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Research Article

# EVALUATION OF ANTICOAGULANT ACTIVITY AQUEOUS AND ETHENOLIC EXTRACTS AND THEIR ISOLATED PHYTOCHEMICALS OF SOME MEDICINAL PLANTS

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**ABSTRACT**

The aqueous and ethanol extracts of leaves and whole plant of four medicinal plants and their isolated phytochemicals are used for evaluation of anticoagulant activity at the different concentrations. The four medicinal plants such as *Enicostemma littorale, Acheranthus aspera*, *Abutilon indicum* and *Tridax procumbens* were selected for study. Ethenolic extract of *Acheranthus aspera* (leaves), *Tridax procumbens* (whole plant) and aqueous extract of *Abutilon indicum* (leaves) and *Acheranthus aspera* (whole plant) exhibited excellent activity. Moreover isolated phytochemicals were subjected for anticoagulant activity.

**Keywords**: Medicinal plants, Aqueous extract, Ethanol extract, Blood coagulation.

## INTRODUCTION

India has long history for the treatment of various diseases using medicinal plants. Indian plants show widespread bioactivity with minimum side effects[1-2]. In contrast to synthetic compounds, herbal products are safer and hence it is preferred for treatment of various ailments[3]. Across the World, large segment population has accepted traditional remedial system that includes use of phytomedicines obtained from different medicinal plants drugs and cosmetics[4]. 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[3, 5]. 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.*

## MATERIAL AND METHOD

### Collection of plants

*Enicostemma littorale, Acheranthus aspera*, *Abutilon indicum* and *Tridax procumbens* was collected from *Satpuda* Mountain in Maharashtra state of India and used freshly for extraction and isolation of their phytochemicals after grinding and the both extracts stored in the refrigerator and used whenever needed[6-7].

### Extraction and isolation

### Ethanol extract

The crushed plant material was placed in thimble of soxhlet apparatus [8-10] 100g and 50g for whole plant and leaves respectively and extraction carried out by using ethanol as solvent (for 12-14 hrs). The extracts were filtered; ethanol was distilled off using rotary evaporator to remove excess solvent. The residues obtained were uses to prepare different concentrations. 100, 200, and 500 µg/ml concentration were prepared using ethanol as solvent[11].

### Aqueous extract

The 25g of crushed whole plant material was soaked in 25 ml, 50 ml, and 100 ml each in distilled water for 24 hrs. The extract was filtered by using muslin cloth. The final volume were corrected to viz. 25 ml, 50 ml, and 100 ml by washing residue with distilled water[11-12] and used for blood coagulation activity. Same procedure adopted for leaves.

### Isolation of Phytochemicals

The phytochemicals were isolated using literature procedure[13]. Whole fresh plant of 35g *E. littorale* was ground in a mixture and homogenized by methanol: water mixture 4:1 (10 X Wt.) for 5 minute. Then it was filtered and the filtrate was evaporated. 2M $H_2SO_4$ was added and extracted with chloroform yielded terpenoids. Aqueous acid layer was made alkaline with $NH_4OH$ and then extracted with chloroform-methanol (3:1, 60 ml) twice, this extract afforded most of the alkaloids whereas remaining aqueous basic layer was evaporated and extracted with methanol yielded quaternary alkaloids. Similarly phytochemicals of remaining all three plants isolated. During purification of quaternary alkaloids, tannins get separated, it was analyzed.

### Phytochemicals Analysis

The phytochemicals were analyzed and identified using known literature procedure[14-15]. Tannins (A): (200 mg Compound in 10 ml distilled water, filtered); 2ml filtrate + 2ml $FeCl_3$ blue-black precipitate indicated the presence of Tannins. Terpenoids (B): 200mg Compound + 2ml acetic anhydride + Conc. $H_2SO_4$ red coloration of solution indicate the presence of Terpenoids. Alkaloids (C) and Quaternary alkaloids (D): (200mg Compound in 10ml methanol, filtered); a 2ml filtrate + 1% HCl + Steam, 1ml filtrate + 6 drops of Wagner reagent/ Dragendroff reagent, brownish precipitate/ orange precipitate indicated the presence of respective alkaloids.

### Dilution of Phytochemicals

The 10, 50 and 100 µg/ml concentrations of isolated phytochemicals were prepared by dilution with appropriate solvents of each plant.

