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HISTOP: ALTERNATIVE PATCH FOR RAPID ARREST OF BLEEDING FROM LEAVES OF *HIBISCUS ROSA-SINENSIS*

Nur Dayana Hassan Cheong, Nuraini Nafisah Jamaludin, Hanisah Ahmad Shapi'I, Yasmin Suraya Mohd Zan, Iadah Elias, Azlin Sham Rambely and Emida Mohamed

Center of Medical Laboratory Technology, Faculty of Health Sciences, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Puncak Alam Selangor, MALAYSIA

E-mail: dayanacheong@gmail.com

ABSTRACT

Uncontrolled haemorrhage leads to over 30% of trauma deaths worldwide and more than half of those are due to delayed medical attention. HiStop is prepared from the extract of the leaves of *Hibiscus rosa-sinensis* or 'bunga raya' which has been found to harbour procoagulant activity. Besides accelerating the clotting process, the leaves extract of *H.rosa-sinensis* has also been reported to possess antimicrobial activity. Hence, when applied to bleeding wounds, HiStop seals the wound, stops bleeding and also act as a barrier to bacterial infections. Preparation of HiStop started with the leaves of *H.rosa-sinensis* being blended with distilled water. Decoction method was then used to concentrate the crude extract. Four percentages of aqueous extract were prepared at 100.0%, 50.0%, 25.0% and 12.5% using normal saline. Then, procoagulant activity was evaluated *in vitro* by using activated partial thromboplastin time (aPTT) and prothrombin time (PT) tests in triplicates. The result revealed a procoagulant trend with the coagulation time decreasing as the percentages increased. All percentages of aqueous extracts showed very highly significant effect on both aPTT and PT tests with their p value obtained were equal to 0.000. Hence, the aqueous extract from *H.rosa-sinensis* leaves has the potential to be used as a natural haemostatic for treatment of minor bleeding. The extract was then lyophilized and dispensed in 'tea bag' like sachets to be applied directly to wound. It is easy to use, flexible and portable for external usage and acts as a temporary control of bleeding wounds, intended for emergency use.

Keywords: Natural sources, medical utilization, *Hibiscus rosa-sinensis*, blood clotting

1. INTRODUCTION

Trauma is the leading cause of death with uncontrolled hemorrhage being its leading source. In many cases, victims bleed to death before care reaches them while some during transport to appropriate care. Therefore, improving the ability to control hemorrhage represents a major hurdle in reducing trauma mortality. Hence, new techniques, devices, and drugs for hemorrhage control are continuously being developed to be applied across the continuum of trauma care. To be truly efficacious, they should be simple to store and use, and must be rapidly effective. In Malaysian folklore medicine, people have used fresh leaves of *Hibiscus rosa-sinensis* (*bunga raya*) to stop minor bleeding. This is accomplished by rubbing the leaves on the injured site until the leaves produce clear, thick and sticky mucus like liquid which were then left on the wound. However, its ability to arrest bleeding is without scientific evidence. To date, *H. rosa-sinensis* has been reported to harbour antioxidant, antipyretic, and antimicrobial properties [1]. It can also reduce blood pressure and helps in wound healing [2]. Thus, in the present work, its potential as a promoter to coagulation was affirmed and was then developed into a hemostatic patch named HiStop for control of hemorrhage in the earliest phases of care. As a bonus, since the leaves

extract of *H.rosa-sinensis* has been reported to possess antimicrobial activity, it also acts as a barrier to bacterial infections.

2. MATERIAL AND METHOD

2.1. Preparation of extract

Matured green leaves of *H. rosa-sinensis* were collected fresh from its habitat in UiTM Selangor, Puncak Alam Campus, Bandar Puncak Alam, Selangor. The aqueous extract was prepared by blending 1 kg of fresh leaves with 2000 mL of distilled water (1:2 w/v) at room temperature (25 °C). The extract was filtered and concentrated via double boiling method prior to storage in a refrigerator at 2 – 8 °C. Part of it was dissolved in PBS at adequate concentrations for the coagulation assays.

2.2. Coagulation assay

The action of *H. rosa-sinensis* on hemostatic system was studied by evaluating its anticoagulant activity via prothrombin time (PT) and activated partial thromboplastin time (aPTT) assays. Both tests were used to assess the coagulant activity of the aqueous extract from *H. rosa-sinensis* leaves at 0%, 12.5%, 25%, 50% and 100% concentrations. The tests were carried out using commercial reagent kits (r² Diagnostics Inc, South Bend USA) while normal control plasma (r² Diagnostics Inc, South Bend USA) was used as samples. To assure the reliability of the assay kits, tests were conducted first with normal control plasma and all assays were done in triplicates. For statistical analysis, the data obtained were analysed by using SPSS version 24.0. The values were expressed as mean ± standard deviation (SD). One Way Analysis of Variance (ANOVA) was used to compare mean of different percentages of aqueous extract between groups of 0%, 12.5%, 25%, 50% and 100% followed by post hoc Dunnett's test. Value was considered as significantly different when *p* value was < 0.05. From the coagulation assays, it was demonstrated that the *H. rosa-sinensis* extract was able to reduce the clotting time in aPTT and PT tests by up to 1.9 and 3 times, respectively [3]. The observation thus demonstrated its pro-coagulant activity. In both tests, the shortest clotting time was observed at 100% concentration of extract therefore it was then used for subsequent part of the project.

2.3. Preparation of patch

Prior to preparation of the HiStop, the extract was freeze-dried and stored at -2 °C until further use. Approximately 1.0 g of the freeze-dried product was then packed into empty tea bag sachets and placed on commercially available sticky tape to hold the patch in place. The patch is easy to use, flexible and portable for external usage. Further work will involve animal study to access its degree of effectiveness as a temporary control of bleeding wounds, intended for emergency use.

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Kelulusan daripada pihak YBhg. Profesor dalam perkara ini amat dihargai.

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