QUALITATIVE AND QUANTITATIVE EVALUATION OF BISINDOLE USAMBARANE ALKALOIDS IN *STRYCHNOS USAMBARENSIS* ROOTS BY HPLC.

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The roots of the African plant *Strychnos usambarensis* Gilg contain a number of alkaloids which form the usambarane group (usambarensine, 3',4'dihydro-usambarensine and their N_b-methylated derivatives) [1]. Some of these alkaloids have been tested on protozoa such as *Plasmodium falciparum, Entamoeba histolytica*, and *Giardia intestinalis*. The results [2,3] showed antiplasmodial properties for 3',4' dihydrousambarensine, antigiardial and antiamoebic properties for usambarensine. Antimitotic activities [4,5] have also been described for these alkaloids. There is no report on an HPLC method allowing the separation, identification and quantitation of bisindolic (usambarane) alkaloids. The present work describes a reversed phase HPLC procedure which enables the qualitative and quantitative evaluation of these alkaloids.

The crude extracts were prepared by methanolic and ethyl acetate extraction. Standard alkaloid solutions were prepared with MeOH. The chromatograph was equipped with a diode-array detector operating at 254 nm. The study was carried out on a RP-8 select B column and the mobile phase was composed of acetonitrile and acetate buffer (pH 3,5). The use of a linear gradient (20 to 35 % of MeCN) was required to have a complete separation in a single chromatographic run. Alkaloids in the sample were identified by characteristics ultraviolet spectra [1] given by the diode-array detector and by relative retention times. In order to find the optimum conditions for extracting bisindole usambarane alkaloids from the roots of *S. usambarensis,* extracts were realised with ethyl acetate and methanol. HPLC analysis of the extracts revealed a higher value of the peak areas for usambarensine and particularly N_b-methylusambarensine in the methanolic extracts. In order to demonstrate the repeatability of the HPLC method, the level of the three major bisindolic alkaloids, dihydrousambarensine, usambarensine and methyl-usambarensine, was analysed in six methanolic extracts of the roots. The major alkaloids found in the roots were Nb-methylusambarensine and usambarensine. Three calibration graphs were prepared. The resulting curves were linear over the range 10 - 100 µg/ml and the correlation coefficients were near 0,999.

In conclusion, the described HPLC method could be used as a rapid and sensitive method for identification, separation and dosage of different compounds of the usambarensine class.

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This work was supported by the National Fund for Scientific Research of Belgium (grant number 3.4519.97) whose M.F. is a research aspirant.