### Blood coagulation study

Blood samples were collected from healthy volunteers, using a disposable polypropylene syringe, and then anti-coagulated using 3.8% tri-sodium citrate in a polypropylene container (9 parts of blood to 1 part of tri-sodium citrate solution). It was immediately centrifuged at 4000 × g for 15 min, and plasma was separated and pooled. The freshly prepared plasma was stored at 4°C until its use. In a test tube 0.1 ml test plasma and Liquiceilin-E (Tulip Diagnostics Pvt Ltd., India) were added and shaken briefly to mix the reagent and plasma. The tube was placed at 37°C for 20 min for incubation. After the incubation, 0.1ml pre-warmed calcium chloride solution was forcibly added into the mixture of plasma and reagent. To this, one ml of aqueous extracts, ethanol extracts and isolated phytochemicals of four plants was added separately in different concentrations and kept at 37°C. A stopwatch was started to record the coagulation time in seconds. The tube was shaken to mix the contents and it was stopped as soon as the clot formation began. The activity is expressed in term of clotting time ratio in relation to control. The steps were repeated three times for each sample, and average of the test value was noted[4]. Normal saline was used in place of the extracts for the negative control, and 50 mg/ml of

commercial heparin for the positive control[6]. Effect of aqueous and ethanol extracts of whole plant and leaves on Prothrombin time (PT) of normal human plasma shows in table 1 and 2 respectively and result of phytochemicals summarized in table-3

**Table 1: Effect of aqueous extracts on Prothrombin time (PT) of normal human plasma.**

| Plant Species | Concentrations (ml) | Prothrombin time (PT) in Second | |
|---|---|---|---|
| | | Leaves | Whole plant |
| Enicostemma littorale | E-1 | 39.9 | 23.0 |
| | E-2 | 28.3 | 19.4 |
| | E-3 | 22.1 | 15.5 |
| Acheranthus aspera | E-1 | 19.2 | 81.9 |
| | E-2 | 12.7 | 70.2 |
| | E-3 | 10.2 | 65.4 |
| Abutilon indicum | E-1 | 63.1 | 48.1 |
| | E-2 | 59.4 | 32.4 |
| | E-3 | 50.5 | 27.9 |
| Tridax procumbens | E-1 | 44.0 | 41.7 |
| | E-2 | 39.3 | 37.4 |
| | E-3 | 30.5 | 32.2 |
| Heparin | 50 mg/ml | 120.8 | |

E-1: 25 g crushed plant material in 25 ml distilled water.

E-2: 25 g crushed plant material in 50 ml distilled water.

E-3: 25 g crushed plant material in 100 ml distilled water.

**Table 2: Effect of ethanol extracts on Prothrombin time (PT) of normal human plasma**

| Plant Species | Concentrations (in ppm) | Prothrombin time (PT) in Second | |
|---|---|---|---|
| | | Leaves | Whole plant |
| | 100 | 5.8 | 9.8 |
| Enicostemma littorale | 200 | 9.5 | 12.2 |
| | 500 | 12.2 | 16.0 |
| | 100 | 32.4 | 12.2 |
| Acheranthus aspera | 200 | 38.2 | 19.4 |
| | 500 | 40.1 | 22.1 |
| | 100 | 9.7 | 11.5 |
| Abutilon indicum | 200 | 16.9 | 20.6 |
| | 500 | 21.3 | 27.3 |
| | 100 | 26.5 | 20.7 |
| Tridax procumbens | 200 | 31.2 | 26.4 |
| | 500 | 35.4 | 32.5 |

**Table 3: Effect of phytochemicals on Prothrombin time (PT) of normal human plasma**

| Plant species | Concentrations (in ppm) | Prothrombin Time (PT) in second | | | |
|---|---|---|---|---|---|
| | | A | B | C | D |
| Enicostemma littorale | 10 | 10.1 | 11.1 | 9.8 | 05.2 |
| | 50 | 12.9 | 18.4 | 12.4 | 09.0 |
| | 100 | 13.2 | 20.9 | 14.8 | 11.6 |
| Acheranthus aspera | 10 | 10.9 | 7.2 | 11.8 | 08.4 |
| | 50 | 18.6 | 9.7 | 16.3 | 13.9 |
| | 100 | 22.3 | 12.0 | 19.0 | 16.7 |
| Abutilon indicum | 10 | 6.4 | 04.1 | 09.9 | 08.7 |
| | 50 | 8.0 | 06.3 | 15.7 | 10.6 |
| | 100 | 10.4 | 08.9 | 18.0 | 14.2 |
| Tridax procumbens | 10 | 8.7 | 06.6 | 10.9 | 09.9 |
| | 50 | 11.9 | 08.6 | 15.8 | 13.1 |
| | 100 | 15.9 | 10.4 | 20.1 | 15.3 |
| Heparin | 50mg/ml | 120.8 | | | |

**RESULTS AND DISCUSSION**

Pharmaceutical importance of phytochemicals depense up on the positive chemical interactions with microorganisms[6]. Aqueous, ethanol extracts (whole plant and leaves) and isolated phytochemicals from four medicinal plants *Enicostemma littorale, Acheranthus aspera*, *Abutilon indicum* and *Tridax procumbens* were tested for blood coagulation effects in normal human plasma and found to be significantly prolonged the Prothrombin time (PT) of normal human plasma.

