Anticoagulant activity, antibacterial activity and toxicity effect of selected plant in Asteracea family

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Introduction

Traditional herbal medicine is used for maintain the health of people since ancient time. Herbal medicinal related drugs are safe and have fewer side effects. Due to that reason the usage of herbal medicine is popular in all around the world. Medicinal plants play major role in various types of medicinal activities (Ramya *et al*., 2009). Among those treatments medicinal plants have the wound healing activity and anticoagulation activity. Plants have the ability of management and treatment of wounds due to its antibacterial activity (Ukwueze *et al*., 2013). Various types of plant have the ability of anticoagulation and such plants claimed in the traditional system still remain to be scientifically investigated (Narjis, 2013). Among such medicinal plants *Emilia sonchifolia*, *Ageratum conyzoides* and *Mikania micrantha* have various medicinal activities. At the same time *Emilia sonchifolia*, *Ageratum conyzoides* and *Mikania micrantha* show some toxicity effect. Due to that reason it is necessary to identify toxic effect of these plants. Zebrafish eggs are used in toxicological and pharmacological activities due to its small size of fish and transparency of eggs and embryos. Changes in the morphology of development of zebrafish embryos can easily observed. Due to that reason zebrafish eggs is used as a toxicological model to test toxic effect of these three plants (Rahman *et al*., 2012).

The aim of this research is to formulate and evaluate the antibacterial activity against *Staphylococcus aureus* which is one of the major wound infective pathogen, anticoagulation activity and toxicity effect of these three plants.

Methodology

Plants were collected from university premises with the consideration of environmental conditions. Powdered plant materials were extracted with methanol, using Soxhlet apparatus at a controlled temperature. The extracts were concentrated to dryness under reduced pressure using rotary vacuum evaporator at 40°C. Various concentration of extract solution were prepared using stock solutions.

Agar well diffusion method was used to test antibacterial activity. MIC was determined for extracts lowest concentration that showed more than or equal 7 mm diameter growth inhibition zone. Prothrombin Test (PT) was used measure coagulation time for test anticoagulation activity. Zebrasfish eggs were used as toxicological model for test toxic activity of three plants.

Results and Discussion

Highest amount of plant extract were showed by leaves part of each plant while roots were given lowest amount of plant extracts.

Table 01: Percentage of leaves extraction yield obtained from three plants

Plant	Percentage of extraction yield (%)
Ageratum conyzoides	9.17
Emilia sonchifolia	13.75
Mikania micrantha	13.33

In antibacterial activity test leaves extracts of three plants were showed highest inhibition zone and root parts were showed lowest inhibition zone. It means that leaves parts of three plants have highest antibacterial activity. Minimum inhibition concentration was obtained by the concentration which was showed inhibition zone more than or equal 0.7cm.

Table 02: Minimum Inhibition Concentration (MIC) of leaves part of three plants

Plant	MIC(mg/mL)
Ageratum conyzoides	12.5
Emilia sonchifolia	12.5
Mikania micrantha	12.5

Statistical analysis was done by using Minitab 15 version. According to the analysis of variance, there were significant relationship between concentration and plant part with inhibition zone (P-value <0.05). According to the mean value comparison highest inhibition zones were showed by leaves parts of each plant and inhibition zones were increased with concentration. Therefore best combination of each plant was leaves with 200 mg/mL.

Tukey comparison was done to pair wise comparison and it was showed that there were significant difference in between each plant part of three plants (P-value <0.05). Dunnet test was proved that there was no other effective plant part other than leaves part of each plant (P-value <0.05).

In anticoagulation activity test, prothrombin time was measured to test anticoagulation activity of each plant part to the analysis of variance, there were significant relationship between concentration and plant part with prothrombin time (P-value <0.05).

According to the mean value comparison highest prothrombin times were showed by flower of *Ageratum conyzoides*, root of *Emilia sonchifolia* and leaves part of *Mikania micrantha* and prothrombin times were increased with concentration. That means flower of *Ageratum conyzoides*, root of *Emilia sonchifolia* and leaves of *Mikania micrantha* have highest anticoagulation activity.

Tukey comparison was done to pair wise comparison and it was showed that there were significant difference in between each plant part of three plants (P-value <0.05). Dunnet test was proved that there were no other effective plant part other than best part of each plant (P-value <0.05).

Acute toxicity was determined by using whole plant extract of each plant and it was determined by probit analysis using LC_{50} value. LC_{50} value was the concentration that was lethal to 50% of the test embryos. When the LC_{50} value in between 500 µg/ml-1000 µg/ml it was considered as least toxic, in between 100 µg/ml to 500 µg/ml moderate toxic, strong in between 0-100 µg/ml and non-toxic above than 1000 µg/ml.

Table 03: LC_{50} values for selected plants

Plant	Antilog value	LC_{50} value(µg/ml)	Toxicity
Ageratum conyzoides	1.68590	48.5177	Toxic
Emilia sonchifolia	1.64740	44.4017	Toxic
Mikania micrantha	1.78230	60.5759	Toxic

Conclusion

Methanolic extracts of *Emilia sonchifolia, Ageratum conyzoides* and *Mikania micrantha* have antibacterial activity against *Staphylococcus aureus*. Leaves parts of three plants have highest antibacterial activity.Aqueous extract of three plants have anticlotting activity by increasing clotting time. Flower of *Ageratum conyzoides*, leaves of *Mikania micrantha* and root of *Emilia sonchifolia* were showed highest anticlotting activity. At the same time these three plant extracts were showed toxic effect on zebrafish embryos. Further studies are recommended to identify and isolate toxic compounds which are responsible for observed toxic activity before they used as medicine for human beings.

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