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Anticoagulant activity of Tridax procumbens Linn review article

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ABSTRACT:
The anticoagulant, antiherpetic, and antibacterial properties of the common Tridax procumbens plant's sulphated polysaccharide were investigated. The T. procumbens sulphated polysaccharide was a powerful anticoagulant with comparable effectiveness to heparin and chondroitin sulphate. In a sample of human blood, Tridax procumbens methanolic extract was tested for anticoagulant action. Moreover, the sulphated polysaccharide extracted from T. procumbens was found non-toxic on Vero cell lines up to the concentration of 200 µg/ml. With an IC(50) value of 100-150 g/ml, sulphated polysaccharide demonstrated detectable antiviral activity against HSV-1. Furthermore, the bacterial strains Vibrio alginolyticus and Vibrio harveyi isolated from oil sardine were severely inhibited by the sulphated polysaccharide from T. procumbens.

KEYWORDS: Tridax Procumbens, anticoagulent .

I. INTRODUCTION

India has long history for the treatment of various diseases using medicinal plants. Indian plants show widespread bioactivity with minimum side effects1-2. In contrast to synthetic compounds, herbal products are safer and hence it is preferred for treatment of various ailments. Across the World, large segment population has accepted traditional remedial system that includes use of phytomedicines obtained from different medicinal plants drugs and cosmetics4. Heparin is commonly used in various surgeries. Beside the pharmaceutical properties such as myocardial infarction, inflammatory and allergic conditions, heparin shows serious side effect like hemorrhage and it is expensive. Therefore, it is necessity and demand of time to explore alternative anticoagulants. The plant are safer source of medicines hence, we undertook the anticoagulation study of extracts and phytochemicals isolated from selected medicinal plants such as, Enicostemma littorale, Acheranthus aspera, Abutilon indicum and Tridax procumbens. Thrombotic disorders such as deep vein thrombosis, pulmonary emboli, ischemic stroke, hypercoagulable states, strokes and heart attacks are the main causes of morbidity and mortality in developed countries .Therefore, anticoagulants play an important role for the prevention and treatment of thromboembolic disorders . Anticoagulant drugs consisting of warfarin heparins, vitamin K antagonists, and their derivatives have been used for the treatment. Although their efficacy remains undisputed, the deleterious life-threatening side effects of these drugs have also been well documented. Herbal anticoagulant therapy can be used as the alternative sources for the development of new anticoagulant agents due to their biological activities. The use of herbal medicine provides an alternative to overcome the limitations of available anticoagulants such as warfarin and heparin which have bleeding complication, as well as uncertainty of the newer anticoagulant drugs dosing in some patient populations such as patient with underlying chronic diseases. This review highlights on documented plants which are used as antithrombotic or anticoagulant as mentioned in folklore medicine. The demand of healthcare need has increased world wide due to emergence of various diseases and failure in irradiation of the existing ailments. Across the World, large segment population has accepted t traditional remedial system that includes use of phytomedicines obtained from different medicinal plants drugs and cosmetics4. Heparin is commonly used in various surgeries. Beside the pharmaceutical properties such as myocardial infarction, inflammatory and allergic conditions, heparin shows serious side effect like hemorrhage and it is expensive. Therefore, it is necessity and demand of time to explore alternative anticoagulants. The plant are safer source of medicines hence, we undertook the anticoagulation study of extracts and phytochemicals isolated from selected medicinal plants such as, Enicostemma littorale, Acheranthus aspera, Abutilon indicum and Tridax procumbens. Thrombotic disorders such as deep vein

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thrombosis, pulmonary emboli, ischemic stroke, hypercoagulable states, strokes and heart attacks are the main causes of morbidity and mortality in developed countries .Therefore, anticoagulants play an important role for the prevention and treatment of thromboembolic disorders . Anticoagulant drugs consisting of warfarin heparins, vitamin K antagonists, and their derivatives have been used for the treatment. Although their efficacy remains undisputed, the deleterious life-threatening side effects of these drugs have also been well documented. Herbal anticoagulant therapy can be used as the alternative sources for the development of new anticoagulant agents due to their biological activities. The use of herbal medicine provides an alternative to overcome the limitations of available anticoagulants such as warfarin and heparin which have bleeding complication, as well as uncertainty of the newer anticoagulant drugs dosing in some patient populations such as patient with underlying chronic diseases. This review highlights on documented plants which are used as antithrombotic or anticoagulant as mentioned in folklore medicine. The demand of healthcare need has increased world wide due to emergence of various diseases and failure in irradiation of the existing ailments. Across the World, large segment population has accepted traditional remedial system that includes use of phyto-medicines, herbal drugs, lifestyle changes and stress managements. WHO report depicts that more than 80% of world's population rely on plants based products to meet their health care needs. It was reported that nearly, 25 to 45% of modern prescriptions contain plant derived drug formulations. The value of plant based prescribed drugs in 1990 was estimated at $15.5 billon which has been reaching to sky since then. Furthermore, about 42% of 25 top selling drugs marketed wide are either directly obtained from natural sources or entities derived from plant products.(1) World Heath Organization has also recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines. [1- 3] 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 value of medicinal plants as potential source of bioactive compounds has been confirmed by various scientific publications. Tridax procumbens is a member of the Asteraceae family of flowering plants and is the fastest growing of the 30 species. It is best known as a widespread weed and pest plant. It is native to tropical America, but has been introduced into tropical, subtropical, and temperate regions around the world. It is designated a poisonous weed in the United States and is in pest status. Some of the medically important species in the Tridax genus are: angustifolia, T. serboana, T. bicolor, T. ignitens, T. dubia, T. erect and T. rosea.