In aqueous (Table-1) - whole plant of four plants extracts showed activity at higher concentrations tested. Whole plant extracts of Acheranthus aspera exhibited grater potency, with prolonged clotting time 81.9 sec, 70.2 sec and 65.4 sec at 25 ml, 50 ml and 100 ml concentrations respectively. Whereas, whole plant extracts of plant

Abutilon indicum and Tridax procumbens demonstrated moderate activity. Among four, whole plants extract of Enicostemma littorale exhibited lower activity. This suggests that in the aqueous whole plant extracts, time taken for blood clotting is higher in Acheranthus aspera but slightly lower than the heparin. So, whole plant extracts of Acheranthus aspera shows better anticoagulant activity.

Similarly, in aqueous extracts of leaves of four plants showed activity at higher concentrations. Leaves extracts of Abutilon indicum exhibited grater activity with prolonged the clotting time 63.1sec, 59.4 sec and 50.5 sec at 25 ml, 50 ml and 100 ml concentrations respectively, followed by leaves extracts of Tridax procumbens and Enicostemma littorale. Whereas, leaves extracts of Acheranthus aspera exhibited lower activity.

Similar procedure was adopted for ethanol extracts (Table-2) of whole plant and leaves of four plants at higher concentrations tested. Anticoagulation study of four plants viz, Abutilon indicum, Tridax procumbens, Enicostemma littorale and Acheranthus aspera was carried out. Tridax procumbens extended the clotting time 20.7 sec, 26.4 sec and 32.5 sec at 100, 200 and 500 ppm concentrations respectively. While Abutilon indicum and Acheranthus aspera showed moderate activity. As compare to four plants, Enicostemma littorale exhibited lower activity. In conclusion, Tridax procumbens showed promising anticoagulant activity just lower than the heparin. Moreover Enicostemma littorale demonstrated lower activity in both aqueous and ethanol extract of whole plant. Similarly anticoagulant activity at higher concentrations of ethanol extracts of leaves of four plants viz, Abutilon indicum, Tridax procumbens, Enicostemma littorale and Acheranthus aspera was examined. The results showed that Acheranthus aspera exhibited better activity by extending clotting time viz, 32.4 sec, 38.2 sec and 40.1 sec at 100, 200 and 500ppm concentrations respectively, followed by Tridax procumbens and Abutilon indicum. Whereas Enicostemma littorale showed poor activity.

Table-3 summarized the isolated phytochemicals of four plants tested for anticoagulant activity, four phytochemicals are isolated (A, B, C and D) from each plant.

Compound A- Compound A of Acheranthus aspera exhibited grater potency, with prolonged clotting time by 10.9 sec, 18.6 sec and 22.3 sec at 10, 50 and 100 ppm concentrations respectively. While Tridax procumbens and Enicostemma littorale and compound A of plant Abutilon indicum showed lower activity.

Compound B- Compound B of Enicostemma littorale extended the clotting time by 11.1 sec, 18.4 sec and 20.9 sec at 10, 50 and 100 ppm concentrations respectively, followed by Acheranthus aspera and Tridax procumbens and compound B of plant Abutilon indicum was found to have lower activity.

Compound C- Compound C of Tridax procumbens prolonged the clotting time by 10.9 sec, 15.8 sec and 20.1 sec at 10, 50 and 100 ppm concentrations respectively, as compare to Acheranthus aspera and Abutilon indicum and compound C of plant Enicostemma littorale demonstrated poor activity.

Compound D- Compound D of Acheranthus aspera found to better anticoagulation activity at 8.4 sec, 13.9 sec and 16.7 sec at 10, 50 and 100 ppm concentrations respectively. Tridax procumbens and Abutilon indicum showed satisfactory activity and compound D of plant Enicostemma littorale exhibited lower activity. In comparison among the four isolated compounds from four plants, Compound B of Enicostemma littorale exhibited better anticoagulant activity. In case Acheranthus aspera Compound A showed better anticoagulant activity as compare to its Compound C and D. similarly for Abutilon indicum compound C found to have better anticoagulant activity followed by its Compound D and A. finally compound C of Tridax procumbens showed better anticoagulant activity followed by its Compound D and A.

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