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Effect of various extracts of leaves of *Tridax procumbens* on human blood clotting time: A comparative *in vitro* study

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ABSTRACT

Scientific reports on the haemostatics well as anticoagulaent effects of Tridax procumbens in human volunteers are relatively scanty even though their uses in wound care in many indigenous tribes around the world have been widely reported. This experiment was therefore designed to scientifically test the possible effects of hydro-alcoholic, pet-ether (60-80°c), and aqueous extracts of leaves of Tridax procumbens on blood coagulation by using Lee -White method. It was observed that blood clotting time was significantly reduced ($P < 0.05$) by ethanolic extract of leaves (LE) and pet-ether extract of leaves (LPT) of Tridax procumbens. Aqueous extract of leaves (LAQ) did not show any activityThis result suggests that the various extracts of Tridax procumbens possess haemostatic properties
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***Keywords**: Tridax procumbens*; Haemostatic activity ; Clotting time; Lee -White method; Ethanolic extract of leaves; Aqueous extracts of leaves; Pet-ether extract of leaves.

INTRODUCTION

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. [4, 5]

Tridax procumbens, Listed as a weed and a pest plant, of Family Asteraceae is found perennially in waste places, road sides and hedges throughout India. It has been known by several names including *Tridax* daisy in English, Jayanti veda in Sanskrit, Ghamra in Hindi, Dagadi pala in Marathi, Herbe caille in French and Thata poodu in

Tamil. 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. [6-8]

Patient with hemostatic disorder, characterized by a tendency to bleeding, constitutes a serious of challenge in medical practice. A number of haemostatic agents have recently been to be used to arrest bleeding before surgical control of the source. The present study aims to open new avenue for the improvement of medicinal uses of *Tridax procumbens* for haemostatic activity. Another important objective of such a study is to bring the haemostatic activity of medicinal plants on a firm scientific footing, raise awareness and add value to the resource and contribute to the socio-economic well being of our country particularly on the national and international level.

In the process of wound healing involve complex series of interactions between different cell types, cytokine mediators and the extracellular matrix. The phases of wound healing include haemostasis, inflammation, proliferation and remodeling [6].

A few Scientific publications have focused on its antimicrobial, wound healing, anti-inflammatory, immunomodulatory and anti-diabetic properties. [9-14] However, there is a paucity of reports on its effect on human blood clotting time. This experiment was therefore designed to scientifically test the possible effect of hydro-alcoholic, pet-ether, aqueous extracts of leaves on human blood clotting time.

METERIALS AND METHODS

o Plant material collection

After obtaining approval from the Institutional Ethics Committee, the study was carried out at
M.G.M. Medical College Kamothe Navi Mumbai, India. The leaves of *Tridax procumbens* were collected from the college campus of M.G.M. Medical College during the monsoon season (months of July and August). The taxonomic identity was confirmed as *Tridax procumbens* belonging to the family Asteraceae with the help of Agarkar Institute, Pune, India (Voucher No.WP-076).

o Preparation of Extract

Plant material was washed thoroughly with tap water and shade dried at room temperature. The leaves were powered in an electronic blender to obtain coarse powder of leaves. It was passed through sieve no. 40 and then stored in a closed container at room temperature for further use. The extraction procedure was carried out in soxhlet apparatus with 100 grams of coarse powder of the leaves of *Tridax procumbens* using hydro-alcohol (500ml), pet-ether (500 ml) and distilled water (500ml) separately for 24 hrs and filter. The concentrated extract was then evaporated to dryness in vacuum oven at temperature 40°c. The dried extract was stored at 4°c in air free sterile container in refrigerator for preliminary phytochemical analysis and further testing on blood coagulation property.

o Phytochemical screening

Various qualitative chemical tests for Carbohydrate, Protein, Alkaloid, Tannins, Saponin, Steroids, Flavonoid, Glycosides, and Mucilage were performed to detect their presence in the extracts. The values are expressed in table 1 and 2. Proximate analysis of dried leaves of plant *Tridax procumbens* was determined as per the Indian Pharmacopoeia.

