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ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 10, Issue, 07, pp.9923-9928, July, 2019

RESEARCH ARTICLE

IN VITRO ANTIBACTERIAL, ANTIDIABETIC AND ANTI-INFLAMMATORY ACTIVITIES OF STEM AND ROOT OF *TRIDAX PROCUMBENS* L.

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ARTICLE INFO

Article History:
Received 19th April, 2019
Received in revised form
14th May, 2019
Accepted 17th June, 2019
Published online 31st July, 2019

Key words:
Antibacterial
Antidiabetic
Anti-inflammatory
Root
Stem
Tridax procumbens

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ABSTRACT

In the present study, *Tridax procumbens* belongs to the family of Asteraceae was selected. *Tridax procumbens* was collected and the stem and root were separated, cleaned and dried under shade. The dried plant materials were ground well into powder. The 20g of powder of stem and root were soaked in 200 ml of benzene and diethyl ether separately and kept at room temperature for 48 hours and then the mixture was filtered and concentrated by using evaporator at 37˚C. The antibacterial activity of extracts of stem and root at the concentration of 5mg/100µl against selected bacterial species *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus epidermis* and *Enterobacter cloacae* was studied. The benzene extract of root showed the maximum zone of inhibition against *Staphylococcus* aureus 12±0.98 mm and *Staphylococcus epidermidis*12±1.26 mm. The diethyl ether extract of root of *Tridax procumbens* showed maximum zone of inhibition against *Staphylococcus* aureus 14±1.43 mm. The extracts of stem and root of *Tridax procumbens* were used for the evaluation of *in vitro* antidiabetic and *in vitro* anti - inflammatory activities at different concentrations. The maximum inhibition 85.71% was found in the benzene extracts of stem and root and in the diethyl ether extract of root of *Tridax procumbens* was. The best anti- inflammatory activity was observed in benzene extract of root and diethyl ether extract of stem at 200 µg/ml. From this study, we confirmed that the antibacterial, *in vitro* antidiabetic and *in vitro* anti-inflammatory potential of the stem and root of *Tridax procumbens*.

***Citation: Thiyagarajan Bharathi* and *Rajangam Udayakumar*, 2019.** "*In vitro* antibacterial, antidiabetic and anti-inflammatory activities of stem and root of *Tridax procumbens* l.", *Asian Journal of Science and Technology*, 09, (07), 9900-9901.

INTRODUCTION

Medicinal plants contain numerous biologically active compounds which are helpful in improving the life and treatment of diseases (Shivananda Nayak, 2006). Natural products are the source of synthetic and traditional herbal medicine. The presence of various life sustaining constituents in plants made scientists to investigate these plants for their uses in treating certain infective diseases and management of chronic wounds. Recently considerable attention has been paid to utilize ecofriendly and biofriendly plant based products for the prevention and cure of different human diseases. Considering the adverse effects of synthetic drugs, the Western population is looking for natural remedies, which are safe and effective. It is documented that most of the World's population has taken in traditional medicine, particularly plant drug for the primary health care (Dubey *et al.,* 2004). *Tridax procumbens* is a common medicinal herb used by ethnomedical practitioners, belonging to *Asteraceae* family. It is native to tropical America, but it has been introduced to tropical, subtropical and mid temperate regions worldwide. The plant is a procumbent herb and is valued for its pharmaceutical properties (Sahoo and Chand, 1998).

The plant is native of tropical America and naturalized in tropical Asia, Africa, Australia, and India. It is a wild herb distributed throughout India. Inflammation is a common reaction of the body caused by various biological and non-biological factors present in the environment. Leaves are used for the treatment of bronchial catarrh, dysentery, diarrhoea and for the restoration of hairs (Gaikwadi *et al.,* 2003). The leaf juice possess antiseptic, insecticidal and antiparasitic properties. It is also used to check hemorrhage from cuts, bruises and wounds (Suseela *et al.,* 2002). Leaf extracts can be used to treat infectious skin diseases in folk medicine. It is well known aurvedic medicine for liver disorders besides gastritis and heart burn (Tiwari *et al.,* 2004). Man has always been surrounded by countless microorganisms. The disease producing microbes are playing a very important role in human life. Pathogenic microorganisms are always trying to develop resistance to various antimicrobial agents used for their control. Scientists are always in search of new antimicrobial agents to control microbes. Medicinal plants are gifts of nature to cure limitless number of diseases among human beings (Bushra Beegum and Ganga Devi, 2003). The abundance of plants on the earth's surfaces has led to an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources

of new antimicrobial agents (Bonjar and Farrokhi, 2004). A few reports have focused on the immense potential of this plant which has antimicrobial, wound healing, anti-inflammatory and immune modulatory properties (Pitout *et al.,* 2008). Madhumeha is a disease in which a patient passes sweet urine and exhibits sweetness all over the body, i.e., in sweat, mucus, breathe, blood, etc. The practical usage of juices of various plants achieved the lowering of blood glucose by 10-20 % (Ivorra *et al.,* 1989). Plant-based drugs have been used against various diseases since long time.

The primitive man used herbs as therapeutic agents and medicament, which they were able to procure easily. The present study aims to open new avenues for the improvement of medicinal uses of *Tridax procumbens* (Compositae) for diabetes. Based on the available literature, there are many plants used as medicine among these, *Tridax procumbens* was selected for this study. Many reports are available including pharmacological activities of *Tridax procumbens*. But, there are no studies on biological activities of stem and root of *Tridax procumbens*. So the present study was aimed to study the antibacterial, *in vitro* antidiabetic and anti-inflammatory activities of benzene and diethyl ether extracts of stem and root of *Tridax procumbens*.

MATERIALS AND METHODS

Details of the medicinal plant: *Tridax procumbens* is an important medicinal plant belongs to the family of Asteraceae. The leaf, flower, stem and root of *Tridax procumbens* are used in the medicinal purposes. The classification of *Tridax procumbens* is as follows: Kingdom - Plantae, Subkingdom - Tracheobionta, Division -Magnoliophyta, Class - Magnoliopsida, Subclass - Asteridae, Order - Asterales, Family -Asteraceae, Genus -*Tridax* and Species - *T. procumbens.* The vernacular names of *Tridax procumbens* is as follows: Tamil - Vettukaaya poondu, English- Tridax daisy, Sanskrit - Jayanti veda, Marathi - Kambarmodi, Hindi - Ghamra, Kannada – Jayanthi and Telugu - Gaddi chemanthi.

Collection of plant materials: The selected plant *Tridax procumbens* was collected from the campus of Government Arts College (Autonomous), Kumbakonam – 612 002, Tamilnadu, India during the months between March and June 2017. The collected plant was identified by Dr. R. Murugan, Assistant Professor and Head, Department of Botany, Government Arts College (Autonomous), Kumbakonam - 612 002, Tamilnadu, India.

Preparation of plant extracts: From the collected plant, the stem and root of *Tridax procumbens* were separated, washed and dried under shade for 10 days at room temperature. The plant sample was made as powder using grinder. The powdered plant material was stored in an air tight container until the time of use. The 20g of powder of stem and root of *Tridax procumbens* was soaked in 200ml of benzene and diethyl ether separately and they were kept in room temperature for 48 hours. After that the mixture was filtered through Whatsman No.1filter paper and then the extracts were concentrated and dried in evaporator at 37˚C until the sticky mass was obtained. The dried extracts were stored at 4˚C until the process for *in vitro* antibacterial, antidiabetic and anti-inflammatory activities.

Antibacterial activity

Selection of microorganisms: The bacterial species *Staphylococcus aureus* (ATCC 25923), *Proteus mirabilis* (ATCC 12453), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus epidermis* (ATCC 12228) and *Enterobacter cloacae* (ATCC 13047) were used in this study. The selected microbes were obtained from Jana Clinical Laboratory, Madurai, Tamilnadu, India and the bacterial stock cultures were maintained in nutrient agar slants at 4˚C until the time of use.

