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Content

Hemostatic activity of the leaves of *Tridax procumbens* Linn

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Various extracts of the leaves of *Tridax procumbens* Linn were screened for hemostatic activity by studying the clotting time of 10 human volunteers employing Lee-White's method performed *in vitro*. Out of the ethanolic extract, fresh leaf juice and petroleum extract, ethanolic extract showed positive activity. The experiment was successfully tested for statistical significance by employing the paired *t*-test.

Key words: Hemostatic, clotting time, extracts, paired *t*-test

INTRODUCTION

Tridax procumbens Linn, commonly known as *Ekdandi* and Mexican Daisy, is a native of tropical America. It also grows wildly in tropical Africa, Asia and Australia and is available in all seasons and in most parts of the country (The Wealth of India, 1985). It is a hardy perennial herb belonging to the family Asteraceae (Saxena and Albert, 2005). It possesses a large number of chemical constituents which have been identified and isolated in flowers as well as other aerial parts of the plant. The flavonoids such as luteolins and quercetins are present in flowers (Ali *et al.*, 1968). The aerial parts (except flowering tops) possess various saturated and unsaturated fatty acids (Gadre and Gabhe, 1988). The plant also possesses phytosterols such as beta-sitosterol, campesterol and stigmasterol, which impart an anti-inflammatory property to it (Gadre and Gabhe, 1993). Recently, a new bis-bithiophene named tridbisbithiophene, along with four known terpenoids: taraxasteryl acetate, beta-amyrenone, lupeol and oleanolic acid has been reported to be present in the plant (Ali and Jahangir, 2002). It exhibits antiseptic, insecticidal and hair growth-promoting properties (Chantraine *et al.*, 1998). More commonly, the plant leaves are used to check haemorrhage from cuts, bruises and wounds (Diwan *et al.*, 1982). Since it has a remarkable influence on wound healing, the present study was undertaken to evaluate its hemostatic activity.

MATERIALS AND METHODS

Materials

The leaves of *Tridax procumbens* Linn were obtained locally and dried for 5 days in sunlight. These were crushed and the coarse powder was then subjected to extraction using a Soxhlet apparatus in ethanol and petroleum ether (60-80°C) separately to prepare the respective extracts. The individual solvents were distilled to get the extract concentrates which were dried in a desiccator (Harborne, 1984). Fresh leaf juice was also prepared to study the same activity .

Methods

As a guide to study the hemostatic activity of these leaves, 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 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 the three extracts were screened for their effect on CT by testing on blood samples from the subjects. Venous blood 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, 0.5 ml of solvents were added to make the respective blanks and in the remaining ones, 0.5 ml of extracts - ethanolic extract (I), petroleum extract (II) and fresh leaf juice (III) - were added. All these test tubes were then placed in water-bath at 37°C. Each of the test tube was removed after 3 min and tilted at an angle of 45°C to see whether clotting had taken place. The test tubes in which clotting had not started were returned to the water-bath and examined at 30-s intervals to see if clotting had occurred. The watch was immediately stopped when there was clotting in a particular test tube and the time was noted in minutes. Likewise, CT was recorded for the remaining samples. Out of the three extracts, extract (I) reduced CT while extracts (II) and (III) increased CT considerably than normal. This procedure

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Table 1: Effect of various extracts of *Tridax procumbens* Linn on clotting time

Subjects	Normal CT (min)	BE CT (min)	E CT (min)	BA CT (min)	A CT (min)	BJ CT (min)	J CT (min)
1	5	5.15	>15	4.25	3.3	5.3	8.15
2	5.45	5.3	>15	3	2.05	6.2	9.05
3	5.15	5.2	>15	2.55	1.95	6.05	8.35
Mean ± SD	5.2 ± 0.13	5.21 ± 0.04	15 ± 0.0	3.26 ± 0.50	2.43 ± 0.43	5.85 ± 0.27	8.51 ± 0.27

CT: clotting time; BE: blank for (II) extract; E: (II) extract; BA: blank for (I) extract; A: (I) extract; BJ: blank for (III); J: (III) fresh leaf juice

Table 2: Statistical analysis for hemostatic activity of *Tridax procumbens* Linn by paired *t*-test

Subjects	BA	A	BA-A
	CT (min)	CT (min)	CT (min)
1	4.25	3.30	0.95
2	3.00	2.05	0.95
3	2.55	1.50	0.60
4	3.05	2.15	0.90
5	2.35	1.50	0.85
6	3.00	2.15	0.85
7	1.95	1.05	0.90
8	2.90	2.05	0.85
9	3.15	2.05	1.10
10	2.15	2.00	1.95
Mean ± SD	2.83 ± 0.20	1.94 ± 0.19	0.89 ± 0.04

CT: clotting time; SD: standard deviation Readings are statistically significant at $P < 0.01$ by paired *t*-test

was carried out *in vitro*, by drawing blood from 10 human volunteers; five males and five females, to minimize subject variation (Godkar, 1994). The results were confirmed for their statistical significance at $P < 0.01$ by the paired *t*-test (Lachman *et al.*, 1991). Observations of CT obtained from the different extracts are depicted in Tables 1 and 2.

RESULTS AND DISCUSSION

The effect of various extracts of the plant on CT was observed and it was found that fresh leaf juice and petroleum ether extract of the plant leaves showed CT that exceeded the normal CT, and so these were rejected from further studies. After testing on the blood samples of 10 subjects, it was observed that CT was reduced only by ethanolic extract. CT for (A) was less than that for control with ethanol (BA) by approximately 1 min in blood samples obtained from all the subjects. Also, CT for (A) was less than that for normal CT by 2-3 min in blood samples of all the subjects.

To relate between clotting time and hemostatic activity, the process of hemostasis was considered, which serially 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 extravascular as well as intravascular clots, respectively.

Clotting time determination is a routine laboratory test, which is carried out when there is coagulation factor deficiency; for example, deficiency of factor VIII, which causes haemophilia. Increase in normal clotting time thus signifies these deficiencies in coagulation. As the ethanolic extract 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, thus affecting haemostasis (Godkar, 1994).

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