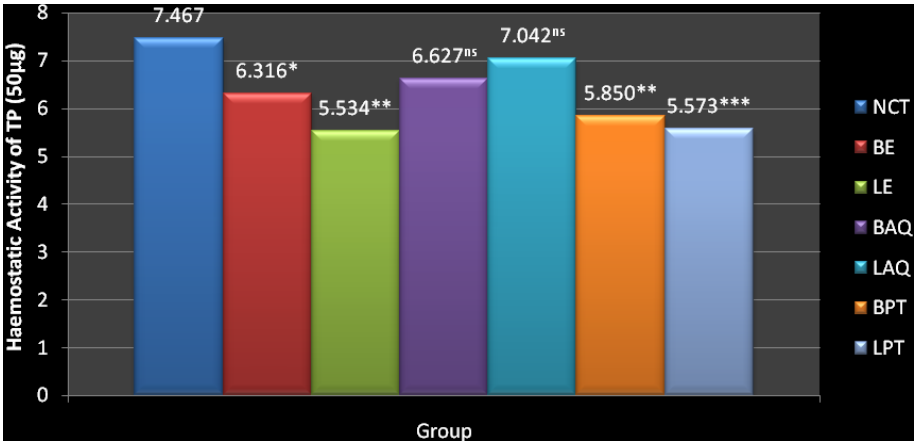
o Evaluation of coagulation activity

After obtaining the written informed consent 45 healthy human volunteer were enrolled in the study**.** Blood samples were collected in aseptic condition from healthy human volunteer of either sex or age between 18-60 years. They had been chosen for this study according to the following criteria: having normal prothrombin time, not suffering from any cardiovascular diseases (hypertension, congestive heart failure), coagulation disorders such as, Hemophilia A or B or diabetes, not recently using nonsteroidal anti-inflammatory drugs, not obese or smokers and free from dyslipidemic disorders. Pregnant women and women on oral contraceptive as well as children were also excluded from the study. The hemostatic activity of various extracts (Hydro-alcoholic, pet-ether (60-80°c) and aqueous of leaves were studied on clotting time (CT) of blood, by using Lee-White method. For the same, venous blood was collected from volunteers in a clean and dry test tube without the addition of an anticoagulant and the time required for clotting was noted (normal clotting time is 5-12 min). Primarily, all extracts were screened for their effect on clotting time (CT) of blood samples obtained from the healthy subject. Pilot study was carried out by using normal Human volunteer. Venous blood from normal Human volunteer (minimum 7) was collected and stop- watch was

started as soon as the blood entered the syringe. A set of seven test tubes were filled, each with blood up to 1 ml mark, the first test tube being for normal CT. In the next three test tubes of the same set, 0.2 ml of solvents were added to make the respective blanks and in the remaining test tube, 0.2 ml of extracts hydro- alcoholic extracts of leaves (I), Pet-ether extracts of leaves (II), aqueous extracts of leaves (III) were added. All these test tubes were placed in the water-bath at 37°±5°c. Each of the test tubes was removed after 3 min and tilted at an angle of 45°c to see whether clotting had been taken place. The test tubes in which clotting had not started were returned to the water-bath and examined at 30 second intervals to see if clotting had occurred. The watch was immediately stopped when clotting in a particular test tube occurred and the time was noted in minutes. Likewise, CT was recorded for the remaining samples. This procedure was carried out in vitro, by drawing blood from at least 7 Normal volunteers for each doses (50, 100, 200, 400 and 800µg) to minimize subject variation. Observation from different extracts will be depicted in Graphical presentation.

