UNIVERSITI TEKNOLOGI MARA

POTENT ANTICOAGULANT FROM NATURAL AND DRIED BUFFALO LEECHES' EXTRACTS IN PROLONGATION OF BLEEDING TIME

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ABSTRACT

This research was conducted on the buffalo leeches from species of *Hirudinaria* manillensis species) that can be found in river, ponds and paddy fields in Malaysia. Leeches are believed to have a potent component of blood anticoagulant similar to hirudin known as bufrudin. Therefore, the main objective of this study was to optimize the extracts of leeches by monitoring the effects of leeches' characteristic, type of extraction chosen and the strength of anticoagulant from results of haematology test. The research design was starting from the selection of leeches followed by the extraction process and ends with blood coagulation test. Briefly, live leeches have been selected form the farm and are categorized by age and colour; otherwise dried leeches were purchased in bulk without knowing the original characteristic of leeches. The hirudin-like compounds were determined from the two main types of extraction processes either by natural (live) or dried leeches. Both types of leeches were extracted under certain controlled parameters. Natural extraction of live leeches is involved only in part of the salivary gland through the bathing or the force-vomited. Meanwhile, dried-leeches extraction involves the process of sonication, boiling or soxhlet using hexane. All extracts were collected for haematology test and several support analyses of HPLC, the concentration of total protein and comparison with other anticoagulants. Overall results of natural extraction have shown that leech's colour or pattern selection is the most influence factor in increasing the strength of anticoagulant, besides age and size. The most significant results were seen from the forced-vomited extracts where the leech at the age more than 8 months and green colour has shown the finest number of prothrombin time (PT) and activated partial thromboplastin time (aPTT) with high total protein concentration. Otherwise for dried-leeches extraction, the soxhlet extraction is most preferable method where their analysis shows satisfaction in any approach before and after extraction process compared to the boiling and sonication. This study also verified that refining or preservation process using solvents is not recommended unless required for the certain purposes because the solvents itself has disturbed the coagulation time in the blood system. The extracts is then compared with other anticoagulants which has been estimated the equivalent strength to hirudin by utmost 1827 ATU/gm for dried leeches and 9850 ATU/gm for natural leech; while the strength of activity equality to heparin below than 10.6 U/mL; and lastly compared rivaroxaban in highest concentration equivalent to 286.6 µg/L for dried leeches; and $6162 \mu g/L$ for natural leech. Some recommendations were stated to develop better research in the future.

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