Staphylococcus aureus: *Staphylococcus aureus* is a Gram-positive, round-shaped bacterium and is frequently found in the nose, respiratory tract and on the skin (Masalha, 2001). *S. aureus* is capable of secreting several exotoxins (Dinges *et al.,* 2000). *S. aureus* is a human pathogen and approximately 30% of the human population is colonized with *S. aureus*. Simultaneously, it is a leading cause of bacteremia and infective endocarditis (IE) as well as osteoarticular, skin and soft tissues, pleuropulmonary, and device-related infections (Wertheim *et al.,* 2005).

Proteus mirabilis: *Proteus mirabilis* is a Gram-negative, facultatively anaerobic and rod-shaped bacterium. *P. mirabilis* causes 90% of all *Proteus* infections in humans. It is widely distributed in soil and water. This organism causes 90% of human infections with *Proteus* species. It is primarily community acquired, and most frequent in the urinary tract. *Proteus mirabilis* is a significant pathogen of the urinary tract. *Proteus mirabilis* is highly virulent and contains many characteristics that aid in its pathogenicity (Kelley Struble *et al.,* 2009).

Pseudomonas aeruginosa: *Pseudomonas aeruginosa* is a common Gram-negative, rod-shaped bacterium (Smith*, 1994)* belongs to the family Pseudomonadaceae. *Pseudomonas* often has a characteristic sweet odor. These pathogens are widespread in nature, inhabiting soil, water, plants, and animals including humans. *Pseudomonas aeruginosa* has become an important cause of infection, especially in patients with compromised host defense mechanisms (Mandell *et al.,* 2000).

Staphylococcus epidermidis: *Staphylococcus epidermidis* is a Gram-positive and one of the leading pathogens of nosocomial infections, particularly associated with foreign body infections. *S. epidermidis* is a facultative anaerobe, it grows best in aerobic conditions. The hosts for the organism are humans and other warm-blooded animals (Nilsson *et al.,* 1998). The nosocomial pathogen causes infections on prosthetic valves, cerebrospinal fluid shunts, joint prosthesis, vascular prostheses, valves, post operative wounds and urinary tract (Parisi, 1985).

Enterobacter cloacae: *Enterobacter cloacae* are rod-shaped, Gram-negative bacteria from the Enterobacteriaceae family. The size of this bacteria ranges from 0.3-0.6 x 0.8-2.0 µm. *Enterobacter cloacae* live in the mesophilic environment with its optimal temperature at 37˚C and use its peritrichous flagella for movement. *Enterobacter cloacae* are nosocomial pathogens that can cause a range of infections such as bacteremia, lower respiratory tract infection, skin and soft tissue infections, urinary tract infections, endocarditis, intra-

abdominal infections, septic arthritis, os eomyelitis, and ophthalmic infections.

Preparation of nutrient agar medium: 28g of nutrient agar was dissolved in 1000 ml of distilled water and boiled to dissolve the agar completely and then sterilized by autoclaving at 15 1bs pressure at 121ºC for 15 minutes (pH 7.4±0.2).

Preparation of inoculum: Stock cultures were maintained at 4°C on nutrient agar slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth, that were incubated at 37ºC for 24hrs.

Screening of antibacterial activity: The antibacterial screening of stem and root extracts of *Tridax procumbens* was carried out by agar well diffusion method (Suseela *et al.,* 2002). Antibacterial activity was tested against Gram positive and Gram negative bacterial species. 5% w/v test sample of stem and root extract of *Tridax procumbens* were prepared separately using 10 ml of sterile N, N'- dimethyl formamide (DMF). From this 100 µl of extracts contain 5mg were taken for antibacterial activity test. The extract of stem and root of *Tridax procumbens* was loaded (5mg/100µl) in the well on preinoculated nutrient agar plates with respective bacterial cultures for the screening of antibacterial acitivity. After incubation, the antibacterial activity was assessed by measuring the diameter of zone of inhibition in millimeter (mm) and reported. Ampicillin (30 µg) was used as a positive control and the solvent DMF was used as negative control for antibacterial experiments.