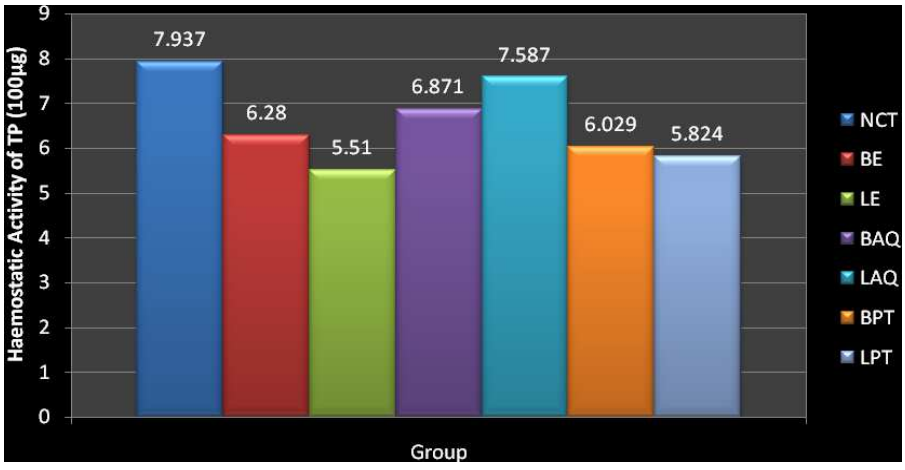
RESULTS

Graph 1: Haemostatic activity of Leaves of *Tridax procumbens* (50µg)



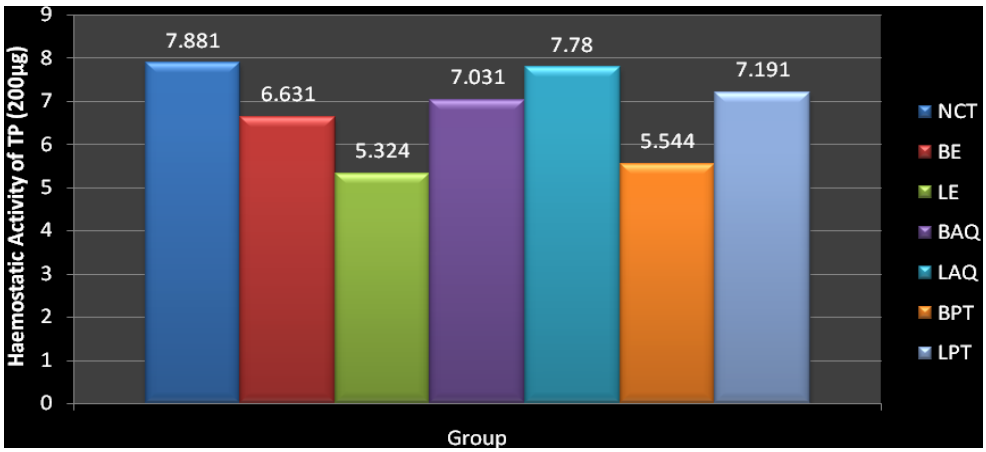
CT- Clotting time, BE- Blank ethanol, LE- Ethanolic extract of leaves, BAQ- Blank aqueous, LAQ- Aqueous extract of leaves, BPT- Blank pet-ether, LPT- Pet-ether extract of leaves.
*Values are expressed as mean±S.E.M. minutes (n = 7).Values are statistically significant at ***P<0.001, **P<0.01, *P<0.05 vs. NCT group (One-way ANOVA followed by Tukey's post hoc test).*

Graph 2: Haemostatic activity of Leaves of *Tridax procumbens* (100µg)