Kingdom	Plantae
Subkingdom	Viridiplantae
Division	Angiosperms
Superdivision	Spermatophytina
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae
Genus	Tridax L-tridax
Species	Tridax Procumbens L.

It is commonly known as **coatbuttons** and **tridax daisy** in English, jayanthi in Kannada, cadillo chisaca in Spanish, herbe caille in French, jayanti veda and "Avanti"[1] in Sanskrit,"Ghajadvu"in Gujarati "Kum minnippacha","Kurikootticheera","Muriyampachila", "Odiyancheera","Railpoochedi,"Sanipoovu","Thelkut hi","Chiravanakku",in Malayalam, bikhalyakarani in Assamese, ghamra in Hindi, Tridhara in Bengali, bish alya karani in Oriya, kambarmodi, Jakhamjudi & tantani in Marathi, gayapaaku & gaddi chemanthi & balapaaku in Telugu,vettukaaya poondu or thatha poo or kinatruppasan in Tamil, Ghaburi in Gujarati[2] kotobukigiku in Japanese and tīn túkkæ in Thai[3] in Urdu it is known as zagh mai hayat.

Tridax procumbens:

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Synonyms of Tridax Procumbens Linn.

Synonyms
Chrysanthemum procumbens
Balbisia canescens
Balbisia divericata
Balbisia peduncalata
Tridax procumbens var. canescsenes
Tridax procumbens var. ovatifolia

Anticoagulent activity:

- Any medication known as an anticoagulant works by keeping blood from clotting when administered to it. Anticoagulants work by inhibiting the production or activity of certain clotting factors that are naturally found in blood.
- These medications are frequently used to stop blood clots (thrombi) from forming in veins or arteries or from growing while circulating in the bloodstream. Anticoagulants, also referred to as blood thinners, are chemicals that stop or slow down blood coagulation, lengthening the clotting time. By interfering with the numerous blood coagulation pathways, anticoagulants are closely related to antiplatelet medications and thrombolytic medications.
- In contrast to anticoagulants, which block the coagulation cascade by clotting factors that follows the initial platelet aggregation, antiplatelet medicines specifically inhibit platelet aggregation (clumping together).
- Anticoagulence is generally used in the treatment of deep-vein thrombosis, in which clots form in so called deep-veins, such as those of the legs.
- They are furthermore used to treat pulmonary embolism, in which a clot obstructs the pulmonary artery or one of each branches ,
- They are also used to treat coronary thrombosis, in which a clot obstructs a coronary artery in the heart.
- Anticoagulents are used in drawing and storing blood to prevent clotting of blood in collection tubes and blood bags.
- It is used in the treatment of Atrial fibrillation commonly forms an atrial appendage clot.
- The advantage of anticoagulation is prevention of reduction of progression of a disease.

Materials and methods :

1. Sample Collection:

The Tridax procumbens plant sample was obtained from the Allagumalai town of District of Tirupur in Tamil Nadu, India. Botanical Survey of India (BSI), Tamil Nadu Agricultural University (TNAU), Coimbatore, verified the authenticity of the plant sample. Tridax procumbens L., an Asteraceae plant, was found in Tamilnadu, India. Its authentication number is BSI/SRC/5/23/2018/TECH/2659.

2. Soxhlet Extraction:

A porous bag or thimble composed of sturdy filter paper and containing a finely pulverised leaf sample are inserted in a Soxhlet apparatus. The vapour from the heated extraction solvent in the flask condenses in the condenser. The sample's container, a thimble, is filled with condensed extractant. The liquid contents of the chamber are drawn into the flask when the liquid level reaches the top of the syphon tube. This procedure continues until there is no longer any solvent left in the syphon tube drops after they have evaporated.

3. Testing of Anti coagulant activity:

Three test tubes, each containing one millilitre of blood, labelled Blank, T1, and T2, were filled with blood drawn from two healthy participants. Blank remained as the control. T1 and T2 were each given 0.2 ml of methanolic extracts of leaf samples. Using a stop watch, the clotting activity of each tube was monitored every 30 seconds. It was noted the clotting time. Another blood sample was used in the experiment.