***In vitro* antidiabetic activity:** *In vitro* antidiabetic activity was estimated by 3, 5-Dinitrosalicylic acid (DNSA) method (Layer *et al.,* 1986). *In vitro* antidiabetic activity of plant extracts of stem and root of *Tridax procumbens* at various concentrations in 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 containing 6mM sodium chloride) containing 0.04 units of α-amylase solution and were incubated at 37˚C for 10min, followed by the addition of 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) in all test tubes. The reaction was stopped with 1.0 ml of 3, 5-DNSA reagent. The test tubes were incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was diluted by adding 10 ml of distilled water and the absorbance was measured at 540 nm. The control samples were also prepared accordingly without any plant extracts and these were compared with the test samples containing various concentration of the plant extract. The results were expressed as % inhibition and it was calculated by using the following formula.

% Inhibitory activity = Abs (Control) – Abs (Extract) / Abs (Control) × 100

***In vitro* anti-inflammatory activity:** *In vitro* anti-inflammatory activity of stem and root of *Tridax procumbens* was estimated (Jagtap *et al.,* 2011). The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffer saline (PBS, pH 6.4) and 2 ml of varying concentrations of benzene and diethyl ether extracts of stem and root of *Tridax procumbens* and the final concentrations become 40, 80, 120, 160 and 200 µg/ml. Similar volume of DMSO (Dimethyl sulfoxide) served as control. Then the mixtures were incubated at 37˚C for 15 min and then heated at 70˚C for 5 min. After cooling, the absorbance was measured at 660 nm using spectrophotometer. Diclofenac sodium at the final concentration of100 µg/ml was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula.

% inhibition = 100 × (Vt / Vc - 1)

Where, Vt = absorbance of test sample, Vc = absorbance of control.

Statistical analysis: The results were subjected to statistical analysis and expressed as mean ± SD of three replicates.

RESULTS AND DISCUSSION

In the present study, the antibacterial activity of benzene and diethyl ether extracts of stem and root of *Tridax procumbens* was carried out and the results were showed in Table 1. The benzene extract of stem of *Tridax procumbens* showed maximum zone of inhibition against *Pseudomonas aeruginosa* 10±1.26 mm and *Enterbacter cloacae* 10±1.36 mm. The extract showed minimum zone of inhibition against *Staphylococcus aureus* 9±1.25 mm, *Proteus mirabilis* 9±0.91 mm and *Staphylococcus epidermidis* 9±1.64 mm at the concentration of 5mg/100µl. The benzene extract of root of *Tridax procumbens* showed maximum zone of inhibition against *Staphylococcus aureus* 12±0.98 mm and *Staphylococcus epidermidis* 12±1.26 mm and the minimum zone of inhibition against *Proteus mirabilis* 11±1.22 mm, *Enterbacter cloacae* 11±0.85 mm and *Pseudomonas aeruginosa* 10±1.37 mm at the concentration of 5mg/100µl.
The diethyl ether extract of stem of *Tridax procumbens* showed antibacterial activity against *Staphylococcus epidermidis* 11±1.64 mm, *Staphylococcus aureus* 11±1.43 mm, *Proteus mirabilis* 10±0.91 mm, *Enterbacter cloacae* 10±1.36 mm and *Pseudomonas aeruginosa* 9±1.26 mm. The diethyl ether extract of root of Tridax *procumbens* showed antibacterial activity against *Staphylococcus aureus* 14±1.43 mm, *Proteus mirabilis* 11±0.96 mm, *Pseudomonas aeruginosa* 10±1.16mm, *Staphylococcus epidermidis* 10±1.38 mm and *Enterbacter cloacae* 9±0.98 mm at the concentration of 5mg/100µl. The benzene and diethyl ether extracts of root of *Tridax procumbens* were showed maximum antibacterial activity against *Staphylococcus aureus.* Similarly, the benzene extract of stem of *Tridax procumbens* showed the maximum zone of inhibition against *Staphylococcus epidermidis* at the concentration of 5mg/100µl. So, the present study confirmed the antibacterial activity of stem and root of *Tridax procumbens.* Traditionally, *Tridax procumbens* has been used in India for wound healing. The juice extracted from the leaves is directly applied on wounds. Its leaf extracts were used for infectious skin diseases in folk medicine. It is used in Ayurvedic medicine for liver disorders, hepatoprotection and gastritis (Wani *et al.,* 2010). The leaves of *Tridax procumbens* including other aerial parts except flowering tops have been claimed to be useful in the treatment of inflammatory conditions. It is also known for several other potential therapeutic activities like antiviral, antioxidant, antidiabetic and antifungal activities. In the Indian traditional medicine, it has been used in bronchial catarrh, diarrhoea and dysentery. For centuries plants have been used for both nutritional and medicinal purposes.