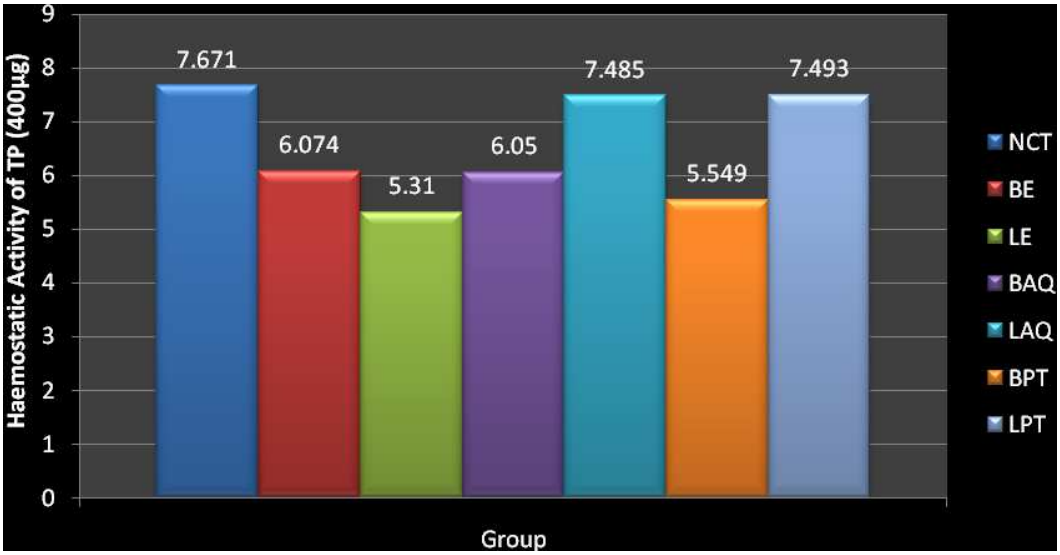
CT- Clotting time, BE- Blank ethanol, LE- Ethanolic extract of leaves, BAQ- Blank aqueous, LAQ- Aqueous extract of leaves, BPT- Blank pet-ether, LPT- Pet-ether extract of leaves.
*Values are expressed as mean±S.E.M. minutes (n = 7).Values are statistically significant at ***P<0.001, **P<0.01, *P<0.05 vs. NCT group (One-way ANOVA followed by Tukey's post hoc test).*

Graph 3: Haemostatic activity of Leaves of *Tridax procumbens* (200µg)



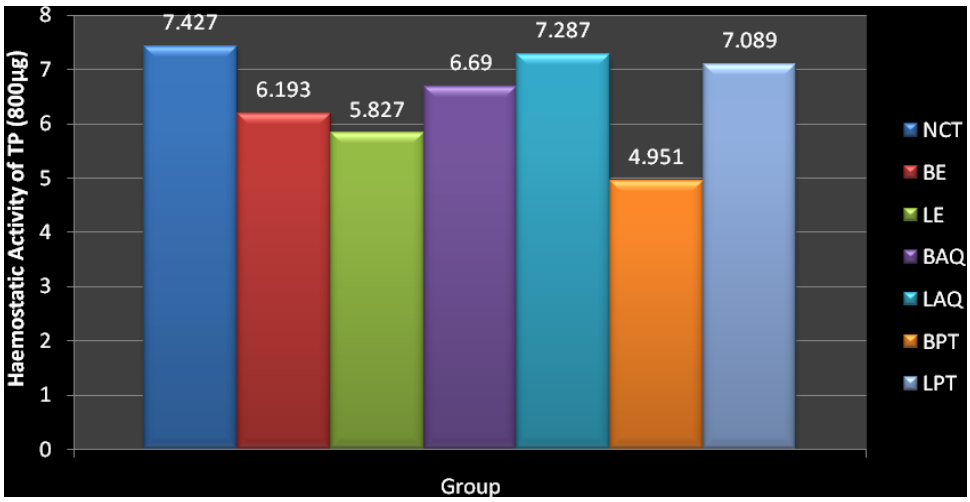
CT- Clotting time, BE- Blank ethanol, LE- Ethanolic extract of leaves, BAQ- Blank aqueous, LAQ- Aqueous extract of leaves, BPT- Blank pet-ether, LPT- Pet-ether extract of leaves.
*Values are expressed as mean±S.E.M. minutes (n = 7).Values are statistically significant at ***P<0.001, **P<0.01, *P<0.05 vs. NCT group (One-way ANOVA followed by Tukey's post hoc test).*

Graph 4: Haemostatic activity of Leaves of *Tridax procumbens* (400µg)



CT- Clotting time, BE- Blank ethanol, LE- Ethanolic extract of leaves, BAQ- Blank aqueous, LAQ- Aqueous extract of leaves, BPT- Blank pet-ether, LPT- Pet-ether extract of leaves.
*Values are expressed as mean±S.E.M. minutes (n = 7).Values are statistically significant at ***P<0.001, **P<0.01, *P<0.05 vs. NCT group (One-way ANOVA followed by Tukey's post hoc test).*

Graph 5: Haemostatic activity of Leaves of *Tridax procumbens* (800µg)