4. MIT Assay:

Numerous in vitro studies evaluating a cell population's reaction to outside influences are based on measurements of cell viability and proliferation. Tetrazolium salt reduction is now recognised as a trustworthy method for assessing cell growth. Dehydrogenase enzymes help metabolically active cells reduce the yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) to produce reducing equivalents like NADH and NADPH. By using spectrophotometric techniques, the resulting intracellular purple formazan can be solubilized and measured.

The MTT Cell Proliferation Assay gauges the rate of cell proliferation and, conversely, the decline in cell viability that results from metabolic processes that cause apoptosis or necrosis. To speed up sample processing, the number of test stages has been reduced to the absolute minimum. In the absence of cells, the MTT Reagent produces low background absorbance values. The linear link between cell number and signal production is established for each kind of cell, enabling precise measurement of changes in the rate of cell proliferation.

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II. Methodology Adopted

Cell culture

The National Centre for Cell Sciences (NCCS), Pune, India, provided the Human Leukemic Cancer Cells, Moult 3. The balanced salt solution (BSS) was adjusted to contain 1.5 g/L Na2CO3, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM l-glutamine, 1.5 g/L glucose, 10 mM (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid) (HEPES), and 10% foetal bovine serum (GIBCO, USA). Streptomycin and penicillin (100 IU/100 g) were changed to 1mL/L. The cells were kept in suspension culture mode at 37°C with 5% CO2 in a humidified CO2 incubator.

Evaluation of cytotoxicity

Through the use of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] test, the inhibitory concentration (IC50) value was calculated. the cancerous cells 48 hours were needed for a 96-well plate to be cultivated (1 104 cells/well) to 75% confluence. The media was changed for a new one that contained serially substance, and the cells were then cultured for a further 48 hours. Each well received 100 mL of the MTT [3-(4,5-dimethylthiozol-2-yl)- 3,5-diphenyl tetrazolium bromide] (Hi-Media) solution before being removed from the culture media and being incubated at 37 °C for 4 hours. The formazan crystals were solubilized by adding 50 L of DMSO to each well after the supernatant had been removed. This process took 10 minutes. A multiwell ELISA plate reader (Thermo Multiskan EX, USA) was used to detect the optical density at 620 nm. The following formula was used to get the percentage of viability using the OD value. Viability is calculated as OD of the experimental sample divided by OD of the experimental control, divided by 100.

MTT Assay (Cytotoxicity)

IC_{50} – Values of respective Compounds (at 24 hrs)

Formula: % of viability = OD value of experimental sample ×100 / OD value of experimental control

III. Discussion:

Tridax procubens leaf extracts (hydro-alcoholic, pet-ether (60-80°c), and aqueous) were tested in vitro using the Lee White Method to see how they affected human clotting time. Using blood samples from healthy human volunteers with normal blood clotting times, it was found that all dosage ranges of the ethanolic extract of the leaves of Tridax procubens significantly slowed down the clotting time, whereas the pet ether extract of the leaves (LPT) only demonstrated activity at doses of 50 and 100 mg. On the other hand, it was discovered that aqueous leaf extract (LAQ) in all doses had no impact on the time it took for healthy human volunteers' blood to clot in vitro In blood samples taken from all the individuals, the clotting times of all the extracts were roughly one minute shorter than those of their respective blanks, which included distilled water, ethanol, and pet ether. Additionally, all extracts' clotting times in blood samples from all the participants evaluated in vitro were 2-3 minutes slower than the typical clotting time (CT). A common laboratory test is blood clotting time determination, which is used to identify abnormalities in blood clotting time caused by a variety of conditions, such as hereditary or acquired coagulation disorders. An increase in the usual clotting time indicates that coagulation disorders are being treated with coagulant-like medications. It can be inferred that the Tridax procumbens leaf extracts studied had hemostatic action because they uniformly shorten the clotting time in blood samples from all patients. All leaf extracts contained typical plant components as mucilage, alkaloids, tannins, flavonoids, and carbohydrates, according to phytochemical analyses. Both ethanolic and aqueous leaf extracts of Tridax procumbens include anthraquinone glycoside, whilst all leaf extracts contain steroids. Typically, these metabolites are what give medicinal plants their pharmacological effects. Tannins, an essential component of plants, control hemostasis, which stops bleeding from harmed or injured arteries by precipitating proteins and forming vascular plugs12. Since mechanisms other than the production of vascular plugs are probably involved, we can confidently assume that the tannins in the extracts contribute to the action in part

IV. Conclusion:

Tridax procumbens extracts have coagulant activities, which suggests that they have a beneficial hemostatic impact. Toxicology tests still need to be improved, though, to make sure that there are no harmful side effects.

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