Table 1. Antibacterial activity of benzene and diethyl ether extracts of stem and root of *Tridax procumbens*

Name of the bacterial Species	Diameter of zone of inhibition (mm)					
	Negative control	Positive control	Name of solvent extract (5% w/v)			
			Benzene extract		Diethyl ether extract	
			Stem	Root	Stem	Root
Staphylococcus aureus	–	16 ± 1.22	9 ± 1.25	12 ± 0.98	11 ± 1.43	14 ± 1.43
Proteus mirabilis	–	15 ± 1.13	9 ± 0.91	11± 1.22	10 ± 0.91	11 ± 0.96
Staphylococcus epidermidis	–	10 ± 1.32	9 ± 1.64	12 ± 1.26	11 ± 1.64	10 ± 1.38
Pseudomonas aeruginosa	–	13 ± 1.25	10 ± 1.26	10 ± 1.37	9 ± 1.26	10 ± 1.16
Enterobacter cloacae	–	15 ± 1.24	10 ± 1.36	11 ± 0.85	10 ± 1.36	9 ± 0.98

Negative control: Ampicillin - 30µg/100µl
Positive control: DMF (N, N' –Dimethyl formamide) -100µl
Values are expressed as mean ± standard deviation of triplicates

Table 2. *In vitro* antidiabetic activity of benzene and diethyl ether extracts of stem and root of *Tridax procumbens*

Concentration of plant extract (µg/ml)	*In vitro* antidiabetic activity (% of inhibition)			
	Benzene extract		Diethyl ether extract	
	Stem	Root	Stem	Root
25	14.28 ± 0.42	14.28 ± 0.22	14.28 ± 0.46	14.28 ± 0.48
50	42.85 ± 0.46	28.57 ± 0.35	28.57 ± 0.87	28.57 ± 0.56
75	57.14 ± 0.81	42.85 ± 0.47	42.85 ± 0.94	42.85 ± 0.72
100	71.42 ± 0.94	57.14 ± 0.49	57.14 ± 0.88	71.42 ± 0.87
125	85.71 ± 1.2	85.71 ± 0.68	71.42 ± 0.92	85.71 ± 0.91

Values are expressed as mean ± standard deviation of triplicates

Table 3. *In vitro* anti-inflammatory activity of benzene and diethyl ether extracts of stem and root of *Tridax procumbens*

Concentration of plant extract (µg/ml)	*In vitro* anti-inflammatory activity (% inhibition)			
	Benzene extract		Diethyl ether extract	
	Stem	Root	Stem	Root
40	3.3± 2.2	10± 3.2	6.6± 2.6	20± 3.2
80	20±3.3	20± 2.7	16.6± 2.7	36.6± 3.0
120	46.6± 3.6	46.6± 3.8	23.3± 3.8	63.3± 2.8
160	63.3± 3.7	70± 3.0	60± 3.6	70± 2.6
200	83.3± 2.8	86.6± 2.5	86.6± 2.5	83.3± 3.2
Standard - 100µg/µl	90±4.2			

Standard – Diclofenac sodium
Values are expressed as mean ± standard deviation of triplicates