CT- Clotting time, BE- Blank ethanol, LE- Ethanolic extract of leaves, BAQ- Blank aqueous, LAQ- Aqueous extract of leaves, BPT- Blank pet-ether, LPT- Pet-ether extract of leaves.
*Values are expressed as mean±S.E.M. minutes (n = 7) .Values are statistically significant at ***P<0.001, **P<0.01, *P<0.05 vs. NCT group (One-way ANOVA followed by Tukey's post hoc test).*

Table 1: Proximate analysis of dried leaves of plant *Tridax procumbens*

S. No	Parameter	(% W/W) (Mean)
1	Water soluble extractive value	28.8 %w/w
2	Alcohol soluble extractive value	6.9%w/w
3	Acid insoluble ash	3.05%w/w
4	Water soluble ash	2.41%w/w
5	Total ash value	11.88%w/w
6	Sulphated ash value	22.5%w/w
7	Moisture content	13%w/w

Table 2: Preliminary phytochemical evaluation of various successive extracts of dried leaves of *Tridax procumbens*

S. No	Chemical Tests	Extracts Leaves		
		PETPL	ETPL	AQTPL
1	Test for Carbohydrate	+	+	+
2	Test for Protein	-	-	-
3	Test for Tannins	+	+	+
4	Test for Flavonoids	+	+	+
5	Test for Glycoside			
a)	Borntrager's test	-	+	+
b)	Modified Borntrager's test	-	+	+
6	Tests for Saponin	-	-	-
7	Tests for Alkaloids	+	+	+
8	Test for Steroids	+	+	+
9	Test for mucilage	+	+	+

'+' Presence of Phytoconstituents.'-' Absence of Phytoconstituents.

DISCUSSION

The effect of various extracts (hydro-alcoholic, pet-ether (60-80°c), aqueous) of leaves of *Tridax procubens* on human clotting time was observed in vitro by Lee White Method. After testing on the blood samples of healthy human volunteer having normal blood clotting time it was observed that all dosage range of ethanolic extract of Leaves of *Tridax procubens* significantly reduced the clotting time while Pet ether extract of leaves (LPT) showed activity only with 50 µg and 100 µg doses. On the other hand it was found that aqueous extract of leaves (LAQ) in all doses did not show any effect on blood clotting time of healthy human volunteer in vitro.

Clotting time of all extracts was less than their respective blanks like distilled water, ethanol and Pet Ether by approximately 1 min in blood samples obtained from all the subjects. Also, clotting time of all extracts was less than that for normal clotting time (CT) by 2-3 min in blood samples of all the subjects tested in vitro.

Blood Clotting time determination is a routine laboratory test, carried out to diagnose abnormality in blood clotting time which is due to various factors including Hereditary or Acquired coagulation disorder. Increase in normal clotting time signifies coagulation disorders are treated with drugs having coagulant properties. As ethanolic and pet-ether extracts of the leaves of *Tridax procumbens* reduces the clotting time uniformly in the blood samples of all the subjects, it can be suggested that the same possesses hemostatic activity,

Phytochemical analysis indicated the presence of typical plant constituents such as carbohydrates, alkaloids, tannins, flavonoids, and mucilage in all extracts of leaves. . Anthraquinone glycoside is present in ethanolic as well as aqueous extracts of leaves while steroids is present in all leaves extracts of *Tridax procumbens*. These metabolites are usually responsible for the pharmacological activities of medicinal plants. Tannins , one of the important constituent of plant is responsible for the haemostatic activity which arrest bleeding from damaged or injured vessels by precipitating proteins and thus to form vascular plugs[12]. We may safely assume that the tannins in the extracts partly contribute to the activity since mechanisms other than vascular plugs formation are likely involved.

Statistical Analysis

Results were analyzed using one way analysis of variance (ANOVA) and expressed as Mean ± SEM. Data was further subjected to Tukey's post hoc test and differences between means accepted significant at $P < 0.05$.

CONCLUSION

The different extracts of *Tridax procumbens* have coagulant properties thus, indicating a positive hemostatic effect. However, work needs to be done on toxicity studies to eliminate any dangerous side effects

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