Girish H.V, Satish S. Antibacterial Activity of Important Medicinal Plants on Human Pathogenic Bacteria- a Comparative Analysis. *World Applied Sciences Journal*, 2008; 5 (3): 267-271.
4. Priya.PV, Radhika K, Siva kumar R, Sri Ramchandra M, Prameela Devi Y, Srinivas Rao A. Evaluation of anti-cancer activity of tridax procumbens flower extracts on pc 3 cell lines. 2011; *An International Journal of Advances In Pharmaceutical Science*, 2 (1):28-30.
5. Elumalai EK, Ramachandran M, thirumalai T, vinothkumar P. Antibacterial activity of various leaf extracts of *Merremia emarginata*. *Asian pacific journal of tropical biomedicine*, 2011; 406-408.

6. Elsaveth R, Yenkareshwarulu G, Manoranjansabat V, Harikrishna, Jyoti CH, Lath K. Phytochemical screening and analytical studies of *Tridax parviflora*. 2013; *Universal journal of pharmacy*, 2 (3): 144-147.
7. Dandge PB, Kasabe PJ and Patil RM. Evaluation of medicinal and nutritional components from the *Eleagnus conferta* fruit. 2011 *Science Research Reporter* 1 (2): 56-60.
8. Aniel Kumar O, Naidu LM. Antibacterial potential of *tridax procumbens* L. against human pathogens. 2011; *An international journal of pharmaceutical sciences*, 2 (2): 21-30.
9. Abba D, Inabo HI, Yakubu SE, Olonitola OS. Phytochemical analysis and antibacterial activity of some powdered herbal preparations marketed in Kaduna metropolis. *Science World Journal*, 2009; 4 (1): 23-26.
10. Salahdeen Hm, Murtala BA. Vasorelaxant effects of aqueous leaf extract of *Tridax procumbens* on aortic smooth muscle isolated from the rat. *Journal of smooth muscle and research*, 2012; 48 (2&3): 37-45.
11. Thakar and Sonawane (2013) Mangrove Infoline Database: A Database of Mangrove Plants with Protein Sequence Information Current Bioinformatics. 8.524- 529.
12. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 2005; 4 (7): 685-688.
13. Vilwanathan R, Kanchi SS, Tiruvengadam D. Effect of *Tridax procumbens* on liver antioxidant defense system during lipopolysaccharide-induced hepatitis in D-galactosamine sensitised rats. *Molecular and Cellular Biochemistry*, 2005; 269: 131-136.
14. Saxena VK, Albert S. (β -Sitosterol-3-O- β -D-xylopyranoside from the flowers of *Tridax procumbens* Linn). *Journal of chemical sciences*, 2005; 117 (3): 263-266.
15. Dhanabalan R, Doss A, Jagadeeswari M, Balachandar S, Kezia E, Parivuguna V, Reena Josephine C.M, Vaidheki R. and Kalamani K. In vitro Phytochemical Screening and Antibacterial Activity of Aqueous and Methanolic Leaf Extracts of *Tridax procumbens* against Bovine Mastitis Isolated *Staphylococcus aureus*. *Ethnobotanical Leaflets*, 2008; 12: 1090-95.
16. Njoku OV, Obi C. (Phytochemical constituents of some selected medicinal plants). *African Journal of Pure and Applied Chemistry*, 2009; 3 (11): 228-233.
17. Hegde K, Joshi AB. Preliminary Phytochemical Screening and Antipyretic Activity of *Carissa spinarum* Root Extract. *Scholars Research Library Der Pharmacia letter*, 2010; 2 (3): 255.
18. Trease G.E., and Evan W.C.: Pharmacognosy, Ed 12, English language Book society, Balliere Tindall, 1983: 309-315 and 706-708.

19. Kokate C.K, Purohit A. P. and Ghokhale S.B.: Pharmacognosy, Nirali Prakashan, Pune, India, 1997.
20. Kale MA, Shahi SR, Somani VG, Shamkuwar PB, Dhake AS. Hemostatic activity of leave of a *Tridax procumbens* Linn. *International journal of green pharmacy*, 2008; 2