The conventional medicine is not cheap and a large population of the people depends on traditional medicine for their healthcare needs. Over the years, these herbal drugs have been shown to be effective (Awe and Omojalasola, 2003). Many plants and their parts are used for the treatment of various diseases in different parts of the world, and are being screened for antimicrobial activities and the results obtained from these scientific studies have aided in the rationalization of medicinal use of these plants (Abo *et al.,* 1999). Similarly, the activity of plant extracts against bacteria have been studied for years, but in a more intensified way during the last three decades. During this period, numerous antimicrobial screening evaluations have been published based on the traditional use of Chinese, African and Asian plant-based drugs (Suffredim *et al.,* 2004). Plants remain the most common source of antimicrobial agents. Many aromatic plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeast (Hulin *et al.,* 1998). Biologically active compounds from natural sources have always been a great interest for scientists working in infectious diseases. 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

(Rhabaso Lhoret and Chiasson, 2004). Alpha amylase and alpha glucosidase are responsible for the hydrolysis of poly and oligosaccharides into monomers or cleavage of bonds between sugars and non carbohydrate aglycan. Thus, this enzyme is involved in a number of important biological processes, such as digestion of carbohydrate into glucose or processing of the oligosaccharide moieties of glycoprotein. There is now a great deal of interest in amylase inhibitors, because these are important biochemical tools for studying the mechanism of enzymes. The search for amylase inhibitors has yielded a number of chemically distinct inhibitors from plants.

In the present investigation, the *in vitro* anti - inflammatory effect of benzene and diethyl ether extracts of stem and root of *Tridax procumbens* was evaluated against denaturation of egg albumin and the results were showed in Table 3. Diclofenac sodium, a standard anti-inflammation drug was used as standard showed 90±4.2% inhibition at the concentration of 100 µg/ml. The *in vitro* anti - inflammatory activity of benzene extracts of stem and root of *Tridax procumbens* was analyzed by using inhibition of albumin denaturation technique at different concentrations 40, 80, 120, 160 and 200 µg/ml were showed 3.3±2.2%, 20±3.3%, 46.67±3.6%, 63.3±3.7% and 83.3±2.8% inhibition by stem extract and 10±3.2%, 20±2.7%, 46.6±3.8, 70±3.0% and 86.6±2.5% inhibition by root extract, respectively. The maximum levels of inhibition 83.3% and 86.6% were observed in benzene extracts of stem and root at 200 µg/ml. The *in vitro* anti- inflammatory activity of diethyl ether extracts of stem and root of *Tridax procumbens* was analyzed at different concentrations 40, 80, 120, 160 and 200 µg/ml were showed 6.6±2.6%, 16.6±2.7%, 23.3±3.8%,

60±3.6% and 86.6±2.5% inhibition by stem extract and 20±3.2%, 36.6± 3.0%, 63.3±2.8, 70±2.6% and 83.3±3.2% inhibition by root extract, respectively. The maximum level of inhibition 86.6% and 83.3% were observed in diethyl ether extracts of stem and root of *Tridax procumbens* at 200 µg/ml. *Tridax procumbens* possess significant anti-inflammatory activity as its action influences exudates leucocytes migration, rat paw oedema and granuloma tissue. The anti-inflammatory action of *Tridax procumbens* may possibly be due to corticotrophic influence as evident from increase in weight (Diwan *et al.,* 1989). The bioactive natural principles have been implicated in counteracting reactive oxidative species (ROS) indicated in the pathogenesis of inflammation and related ailments in biological systems (Nia *et al.,* 2003). Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, and it is a prective response involving immune cells, blood vessels, and molecular mediators. *Denaturation of protein is a well documented cause of inflammation. As a part of the investigation on the mechanism of anti-inflammation activity, the abiltity of stem and root extract of Tridax procumbens to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation.*

Conclusion

The present study confirmed that the *in vitro* antibacterial, antidiabetic and anti-inflammatory activities of stem and root of *Tridax procumbens*. In future, this study may be useful in the identification of new potent antibacterial, antidiabetic and anti-inflammatory agents from stem and root of *Tridax procumbens*.

Acknowledgement: The authors are thankful to the Principal, Government Arts College (Autonomous), Kumbakonam – 612 002, Tamilnadu, India for granting permission and also thankful to the authorities of Research Laboratory, Department of Biochemistry, Government Arts College (Autonomous), Kumbakonam – 612 002, Tamilnadu, India for provided facilities to do this research work.

Competing interest: Authors declare that no competing interests exist.

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